Phyton-International Journal of Experimental Botany
DOI:10.32604/phyton.2020.011979
Review

Flesh Color Diversity of Sweet Potato: An Overview of the Composition, Functions, Biosynthesis, and Gene Regulation of the Major Pigments

Hanna Amoanimaa-Dede, Chuntao Su, Akwasi Yeboah, Chunhua Chen, Shaoxia Yang, Hongbo Zhu* and Miao Chen*

Department of Biotechnology, College of Agricultural Sciences, Guangdong Ocean University, Zhanjiang, 524088, China
*Corresponding Authors: Hongbo Zhu. Email: tdzhu@126.com; Chen Miao. Email: czchenmiao@126.com
Received: 08 June 2020; Accepted: 31 July 2020

Abstract: Sweet potato is a multifunctional root crop and a source of food with many essential nutrients and bioactive compounds. Variations in the flesh color of the diverse sweet potato varieties are attributed to the different phytochemicals and natural pigments they produce. Among them, carotenoids and anthocyanins are the main pigments known for their antioxidant properties which provide a host of health benefits, hence, regarded as a major component of the human diet. In this review, we provide an overview of the major pigments in sweet potato with much emphasis on their biosynthesis, functions, and regulatory control. Moreover, current findings on the molecular mechanisms underlying the biosynthesis and accumulation of carotenoids and anthocyanins in sweet potato are discussed. Insights into the composition, biosynthesis, and regulatory control of these major pigments will further advance the biofortification of sweet potato and provide a reference for breeding carotenoid- and anthocyanin-rich varieties.

Keywords: Anthocyanin; biosynthesis; carotenoid; flesh color; sweet potato

1 Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam.] is a dicot perennial *Convolvulaceae* plant cultivated as an annual crop. The main areas of production include Africa, Asia, and the Pacific with Central and South America as its center of origin [1,2]. It is an economically important food crop ranking seventh with regards to global production, mostly used as an energy source, animal feed, staple food, raw material for industries, and alcohol production [3–5]. As a multifunctional food crop, its enlarged edible storage root and leaves contain considerable amounts of essential nutrients including minerals, vitamins, carbohydrate, and dietary fiber in addition to other extra-nutritional components such as caffeoylquinic acids, anthocyanin, and carotenoids [6,7]. Sweet potato is genetically diverse and highly heterozygous because of the large chromosome number, polyploidy (2n = 6x = 90), and mating systems (outcrossing and self-incompatibility) [6,8].

The numerous sweet potato varieties are distinguished by their flesh and skin colors (white, yellow, orange, and purple). Variations in the flesh color of the diverse sweet potato varieties are attributed to the pigments produced and the phytochemical composition of the storage root [9,10]. For example, the

orange- and purple-fleshed varieties of sweet potato accumulate varied compositions of carotenoid and anthocyanin resulting in the orange and purple colors respectively. The other colors have little to no pigments resulting in the lighter colors. The main sweet potato pigments are hence anthocyanin and carotenoids, both known to function as antioxidants with many beneficial effects on both plants and animals. With the orange- and purple-fleshed varieties of sweet potato being the major accumulators of carotenoid and anthocyanin, pigment studies focus on these two types with little information available for the other colors. The nutritional composition, cultivar adaptability, extra-nutritional constituents, and morphological traits may also influence varietal differences [11].

Carotenoids and anthocyanin are the main natural pigments in sweet potato storage root responsible for the orange and purple colors respectively. These pigments are known for their antioxidant activity which scavenges free radicals and offers protection against many age-related degenerative diseases and other chronic disorders [12–14]. For instance, the purple- and orange-fleshed sweet potato varieties are reported to provide varied health-promoting functions which are attributed to their high anthocyanin and carotenoids contents [15,16]. In plants, these pigments prevent photo-oxidative damage, facilitate pollination and seed dispersal, and offer protection against various abiotic stress [17–19]. Due to the versatile functions of carotenoids and anthocyanin in the food, cosmetic, pharmaceutical industries, and in human health, biosynthesis, regulatory control, and accumulation have been of research focus. The unique composition of these natural pigments and the many health benefits make it worth studying.

As reviewed in Amoanimaa-Dede et al. [20], anthocyanins are biosynthesized in the phenylpropanoid pathway. The synthesis primarily occurs in the cytoplasm from where they are transported to the vacuole in either of the three proposed mechanisms; glutathione S-transferase, membrane and vesicles transporters for onward sequestration to form colored pigments in diverse plant tissues [21,22]. The MBW complex (MYB-bHLH and WD40 protein) regulates anthocyanin biosynthesis [23]. On the other hand, carotenoids are synthesized in the chloroplasts and chromoplasts [18] through the methylerythritol-4-phosphate (MEP) pathway and accumulated in the plastid [24]. Several genes such as PSY, PDS, LCY-ε, CHY-β, and LCY-β [18,25–27] regulate carotenoid biosynthesis in sweet potato whereas its accumulation is controlled by the *IbCCD1*, *IbCCD4*, and *IbOr* genes. Though the molecular mechanism underlying the biosynthesis, gene regulation, and accumulation of these pigments have been studied extensively in sweet potato, it remains slightly implicit.

Focusing on recent researches, this review updates current knowledge on these natural pigments emphasizing the regulatory control of genes involved in the biosynthesis and accumulation of these all-important sweet potato pigments. This will further facilitate understanding and provide better strategies for breeding carotenoid- and anthocyanin-rich sweet potato varieties.

2 Diversity of Sweet Potato Varieties

In addition to genetic diversity, sweet potato varieties are diversified in terms of flesh and skin colors, size, shape, texture, and taste of the storage root [28,29]. The color, width, thickness, and shape of leaves may also distinguish the various sweet potato varieties [30]. The inside (flesh) and skin colors are mainly white, cream, yellow, orange, pink, red, and purple (Fig. 1) with their diversity attributed to their varying nutritional and phytochemical components [9,10]. The levels of these components determine the flesh color and conditions such as storage, extraction method, processing techniques, and analysis may affect their compositions [31]. Thus, the darker the color, the higher the amount of pigment it contains. The yellow- and cream-fleshed varieties contain phenols and β-carotenes whereas the red-fleshed has anthocyanins that greatly influence their flesh colors but their relative quantities are incomparable to that of the orange and purple-fleshed varieties which accumulate high levels of carotenoids and anthocyanin respectively, influencing their antioxidant properties [16]. Hence, the orange and purple-fleshed varieties are the main focus of this review due to the high carotenoid and anthocyanin contents. These pigments are considered

as the main pigments in sweet potato because of their high antioxidant properties and the many beneficial effects on human health. In summary, sweet potato is highly diversified due to its varied flesh and skin colors attributed to the different phytochemical components.

Figure 1: Different flesh colors of sweet potato: a. White-fleshed; b. Purple-fleshed; c. Yellow-fleshed d. Orange-fleshed

2.1 Orange-Fleshed Sweet Potato

Orange-fleshed sweet potato is a nutrient-rich crop with appreciable amounts of carotenoids which provides the characteristic orange color. It is a cheap source of dietary antioxidants with many physiological functions including anti-inflammation, anti-mutagenic, anti-cancer, anti-oxidation, anti-diabetic, and cardiovascular disease prevention properties [14,32].

Carotenoids including α- and β-carotenes have been identified in orange-fleshed sweet potato but the amount of β-carotene is relatively higher compared to other carotenoid-rich fruits and vegetables such as carrot, mango, and tomato [33–36]. For example, high β-carotene content of about 20–30 mg/100 g and 276.98 μg/g has been recorded in orange-fleshed sweet potato [37–39]. However, the amount of vitamin A correlates with the color intensity of sweet potato, thus, the darker the orange color, the higher the β-carotene content. β-carotene has potent pro-vitamin A activity which the body converts to vitamin A. Vitamin A promotes health by boosting the immune system, improving overall skin and eye health, and for good vision [40]. About 100–150 g of orange-fleshed sweet potato may provide the daily Vitamin A needs of children and prevent night blindness [41]. Studies have revealed that the consumption of a medium-size orange-fleshed sweet potato can double the required daily needs of vitamin A. The retention capacity of about 80% β-carotene in boiled orange-fleshed sweet potato remains unmatched [42], hence described as a "superfood" that promotes health [43,44].

As a food security crop, the orange-fleshed sweet potato could supplement as an alternative source of staple food in areas with increasing population and nutritional deficits and for resource-poor farmers [45]. Hence, this biofortified food crop with a good supply of vitamin A can serve as a beacon of hope to battle vitamin A deficiency in underdeveloped countries and also scuffle malnutrition in rural communities [46]. In consequence, the orange-fleshed sweet potato has been incorporated into the vitamin A deficiency prevention program in Africa [47] due to its cheap source of vitamin A.

2.2 Purple-Fleshed Sweet Potato

Purple-fleshed sweet potato is a nutritious crop with high levels of anthocyanin which provides its distinctive skin and flesh colors. Anthocyanin is a natural hydro-soluble pigment that provides the purple, red, and blue coloration of flowers, leaves, fruits, and other plant parts. The purple and red coloration of the leaves, stem, and storage roots of sweet potato results from the accumulation of acylated anthocyanins

[48]. Peonidin and cyanidin, acylated with either hydroxybenzoic, ferulic, or caffeic acids are the main anthocyanins among the 39 anthocyanins identified in purple-fleshed sweet potato [11,30]. In recent years, anthocyanins from purple-fleshed sweet potato have extensively been studied due to their potential beneficial health effects on humans. Purple-fleshed sweet potato anthocyanin has good bioactivity and scavenges free radicals [1] contributing to its diverse biological and antioxidant activities. The antioxidants also act as a good protective agent against inflammations, cancers, diabetes, tumors, and hypoglycemia [49–51]. Furthermore, the anthocyanin from purple-fleshed sweet potato has high heat and light stability owing to its acylated forms, hence used as natural food additives [52]. Purple-fleshed sweet potato anthocyanin reduced inflammations caused by oxidative stress and decreased oxidative stressors confirming its free radical scavenging ability, thus, the strong antioxidant ability of purple-fleshed sweet potato [16]. The robust anti-mutagenic properties of the purple-fleshed sweet potato anthocyanin attributed to its radical scavenging activity decreased the risk of hypertension and liver injury in rats [53]. Again, the purple-fleshed sweet potato anthocyanin was revealed to have resilient anti-microbial and anti-inflammation properties in addition to its ability to protect against colorectal cancer, UV light, and reduce memory loss [49]. The daily intake of 400 mg beverage prepared from purple-fleshed sweet potato protected the liver from oxidative stresses [54]. Hence, the many physiological activities of purple-fleshed sweet potato make it a health-promoting functional food.

3 Major Pigments in Sweet Potato

Pigments are mainly produced by plants and are responsible for the color variations observed in many plant tissues. Natural pigments accumulate in different plant parts including flowers, fruits, leaves, and stems. Generally, these natural pigments provide several physiological activities with many beneficial health effects [55]. The relative quantity of pigments accumulated mainly determines the colors produced by the various plant tissues. However, the concentration of pigments correlates directly to the color intensity [56]. The storage root of sweet potato is the main repository organ of natural pigments that provide the different flesh colors (white, yellow, orange, and purple) compared to other crops [57].

Carotenoids and anthocyanins are the major sweet potato pigments which provide the yellow, orange, and purple colors. These pigments are synthesized through different metabolic pathways with different structural and regulatory genes regulating their biosynthesis and accumulation. They also accumulate in different sweet potato genotypes, the orange- and purple-fleshed respectively. As a result, it may be likely to observe no form of interaction between their biosynthesis and accumulation. However, there is evidence of transgenic sweet potato accumulating both color pigments in a single storage root. For instance, Park et al. [58] produced a dual-pigmented transgenic sweet potato through *Agrobacterium*-mediated transformation. The transgenic plants expressing the *IbMYB1* gene (a key regulator of anthocyanin biosynthesis in sweet potato), accumulated high levels of both anthocyanins and carotenoids in a single storage root. Overexpression of the gene slightly increased most carotenoid biosynthetic genes, such as phytoene desaturase, lycopene ε-cyclase, zeta-carotene desaturase, and lycopene β-cyclase in transgenic plants than in control plants, suggesting that *IbMYB1* expression might affect carotenoid biosynthesis-related gene expression. The above result indicates a possible interaction in the regulation of carotenoid and anthocyanin biosynthesis but needs to be clarified through further research.

3.1 Carotenoids

Carotenoids are lipid-soluble natural pigments responsible for the yellow, orange, and red colors of flowers, vegetables, seeds, fruits, and roots [59]. They are synthesized only in photosynthetic organisms including plants, some bacteria, fungi, and algae. Carotenoids are the most abundant of all plant pigments [60]. The storage root of sweet potato has an excellent supply of carotenoids including β-cryptoxanthin, violaxanthin, β-carotene, lycopene, and zeaxanthin [26,61] among which β-carotene is the main

carotenoid with the highest pro-vitamin A activity both in terms of widespread distribution and bioavailability [47,57]. Sweet potato leaves have an adequate supply of carotenoids including lutein, neoxanthin, β-carotene, and violaxanthin [19] with the composition equivalent to other plant chloroplasts [62]. Research evidence revealed that orange-fleshed sweet potato has high β-carotene content, thus about 80% of the carotenoids being *trans*-β-carotenes [63,64]. However, other studies also reported the presence of lutein and zeaxanthin in the orange-fleshed sweet potato which provides the orange color [65]. Inclusion of orange-fleshed sweet potato in the diet provides dietary pro-vitamin A due to the high β-carotene content [19], hence used as a model crop for many small-scale research to increase vitamin A status [45,66]. The carotenoids identified in orange-fleshed sweet potato with their concentrations reported by different authors have been presented in Tab. 1.

Table 1: Carotenoids in orange-fleshed sweet potato reported by different authors

Carotenoids		Quantity (μg/g)	Reference
Total carotene		61.77 (db)	[34]
Carotenes	α-carotene	13.11 (db)	[34]
		1–15 (fw)	[67]
	β-carotene	48.66 (db)	[34]
		44.9–226 (fw)	[16]
		34.6–83.3 (db)	[68]
		0.96–13.6 (fw)	[42]
		20–364 (db)	[37]
		132–194 (fw)	[69]
		6.2–231 (fw)	[70]
		67–131 (fw)	[67]
		85.36–177.16 (fw)	[71]
		67.33–315.71 (db)	[37]
Xanthophylls	β-cryptoxanthin	21.2 (db)	[36]
		0.66–2.0 (fw)	[72]
	Lutein	120–148 (fw)	[73]
		1–4 (db)	[65]
		0.2–5.48 (fw)	[72]
	Zeaxanthin	242–2,055 (fw)	[73]
		1–2 (db)	[65]
		15–155 (fw)	[42]
		41.7–251 (db)	[33]
		19.31–61.94 (fw)	[74]
		570 (db)	[36]
		5.5–72.4 (fw)	[75]
	Violanxanthin	0.9–6.8 (fw)	[72]

*fw: fresh weight *db: dry weight

3.1.1 Composition of Carotenoids

Carotenoids are the most prevalent class of isoprenoid pigments synthesized by plants, algae, bacteria, and fungi that undergo photosynthesis [76,77]. Carotenoids are synthesized from C_{40} isoprenoids which consist of 3 to 15 conjugated double bonds of polyene chains. At the molecule center of these compounds are eight inverted isoprenoid units. All carotenoids are byproducts of lycopene ($C_{40}H_{56}$) undergoing series of reactions including cyclization, methyl migration, hydrogenation, double-bond migration, oxygen insertion, dehydrogenation, chain shortening, and/or chain elongation [55,78]. Chemically, carotenoids are classified into two classes; xanthophylls and carotenes based on their functional groups. Carotenes are hydrocarbons made up of carbon and hydrogen (examples are β-carotene and lycopene) and oxycarotenoids (xanthophylls) originally known as phylloxanthins consist of carbon, hydrogen, and oxygen (example: lutein and zeaxanthin) [79]. Carotenoids may also be characterized as primary and secondary carotenoids. Primary carotenoids are needed by plants for photosynthesis e.g., α-carotene, β-carotene, and neoxanthin whereas secondary carotenoids are confined in flowers and fruits e.g., zeaxanthin, β-cryptoxanthin, antheraxanthin, and violaxanthin [55]. The over 700 carotenoids identified and characterized in nature are synthesized by almost 20 biosynthetic enzymes out of which only fifty (50) carotenoids have pro-vitamin A activity [55,80]. Research evidence suggests that the β-branch carotenoids including, β-carotene, violaxanthin, zeaxanthin, and β-cryptoxanthin are the mainstream carotenoids in sweet potato [18]. Fig. 2 shows the structures of the carotenoids identified in orange-fleshed sweet potato.

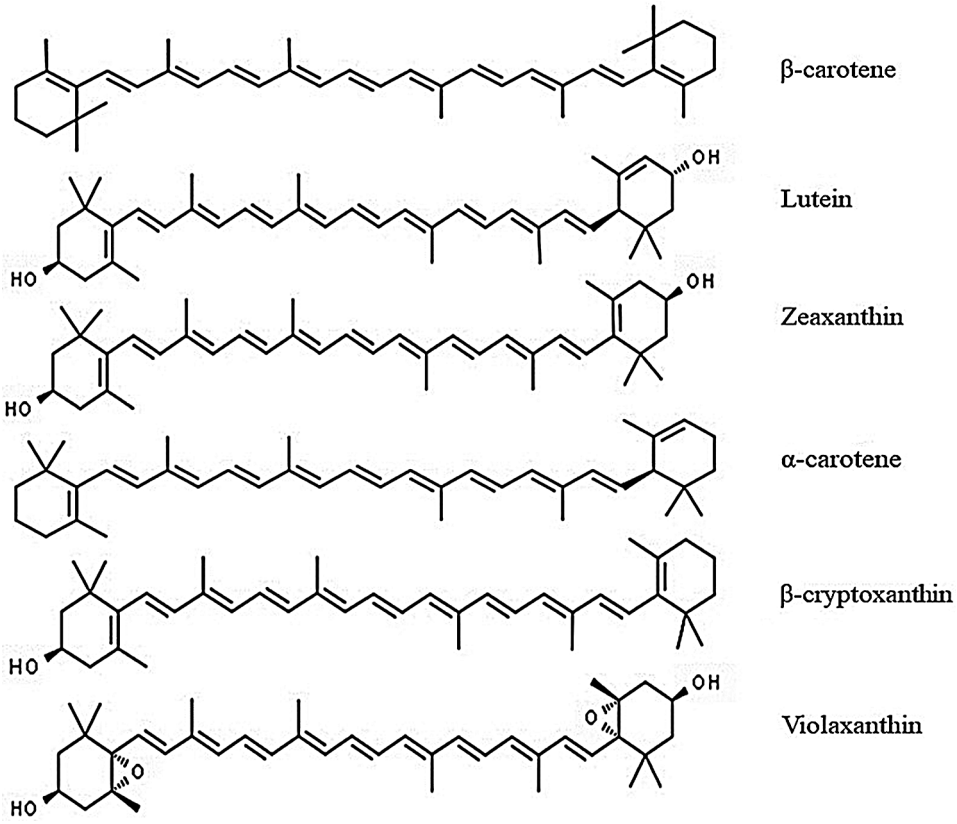

Figure 2: Structures of common carotenoids in orange-fleshed sweet potato

3.1.2 Functions of Carotenoids

Carotenoids are naturally occurring pigments prevalent in plants with their accumulation in flowers, fruits, and other plant parts, giving those parts the yellow, orange, and red colors [59]. Carotenoids are mostly found in the chloroplast and chromoplast with several functions in both plants and animals. In plants, these molecules serve as accessory pigments for harvesting light in photosynthetic reaction sites and inhibit photo-oxidative damage to cells and tissues. Mostly, they combine with chlorophyll to absorb blue-green wavelengths, thereby protecting cells from superfluous light, increase tolerance to herbicide and salt stress and also safeguard photosynthetic apparatus from photo-oxidative damage [18].

Generally, exposure to stress leads to the production of free radicals by reactive oxygen species (ROS) causing oxidative damage which is mostly inhibited by antioxidants [81,82]. Carotenoids exhibit antioxidant properties that inhibit the harmful effects of various environmental stresses such as high temperature, resilient light, ultra-violet radiation, and drought in plants [83,84]. This ability was reported in genes that code for carotenoid. For instance, *IbPSY1*, a carotenoid gene was reported to be crucial in plants' resistance to abiotic stress *in vivo*. In some plant species (daffodil, maize, potato), the upregulation of the *PSY* gene was reported to highly increase carotenoid levels [11], this then explains the ability of the *IbPSY1* gene to increase tolerance to environmental stress including drought, salinity, and high/low temperature which may negatively affect growth and yield of sweet potato. Another gene observed to improve sweet potato resistance to environmental stress is *IbOr*, a gene involved in carotenoid accumulation, and its overexpression improves resistance to heat stress and oxidative damage [19,85]. These genes can therefore be beneficial for engineering plants with improved tolerance to abiotic stress and can be proposed to be essential in the defense response mechanism of plants to abiotic stresses. Similarly, the upregulation of the *IbOr* gene in transgenic potato and alfalfa increased tolerance to certain abiotic stress such as salinity, drought and heat stress [86,87].

The brightly colored parts of plants resulting from carotenoid accumulation, aids in pollination and seed dispersal by attracting pollinators and other agents of dispersal [19]. Similar antioxidant properties of carotenoids exhibited in plants have also been observed in animals that feed on plants with carotenoids. Carotenoids have positive impacts on human health as they are sources of dietary antioxidants which reduces the risk of many age-related illnesses including macular degeneration, cancer, and cardiovascular diseases [2,18]. Recently, orange-fleshed sweet potato has been on the research spotlight due to the high carotenoid content particularly β-carotene.

Orange-fleshed sweet potato is considered among the main sources of vitamin A to animals and humans that cannot synthesize vitamin A but can only obtain them through their diet [88,89]. Owing to that, several studies recommend the daily intake of orange-fleshed sweet potato which helps increase the levels of vitamin A and improve general well-being [90,91]. For instance, the daily intake of orange-fleshed sweet potato significantly elevated the vitamin A status of men, women, and children in some developing nations including Bangladeshi, Kenya, and Mozambique respectively [45,66,90]. Orange-fleshed sweet potato has also been revealed to be used to prevent blindness and maternal mortality resulting from vitamin A deficiency in most developing countries [92]. This then explains the incorporation of sweet potato as an excellent food source to fight vitamin A deficiency especially in underdeveloped countries, hence, its integration in the vitamin A deficiency prevention program [93].

Carotenoids are also used as additives to improve pigmentation and to prevent UV radiation damage. In ornamental fishes, dietary astaxanthin, a carotenoid is used on commercial bases as a natural colorant [94] and also used to improve the pigmentation of egg yolks [95]. Humans have also benefited from the photo-protective properties of carotenoids through their inclusion in cosmetic products to impede damages from UV radiation.

3.1.3 Carotenoid Biosynthetic Pathway

The pathway for carotenoid biosynthesis together with its metabolic enzymes in higher-order plants is well elucidated using standard biochemical analyses such as specific inhibitors, mutant characterization, and

labeled precursors [92]. Though the biosynthesis of carotenoids differs from one species to the other, all photosynthetic florae and algae share a common metabolic pathway. Carotenoids are synthesized in the chloroplasts and chromoplasts [18] through the methylerythritol-4-phosphate (MEP) pathway (Fig. 3) and are metabolized and accumulated in the plastid [24]. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are catalyzed by geranylgeranyl pyrophosphate synthase (GGPS) to produce two geranylgeranyl diphosphate (GGPP) molecules. Phytoene synthase (PSY) catalyzes the condensation of the two GGPP molecules to produce phytoene, the first C_{40} carotenoid in the pathway. Phytoene consists of three conjugated double bonds and its chemical diversification causes variations in carotenoids [96]. Subsequently, the addition of conjugated bonds by ζ-carotene desaturase (ZDS) and phytoene desaturase (PDS) yields lycopene from phytoene. Here a two-branched pathway is produced: the α-branch pathway which converts α-carotene to lutein and the β-branch pathway where β-carotene is converted to neoxanthin [97,98]. Further modifications of the α- and β-carotene by hydroxylation, ketolation, epoxidation, glycosylation, and oxygen cleavage reactions provide a range of structural features [99]. However, the merging of the polar groups (epoxy, keto, and hydroxyl) may biologically alter the functions of carotenoids [100]. CHY-ε (α-carotene ε-ling hydroxylase) catalyzes the production of lutein in the α-branch pathway whereas CHY-β (β-hydroxylase) catalyzes the hydroxylation of β-carotene to produce zeaxanthin in the β-branch pathway. Zeaxanthin epoxidase (ZEP) further converts zeaxanthin to violaxanthin. Neoxanthin synthase (NXS) acts on the violaxanthin so formed and converts it to neoxanthin [101].

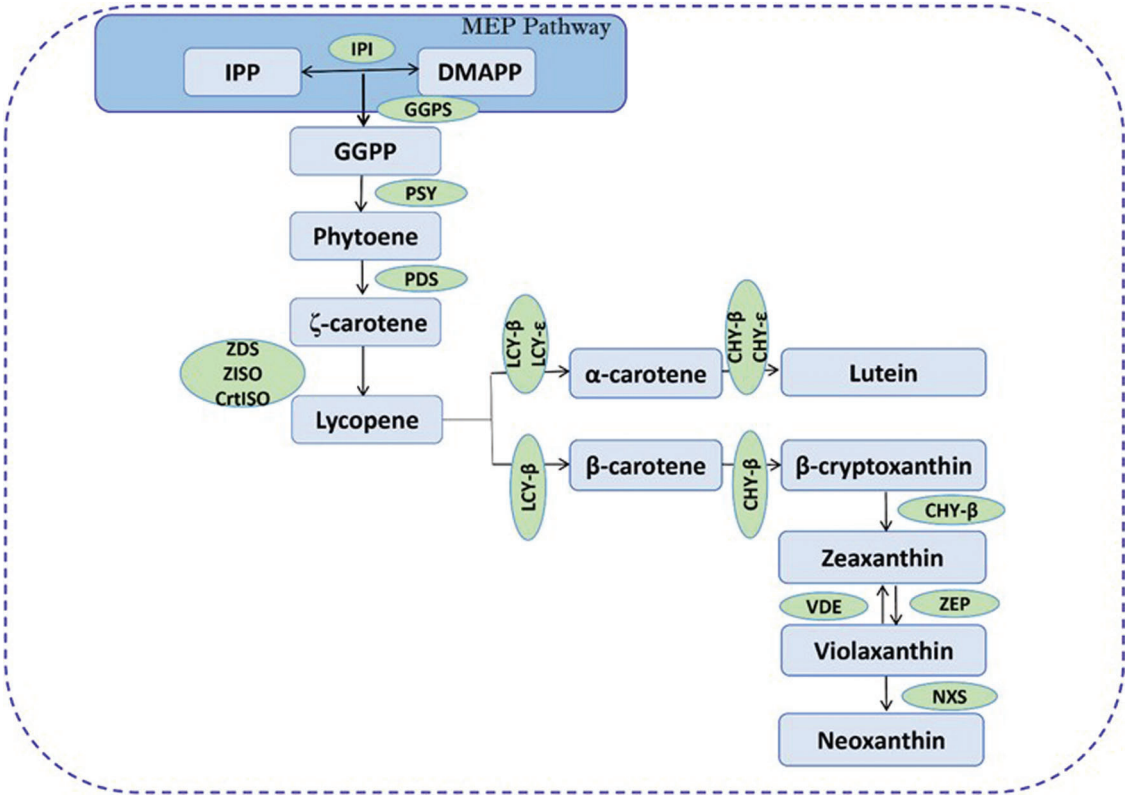

Figure 3: Carotenoid biosynthetic pathway and regulatory enzymes in plants. Adapted from Kim et al. [105]. Names of the regulatory enzyme are abbreviated as follows; MEP: methylerythritol 4-phosphate; LCY-ε: lycopene ε-cyclase; DMAPP: dimethylallyl pyrophosphate; ZISO: 15-cis-ζ-carotene isomerase; GGPP: geranylgeranyl diphosphate; IPP: isopentenyl pyrophosphate; PSY: phytoene synthase; GGPS: geranylgeranyl diphosphate synthase; PDS: phytoene desaturase; IPI: isopentenyl pyrophosphate isomerase; ZDS: ζ-carotene desaturase; VDE: violaxanthin de-epoxidase; CrtISO: carotenoid (pro-lycopene) isomerase; CHY-ε: carotenoid ε-hydroxylase; LCY-β: lycopene β-cyclase; CHY-β: carotenoid β-hydroxylase; ZEP: zeaxanthin epoxidase; NXS: neoxanthin synthase

GGPP is an essential metabolic intermediary and a precursor of tocopherols, diterpenoids, chlorophylls, gibberellins, and carotenoids [102]. The *IbGGPS* gene recently cloned from sweet potato storage root and transformed in Arabidopsis increased tolerance to osmotic stress and the total carotenoid content in *IbGGPS* overexpressing Arabidopsis [27]. In *IbGGPS*-overexpressing plants, α-carotene and lutein (α-branch carotenoids) were upregulated, while levels of zeaxanthin and β-cryptoxanthin (β-branch carotenoids) were significantly reduced. Therefore, the upregulation of *IbGGPS* in sweet potato may perhaps improve the quantity of α-branch carotenoids.

The lycopene cyclase genes, LCY-β, and LCY-ε are involved in the biosynthesis of the branch components of carotenoids in diverse plant species. Regulating the expression levels of these genes (LCY-ε and LCY-β) may affect the relative activity and production of cyclic carotenoid genes associated with lutein synthesis in some plants including rice, Arabidopsis, and tomato [103,104]. Both genes are interrelated in producing α and β branch carotenoids in that, the overexpression of one can suppress the other. LCY-β is reported as a vital enzyme associated with the synthesis of both α- and β-branch carotenoids, like α-carotene and β-carotene.

According to Haskell et al. [26], the LCY-β gene functions to increase carotenoid content, oxidative ability, and resistance to abiotic stress in sweet potato. It also increases the production of β-branch carotenoids including zeaxanthin, β-carotene, violaxanthin, and β-cryptoxanthin. In sweet potato, the downregulation of *IbLCY-ε* in non-embryogenic calli of light orange-fleshed sweet potato cv. Yulmi increased the content of β-branch carotenoids resulting in an orange coloration of the ensuing transgenic calli [25].

Other research has also reported that silencing *IbLCY-ε* or *IbCHY-β* during carotenoid metabolic engineering can result in increased β-carotene and total carotenoid content in sweet potato. According to Kim et al. [18], the suppression of *IbCHY-β* in the catalytic hydroxylation of β-carotene to yield β-cryptoxanthin which is further converted to zeaxanthin in sweet potato increased the β-carotene and the overall total carotenoids content. Based on these results, it can be established that *CHY-β* and *LCY-β* are the primary regulatory enzymes involved in carotenoid biosynthesis in sweet potato making β-carotene the main cellular carotenoid in sweet potato. Though the pathway for carotenoid biosynthesis is well elucidated in all higher plants, it is slightly implicit in sweet potato. Therefore, the pathway for the biosynthetic of carotenoids needs to be further characterized in sweet potato.

3.1.4 Gene Regulation of Carotenoid Biosynthesis

A key determining factor of carotenoid content is the regulation of essential biosynthetic genes [106]. Regulation of these genes and the allelic variation of genes in the biosynthetic pathway may influence the different accumulation levels of carotenoids [107,108]. Fluctuations in the levels of these genes have been associated with the development of some crops with increased carotenoid content. Most of the genes have been reported to be involved in the regulation of the three key processes (biosynthesis, degeneration, and storage) in carotenoid accumulation at different stages of plant growth [109]. Genes coding for practically all enzymes involved in the biosynthesis of carotenoids have been isolated from bacteria, fungi, and plants (Fig. 3) [92]. Carotenoid biosynthetic genes in sweet potato including PSY, GGPS, CrtISO, PDS, LCY-ε, ZDS, ZEP, CHY-β, and LCY-β have been cloned and characterized [18,25–27].

Research evidence on the specific gene that regulates the accumulation of carotenoids in plants is limited. However, the *Or* (orange) gene is a gene of interest and has been researched in several plant species including sorghum, cauliflower, melon, alfalfa, potato, Arabidopsis, and sweet potato. *Orange* denotes an extraordinary group of regulatory genes that facilitate the accumulation of carotenoids which are highly conserved in diverse species and exhibit functions like preserving homeostasis of carotenoids, maintaining photosynthesis, and regulating carotenoid biosynthesis [110,111].

The overexpression of the *Or* gene increased carotenoid accumulation in plants including *Brassica oleracea* var. botrytis [112], *Cucumis melo* [113], *Solanum tuberosum* and *Arabidopsis thaliana* [114]. In sweet potato, the *Orange* gene (*IbOr*) originally cloned from the sweet potato cv. Sinhwangmi (orange-fleshed) based on the *BoOr* sequence, induced the accumulation of carotenoids in various tissues (leaves, stem, and storage root) [25]. However, the gene expression levels vary in different parts of the various sweet potato varieties. It is highly expressed in the storage root of the orange-fleshed varieties whilst in other colored varieties (white, orange, and purple), its expression is highly observed in the leaves [110]. The *IbOr* functions to aid the buildup of carotenoids and regulate carotenoid homeostasis in sweet potato. For instance, the overexpression of *IbOr* increased the carotenoid content in transgenic sweet potato, alfalfa, and potato plants compared to non-transgenic plants [77,86,89]. According to Park et al. [77], *IbOr-Ins* successfully altered a purple-fleshed sweet potato to yield carotenoids and anthocyanin in a single tuber. *IbOr* transformed plants had superior carotenoid content compared to non-transformed plants. However, the levels of carotenoid accumulation correlate with the transcription levels of *IbOr*.

The orange gene has also been reported to interact with PSY and carotenoid cleavage dioxygenases (CCDs). In sweet potato, *IbOr* interrelates with *IbPSY* to enable higher stability of *IbPSY* through the holdase chaperone activity of *IbOr* [85] which offers a substitute and complement strategy for increased carotenoid levels, chromoplast differentiation and PSY stabilization [115]. Apparently, carotenoid catabolism negatively regulates accumulation. In potato, the accumulation of carotenoid was controlled negatively by *CCD1* and *CCD4* [116]. However, the purple-fleshed sweet potato expressing higher levels of *IbOr* also contained increased levels of *CCD1*, *CCD4*, and *NCED* transcripts [77] which proposes the ability of carotenoid catabolism genes (*IbCCD1* and/or *IbCCD4*) to increase carotenoid accumulation in sweet potato. This is however inconclusive and exposes us to the complex mechanism involved in carotenoid accumulation, regulated by the molecular function of the *IbOr* gene. The interrelation between carotenoid accumulation and catabolism needs to be further elucidated.

3.2 Anthocyanins

Anthocyanins are a subclass of flavonoid compounds and an essential water-soluble natural pigment in vascular plants, responsible for the wide-ranging colors in several plant species [117]. They are existent in diverse plant tissues including flowers, fruits, and storage organs like the root and stem. Anthocyanins occur naturally as glycosides of anthocyanidins attached to different sugar moieties [118] and are highly appreciated for their anti-oxidant activities which provide several health benefits such as anti-cancer, anti-inflammatory, anti-diabetic, anti-mutagenic, and cardiovascular diseases prevention properties [119,120].

Purple-fleshed sweet potato mounts up high levels of anthocyanins in their storage roots, with anthocyanin 3-*O*-sophoroside and its derivatives as the major compounds [121]. Anthocyanins from purple-fleshed sweet potato are non-toxic, resource-rich, and unscented bioactive compounds with stable physicochemical properties compared to anthocyanins from other plant sources including cherry, strawberry, and grapes [122]. Because these anthocyanins are acylated, they have high stability against heat and UV radiations, hence used as natural food additives [52]. The various anthocyanins identified by different authors in purple-fleshed sweet potato are summarized in Tab. 2.

3.2.1 Composition of Anthocyanin

Anthocyanin, a major plant secondary metabolite is a subclass of flavonoid compounds made of mono- or di-glycosylated aglycones of anthocyanidins attached to a sugar moiety [123]. The molecular structure of anthocyanin mainly exists as glycosides of poly-hydroxyl or poly-methoxyl derivative of the flavylium (2-phenylbenzopyrylium) cation which consist of a double aromatic ring [A and B], divided by a heterocyclic ring [C] (Fig. 4) [124]. The presence of a positive charge on the C-ring distinguishes anthocyanins from other flavonoids. Anthocyanins are natural plant pigments with varied and complex structures. The structural variation of the various anthocyanins is attributed to the number of the sugar moiety, type of functional group, and the natural acyl group present [49].

Table 2: Structural identification of anthocyanins in purple-fleshed sweet potato by different authors

Anthocyanidin	Structure of anthocyanin	Method of identification	Reference
Cyanidin	Cyanidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside	HPLC-DAD/ESI-MS^2 HPLC-DAD/ESI-MS/MS and LC-MS	[118,119,124,125,127,128]
	Cyanidin 3-caffeoyl sophoroside-5-glucoside	HPLC-DAD/ESI-MS/MS	[119]
	Cyanidin 3-(6″,6‴-dicaffeoyl sophoroside)-5-glucoside	HPLC-MS/MS and HPLC-DAD/ESI-MS/MS	[119,124,128]
	Cyanidin 3-(6″-caffeoyl-6‴-feruloyl sophoroside)-5-glucoside	ODS-HPLC and HPLC-ESI-/MS–MS	[119,123]
	Cyanidin 3-O-(6-O-(E)-caffeoyl-(2-O-(6-O-(E)-feruloyl)-β-D-glucopyranosyl)-β-D-glucopyranoside)	MS and NMR	[121]
	Cyanidin 3-*p*-hydroxybenzoyl sophoroside-5-glucoside	HPLC-ESI-/MS–MS, HPLC-DAD and HPLC-ESI-QTOF-MS/MS	[124,129]
	Cyanidin 3-(6,6′-caffeoyl-*p*-hydroxybenzoyl sophoroside)-5-glucoside	HPLC-MS/MS, HPLC-DAD and HPLC-ESI-QTOF-MS/MS	[124,128,129]
	Cyanidin 3-sophoroside-5-glucoside	ODS-HPLC and HPLC-MS/MS	[123,127]
	Cyanidin 3-caffeoyl-feruloyl sophoroside-5-glucoside	HPLC-DAD and LC-MS	[124,125]
	Cyanidin 3-caffeoyl-vanilloyl sophoroside-5-glucoside	UPLC-PDA and UPLC-QTOF-MS/MS	[112]
	Cyanidin 3-feruloyl sophoroside-5-glucoside	HPLC-MS/MS	[127]
	Cyanidin 3-(6‴-caffeoyl sophoroside)-5-glucoside	HPLC-MS/MS and HPLC-DAD and ESI-MSn	[127,128]
Peonidin	Peonidin 3-caffeoyl sophoroside-5-glucoside	HPLC-PDA-ESI-MS^n, HPLC-DAD/ESI-MS/MS	[117,119,122]

(Continued)

Table 2 (continued).

Anthocyanidin	Structure of anthocyanin	Method of identification	Reference
	Peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside	HPLC-DAD/ESI-MS^2, LC-MS and HPLC-MS/MS	[118,119,122,125,126,127]
	Peonidin 3-(6″-caffeoyl-6‴feruloyl sophoroside)-5-glucoside	ODS-HPLC and HPLC-DAD/ESI-MS2	[118,123]
	Peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside	HPLC-DAD/ESI-MS/MS, LC-MS and HPLC-MS/MS	[119,125,126,127]
	Peonidin 3-caffeoyl-vanilloyl sophoroside-5-glucoside	UPLC-PDA and UPLC-QTOF-MS/MS	[112]
	Peonidin 3-dicaffeoyl sophoroside-5-glucoside	HPLC-DAD and HPLC-MS/MS	[126]
	Peonidin 3-O-(6-O-(*E*)-caffeoyl-(2-O-(6-O-*p*-hydroxybenzoyl)-β-D-glucopyranosyl)-β-D-glucopyranoside)-5-O-(β-D-glucopyranoside) Peonidin 3-O-(6-O-(E)- caffeoyl-(2-O-(6-O-(*E*)- feruloyl)-β-D-glucopyranosyl)-β-D-glucopyranoside)-5-O-(β-D-glucopyranoside)	UPLC-QTOF-MS/MS and ^1H and ^{13}C-NMR	[126]
	Peonidin 3-caffeoyl-*p*-coumaryl sophoroside-5-glucoside	HPLC-MS/MS	[124]
	Peonidin 3-O-(6-O-(E)-caffeoyl-(2-O-(6-O-acyl)-D-glucopyranosyl)-β-D glucopyranosides) Peonidin 3-O-(6-O-*p*-hydroxybenzoyl-(2-O-(6-O-acyl)-D-glucopyranosyl-β-D glucopyranosides) Peonidin 3-O-(6-O-(E)-feruloyl-(2-O-(6-O-acyl)-D-glucopyranosyl-β-D glucopyranosides)	MS and NMR	[121]
	Peonidin 3-sophoroside-5-glucoside	ODS-HPLC and HPLC-MS/MS	[123,127]
Delphinidin	Delphinidin 3, 5-diglucoside	HPLC-PDA-ESI-MS^n	[117]

Table 2 (continued).

Anthocyanidin	Structure of anthocyanin	Method of identification	Reference
Pelargonidin	Pelargonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside	HPLC-MS/MS, HPLC-DAD and HPLC-ESI-QTOF-MS/MS	[127,129]
	Pelargonidin 3-sophoroside-5-glucoside	HPLC-MS/MS	[127]
	Pelargonidin 3-caffeoyl-*p*-coumaryl sophoroside-5-glucoside	HPLC-DAD and HPLC-ESI-QTOF-MS/MS	[129]

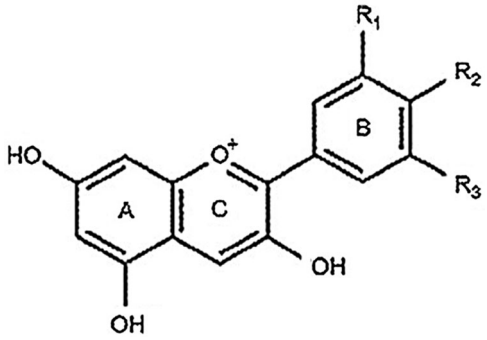

Figure 4: General structure of anthocyanin

The basic anthocyanin structure consists of aglycone base (anthocyanidin), two (2) or three (3) chemical units, sugars, and organic acids as in acylated anthocyanins [125]. Among the over twenty-six (26) anthocyanidins discovered in nature, only six (6) main types; petunidin, cyanidin, malvidin, pelargonidin, delphinidin, and peonidin are found in plants [126,127]. The six (6) main types are mostly responsible for the diverse color variations in plants (Tab. 3). These anthocyanidins usually work together with genes and enzymes to regulate the various colors of anthocyanins. According to Tanaka and Brugliera [128], the enzymes F3′H and F3′5′H which determines the hydroxylation pattern of the B-ring by different substitution patterns at R1, R2, and R3, influences the diversity and color variations of anthocyanins (Fig. 4; Tab. 3). At present, there are over 600 kinds of anthocyanins identified in plants [127].

Sweet potato cell lines, storage root, and leaves have adequate supply of anthocyanin which provides the characteristic purple color. These anthocyanins are seen as mono-, di-, and non-acylated forms with peonidin, cyanidin, or pelargonidin aglycones [129]. Previous research has identified several anthocyanins in purple-fleshed sweet potato as peonidin and cyanidin-based anthocyanins acylated with hydroxybenzoic, ferulic, or caffeic acids [49]. However, some studies identified peonidin 3-caffeoyl-feruloyl sophoroside-5-glucoside and peonidin 3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside as the major anthocyanins [130,131]. These anthocyanins are acylated and this influences their high stability and physiological activities [132]. However, cyanidin-based anthocyanins have strong anti-oxidant activity compared to peonidin-based anthocyanins mainly due to its additional hydroxyl group [133].

Table 3: Substitution pattern of the common anthocyanins in plants

Aglycones	Substitution pattern			Visible color
	R1	R2	R3	
Cyanidin	OH	OH	H	Orange-red
Delphinidin	OH	OH	OH	Purple
Malvidin	OCH_3	OH	OCH_3	Blue-red
Pelargonidin	H	OH	H	Orange
Peonidin	OCH_3	OH	H	Orange-red
Petunidin	OCH_3	OH	OH	Purple

3.2.2 Functions of Anthocyanins

Anthocyanins are among the major secondary metabolites, responsible for distinctive colors in plants [134,135]. As a water-soluble natural pigment, anthocyanin plays a significant role in both plants and animals, especially in human health. In plants, anthocyanins aid reproduction by alluring insect pollinators. The brightly colored parts of plants resulting from the accumulation of anthocyanin attract insect pollinators which aid in pollination and seed dispersal [17,136]. Although some of these insects are essential in influencing the reproductive ability of the plant, others act as pathogens that infest plants with diseases. Anthocyanins are effective in reducing the infestations from these pathogenic insects. For example, tomato fruits enriched in anthocyanin exhibited tolerance to gray mold [137]. Also, large numbers of African bollworm died and pupation delayed in tropical armyworm when fed with anthocyanin-rich leaves relative to those fed with green leaves [138].

Anthocyanins also safeguard plants against some biotic and abiotic stress which may offer them better adaptation to climatic changes [139]. Although much has been reported on anthocyanin-related stress response in diverse plant species, little information is available in sweet potato. Dihydroflavonol-4-reductase (DFR), a gene involved in the biosynthesis of several flavonoids including anthocyanins was reported to influence sweet potato tolerance to cold stress [140] with the increase attributed to the enhanced antioxidant ability. Research has reported that the enhanced antioxidant activity of purple-fleshed sweet potato was due to its resilient ability to scavenge free radicals [141]. These findings then propose the role of anthocyanins in the maintenance of ROS homeostasis as the sweet potato grows and develops.

As photo-protective agents, anthocyanins protect the photosynthetic tissues by absorbing excess visible ultraviolet radiation and also act as scavengers of free radicals [142]. Furthermore, anthocyanins accumulate in immature non-reproductive tissues and light-exposed parts of fruits to offer protection against photo-inhibition and photo-bleaching under light stress without considerably affecting the process of photosynthesis [143,144].

Despite its countless roles in plants, anthocyanins have beneficial health effects on mammals owing to their antioxidant properties. Purple-fleshed sweet potato has high anthocyanin content and subsequently high antioxidant properties which influences its health-promoting functions [49]. These antioxidant properties enable the scavenging of free radicals associated with aging and degenerative diseases [145]. Typically, anthocyanins are administered to animals through their diets with dietary anthocyanins offering protection against cardiovascular diseases, cancer, and other chronic disorders [146]. Some studies have attributed the shielding effects of dietary anthocyanins to their antioxidative and anticancer properties [147,148]. In an experiment on rats, it was observed that feeding rats with purple-fleshed sweet potato anthocyanin reduced

hepatoxin-induced liver injury [53]. This was reported to be due to the enhanced expression of some antioxidant enzymes like glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) in the liver.

Also, the anti-aging and anti-oxidative properties of anthocyanins make it safe for the manufacturing of natural skin-care products in the cosmetic industry [135]. This inhibits the impact of UV radiation on the skin, hence, reducing inflammations and diseases. Furthermore, anthocyanins from purple-fleshed sweet potato are used as a replacement for some synthetic pigments in cosmetic products like shampoos, rouge, creams, and lipsticks among others. In the pharmaceutical industry, purple-fleshed sweet potato anthocyanins are used as potential components for the production of pharmaceuticals such as anti-neoplastic and anti-inflammatory agents due to their antioxidant properties [57]. As a non-toxic natural pigment, purple-fleshed sweet potato anthocyanin can be used to substitute synthetic pigments in the production of colored medicines as the long-term effect of these synthetic pigments could be detrimental to the human body [149].

Purple-fleshed sweet potato anthocyanin is used as a functional ingredient in food processing industries as preservatives and sources of natural colorants with excellent color potency [150,151]. Purple-fleshed sweet potato anthocyanin exhibited high stability when added to beverages and prolonged the shelf life than pigments from grapes and blackberries [152]. Anthocyanins from purple-fleshed sweet potato can proliferate the growth of helpful bacteria especially those utilized in probiotics and as well inhibit the growth of harmful ones. According to Sun et al. [153], peonidin-based anthocyanins proliferate the *Bifidobacterium spp. (bifidum*, *adolescentis*, *infantis)* and *Lactobacillus acidophilus* whiles inhibiting the growth of *Salmonella typhimurium* and *Staphylococcus aureus*. Similarly, crude anthocyanin derived from purple-fleshed sweet potato inhibits the growth of *Bacteroides, Prevotella*, and *Clostridium histolyticum* [154] proposing the ability of anthocyanins to be involved in prebiotic-like activity by modulating intestinal microbiota. In effect, purple-fleshed sweet potato anthocyanins are essential due to their diversified functions.

3.2.3 Anthocyanin Biosynthetic Pathway

Anthocyanins are synthesized in the cytosolic side of the endoplasmic reticulum through the phenylpropanoid pathway (Fig. 5). Phenylalanine ammonia lyase (PAL) deaminates phenylalanine to produce trans-cinnamic acid [58]. Cinnamate 4-hydroxylase converts trans-cinnamic acid to *p*-coumaric acid, however, tyrosine ammonia lyase (TAL) catalyzes the production of *p*-coumaric acid from tyrosine in some plants [155,156]. Co-enzyme A combined with *p*-coumaric acid is catalyzed by 4-coumarate-CoA ligase (4CL) to yield *p*-coumaroyl-CoA [157]. Chalcone synthase (CHS) converts the condensed *p*-coumaroyl-CoA alongside three molecules of malonyl-CoA to yield chalcone [158] which is further converted by chalcone isomerase (CHI) to flavanone naringenin. Flavanone 3-hydroxylase (F3H) catalyzes the synthesis of flavonol dihydrokaempferol (DHK or aromadendrin) from flavanone naringenin. Dihydroquercetin (taxifolin) and dihydromyricetin (ampelopsin), the two dihydroflavonols are synthesized from DHK by flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′5′-hydroxylase (F3′5′H) respectively. The three dihydroflavonols are converted by dihydroflavonol 4-reductase (DFR) to colorless leucoanthocyanidins and subsequently to colored anthocyanidins (delphinidin, cyanidin, and pelargonidin) by anthocyanidin synthase (ANS)/leucoanthocyanidin dioxygenase (LDOX). Finally, methyltransferases (OMT) and acetylates cling unto anthocyanidins which are further converted to anthocyanin 3-*O*-glucoside (a chemically constant hydro-soluble pigment) by 3-*O*-glycosyl transferases (3GT) [159].

Anthocyanins after their synthesis are transported from the cytosol to the vacuole for storage. Vacuolar sequestration is crucial to prevent anthocyanins from being oxidized [160] and to perform its function as bioactive pigments. Though anthocyanin biosynthesis and regulatory genes are well characterized, the mechanism involved in its translocation from the cytosol to the vacuole in plants is still debatable [161,162]. The Multidrug toxic compound extrusion (MATE) protein and ATP-binding cassette (ABC) transporters confined in the tonoplast help link anthocyanins to glutathione S-transferase (GSTF) for

effective segregation into the vacuole and may also cling unto anthocyanoplasts, a pre-vacuolar segment proceeding to the vacuole [163,164]. Acylated anthocyanins accumulate at high levels inside the vacuole to form AVI (anthocyanic vacuolar inclusions) in some species [165].

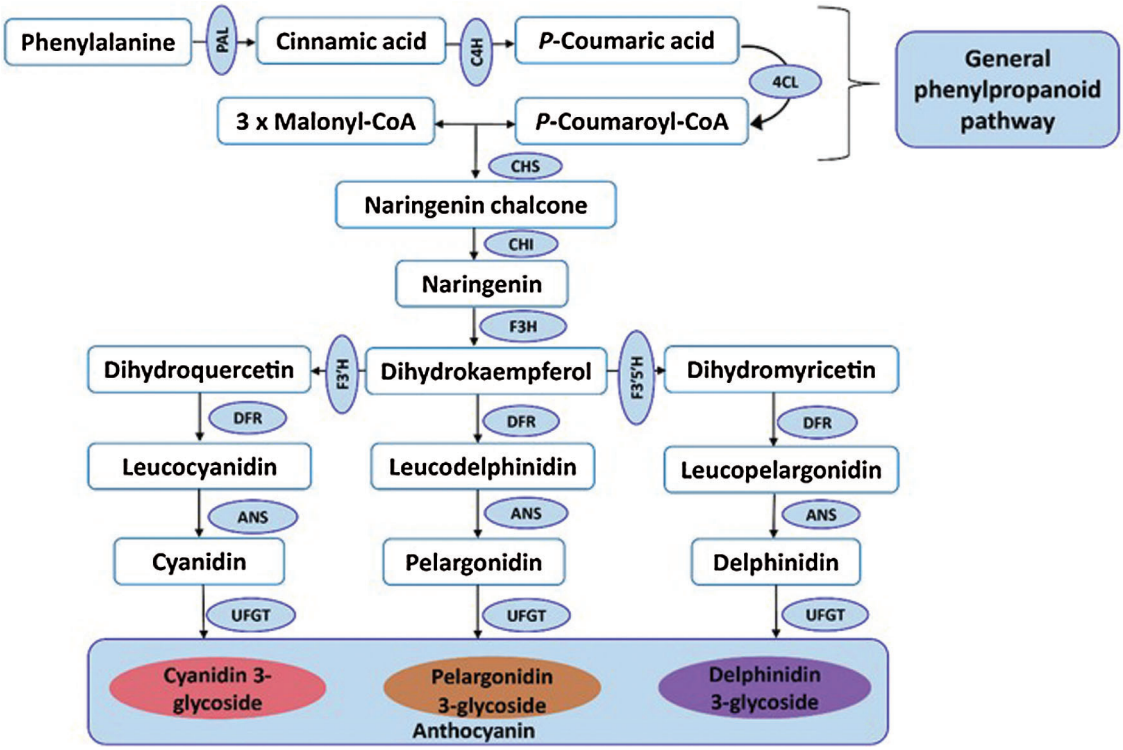

Figure 5: Anthocyanin biosynthetic pathway and regulatory enzymes in plants. Adapted from Amoanimaa-Dede et al. [20]. Names of the regulatory enzyme are abbreviated as follows; PAL: Phenylalanine Ammonia Lyase; CHI: Chalcone Isomerase; F3′5′H: Flavonoid 3′5′-Hydroxylase; C4H: Cinnamate 4-Hydroxylase; DFR: Dihydroflavonol 4-Reductase; 4CL: 4-Coumarate-CoA Ligase; F3′H: Flavonoid 3′-Hydroxylase; CHS: Chalcone Synthase; ANS: Anthocyanidin Synthase; F3H: Flavanone 3-Hydroxylase; UFGT: UDP-glucose Flavonoid 3-O-glucosyl Transferase

DFR is an essential structural gene and its substrate specificity regulates the structure and color of anthocyanins. Characterization of *IbDFR* revealed its expression to be also associated with both biosynthesis and accumulation. According to Wang et al. [140], expression levels of *IbDFR* in the leaves, stem, and root correlated with anthocyanin accumulation in these plant parts. In purple-fleshed sweet potato, a decrease in the expression of *IbDFR* also impacted the flux dissemination of flavonoids like proanthocyanidins and flavonols. In Arabidopsis, the *transparent testa* (*tt*) loci encode several flavonoid (anthocyanin) biosynthetic enzymes such as CHI, DFR, and CHS at the *tt5, tt3 and tt4* loci respectively. However, mutations in genes encoding these anthocyanin biosynthetic enzymes eliminate anthocyanin synthesis [166,167]. For example, Dong et al. [168] observed no pigment accumulation (anthocyanin and brown tannins in the hypocotyl and seed coat respectively) in Arabidopsis *tt* mutants compared to the seeds of the wild-type, suggesting the absence of biosynthetic enzyme (CHS, CHI, and DFR) activity. Introduction of *IbDFR* into the hypocotyls, cotyledons, and seed coat of Arabidopsis *tt3* mutants gave the hypocotyls and cotyledons a purple color and restored the pigments in the seed coat, proposing the biosynthetic function of the *IbDFR* gene.

Glutathione S-transferases (GSTs) function to detoxify xenobiotics (heterocyclic compounds) by connecting glutathione to a substrate to form a glutathione S-conjugate. According to Marrs et al. [169], these enzymes catalyze the conjugation of glutathione (GSH) to anthocyanins to form anthocyanin-GSH conjugates for onward sequestration into vacuoles by the glutathione pump, proposing that anthocyanin may be an endogenous substrate for the glutathione pump. GST plays an integral role in the intracellular transport of anthocyanin by coupling its synthesis and accumulation in the vacuole. GSTs also function as carrier proteins by physically binding to anthocyanins to facilitate the vacuolar sequestration of anthocyanin from the cytoplasm, though their functions remain indistinct [170]. For instance, Kitamura et al. [171] and Sun et al. [172] observed the localization of GSTs from other plants and Arabidopsis tt19 that accrue high proanthocyanidins than anthocyanins in the cytoplasm of undeveloped seed coats. Recently, Marrs et al. [160] and Alfenito et al. [173] reported the inability of *Petunia hybrida* (petunia) and *Zea mays* (maize) mutants to accumulate anthocyanins in their vacuoles due to the lack of GST. This suggests the function of GST as flavonoid binding protein, hence, confirming its involvement in anthocyanin accumulation. Again, GSTs are allied to high-anthocyanin producing membranes in the plant cell, possibly the vacuole and endoplasmic reticulum [172]. These results confirm the function of GSTs as carrier proteins and thus, the glutathionylation of flavonoids might not be catalyzed by GSTs due to their inability to conjugate GSH to anthocyanins [161] which therefore contrast with the report by Marrs et al. [169]. It is therefore noteworthy that, there is no evidence of anthocyanin-GSH conjugates in plants [170].

GST genes involved in anthocyanin accumulation have been identified in several plant species including strawberry, cyclamen, litchi, and grapevine [174–177]. For example, Hu et al. [175] reported the involvement of *LcGST4* in the accumulation of anthocyanin in the fruit *Litchi chinensis* (litchi) and its overexpression in pigmented tissues. The results revealed that the expression of *LcGST4* was regulated by the *LcMYB1* gene. MYB gene family is a part of a larger family of transcriptional factors that regulate anthocyanin biosynthesis and accumulation in plants. For instance, the *IbMYB1* gene regulates anthocyanin biosynthesis in sweet potato [58] whereas, in Arabidopsis, *IbMYB1a* increased anthocyanin accumulation in transgenic plants [178].

In sweet potato, a GST encoding gene, *IbGSTF4* is reported to be involved in the accumulation of anthocyanin. For instance, the *IbGSTF4* gene after characterization was found to be highly expressed in pigmented stems, leaves, and storage root with its expression correlating with the accumulation of anthocyanin. In the same study, the varied expression profiles of *IbGSTF4* in the Arabidopsis tt19 (a knockout mutant of anthocyanin-related GST) gave the cotyledon and hypocotyl a purple color, suggesting *IbGSTF4* participation in anthocyanin accumulation in sweet potato [22]. However, a dual luciferase assay pointed out that the *IbMYB1* gene could not directly regulate the expression of *IbGSTF4* which conflicts with the report by Hu et al. [175]. This then suggests that the regulation of anthocyanin biosynthesis and sequestration may involve other MYB regulatory factors [22], thus, proposing a complex regulatory mechanism of anthocyanin vacuolar sequestration and accumulation in sweet potato which needs to be elucidated through further research.

3.2.4 Gene Regulation of Anthocyanin Biosynthesis

The primary regulatory genes involved in anthocyanin biosynthesis have been studied extensively and sequestered in many plant species [124]. The transcriptional factors regulating the biosynthesis of anthocyanins are WD40-type co-regulators (WD40), R2R3-MYB protein, and a basic helix-loop-helix (bHLH, MYC) protein [179].

Structural and regulatory genes are the two main types of biosynthetic genes. The structural genes encode the enzymes which catalyze every reaction step while the regulatory genes encode transcriptional components that regulate structural gene expression [180,181]. Structural genes involved in anthocyanin biosynthesis are homogeneously expressed and their expression levels are dependent on the concentration [182]. There are two divisions of structural genes in dicot plants i.e., early (CHI, CHS, FLS, F3′H, and

F3H,) and late (UFGT, ANS/LDOX, and DFR) biosynthetic genes [181]. These genes operate under the MYB-bHLH-WD40 (MBW) regulatory network made up of the MYB, basic helix-loop-helix (bHLH) and WD40 replicate families. For instance, the MYB domain C1 protein which regulates anthocyanin biosynthesis in maize requires a bHLH partner to activate the flavonoid structural genes and the dihydroflavonol reductase (DFR) promoter, although the MYB domain P protein which controls phlobaphene to stimulate the promoter lacks a bHLH partner [183]. These MYB proteins have a central responsibility of regulating the biosynthesis of secondary metabolites, signal transduction, resistance to diseases as well as growth and developmental fluctuations [181]. As reviewed in Amoanimaa-Dede et al. [20], the structurally conserved MYB genes comprise 100–160 bp DNA-binding regions with one or more replications. The R2R3 MYB genes with two repeats are the predominant group of MYB genes involved in the flavonoid pathway in plants. Therefore, the intensity of anthocyanin synthesis solely depends on the expression of structural genes that are related to a specific species [184].

In sweet potato, some structural and transcription factor genes have been characterized, with most of the genes functioning in both anthocyanin biosynthesis and accumulation. The *IbMADS10* is a vital regulatory gene involved in anthocyanin biosynthesis of sweet potato [185]. Two MYB genes (*IbMYB1* and *IbMYB2*) isolated from the storage root of purple-fleshed sweet potato cv. Ayamurasaki regulates anthocyanin biosynthesis in sweet potato [186]. According to Mano et al. [186], the *IbMYB1* transcription factor from the MYB-family facilitates the accumulation of anthocyanins in sweet potato storage roots. Park et al. [58] reported that the overexpression of the *IbMYB1* gene effectively caused the accumulation of anthocyanin in the storage root of an orange-fleshed sweet potato with high carotenoid content thereby increasing the radical scavenging activity.

Current research has identified some post-transcriptional modulators mostly miRNA including ib-miR164c, ib-miR160e-5p, ib-miR172e-3p, and ib-miR166m [187] to be involved in anthocyanin biosynthesis. These miRNA target genes are involved in auxin signaling. Auxin can inhibit the expression of the MBW complex which intends to regulate anthocyanin biosynthesis [36]. The ib-miR159, ib-miR319, ib-miR858, and ib-miR156 also regulated the MYB genes whereas the SPL gene was targeted by ib-miR156 and its upregulation reduced the expression of *ibSPL* in purple-fleshed sweet potato. The ib-miR156a-5p was also reported to cling unto *ibSPL* genes proposing that ib-miR156 may increase the biosynthesis of anthocyanin via structural gene regulation in the phenylpropanoid pathway.

Though there is post-transcriptional modulation of anthocyanin biosynthesis, the primary level at which anthocyanin biosynthesis is inducted or shut down in plants is controlled by the expression of biosynthetic genes [188]. From the above results, it can be deduced that the molecular regulation of anthocyanin biosynthesis and accumulation is complex both at the transcriptional and post-transcriptional levels. One keen observation made was that most molecular results were more tailored to individual research (transcriptome sequencing). This is due to the lack of a reference genome (the only one sequenced "Taizhong 6" is incomplete and inaccurate hence not representative of hexaploid sweet potato). The ability to sequence the reference genome will go a long way to improve molecular research in sweet potato.

4 Concluding Remarks and Perspectives

Sweet potato is a multifunctional food crop with rich nutritional composition and bioactive compounds. Several cultivated sweet potato varieties differ with flesh and skin colors (white, yellow, orange, red, and purple) of the storage root. Variations in phytochemicals and nutritional compositions, the pigments produced and the morphological traits may also distinguish the various sweet potato varieties. Carotenoid and anthocyanin are the major natural pigments in sweet potato known for their antioxidative properties which scavenge free radicals and protect both plants and animals from oxidative damage. The *IbGGPS*, *IbLCY-ε*, and *IbCHY-β* genes regulate carotenoid biosynthesis while *IbCCD1*, *IbCCD4*, and *IbOr* control its accumulation. Anthocyanin biosynthesis and accumulation are both regulated by the *IbMYB*, *IbDFR*,

and *IbGSTF3* genes. Besides, some post-transcriptional modulators basically miRNAs were revealed to be involved in anthocyanin biosynthesis. Further characterization of the biosynthesis and regulatory mechanism of carotenoids and anthocyanins will be beneficial to unravel the complex mechanism of carotenoid accumulation regulated by the molecular function of the *IbOr* gene. This will help elucidate the interrelation between carotenoid accumulation and catabolism. Although the molecular mechanism underlying the biosynthesis and regulatory control of carotenoids and anthocyanin is extensively studied in sweet potato, a lot is still unknown. Furthermore, the limited report on the role of anthocyanin in sweet potato stress response mechanism calls for further research.

Many innovative biotechniques such as CRISPR/Cas9 through synthetic transcription factor detection and gene activation, could modify the expression of targeted genes [189]. Although this technology has been used extensively for crop improvement, little is known about its application in sweet potato. For sweet potato pigmentation, transgenic technologies (genetic modification and metabolic engineering) have been used to further increase carotenoid and anthocyanin content by modifying the expression of single genes through *Agrobacterium*-mediated transformation. Also, the identification and development of synthetic transcription factors in sweet potato might increase the accumulation of carotenoids and anthocyanins. Therefore, CRISPR-Cas9-mediated genome editing technique may be significantly useful for the biofortification of sweet potato. The lack of a reference genome makes genetic and molecular studies very challenging, hence, whole-genome sequencing is suggested to improve molecular research in sweet potato. Overall, the complex molecular regulation of anthocyanin biosynthesis and accumulation both at the transcriptional and post-transcriptional levels due to the inconsistencies in previous reports should be addressed through further research. Understanding the biosynthesis and gene regulation of these major sweet potato pigments may provide appropriate resources and better schemes for breeding sweet potato varieties with high anthocyanin and carotenoid contents.

Acknowledgement: The authors are thankful to Ms. Linda Adzigbli for the critical review and useful suggestions during the manuscript preparation.

Funding Statement: This study was supported by the NSFC-Guangdong Natural Science Foundation Joint Project (U1701234), Strategic Leading Science & Technology Programme (XDA13020604), Program for Scientific Research Start-up Funds of Guangdong Ocean University, and Studies on Resistance Resources and Molecular Mechanisms of Sweet potato Weevil in South China (U1701234).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report that could influence the work reported in this paper.

References

1. El Sheikha, A. F., Ray, R. C. (2017). Potential impacts of bioprocessing of sweet potato. *Critical Reviews in Food Science and Nutrition, 57,* 455–471. DOI 10.1080/10408398.2014.960909.
2. Truong, V., Avula, R., Pecota, K., Yencho, G. (2018). Sweetpotato production, processing, and nutritional quality. In: Siddiq, M., Uebersax, M. A. (eds.), *Handbook of vegetables and vegetable Processing*, 2nd ed., pp. 811–838. USA: John Wiley & Sons, Ltd.
3. Lebot, V. (2009). *Tropical root and tuber crops: Cassava, sweetpotato, yams and aroids*, pp. 99–274. Oxfordshire, UK: CABI.
4. Zhao, J., Chen, Z., Jin, Z., Buwalda, P., Gruppen, H. et al. (2015). Effects of granule size of cross-linked and hydroxypropylated sweet potato starches on their physicochemical properties. *Journal of Agricultural and Food Chemistry, 63(18),* 4646–4654. DOI 10.1021/jf506349w.
5. Trancoso-Reyes, N., Ochoa-Martínez, L. A., Bello-Pérez, L. A., Morales-Castro, J., Estévez-Santiago, R. et al. (2016). Effect of pre-treatment on physicochemical and structural properties, and the bioaccessibility of β-carotene in sweet potato flour. *Food Chemistry, 200,* 199–205. DOI 10.1016/j.foodchem.2016.01.047.

6. Katayama, K., Kobayashi, A., Sakai, T., Kuranouchi, T., Kai, Y. (2017). Recent progress in sweetpotato breeding and cultivars for diverse applications in Japan. *Breeding Science, 67(1),* 3–14. DOI 10.1270/jsbbs.16129.
7. Yang, Y., Wang, Y., Jia, L., Yang, G., Xu, X. et al. (2018). Involvement of an ABI-like protein and a Ca^{2+}-ATPase in drought tolerance as revealed by transcript profiling of a sweetpotato somatic hybrid and its parents Ipomoea batatas (L.) Lam. and I. triloba L. *PLoS One, 13(2),* e0193193. DOI 10.1371/journal.pone.0193193.
8. Liu, Q. (2017). Improvement for agronomically important traits by gene engineering in sweetpotato. *Breeding Science, 67(1),* 15–26. DOI 10.1270/jsbbs.16126.
9. Rose, I. M., Vasanthakaalam, H. (2011). Comparison of the nutrient composition of four sweet potato varieties cultivated in Rwanda. *American Journal of Food and Nutrition, 1(1),* 34–38. DOI 10.5251/ajfn.2011.1.1.34.38.
10. Tanaka, M., Ishiguro, K., Oki, T., Okuno, S. (2017). Functional components in sweetpotato and their genetic improvement. *Breeding Science, 67(1),* 52–61. DOI 10.1270/jsbbs.16125.
11. Shao, H., Yong, B., Xu, P., Zheng, H., Liao, R. et al. (2018). Phytoene synthase gene (PSY) from sweet potato (Ipomoea batatas Lam.) enhances tolerance to abiotic stress. *Brazilian Archives of Biology and Technology, 61,* 1. DOI 10.1590/1678-4324-2018160558.
12. Oki, T., Masuda, M., Furuta, S., Nishiba, Y., Terahara, N. et al. (2002). Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *Journal of Food Science, 67(5),* 1752–1756. DOI 10.1111/j.1365-2621.2002.tb08718.x.
13. Swamy, A., Omwenga, J. (2014). Analysis of phytochemical composition of white and purple sweet potato (Ipomoea batatas [L.] Lam.) root. *Indian Journal of Advanced Plant Research, 1,* 19–22.
14. Grace, M. H., Truong, A. N., Truong, V. D., Raskin, I., Lila, M. A. (2015). Novel value-added uses for sweet potato juice and flour in polyphenol-and protein-enriched functional food ingredients. *Food Science & Nutrition, 3(5),* 415–424. DOI 10.1002/fsn3.234.
15. Van Jaarsveld, P., Harmse, E., Nestel, P., Rodriguez-Amaya, D. (2006). Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. *Journal of Food Composition and Analysis, 19(4),* 321–329. DOI 10.1016/j.jfca.2004.10.007.
16. Teow, C. C., Truong, V. D., McFeeters, R. F., Thompson, R. L., Pecota, K. V. et al. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. *Food Chemistry, 103(3),* 829–838. DOI 10.1016/j.foodchem.2006.09.033.
17. Shang, Y., Venail, J., Mackay, S., Bailey, P. C., Schwinn, K. E. et al. (2011). The molecular basis for venation patterning of pigmentation and its effect on pollinator attraction in flowers of Antirrhinum. *New Phytologist, 189(2),* 602–615. DOI 10.1111/j.1469-8137.2010.03498.x.
18. Kim, S. H., Ahn, Y. O., Ahn, M. J., Lee, H. S., Kwak, S. S. (2012). Down-regulation of β-carotene hydroxylase increases β-carotene and total carotenoids enhancing salt stress tolerance in transgenic cultured cells of sweetpotato. *Phytochemistry, 74,* 69–78. DOI 10.1016/j.phytochem.2011.11.003.
19. Kang, L., Park, S. C., Ji, C. Y., Kim, H. S., Lee, H. S. et al. (2017). Metabolic engineering of carotenoids in transgenic sweetpotato. *Breeding Science, 67(1),* 27–34. DOI 10.1270/jsbbs.16118.
20. Amoanimaa-Dede, H., Hongbo, Z., Kyereko, W., Yeboah, A., Agyenim-Boateng, K. (2019). Structure, functions and biosynthetic pathway of naturally occurring anthocyanin in sweet potato-a review. *International Journal of Plant Biochemistry and Physiology, 7,* 234.
21. Zhao, J. (2015). Flavonoid transport mechanisms: how to go, and with whom. *Trends in Plant Science, 20(9),* 576–585. DOI 10.1016/j.tplants.2015.06.007.
22. Kou, M., Liu, Y. J., Li, Z. Y., Zhang, Y. G., Tang, W. et al. (2019). A novel glutathione S-transferase gene from sweetpotato, IbGSTF4, is involved in anthocyanin sequestration. *Plant Physiology and Biochemistry, 135,* 395–403. DOI 10.1016/j.plaphy.2018.12.028.
23. Feller, A., Machemer, K., Braun, E. L., Grotewold, E. (2011). Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *Plant Journal, 66(1),* 94–116. DOI 10.1111/j.1365-313X.2010.04459.x.
24. Phillips, M. A., León, P., Boronat, A., Rodríguez-Concepción, M. (2008). The plastidial MEP pathway: unified nomenclature and resources. *Trends in Plant Science, 13(12),* 619–623. DOI 10.1016/j.tplants.2008.09.003.

25. Kim, S. H., Kim, Y. H., Ahn, Y. O., Ahn, M. J., Jeong, J. C. et al. (2013). 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, 147(4),* 432–442. DOI 10.1111/j.1399-3054.2012.01688.x.
26. Kim, S. H., Jeong, J. C., Park, S., Bae, J. Y., Ahn, M. J. et al. (2014). Down-regulation of sweetpotato lycopene β-cyclase gene enhances tolerance to abiotic stress in transgenic calli. *Molecular Biology Reports, 41(12),* 8137–8148. DOI 10.1007/s11033-014-3714-4.
27. Chen, W., He, S., Liu, D., Patil, G. B., Zhai, H. et al. (2015). A sweetpotato geranylgeranyl pyrophosphate synthase gene, IbGGPS, increases carotenoid content and enhances osmotic stress tolerance in Arabidopsis thaliana. *PLoS One, 10(9),* e0137623. DOI 10.1371/journal.pone.0137623.
28. Flores, G., Wu, S. B., Negrin, A., Kennelly, E. J. (2015). Chemical composition and antioxidant activity of seven cultivars of guava (Psidium guajava) fruits. *Food Chemistry, 170,* 327–335. DOI 10.1016/j.foodchem.2014.08.076.
29. Shekhar, S., Mishra, D., Buragohain, A. K., Chakraborty, S., Chakraborty, N. (2015). Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (Ipomoea batatas L.). *Food Chemistry, 173,* 957–965. DOI 10.1016/j.foodchem.2014.09.172.
30. Hue, S., Chandran, S., Boyce, A. (2010). Variations of leaf and storage roots morphology in Ipomoea batatas L. (Sweet potato) cultivars in Proceedings of Asia Pacific Symposium on Postharvest Research, Education and Extension. *Acta Horticulturae, 943,* 73–79.
31. Anastácio, A., Carvalho, I. S. (2013). Spotlight on PGI sweet potato from europe: study of plant part, time and solvent effects on antioxidant activity. *Journal of Food Biochemistry, 37,* 628–637.
32. Mei, X., Mu, T. H., Han, J. J. (2010). Composition and physicochemical properties of dietary fiber extracted from residues of 10 varieties of sweet potato by a sieving method. *Journal of Agricultural and Food Chemistry, 58(12),* 7305–7310. DOI 10.1021/jf101021s.
33. Tomlins, K., Owori, C., Bechoff, A., Menya, G., Westby, A. (2012). Relationship among the carotenoid content, dry matter content and sensory attributes of sweet potato. *Food Chemistry, 131(1),* 14–21. DOI 10.1016/j.foodchem.2011.07.072.
34. Stinco, C. M., Benítez-González, A. M., Hernanz, D., Vicario, I. M., Meléndez-Martínez, A. J. (2014). Development and validation of a rapid resolution liquid chromatography method for the screening of dietary plant isoprenoids: carotenoids, tocopherols and chlorophylls. *Journal of Chromatography A, 1370,* 162–170. DOI 10.1016/j.chroma.2014.10.044.
35. Gul, K., Tak, A., Singh, A., Singh, P., Yousuf, B. et al. (2015). Chemistry, encapsulation, and health benefits of β-carotene-a review. *Cogent Food & Agriculture, 1,* 1018696.
36. Kim, H. J., Park, W. S., Bae, J. Y., Kang, S. Y., Yang, M. H. et al. (2015). Variations in the carotenoid and anthocyanin contents of Korean cultural varieties and home-processed sweet potatoes. *Journal of Food Composition and Analysis, 41,* 188–193. DOI 10.1016/j.jfca.2015.01.012.
37. Tumuhimbise, G. A., Namutebi, A., Muyonga, J. H. (2009). Microstructure and *in vitro* beta carotene bioaccessibility of heat processed orange fleshed sweet potato. *Plant Foods for Human Nutrition, 64(4),* 312–318. DOI 10.1007/s11130-009-0142-z.
38. Padmaja, G., Sheriff, J. T., Sajeev, M. S. (2012). Food uses and nutritional benefits of sweet potato. *Fruit, Vegetable and Cereal Science and Biotechnology, 6,* 115–123.
39. Kurabachew, H. (2015). The role of orange fleshed sweet potato (Ipomea batatas) for combating vitamin A deficiency in Ethiopia: a review. *International Journal of Food Science and Nutrition Engineering, 5(3),* 141–146.
40. Sommer, A., West, K. P. Jr, Schwab, L. (1997). Vitamin A deficiency: health, survival, and vision. *American Journal of Ophthalmology, 123,* 274.
41. Low, J. W. (2013). Biofortified crops with a visible trait: the example of orange-fleshed sweet potato in sub-Saharan Africa. In: Preedy, V. R., Srirajaskanthan, R., Patel, V. (eds.), *Handbook of Food Fortification and Health*, NewYork, NY: Humana Press.
42. Vimala, B., Nambisan, B., Hariprakash, B. (2011). Retention of carotenoids in orange-fleshed sweet potato during processing. *Journal of Food Science and Technology, 48(4),* 520–524. DOI 10.1007/s13197-011-0323-2.

43. Jacobi, D. (2014). *The superfoods cookbook: nutritious meals for any time of day using nature's healthiest foods*. USA: Weldon Owen.
44. Oliver, J. (2016). *Everyday super food*. UK: Penguin.
45. Low, J. W., Arimond, M., Osman, N., Cunguara, B., Zano, F. et al. (2007). A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. *Journal of Nutrition, 137(5),* 1320–1327. DOI 10.1093/jn/137.5.1320.
46. Kidane, G., Abegaz, K., Mulugeta, A., Singh, P. (2013). Nutritional analysis of vitamin A enriched bread from orange flesh sweet potato and locally available wheat flours at Samre Woreda, Northern Ethiopia. *Current Research in Nutrition and Food Science Journal, 1(1),* 49–57. DOI 10.12944/CRNFSJ.1.1.05.
47. Burri, B. J. (2011). Evaluating sweet potato as an intervention food to prevent vitamin A deficiency. *Comprehensive Reviews in Food Science and Food Safety, 10(2),* 118–130. DOI 10.1111/j.1541-4337.2010.00146.x.
48. Fan, G., Han, Y., Gu, Z., Chen, D. (2008). Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM). *LWT-Food Science and Technology, 41(1),* 155–160. DOI 10.1016/j.lwt.2007.01.019.
49. Lim, S., Xu, J., Kim, J., Chen, T. Y., Su, X. et al. (2013). Role of anthocyanin-enriched purple-fleshed sweet potato p40 in colorectal cancer prevention. *Molecular Nutrition & Food Research, 57(11),* 1908–1917. DOI 10.1002/mnfr.201300040.
50. Jawi, I. M., Wita, I. W., Suprapta, D. N. (2014). Aqueous extract of purple sweet potato tuber increases sod and decreases VCAM-1 expression by increasing Nrf2 expression in *the Aortic Endothelia of Hypercholesterolemic Rabbits*. *Journal of Biology, Agriculture and Healthcare, 4(10),* 76–84.
51. Hu, Y., Deng, L., Chen, J., Zhou, S., Liu, S. et al. (2016). An analytical pipeline to compare and characterise the anthocyanin antioxidant activities of purple sweet potato cultivars. *Food Chemistry, 194,* 46–54. DOI 10.1016/j.foodchem.2015.07.133.
52. Montilla, E. C., Hillebrand, S., Winterhalter, P. (2011). Anthocyanins in purple sweet potato (Ipomoea batatas L.) varieties. *Fruit, Vegetable and Cereal Science and Biotechnology, 5,* 19–23.
53. Zhang, Z. F., Fan, S. H., Zheng, Y. L., Lu, J., Wu, D. M. et al. (2009). Purple sweet potato color attenuates oxidative stress and inflammatory response induced by d-galactose in mouse liver. *Food and Chemical Toxicology, 47(2),* 496–501. DOI 10.1016/j.fct.2008.12.005.
54. Suda, I., Ishikawa, F., Hatakeyama, M., Miyawaki, M., Kudo, T. et al. (2008). Intake of purple sweet potato beverage affects on serum hepatic biomarker levels of healthy adult men with borderline hepatitis. *European Journal of Clinical Nutrition, 62(1),* 60–67. DOI 10.1038/sj.ejcn.1602674.
55. Delgado-Vargas, F., Jiménez, A., Paredes-López, O. (2000). Natural pigments: carotenoids, anthocyanins, and betalains—characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition, 40(3),* 173–289. DOI 10.1080/10408690091189257.
56. Takahata, Y., Noda, T., Nagata, T. (1993). HPLC determination of β-carotene content of sweet potato cultivars and its relationship with color values. *Japanese Journal of Breeding, 43(3),* 421–427. DOI 10.1270/jsbbs1951.43.421.
57. Bovell-Benjamin, A. C. (2007). Sweet potato: a review of its past, present, and future role in human nutrition. *Advances in Food and Nutrition Research, 52,* 1–59.
58. Park, S. C., Kim, Y. H., Kim, S. H., Jeong, Y. J., Kim, C. Y. et al. (2015). Overexpression of the IbMYB1 gene in an orange-fleshed sweet potato cultivar produces a dual-pigmented transgenic sweet potato with improved antioxidant activity. *Physiologia Plantarum, 153(4),* 525–537. DOI 10.1111/ppl.12281.
59. Namitha, K., Negi, P. (2010). Chemistry and biotechnology of carotenoids. *Critical Reviews in Food Science and Nutrition, 50(8),* 728–760. DOI 10.1080/10408398.2010.499811.
60. Butnariu, M. (2016). Methods of analysis (extraction, separation, identification and quantification) of carotenoids from natural products. *Journal of Ecosystem and Ecography, 6(2),* 193. DOI 10.4172/2157-7625.1000193.
61. Ishiguro, K., Yoshinaga, M., Kai, Y., Maoka, T., Yoshimoto, M. (2010). Composition, content and antioxidative activity of the carotenoids in yellow-fleshed sweetpotato (Ipomoea batatas L.). *Breeding Science, 60(4),* 324–329. DOI 10.1270/jsbbs.60.324.

62. Botella-Pavía, P., Rodríguez-Concepción, M. (2006). Carotenoid biotechnology in plants for nutritionally improved foods. *Physiologia Plantarum, 126(3),* 369–381. DOI 10.1111/j.1399-3054.2006.00632.x.
63. Bengtsson, A., Namutebi, A., Alminger, M. L., Svanberg, U. (2008). Effects of various traditional processing methods on the all-trans-β-carotene content of orange-fleshed sweet potato. *Journal of Food Composition and Analysis, 21(2),* 134–143. DOI 10.1016/j.jfca.2007.09.006.
64. Bechoff, A., Dufour, D., Dhuique-Mayer, C., Marouzé, C., Reynes, M. et al. (2009). Effect of hot air, solar and sun drying treatments on provitamin A retention in orange-fleshed sweetpotato. *Journal of Food Engineering, 92(2),* 164–171. DOI 10.1016/j.jfoodeng.2008.10.034.
65. Donado-Pestana, C. M., Salgado, J. M., Oliveira Rios, A., Santos, P. R., Jablonski, A. (2012). Stability of carotenoids, total phenolics and *in vitro* antioxidant capacity in the thermal processing of orange-fleshed sweet potato (Ipomoea batatas Lam.) cultivars grown in Brazil. *Plant Foods for Human Nutrition, 67(3),* 262–270. DOI 10.1007/s11130-012-0298-9.
66. Haskell, M. J., Jamil, K. M., Hassan, F., Peerson, J. M., Hossain, M. I. et al. (2004). Daily consumption of Indian spinach (Basella alba) or sweet potatoes has a positive effect on total-body vitamin A stores in Bangladeshi men. *American Journal of Clinical Nutrition, 80(3),* 705–714. DOI 10.1093/ajcn/80.3.705.
67. Huang, A., Tanudjaja, L., Lum, D. (1999). Content of alpha-, beta-carotene, and dietary fiber in 18 sweetpotato varieties grown in Hawaii. *Journal of Food Composition and Analysis, 12(2),* 147–151. DOI 10.1006/jfca.1999.0819.
68. Shih, M. C., Kuo, C. C., Chiang, W. (2009). Effects of drying and extrusion on colour, chemical composition, antioxidant activities and mitogenic response of spleen lymphocytes of sweet potatoes. *Food Chemistry, 117(1),* 114–121. DOI 10.1016/j.foodchem.2009.03.084.
69. Jaarsveld, P., Marais, D. W., Harmse, E., Nestel, P., Rodriguez-Amaya, D. (2006). Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. *Journal of Food Composition and Analysis, 19(4),* 321–329. DOI 10.1016/j.jfca.2004.10.007.
70. Wu, X., Sun, C., Yang, L., Zeng, G., Liu, Z. et al. (2008). β-carotene content in sweet potato varieties from China and the effect of preparation on β-carotene retention in the Yanshu No. 5. *Innovative Food Science & Emerging Technologies, 9(4),* 581–586. DOI 10.1016/j.ifset.2008.06.002.
71. Oki, T., Nagai, S., Yoshinaga, M., Nishiba, Y., Suda, I. (2006). Contribution of β-carotene to radical scavenging capacity varies among orange-fleshed sweet potato cultivars. *Food Science and Technology Research, 12(2),* 156–160. DOI 10.3136/fstr.12.156.
72. Drapal, M., Fraser, P. D. (2019). Determination of carotenoids in sweet potato (Ipomoea batatas L., Lam) tubers: implications for accurate provitamin A determination in staple sturdy tuber crops. *Phytochemistry, 167,* 112102. DOI 10.1016/j.phytochem.2019.112102.
73. Brown, C., Edwards, C., Yang, C. P., Dean, B. (1993). Orange flesh trait in potato: inheritance and carotenoid content. *Journal of the American Society for Horticultural Science, 118(1),* 145–150. DOI 10.21273/JASHS.118.1.145.
74. Islam, S. N., Nusrat, T., Begum, P., Ahsan, M. (2016). Carotenoids and β-carotene in orange fleshed sweet potato: a possible solution to vitamin A deficiency. *Food Chemistry, 199,* 628–631. DOI 10.1016/j.foodchem.2015.12.057.
75. Mohammad, K. A., Ziaul, H., Sheikh, N. (2016). Comparison of the proximate composition, total carotenoids and total polyphenol content of nine varieties of orange-fleshed sweet potato grown in Bangladesh. *Foods, 5(3),* 64. DOI 10.3390/foods5030064.
76. Ruiz-Sola, M. Á., Rodríguez-Concepción, M. (2012). *Carotenoid biosynthesis in Arabidopsis: a colorful pathway.* USA: American Society of Plant Biologists.
77. Park, S. C., Kim, S. H., Park, S., Lee, H. U., Lee, J. S. et al. (2015). Enhanced accumulation of carotenoids in sweetpotato plants overexpressing IbOr-Ins gene in purple-fleshed sweetpotato cultivar. *Plant Physiology and Biochemistry, 86,* 82–90. DOI 10.1016/j.plaphy.2014.11.017.
78. Goodwin, T. (2012). *The biochemistry of the carotenoids: volume I plants*. USA: Springer Science & Business Media.

79. Bechoff, A. (2010). *Investigating carotenoid loss after drying and storage of orange-fleshed sweet potato (Ph.D. Thesis)*. University of Greenwich, UK.
80. Britton, C. G., Liaaen-Jensen, S., Pfander, H. (2004). Carotenoids handbook. *Photosynthetica, 42(2),* 186. DOI 10.1023/B:PHOT.0000040641.40049.19.
81. Wei, L. B., Zhang, H. Y., Zheng, Y. Z., Guo, W. Z., Zhang, T. Z. (2008). Developing EST-derived microsatellites in sesame (Sesamum indicum L.). *Acta Agronomica Sinica, 34(12),* 2077–2084. DOI 10.1016/S1875-2780(09) 60019-5.
82. Römer, S., Fraser, P. D. (2005). Recent advances in carotenoid biosynthesis, regulation and manipulation. *Planta, 221(3),* 305–308. DOI 10.1007/s00425-005-1533-5.
83. Havaux, M. (2014). Carotenoid oxidation products as stress signals in plants. *Plant Journal, 79(4),* 597–606. DOI 10.1111/tpj.12386.
84. Wu, J., Ji, J., Wang, G., Wu, G., Diao, J. et al. (2015). Ectopic expression of the Lycium barbarum β-carotene hydroxylase gene (chyb) enhances drought and salt stress resistance by increasing xanthophyll cycle pool in tobacco. *Plant Cell, Tissue and Organ Culture, 121(3),* 559–569. DOI 10.1007/s11240-015-0725-3.
85. Park, S., Kim, H. S., Jung, Y. J., Kim, S. H., Ji, C. Y. et al. (2016). Orange protein has a role in phytoene synthase stabilization in sweetpotato. *Scientific Reports, 6(1),* 33563. DOI 10.1038/srep33563.
86. Wang, Z., Ke, Q., Kim, M. D., Kim, S. H., Ji, C. Y. et al. (2015). Transgenic alfalfa plants expressing the sweetpotato Orange gene exhibit enhanced abiotic stress tolerance. *PLoS One, 10(5),* e0126050. DOI 10.1371/ journal.pone.0126050.
87. Cho, K. S., Han, E. H., Kwak, S. S., Cho, J. H., Im, J. S. et al. (2016). Expressing the sweet potato orange gene in transgenic potato improves drought tolerance and marketable tuber production. *Comptes Rendus Biologies, 339(5–6),* 207–213. DOI 10.1016/j.crvi.2016.04.010.
88. Moran, N. A., Jarvik, T. (2010). Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science, 328(5978),* 624–627. DOI 10.1126/science.1187113.
89. Goo, Y. M., Han, E. H., Jeong, J. C., Kwak, S. S., Yu, J. et al. (2015). Overexpression of the sweet potato IbOr gene results in the increased accumulation of carotenoid and confers tolerance to environmental stresses in transgenic potato. *Comptes Rendus Biologies, 338(1),* 12–20. DOI 10.1016/j.crvi.2014.10.006.
90. Jaarsveld, P. J., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J. et al. (2005). β-Carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *American Journal of Clinical Nutrition, 81(5),* 1080–1087. DOI 10.1093/ajcn/81.5.1080.
91. Low, J. W., Mwanga, R. O., Andrade, M., Carey, E., Ball, A. M. (2017). Tackling vitamin A deficiency with biofortified sweetpotato in sub-Saharan Africa. *Global Food Security, 14,* 23–30. DOI 10.1016/j.gfs.2017.01.004.
92. Arizio, C. M., Tártara, S. C., Manifesto, M. M. (2014). Carotenoids gene markers for sweetpotato (Ipomoea batatas L. Lam): applications in genetic mapping, diversity evaluation and cross-species transference. *Molecular Genetics and Genomics, 289(2),* 237–251. DOI 10.1007/s00438-013-0803-3.
93. Mitra, S. (2012). Nutritional status of orange-fleshed sweet potatoes in alleviating vitamin A malnutrition through a food-based approach. *Journal of Nutrition and Food Science, 2,* 160.
94. Ambati, R., Phang, S. M., Ravi, S., Aswathanarayana, R. (2014). Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. *Marine Drugs, 12(1),* 128–152. DOI 10.3390/ md12010128.
95. Shah, M., Mahfuzur, R., Liang, Y., Cheng, J. J., Daroch, M. (2016). Astaxanthin-producing green microalga Haematococcus pluvialis: from single cell to high value commercial products. *Frontiers in Plant Science, 7,* 531.
96. Ajikumar, P. K., Tyo, K., Carlsen, S., Mucha, O., Phon, T. H. et al. (2008). Terpenoids: opportunities for biosynthesis of natural product drugs using engineered microorganisms. *Molecular Pharmaceutics, 5(2),* 167–190. DOI 10.1021/mp700151b.
97. Dall'Osto, L., Fiore, A., Cazzaniga, S., Giuliano, G., Bassi, R. (2007). Different roles of α- and β-branch xanthophylls in photosystem assembly and photoprotection. *Journal of Biological Chemistry, 282(48),* 35056–35068. DOI 10.1074/jbc.M704729200.

98. Neela, S., Fanta, S. W. (2019). Review on nutritional composition of orange-fleshed sweet potato and its role in management of vitamin A deficiency. *Food Science & Nutrition, 7(6),* 1920–1945. DOI 10.1002/fsn3.1063.
99. Barredo, J. L. (2012). *Microbial carotenoids from bacteria and microalgae: methods and protocols*. USA: Springer.
100. Britton, G. (2008). Functions of intact carotenoids. In: Britton, G., Liaaen-Jensen, S., Pfander, H. (eds.), *Carotenoids*, pp. 189–212. Switzerland: Birkhäuser Basel.
101. Cazzonelli, C. I., Pogson, B. J. (2010). Source to sink: regulation of carotenoid biosynthesis in plants. *Trends in Plant Science, 15(5),* 266–274. DOI 10.1016/j.tplants.2010.02.003.
102. Lange, B. M., Ghassemian, M. (2003). Genome organization in Arabidopsis thaliana: a survey for genes involved in isoprenoid and chlorophyll metabolism. *Plant Molecular Biology, 51(6),* 925–948. DOI 10.1023/A:1023005504702.
103. Yu, Q., Beyer, P. (2012). Reaction specificities of the ε-ionone-forming lycopene cyclase from rice (*Oryza sativa*) elucidated *in vitro*. *FEBS Letters, 586(19),* 3415–3420. DOI 10.1016/j.febslet.2012.07.060.
104. Giorio, G., Yildirim, A., Stigliani, A. L., D'Ambrosio, C. (2013). Elevation of lutein content in tomato: a biochemical tug-of-war between lycopene cyclases. *Metabolic Engineering, 20,* 167–176. DOI 10.1016/j.ymben.2013.10.007.
105. Kim, H. S., Wang, W., Kang, L., Kim, S. E., Lee, C. J. et al. (2020). Metabolic engineering of low-molecular-weight antioxidants in sweetpotato. *Plant Biotechnology Reports, 14(2),* 193–205. DOI 10.1007/s11816-020-00621-w.
106. Yuan, H., Zhang, J., Nageswaran, D., Li, L. (2015). Carotenoid metabolism and regulation in horticultural crops. *Horticulture Research, 2(1),* 1–11. DOI 10.1038/hortres.2015.36.
107. Clotault, J., Geoffriau, E., Lionneton, E., Briard, M., Peltier, D. (2010). Carotenoid biosynthesis genes provide evidence of geographical subdivision and extensive linkage disequilibrium in the carrot. *Theoretical and Applied Genetics, 121(4),* 659–672. DOI 10.1007/s00122-010-1338-1.
108. Yuan, H., Owsiany, K., Sheeja, T., Zhou, X., Rodriguez, C. et al. (2015). A single amino acid substitution in an ORANGE protein promotes carotenoid overaccumulation in Arabidopsis. *Plant Physiology, 169(1),* 421–431. DOI 10.1104/pp.15.00971.
109. Nisar, N., Li, L., Lu, S., Khin, N. C., Pogson, B. J. (2015). Carotenoid metabolism in plants. *Molecular Plant, 8 (1),* 68–82. DOI 10.1016/j.molp.2014.12.007.
110. Kim, H. S., Ji, C. Y., Lee, C. J., Kim, S. E., Park, S. C. et al. (2018). Orange: a target gene for regulating carotenoid homeostasis and increasing plant tolerance to environmental stress in marginal lands. *Journal of Experimental Botany, 69(14),* 3393–3400. DOI 10.1093/jxb/ery023.
111. Wang, Z., Xu, W., Kang, J., Li, M., Huang, J. et al. (2018). Overexpression of alfalfa Orange gene in tobacco enhances carotenoid accumulation and tolerance to multiple abiotic stresses. *Plant Physiology and Biochemistry, 130,* 613–622. DOI 10.1016/j.plaphy.2018.08.017.
112. Lopez, A. B., Van Eck, J., Conlin, B. J., Paolillo, D. J., O'neill, J. et al. (2008). Effect of the cauliflower *Or* transgene on carotenoid accumulation and chromoplast formation in transgenic potato tubers. *Journal of Experimental Botany, 59(2),* 213–223. DOI 10.1093/jxb/erm299.
113. Tzuri, G., Zhou, X., Chayut, N., Yuan, H., Portnoy, V. et al. (2015). A 'golden' SNP in CmOr governs the fruit flesh color of melon (Cucumis melo). *Plant Journal, 82(2),* 267–279. DOI 10.1111/tpj.12814.
114. Lu, S., Van Eck, J., Zhou, X., Lopez, A. B., O'Halloran, D. M. et al. (2006). The cauliflower *Or* gene encodes a DnaJ cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. *Plant Cell, 18(12),* 3594–3605. DOI 10.1105/tpc.106.046417.
115. Welsch, R., Zhou, X., Yuan, H., Álvarez, D., Sun, T. et al. (2018). Clp protease and *Or* directly control the proteostasis of phytoene synthase, the crucial enzyme for carotenoid biosynthesis in Arabidopsis. *Molecular Plant, 11(1),* 149–162. DOI 10.1016/j.molp.2017.11.003.
116. Zhou, X., McQuinn, R., Fei, Z., Wolters, A. M. A., Van Eck, J. et al. (2011). Regulatory control of high levels of carotenoid accumulation in potato tubers. *Plant, Cell & Environment, 34(6),* 1020–1030. DOI 10.1111/j.1365-3040.2011.02301.x.

117. Glover, B. J., Martin, C. (2012). Anthocyanins. *Current Biology, 22(5),* R147–R150. DOI 10.1016/j.cub.2012.01.021.

118. He, W., Zeng, M., Chen, J., Jiao, Y., Niu, F. et al. (2016). Identification and quantitation of anthocyanins in purple-fleshed sweet potatoes cultivated in China by UPLC-PDA and UPLC-QTOF-MS/MS. *Journal of Agricultural and Food Chemistry, 64(1),* 171–177. DOI 10.1021/acs.jafc.5b04878.

119. Sancho, R. A. S., Pastore, G. M. (2012). Evaluation of the effects of anthocyanins in type 2 diabetes. *Food Research International, 46(1),* 378–386. DOI 10.1016/j.foodres.2011.11.021.

120. Miguel, M. G. (2011). Anthocyanins: Antioxidant and/or anti-inflammatory activities. *Journal of Applied Pharmaceutical Science, 1,* 7–15.

121. Tian, Q., Konczak, I., Schwartz, S. J. (2005). Probing anthocyanin profiles in purple sweet potato cell line (Ipomoea batatas L. Cv. Ayamurasaki) by high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 53(16),* 6503–6509. DOI 10.1021/jf050671m.

122. Zhao, J. G., Yan, Q. Q., Lu, L. Z., Zhang, Y. Q. (2013). *In vivo* antioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato. *Nutrition Research and Practice, 7(5),* 359–365. DOI 10.4162/nrp.2013.7.5.359.

123. Lucioli, S. (2012). Anthocyanins: mechanism of action and therapeutic efficacy. In: Capasso, A. (ed.), *medicinal plants as antioxidant agents: understanding their mechanism of action and therapeutic efficacy*, pp. 27–57. Kerala, India: Research Signpost.

124. Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends in Plant Science, 18(9),* 477–483. DOI 10.1016/j.tplants.2013.06.003.

125. Sui, X. (2017). Changes in the color, chemical stability and antioxidant capacity of thermally treated anthocyanin aqueous solution over storage. In: *impact of food processing on anthocyanins*, pp. 49–65. *Springer Theses (Recognizing Outstanding Ph.D. Research)*. Singapore: Springer.

126. Zhao, C. L., Chen, Z. J., Bai, X. S., Ding, C., Long, T. J. et al. (2014). Structure-activity relationships of anthocyanidin glycosylation. *Molecular Diversity, 18(3),* 687–700. DOI 10.1007/s11030-014-9520-z.

127. Moglia, A., Lanteri, S., Comino, C., Hill, L., Knevitt, D. et al. (2014). Dual catalytic activity of hydroxycinnamoyl-coenzyme A quinate transferase from tomato allows it to moonlight in the synthesis of both mono-and dicaffeoylquinic acids. *Plant Physiology, 166(4),* 1777–1787. DOI 10.1104/pp.114.251371.

128. Tanaka, Y., Brugliera, F. (2013). Flower colour and cytochromes P450. *Philosophical Transactions of the Royal Society B, 368(1612),* 20120432. DOI 10.1098/rstb.2012.0432.

129. Kim, H. W., Kim, J. B., Cho, S. M., Chung, M. N., Lee, Y. M. et al. (2012). Anthocyanin changes in the Korean purple-fleshed sweet potato, Shinzami, as affected by steaming and baking. *Food Chemistry, 130(4),* 966–972. DOI 10.1016/j.foodchem.2011.08.031.

130. Wang, L., Zhao, Y., Zhou, Q., Luo, C. L., Deng, A. P. et al. (2017). Characterization and hepatoprotective activity of anthocyanins from purple sweet potato (Ipomoea batatas L. cultivar Eshu No. 8). *Journal of Food and Drug Analysis, 25(3),* 607–618. DOI 10.1016/j.jfda.2016.10.009.

131. Luo, C. L., Zhou, Q., Yang, Z. W., Wang, R. D., Zhang, J. L. (2018). Evaluation of structure and bioprotective activity of key high molecular weight acylated anthocyanin compounds isolated from the purple sweet potato (Ipomoea batatas L. cultivar Eshu No. 8). *Food Chemistry, 241,* 23–31.

132. Kamiloglu, S., Pasli, A. A., Ozcelik, B., Van Camp, J., Capanoglu, E. (2015). Colour retention, anthocyanin stability and antioxidant capacity in black carrot (Daucus carota) jams and marmalades: effect of processing, storage conditions and *in vitro* gastrointestinal digestion. *Journal of Functional Foods, 13,* 1–10. DOI 10.1016/j.jff.2014.12.021.

133. Xu, J., Su, X., Lim, S., Griffin, J., Carey, E. et al. (2015). Characterisation and stability of anthocyanins in purple-fleshed sweet potato P40. *Food Chemistry, 186,* 90–96. DOI 10.1016/j.foodchem.2014.08.123.

134. He, J., Giusti, M. M. (2010). Anthocyanins: natural colorants with health-promoting properties. *Annual Review of Food Science and Technology, 1(1),* 163–187. DOI 10.1146/annurev.food.080708.100754.

135. Mu, T., Sun, H., Zhang, M., Wang, C. (2017). *Sweet potato processing technology.* USA: Academic Press.

136. Hoballah, M. E., Gübitz, T., Stuurman, J., Broger, L., Barone, M. et al. (2007). Single gene-mediated shift in pollinator attraction in Petunia. *Plant Cell, 19(3),* 779–790. DOI 10.1105/tpc.106.048694.

137. Zhang, Y., Butelli, E., De Stefano, R., Schoonbeek, H. J., Magusin, A. et al. (2013). Anthocyanins double the shelf life of tomatoes by delaying overripening and reducing susceptibility to gray mold. *Current Biology, 23 (12),* 1094–1100. DOI 10.1016/j.cub.2013.04.072.

138. Malone, L. A., Barraclough, E. I., Wang, K. L., Stevenson, D. E., Allan, A. C. (2009). Effects of red-leaved transgenic tobacco expressing a MYB transcription factor on two herbivorous insects, Spodoptera litura and Helicoverpa armigera. *Entomologia Experimentalis et Applicata, 133(2),* 117–127. DOI 10.1111/j.1570-7458.2009.00910.x.

139. Ahmed, N. U., Park, J. I., Jung, H. J., Yang, T. J., Hur, Y. et al. (2014). Characterization of dihydroflavonol 4-reductase (DFR) genes and their association with cold and freezing stress in Brassica rapa. *Gene, 550(1),* 46–55. DOI 10.1016/j.gene.2014.08.013.

140. Wang, H., Fan, W., Li, H., Yang, J., Huang, J. et al. (2013). Functional characterization of dihydroflavonol-4-reductase in anthocyanin biosynthesis of purple sweet potato underlies the direct evidence of anthocyanins function against abiotic stresses. *PLoS One, 8(11),* e78484. DOI 10.1371/journal.pone.0078484.

141. Kano, M., Takayanagi, T., Harada, K., Makino, K., Ishikawa, F. (2005). Antioxidative activity of anthocyanins from purple sweet potato, Ipomoera batatas cultivar Ayamurasaki. *Bioscience, Biotechnology, and Biochemistry, 69(5),* 979–988. DOI 10.1271/bbb.69.979.

142. Zhang, Y., Butelli, E., Martin, C. (2014). Engineering anthocyanin biosynthesis in plants. *Current Opinion in Plant Biology, 19,* 81–90. DOI 10.1016/j.pbi.2014.05.011.

143. Li, P., Cheng, L. (2008). The shaded side of apple fruit becomes more sensitive to photoinhibition with fruit development. *Physiologia Plantarum, 134(2),* 282–292. DOI 10.1111/j.1399-3054.2008.01131.x.

144. Zhu, H., Zhang, T. J., Zheng, J., Huang, X. D., Yu, Z. C. et al. (2018). Anthocyanins function as a light attenuator to compensate for insufficient photoprotection mediated by nonphotochemical quenching in young leaves of Acmena acuminatissima in winter. *Photosynthetica, 56(1),* 445–454. DOI 10.1007/s11099-017-0740-1.

145. Pereira, R. J., Cardoso, M. G. (2012). Metabólitos secundários vegetais e benefícios antioxidantes. *Journal of Biotechnology and Biodiversity, 3(4),* 146–152.

146. Wang, L. S., Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters, 269(2),* 281–290. DOI 10.1016/j.canlet.2008.05.020.

147. Bontempo, P., De Masi, L., Carafa, V., Rigano, D., Scisciola, L. et al. (2015). Anticancer activities of anthocyanin extract from genotyped Solanum tuberosum L. "Vitelotte". *Journal of Functional Foods, 19,* 584–593. DOI 10.1016/j.jff.2015.09.063.

148. Ramirez, J. E., Zambrano, R., Sepúlveda, B., Kennelly, E. J., Simirgiotis, M. J. (2015). Anthocyanins and antioxidant capacities of six Chilean berries by HPLC-HR-ESI-ToF-MS. *Food Chemistry, 176,* 106–114. DOI 10.1016/j.foodchem.2014.12.039.

149. Li, Y. Q. (2008). Application prospect of the purple sweet potato anthocyanin. *Journal of Anhui Agricultural Sciences,* 29.

150. Stintzing, F. C., Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science & Technology, 15(1),* 19–38. DOI 10.1016/j.tifs.2003.07.004.

151. He, W., Zeng, M., Chen, J., Jiao, Y., Niu, F. et al. (2015). Identification and quantitation of anthocyanins in purple-fleshed sweet potatoes cultivated in China by UPLC-PDA and UPLC-QTOF-MS/MS. *Journal of Agricultural and Food Chemistry, 64(1),* 171–177. DOI 10.1021/acs.jafc.5b04878.

152. Bassa, L., Francis, F. (1987). Stability of anthocyanins from sweet potatoes in a model beverage. *Journal of Food Science, 52(6),* 1753–1754. DOI 10.1111/j.1365-2621.1987.tb05927.x.

153. Sun, H., Zhang, P., Zhu, Y., Lou, Q., He, S. (2018). Antioxidant and prebiotic activity of five peonidin-based anthocyanins extracted from purple sweet potato (Ipomoea batatas (L.) Lam.). *Scientific Reports, 8(1),* 1–12. DOI 10.1038/s41598-017-17765-5.

154. Zhang, X., Yang, Y., Wu, Z., Weng, P. (2016). The modulatory effect of anthocyanins from purple sweet potato on human intestinal microbiota *in vitro*. *Journal of Agricultural and Food Chemistry, 64(12),* 2582–2590. DOI 10.1021/acs.jafc.6b00586.

155. Yoo, H., Widhalm, J. R., Qian, Y., Maeda, H., Cooper, B. R. et al. (2013). An alternative pathway contributes to phenylalanine biosynthesis in plants via a cytosolic tyrosine: phenylpyruvate aminotransferase. *Nature Communications, 4(1),* 2833. DOI 10.1038/ncomms3833.

156. Manela, N., Oliva, M., Ovadia, R., Sikron-Persi, N., Ayenew, B. et al. (2015). Phenylalanine and tyrosine levels are rate-limiting factors in production of health promoting metabolites in Vitis vinifera cv. Gamay Red cell suspension. *Frontiers in Plant Science, 6,* 538.

157. Nishiyama, Y., Yun, C. S., Matsuda, F., Sasaki, T., Saito, K. et al. (2010). Expression of bacterial tyrosine ammonia-lyase creates a novel p-coumaric acid pathway in the biosynthesis of phenylpropanoids in Arabidopsis. *Planta, 232(1),* 209–218. DOI 10.1007/s00425-010-1166-1.

158. Falcone Ferreyra, M. L., Rius, S., Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science, 3,* 222.

159. Sasaki, N., Nishizaki, Y., Ozeki, Y., Miyahara, T. (2014). The role of acyl-glucose in anthocyanin modifications. *Molecules, 19(11),* 18747–18766. DOI 10.3390/molecules191118747.

160. Marrs, K. A., Alfenito, M. R., Lloyd, A. M., Walbot, V. (1995). A glutathione S-transferase involved in vacuolar transfer encoded by the maize gene Bronze-2. *Nature, 375(6530),* 397–400. DOI 10.1038/375397a0.

161. Zhao, J., Dixon, R. A. (2010). The 'ins' and 'outs' of flavonoid transport. *Trends in Plant Science, 15(2),* 72–80. DOI 10.1016/j.tplants.2009.11.006.

162. Grotewold, E., Davies, K. (2008). Trafficking and sequestration of anthocyanins. *Natural Product Communications, 3(8),* 1251–1258. DOI 10.1177/1934578X0800300806.

163. Chanoca, A., Kovinich, N., Burkel, B., Stecha, S., Bohorquez-Restrepo, A. et al. (2015). Anthocyanin vacuolar inclusions form by a microautophagy mechanism. *Plant Cell, 27(9),* 2545–2559. DOI 10.1105/tpc.15.00589.

164. Kallam, K., Appelhagen, I., Luo, J., Albert, N., Zhang, H. et al. (2017). Aromatic decoration determines the formation of anthocyanic vacuolar inclusions. *Current Biology, 27(7),* 945–957. DOI 10.1016/j.cub.2017.02.027.

165. Markham, K. R., Gould, K. S., Winefield, C. S., Mitchell, K. A., Bloor, S. J. et al. (2000). Anthocyanic vacuolar inclusions—their nature and significance in flower colouration. *Phytochemistry, 55(4),* 327–336. DOI 10.1016/S0031-9422(00)00246-6.

166. Shirley, B. W., Kubasek, W. L., Storz, G., Bruggemann, E., Koornneef, M. et al. (1995). Analysis of Arabidopsis mutants deficient in flavonoid biosynthesis. *Plant Journal, 8(5),* 659–671. DOI 10.1046/j.1365-313X.1995.08050659.x.

167. Pelletier, M. K., Burbulis, I. E., Winkel-Shirley, B. (1999). Disruption of specific flavonoid genes enhances the accumulation of flavonoid enzymes and end-products in Arabidopsis seedlings. *Plant Molecular Biology, 40(1),* 45–54. DOI 10.1023/A:1026414301100.

168. Dong, X., Braun, E. L., Grotewold, E. (2001). Functional conservation of plant secondary metabolic enzymes revealed by complementation of Arabidopsis flavonoid mutants with maize genes. *Plant Physiology, 127(1),* 46–57. DOI 10.1104/pp.127.1.46.

169. Marrs, K. A. (1996). The functions and regulation of glutathione S-transferases in plants. *Annual Review of Plant Biology, 47(1),* 127–158. DOI 10.1146/annurev.arplant.47.1.127.

170. Mueller, L. A., Goodman, C. D., Silady, R. A., Walbot, V. (2000). AN9, a petunia glutathione S-transferase required for anthocyanin sequestration, is a flavonoid-binding protein. *Plant Physiology, 123(4),* 1561–1570. DOI 10.1104/pp.123.4.1561.

171. Kitamura, S., Matsuda, F., Tohge, T., Yonekura-Sakakibara, K., Yamazaki, M. et al. (2010). Metabolic profiling and cytological analysis of proanthocyanidins in immature seeds of Arabidopsis thaliana flavonoid accumulation mutants. *Plant Journal, 62(4),* 549–559. DOI 10.1111/j.1365-313X.2010.04174.x.

172. Sun, Y., Li, H., Huang, J. R. (2012). Arabidopsis TT19 functions as a carrier to transport anthocyanin from the cytosol to tonoplasts. *Molecular Plant, 5(2),* 387–400. DOI 10.1093/mp/ssr110.

173. Alfenito, M. R., Souer, E., Goodman, C. D., Buell, R., Mol, J. et al. (1998). Functional complementation of anthocyanin sequestration in the vacuole by widely divergent glutathione S-transferases. *Plant Cell, 10(7),* 1135–1149. DOI 10.1105/tpc.10.7.1135.

174. Kitamura, S., Akita, Y., Ishizaka, H., Narumi, I., Tanaka, A. (2012). Molecular characterization of an anthocyanin-related glutathione S-transferase gene in cyclamen. *Journal of Plant Physiology, 169(6),* 636–642. DOI 10.1016/j.jplph.2011.12.011.

175. Hu, B., Zhao, J., Lai, B., Qin, Y., Wang, H. et al. (2016). LcGST4 is an anthocyanin-related glutathioneS-transferase gene in Litchi chinensis Sonn. *Plant Cell Reports, 35(4),* 831–843. DOI 10.1007/s00299-015-1924-4.

176. Pérez-Díaz, R., Madrid-Espinoza, J., Salinas-Cornejo, J., González-Villanueva, E., Ruiz-Lara, S. (2016). Differential roles for VviGST1, VviGST3, and VviGST4 in proanthocyanidin and anthocyanin transport in Vitis vinifera. *Frontiers in Plant Science, 7,* 1166.

177. Luo, H., Dai, C., Li, Y., Feng, J., Liu, Z. et al. (2018). Reduced anthocyanins in petioles codes for a GST anthocyanin transporter that is essential for the foliage and fruit coloration in strawberry. *Journal of Experimental Botany, 69(10),* 2595–2608. DOI 10.1093/jxb/ery096.

178. Chu, H., Jeong, J. C., Kim, W. J., Chung, D. M., Jeon, H. K. et al. (2013). Expression of the sweetpotato R2R3-type IbMYB1a gene induces anthocyanin accumulation in Arabidopsis. *Physiologia Plantarum, 148(2),* 189–199. DOI 10.1111/j.1399-3054.2012.01706.x.

179. Allan, A. C., Hellens, R. P., Laing, W. A. (2008). MYB transcription factors that colour our fruit. *Trends in Plant Science, 13(3),* 99–102. DOI 10.1016/j.tplants.2007.11.012.

180. Gonzali, S., Mazzucato, A., Perata, P. (2009). Purple as a tomato: towards high anthocyanin tomatoes. *Trends in Plant Science, 14(5),* 237–241. DOI 10.1016/j.tplants.2009.02.001.

181. Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C. et al. (2010). MYB transcription factors in Arabidopsis. *Trends in Plant Science, 15(10),* 573–581. DOI 10.1016/j.tplants.2010.06.005.

182. Crifò, T., Puglisi, I., Petrone, G., Recupero, G. R., Piero, A. R. L. (2011). Expression analysis in response to low temperature stress in blood oranges: implication of the flavonoid biosynthetic pathway. *Gene, 476(1–2),* 1–9. DOI 10.1016/j.gene.2011.02.005.

183. Xu, W., Dubos, C., Lepiniec, L. (2015). Transcriptional control of flavonoid biosynthesis by MYB-bHLH–WDR complexes. *Trends in Plant Science, 20(3),* 176–185. DOI 10.1016/j.tplants.2014.12.001.

184. Pervaiz, T., Songtao, J., Faghihi, F., Haider, M. S., Fang, J. (2017). Naturally occurring anthocyanin, structure, functions and biosynthetic pathway in fruit plants. *International Journal of Plant Biochemistry and Physiology, 5,* 1–9.

185. Lalusin, A. G., Nishita, K., Kim, S. H., Ohta, M., Fujimura, T. (2006). A new MADS-box gene (IbMADS10) from sweet potato (Ipomoea batatas (L.) Lam) is involved in the accumulation of anthocyanin. *Molecular Genetics and Genomics, 275(1),* 44–54. DOI 10.1007/s00438-005-0080-x.

186. Mano, H., Ogasawara, F., Sato, K., Higo, H., Minobe, Y. (2007). Isolation of a regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato. *Plant Physiology, 143(3),* 1252–1268. DOI 10.1104/pp.106.094425.

187. He, L., Tang, R., Shi, X., Wang, W., Cao, Q. et al. (2019). Uncovering anthocyanin biosynthesis related microRNAs and their target genes by small RNA and degradome sequencing in tuberous roots of sweetpotato. *BMC Plant Biology, 19(1),* 232. DOI 10.1186/s12870-019-1790-2.

188. Brugliera, F., Tao, G. Q., Tems, U., Kalc, G., Mouradova, E. et al. (2013). Violet/blue chrysanthemums-metabolic engineering of the anthocyanin biosynthetic pathway results in novel petal colors. *Plant and Cell Physiology, 54(10),* 1696–1710. DOI 10.1093/pcp/pct110.

189. Kabadi, A. M., Gersbach, C. A. (2014). Engineering synthetic TALE and CRISPR/Cas9 transcription factors for regulating gene expression. *Methods, 69(2),* 188–197. DOI 10.1016/j.ymeth.2014.06.014.