Development of fruit color in Solanaceae: a story of two biosynthetic pathways

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Abstract
This review highlights the major differences between the regulation of two important pathways namely anthocyanin and carotenoid pathways, responsible for fruit color generation in Solanaceae mediated by transcription factors (TFs). The anthocyanin pathway is regulated by a common set of TFs (MYB, MYC and WD40) belonging to specific families of DNA-binding proteins. Their regulation is aimed at controlling the type and amount of pigments produced and the physiological conditions (like pH) at which they are finally stored. In the carotenoid pathway, the color diversity depends on the quantity of pigment produced and the point where the pathway is arrested. TFs in the latter case are accordingly found to influence the sequestration and degradation of these pigments, which determines their final concentration in the tissue. TFs (phytochrome interacting factors, MADS-BOX, HB-ZIP and B-ZIP) also regulate important rate-determining steps, which decide the direction in which the pathway proceeds and the point at which it is terminated. In the absence of a clear pattern of TF-mediated regulation, it is suggested that the carotenoid pathway is more significantly influenced by other regulatory methods which need to be explored. It is expected that common factors affecting these pathways are the ones acting much before the initiation of the biosynthesis of respective pigments.

Keywords: pigments; Solanaceae; carotenoids; anthocyanins; transcription factors; regulation

INTRODUCTION
Secondary metabolites are the organic compounds that are not essential for the growth of an organism but provide additional advantages. These compounds are produced by large number of plants where they act as attractants (carotenoids and anthocyanins) for pollinators and foragers so as to ensure pollination and seed dispersal, provide defense against predators [1, 2], induce resistance to a variety of environmental stresses, etc. Thus, secondary metabolism represents an adaptation of plants, expected to have been modified, by a large number of natural genetic variations (intra/interspecific) during evolution, so that they can survive better under harsh environmental conditions. Besides being a signature of the adaptation, natural variations also provide evidence regarding the genetic regulation of the complex traits in an organism.

MAJOR PIGMENTS IN SOLANACEAE
Among all plant families, Solanaceae serves as a model family to understand the evolutionary and comparative genomics of secondary metabolism. The Solanaceae comprises about 96 genera and 3000 species [3], which include food plants (tomato, brinjal and potato), medicinal plants...
(Withania and Datura), ornamentals (Physalis and Petunia), desert dwellers (Solanum surratense), aquatic plants (Solanum tampicense) and vegetable crops (tomato, potato, brinjal and pepper). It ranks third among economically important plant families [4].

Pigments represent a subtype of secondary metabolites that have direct influence on the survivability of a plant. Fruits of the plants belonging to Solanaceae have been found to contain two important types of pigments anthocyanins and carotenoids, which belong to two important classes of secondary metabolites, flavonoids (phenylpropanoid pathway) and isoprenoids (DOXP/MEP and MVA pathway), respectively [5–7] (Figure 1).

Anthocyanins represent the largest class of water soluble vacuolar flavonoids, products of phenylpropanoid pathway, which impart red/orange to violet/blue coloration to the fruits and flowers according to the pH [8]. These act as photoprotectants in photosynthetic tissues and attractants in non-photosynthetic tissues [9–11]. Anthocyanin biosynthesis and regulation has been studied in many plants such as Petunia, Fragaria ananassa (strawberry), Solanum tuberosum (potato), S. melongena (brinjal), Arabidopsis, etc. [8, 10–16]. Phenylalanine is the initial precursor of the flavonoid biosynthetic pathway from where different types of flavonoids are synthesized utilizing various enzymes [17] (Figure 2). Capsicum and Petunia of Solanaceae represent model fruit and flower, respectively, which have been used to understand the complex biology of anthocyanin biosynthetic pathway.

Carotenoids are C40 isoprenoid molecules synthesized within plastids by two pathways in plants: MVA and MEP/DOXP [18–20]. These are synthesized in all photosynthetic organisms (plants, algae and bacteria) as well as some non-photosynthetic bacteria and fungi [21, 22]. Similar to anthocyanins, carotenoids act as photoprotectants from oxidative damage in photosynthetic tissues, whereas in chromoplast tissues, they act as attractants for pollination and seed dispersal [18, 23, 24]. Animals do not synthesize carotenoids in their body; they derive it from their diet, where β-carotene serves as a precursor for the vitamin A synthesis [25, 26]. Carotenoids have been extensively studied in Solanaceae plants such as tomato, pepper, etc. [18, 27–29]. Geranyl-geranyl pyrophosphate is the precursor of carotenoid biosynthetic pathway which leads to the synthesis of large number of carotenoids such as lycopene, β-carotene, lutein, zeaxanthin, etc. [30, 31] (Figure 2). Tomato represents a model plant of Solanaceae, which has been used for the study of structural and functional genomics as well as metabolic engineering of carotenoid biosynthetic pathway [32–34].

This review focuses on understanding the role of transcription factors (TFs) in the differential regulation of carotenoids and anthocyanins that are responsible for determining the fruit color in various members of the Solanaceae family.

**DIVERSITY IN FRUIT COLOR WITHIN SOLANACEAE**

Diversity of fruit color within family Solanaceae has been attributed to the presence of either anthocyanins (purple, blue and red fruits) or carotenoids (red, orange or yellow fruits) as dominant pigments (or their absence hence imparting green color) in the ripe fruits (Figure 3). Both these pathways are isolated from each other. It is unlikely that the machinery of biosynthesis of one of the pigments is entirely absent from the plants in which they are not produced, because this would imply major gene losses from the genomes. Solanaceae in particular is known to be a moderately evolving family, wherein translocations and paracentric inversions have been major modes of genome evolution [35]. This is evident from the cases where environmental conditions have been found to induce expression of pigments...
that are normally not expressed in them. For instance, cold induces expression of anthocyanins in tomato fruits, which normally express only carotenoids. Moreover, both these end products share their precursors with other important secondary metabolites such as lignin, tannins and phytoalexins, which are important to generate strong responses against environmental stresses [35]. So, their production may indirectly be affected by the plant’s response to environmental conditions even if it is mediated by other compounds. Such instances indicate that the nature of processes determining the dominant pigment in a plant may be reversible. Hence, it can be expected that very few regulatory factors determine the functional state of either of the pathways at a very early point.

To arrive at a conclusion, it is important to understand how these pathways are individually regulated and what mechanisms are responsible for generating variations in both anthocyanin and carotenoid-based coloration. Therefore, many studies carried out in plants other than those belonging to Solanaceae family and tissues other than fruits have also been reviewed in this article to give more information regarding the regulatory events associated with each of these two pathways.

**Diversity at structural level**

In case of anthocyanin pathway, it has been observed that the evolution of structural genes is highly constrained. Among these genes, the ones present upstream are subject to negligible variations, possibly due to their involvement in multiple pathways [36]. Livingstone and Anderson [37] suggested a similar pattern of evolution of carotenoid biosynthetic genes, which stated that upstream genes are under strong selective constraint due to different metabolic flux as compared with the downstream enzymes. Ramsay et al. [38] demonstrated that upstream genes of plant terpenoid biosynthetic pathway are under very high selective driving forces and tend to evolve slowly than the downstream genes, because of being utilized for the synthesis of large number of end products, e.g. carotenoids, phytoalexins and various plant developmental hormones. Similar constraints were expressed by Clotault et al. [39] in carrot and other dicots.

Studies have shown that the Solanaceae genome is amenable to variations at a moderate rate. Wu and Tanksley [40] found that when genome maps of tomato, potato, brinjal and pepper were compared, rearrangements per chromosome/million years occurred at 0.03–0.12 rates. Also it was explained
that Solanaceae family has evolved mainly in the absence of polyploidization, e.g. tomato, brinjal and pepper have the same chromosome number \((2n = 24)\). Some tetraploids of potato and tobacco are available, but this modification has occurred recently and still diploid species have been found in wild varieties. Doganlar et al. [35] studied evolution of Solanaceae plants at genetic level and found that the fruit color has been affected during evolution by domestication. It has been seen that most wild relatives of *Solanum melongena* (brinjal) have green fruits when half ripe, whereas its cultivated varieties display variety of colors due to the presence or absence of anthocyanin and chlorophyll pigments in the fruit tissue.

Anthocyanin accumulation is determined by a major locus at linkage group 10 of brinjal, which has been attributed to about 93% phenotypic variations in anthocyanin content of fruits and different plant tissues [35]. Additional loci for anthocyanin content were found in the genome-like linkage group 6, but they showed very less effect when compared with the Quantitative Trait Loci (QTL) present on linkage group 10. The diverse phenotypic expression of the conserved anthocyanin-related regions in brinjal, tomato and potato indicates that the same anthocyanin gene targets might have experienced different mutations. These mutations affected their regulation hence resulting in the difference in the kind of pigment that is primarily being expressed in the contemporary varieties of these species. The linkage group 10 locus of tomato may have experienced some kinds of mutations during domestication due to which tomato plants express only low levels

Figure 3: Fruit color diversity in various plants of family Solanaceae. (A) Brinjal, pepper and tomato. (B) Variation in fruit color in tomato. (A colour version of this figure is available online at: http://bfg.oxfordjournals.org)
of anthocyanin [35]. However, there is a possibility that the nature of these mutations is reversible as seen in cold-stressed tomato and eggplant that express high amount of anthocyanins. Variations occurring at structural level do not offer such flexibility. Hence, it can be concluded that regulatory genes play a major part in the evolution of fruit color. The regulation of these traits is largely attributed to regulatory elements such as transcription factors (TFs) or post-transcriptional gene silencing (PTGS) mechanisms to allow these plants to get adapted rapidly to the dynamically changing environmental conditions.

Diversity at functional level
Color diversity in Solanaceae is controlled by a set of regulatory genes called as TFs. TFs bind to the cis-regulatory elements, i.e. either enhancer or promoter region of DNA adjacent to the genes, that they regulate at functional level. Depending on the TFs, the transcription is either up- or downregulated. TFs have been extensively studied in chili pepper [41, 42], tomato [43–45], Petunia [11, 46–48], potato [49, 50] and tobacco [51]. Large number of TFs have been identified in the model plant Arabidopsis [52], apple [53] and animals [54].

TFs REGULATING ANTHOCYANIN PATHWAY: MYB, MYC AND WD40
Regulation of expression of structural genes of anthocyanin biosynthetic pathway is carried out by a ternary complex of three TFs namely MYB, MYC and WD40 [55, 56]. In animals, MYBs have been found to regulate the cell proliferation and differentiation [57, 54]. On the other hand, plant MYB genes are structurally and functionally more variable and play diverse roles in plant growth and development, e.g. signal transduction [58], abiotic stress [59] and regulation of carotenoids and flavonoid biosynthetic pathways [48, 55, 56, 60]. MYBs contain a common binding domain (about 52 amino acids) that consists of 1–3 imperfect helix-turn-helix repeats abbreviated as R1, R2 and R3 [52].

Capsicum annuum (pepper) displays a wide spectrum of anthocyanin pigmentation, which makes it a suitable model for the study of anthocyanins. When transcript levels of TFs were studied in fruits, flowers and foliar tissue by real-time polymerase chain reaction, it was found that MybA and Myc transcript levels are higher in fruits and flowers, whereas no differential expression was seen in foliar tissues [55]. No differential expression was observed in transcript levels of WD40 genes in pigmented as well as non-pigmented floral tissues and fruit but anthocyanin structural gene (chs, dfr and ans) transcript levels were higher in anthocyanin-pigmented tissues than in non-anthocyanin pigmented tissues, showing that WD40 does not have much influence on anthocyanin biosynthesis. mybA:myc transcript ratio in Capsicum annum was ~4 fold greater in fruit and flower than foliar tissues [55]. Rausher et al. [36] explained that regulatory genes tend to evolve more quickly than the structural genes they regulate. This shows that the color diversity is mediated more by regulatory genes than the structural ones [61].

In Petunia, R2R3-MYB TF namely DEEP PURPLE (DPL), PURPLE HAZE (PHZ), AN2, AN4 are involved in the regulation of anthocyanin synthesis [11, 48, 62]. Besides the basic helix-loop-helix (bHLH) factor, ANTHOCYANIN 1 (AN1) and WD-repeat protein (AN11) are also necessary for vegetative pigmentation [48]. In Petunia, differential expression of these TFs was observed [48]. AN2 regulates the petal limb color, whereas AN4 controls flower tube and anther pigmentation. DPL is associated with flower tube vein pigmentation, whereas PHZ regulates the extended floral pigmentation. AN 11 is expressed throughout all major plant organs [63], whereas differential expression of AN1 was observed in Petunia [46, 64]. Together all these TF (AN4, AN1, AN11, AN2, DPL and PHZ) differentially regulate the anthocyanin pigmentation in petunia flower leading to variable anthocyanin coloration in flowers, e.g. purple, white, deep blue, pink and many more.

MYB proteins also showed differential regulation in two cultivars of tomato namely VFNT cherry (VC), a small fruited tomato containing 200 μg/g fresh weight lycopene in the ripe fruit and Ailsa craig (AC), a medium fruited tomato containing 70.5 μg/g fresh weight lycopene in the ripe fruit [60]. MYB TC85864 was more abundant in red ripe fruit of VC than at other stages, whereas it was more abundant at the mature green stage of AC than at other stages. This shows that MYB also regulates the carotenoid biosynthetic pathway in tomato.

Rate of evolution of regulatory genes involved in anthocyanin pathway has been found to be higher than that of the structural genes [65]. Rosinski and Atchley [66] and Jin and Martin [67] deciphered the evolution of MYB TF. They proposed that a single original MYB repeat was replicated to give rise to
R2 and R3 repeat MYB proteins. MYB proteins of plants are thought to have evolved following deletion of the R1 to give R2R3 family and the subgroups within this MYB family are thought to have arisen by duplication of entire genes. This indicates that the regulatory genes often undergo dynamic changes to evolve. It can be concluded that the anthocyanin pathway is primarily regulated by means of a common set of TFs, whose variants are found in different plants. The genes involved in this regulation are more or less similar (Table 1) and encode protein variants belonging to specific families.

Anthocyanin accumulation is known to occur transiently at various developmental stages and in response to certain environmental factors including light (visible and UV radiations), osmotic stresses, low temperature, etc. [68]. In the context of foliar tissue, it has been observed in young and expanding foliage as well as in the autumnal foliage of deciduous trees. Although being associated with decreased photosynthetic capability, this strategy is likely to confer adaptive advantage against nutritional deficiency, UV radiations and herbivores [69]. Among the various environmental factors affecting anthocyanin accumulation, the influence of light has been well demonstrated. Recently, novel alleles encoding mutant anthocyanin biosynthetic and light signaling genes have been identified in Petunia [70]. A novel Flavonone-3-hydroxylase (F3H) cDNA has been isolated from the desert plant Reaumuria soongorica, which showed increased expression under UV and drought stress [71].

Many studies have revealed how various environmental signals and developmental regulatory networks interact with the specific regulatory factors that are known to influence anthocyanin production and accumulation in plant tissues (see Jaakola [72]). Both developmental and environmental factors are known to influence anthocyanin accumulation by either directly influencing the expression of structural genes or by influencing the accumulation or degradation of the basic regulatory complex. Recently, a R2R3-MYB TF encoding gene LcMYB1 was discovered in Litchi chinensis whose expression was found to enhance upon exposure to sunlight and abscisic acid treatment. The expression of this gene was also observed to be strongly correlated with tissue anthocyanin content and LcUFGT expression [73]. This study also reported that the mechanism of anthocyanin accumulation in reproductive tissue, unlike their vegetative counterparts, is independent of endogenous levels of MYB and bHLH TFs and is associated with direct upregulation of specific structural genes.

Environmental factors may also influence the level of anthocyanins by regulating the level of carbon supply in the anthocyanin biosynthetic pathway. In grapes, UV irradiation was found to increase anthocyanin accumulation, attributed to increased carbon supply for anthocyanin biosynthesis due to promotion of shikimate pathway [74]. This study also reported that the effect of UV rays is dependent on the developmental stage of the fruit. UV-A radiations were reported to act as a stimulus for upregulating the shikimate pathway genes in 3-week old grape berries, whereas UV-B and UV-C influence this expression in 11-week old berries. Similar modulations in the expression of three structural genes (VvANR, VvLAR1 and VvLAR2) and three regulatory genes (MYB5a, MYB5b and MYBPA1) were observed in response to UV radiations [75]. These observations suggest the existence of an extensive crosstalk between the basic regulators of anthocyanin pathway as well as environmental and developmental regulators, which need to be further understood.

<table>
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<th>Table I: Gene families regulating carotenoids and anthocyanins</th>
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<td>WD-Repeat</td>
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<td>HB-ZIP</td>
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<td>B-ZIP</td>
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<td>AP 2</td>
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<td>UV-damaged DNA-binding protein</td>
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<td>CRYPTOCHROMES</td>
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Note: Summary of the TFs regulating the two pathways in Solanaceae. Common TFs regulating the two pathways belong to the families: MYB, bHLH, damaged DNA-binding proteins and cryptochromes. These common TFs act as initial sensors of various environmental stimuli like light, DNA damage, wounding, etc., which in turn affects these two biosynthetic pathways.
REGULATION OF CAROTENOID PATHWAY

Regulation of carotenoid-based coloration is not as clearly understood. Three major mechanisms regulating the amount of carotenoids in plant tissues include:

(i) Transcriptional and post-transcriptional regulation of biosynthesis.
(ii) Transcriptional and post-transcriptional regulation of degradation.
(iii) Regulation involving lipo-protein sequestering structures.

TARGETS FOR REGULATORY MECHANISMS

Phytoene synthase (psy) gene

Structural genes encoding carotenoid biosynthetic enzymes have been identified but their regulation is obscure. The psy gene forms an important regulatory control point and is found to be a frequent target of various members of the phytochrome family. RAP2.2, a light-induced TF has been found to bind to the promoter region of this gene. However, modulation of this TF has been found to cause small pigment alterations [76].

Carotenoid isomerase (crtiso) gene

Another important enzyme that is a major target of various regulatory mechanisms is the crtiso gene, whose activity has been found to influence the carotenoid composition of various tissues. This gene converts poly-cis forms of carotenes into poly-trans forms [79].

MAJOR REGULATORY MECHANISMS

Regarding regulation of biosynthesis, TFs directly affecting the carotenoid pathway are poorly understood till now. However, there are many other factors that show indirect effect on the amount and composition of carotenoids in various tissues. Most of these factors are known to have broad ripening effects instead of specifically regulating the fruit metabolic pathways. These factors include RIN-MADS [83], CNR-SQUAMOSA promoter binding protein [84], TAGL1-MADS BOX [85], LeHB-1 HB zip [77] and SIAP2a (AP2 gene) [86, 87] (Table 1). Apart from these, various ethylene response factors and phytochrome interacting factors (PIFs) have been found to have a major effect on carotenoid content, as discussed below.

Light induced regulation

PIFs are a family of proteins that mediate light-induced signaling in plants. They act downstream to phytochromes (PHY), which function as photoreceptors within the cell. PIF1 (a bHLH TF) and related PIF families have been found to repress the gene encoding phytoene synthase (psy) and hence downregulating the expression of carotenoids. PIF1 has been found to bind to the psy promoter region, thereby repressing its expression [88]. All light responsive genes, including psy are characterized by the presence of a light responsive element in their promoter, which contains conserved G1 and G2 motifs and Z-box. Other light-responsive TFs that are needed by PHY in downstream signaling steps include the HY5, which is a b-zip TF. Lehy5 transgenic plants, which contained an RNAi of PHY-A activated hy5 gene, have been found to have 24-31%
less leaf chlorophyll and a reduced chlorophyll and carotenoid content in its immature fruits [89].

Another class of light-induced regulators include the cryptochrome, CRY, which is also a photoreceptor. CRY1 is found to localize into nucleus when plants are grown in light. It is exported to the cytoplasm in case of dark conditions [90–92]. CRY2, which belongs to same family as CRY1, is localized in the nucleus during both light and dark periods [90]. Overexpression of cry2 gene has been found to repress cry gene and cause overproduction of flavonoids and lycopene in fruits [93]. All these light-controlled TFs are further affected by COP1, which is a ring finger ubiquitin ligase protein associated with the signalosome complex involved in protein degradation. CRY and PHY, which induce these TFs, have been found to inactivate COP1 through direct contact [94, 95]. In presence of light, COP1 undergoes conformational change and releases TFs like HY5, HFR1, etc. that are otherwise colocalized with it [96–98]. Decreased COP1 content has been found to increase chlorophyll and carotenoid content in an RNAi containing transgenic tomato. Other negative regulators of light-mediated gene expression include DET1, whose silencing leads to carotenoid accumulation in tomato fruits [99] and DDB1, which is a UV-damaged DNA-binding protein. DDB1 along with DET1 leads to ubiquitination of TFs by interacting with the signalosome, leading to their degradation. HP1 and HP2 represent the tomato orthologs of DDB1 and DET1. Tomato mutants hp1 and hp2 are characterized by high amount of carotenoids in their fruits, anthocyanin accumulation and excessive response to light [100].

**TF-mediated regulation: The MADS-BOX Family**

One of the important aspects of carotenoid regulation includes dependence on TFs encoded by an important family of genes known as the MADS-box genes. MADS-box genes are involved in regulating many developmental processes in plants, such as meristem growth, flower, fruit and root development [101, 102]. MADS-box family has been extensively studied in tomato [103–106] and Petunia [102, 107]. Seven MADS-box genes have been identified to be effective during fruit development in tomato. MADS box protein consists of 56 amino acids, which are involved in the recognition and binding to DNA sequences, which they regulate.

Similar to MYB, MADS-box is involved in diverse functions in plants [108–111].

One of the essential MADS-box TFs that regulates the carotenoid biosynthesis in tomato is the RIPENING INHIBITOR (RIN) [106]. RIN interacts with the promoters of genes involved in carotenoid biosynthesis and affects the level of carotenoids in tomato by differential regulation. Pan et al. [112] demonstrated by chromatin immunoprecipitation assay that RIN interacts with the promoters of genes encoding rate-limiting activities mainly Phytoene synthase 1 (psy1). Similarly, by using RNAi (RNA interference) technology, Pan et al. [112] demonstrated that AGAMOUS genes encoding MADS-box TF regulate the carotenoid biosynthesis by interacting with the promoters of lycopene–β-cyclase gene (cyc-β) and carotenoid isomerase (crtiso) gene.

Tomato has two representatives of this AGAMOUS lineage namely TOMATO AGAMOUS (TAG1) and TAG-LIKE1 (TAGL1) having different functions in plant development. TAG1 has been linked to carotenoid biosynthesis. Taking all the factors into account, different TFs regulate the carotenoid biosynthetic pathway in different ways by targeting different structural genes. RIN has high expression at the turning and red ripe stages in the VC and AC tomato cultivars, which show that RIN TF is important to fruit ripening, whereas there is differential expression of other MADS-box TF like TAG1, TM6 in these cultivars [60]. Along with other factors like nor, crn, hb-1, RIN also acts upstream to ethylene regulated biochemical events. Tomato plants possessing a mutant version of rin gene show a non-ripening phenotype. These mutants show various deficiencies including inhibition of carotenoid biosynthesis genes [113]. Another mutant nr (never ripe) shows ethylene insensitivity but has functional rin gene.

**Carotenoid degradation**

A major aspect of regulation of carotenoid content includes control of carotenoid degradation. Enzymes involved in carotenoid degradation and cleavage activities affect the final amount of carotenoids in plant tissues and also result in the formation of other important metabolites. Carotenoid cleavage dioxygenases (ccd), for instance, have a regulatory role and also mediate strigolactone production by producing a hormonal intermediate [114]. Another such enzyme NCED (9-cis-epoxycarotenoid dioxygenase) has been found to be involved in production of abscisic acid (ABA) [114, 115]. Stigma-specific ccd
genes from saffron have been isolated that have same function but differ in sub-cellular localization. Another \textit{ccd} homolog from \textit{Chrysanthemum} namely \textit{Cmccd4a} is strongly expressed in white petals. It was found that in these tissues, carotenoids were produced but were degraded into colorless apocarotenoids [116]. Hence, carotenogenesis, along with carotenoid retention is important for color generation in tissues.

**Carotenoid retention and sequestration**

Retention of carotenoids or carotenoid accumulation is generally preceded by hydroxylation and subsequent esterification. Upregulation of \(\beta\)-carotene hydroxylase activity has been associated with carotenoid accumulation in many cases including tomato [117]. It has also been observed that free, soluble forms of phytoene synthase and phytoene desaturase are non-functional, whereas membrane associated forms, after hydroxylation are active [118].

Another major mechanism affecting the carotenoid accumulation in plants is the presence of sequestering structures within the cells. Although trying to develop carotenoid-rich cultivars of various important food crops, it was observed that altering gene expression to achieve desired carotenoid concentration was not an effective approach [119, 120]. In many plants, carotenoid accumulation was found to be controlled by the creation of a metabolic sink that sequestered these pigments. It was therefore suggested that the combined manipulation of the sink and the catalytic activity should be done to enhance carotenoid content of the tissues [121]. The sequestering mechanism involves creation of novel substructures within the chromoplast. These structures are referred to as carotenoid sequestering structures [122, 123]. These structures not only serve the purpose of accumulating carotenoids but also prevent the end products of the pathway from overloading the synthesis site and hence inhibiting the synthesis in any way [124–126].

Li and Eck [121] found that the mutant \(Or\) gene, that encodes a DnaJ cysteine-rich domain containing protein, conferred orange–red color in the curd tissue of cauliflower. This accumulation of \(\beta\)-carotene was found to be a result of differentiation of proplastids/non-colored plastids into chromoplasts in apical shoot and inflorescence meristem, rather than being a result of alterations in the biosynthesis pathway. Cytological effect of this transgene revealed the formation of large membranous chromoplasts within the cells of transformants [127]. The total carotenoid level within the transformants was found to be \(\sim 6\) times higher than the non-transformed and vector only controls in experiments where this gene was expressed in potato tubers under the effect of a GBSS promoter [121]. The transformed tubers showed orange–yellow flesh. These \(Or\) transgenic tubers were also found to have additional orange bodies that included intact chromoplasts and orange–colored helical sheets and fragments that had been released from chromoplasts. It has also been pointed out that increased level of carotenoids does not normally lead to the formation of these structures as observed in a high carotenoid breeding line of potato [128]. Hence, this strategy can be useful in enhancing the carotenoid content of those tissues that contain all genes of the carotenoid pathway in functional state. The enhanced sink capacity also provides a pulling force that draws the flux through the pathway, hence leading to an elevated level of carotenoids in the tissues.

**INFERENCE**

As is clear from above discussion, the regulation of carotenoids biosynthesis is not as straightforward as in case of anthocyanins. It seems that the regulatory factors mediating the crosstalk between these two pathways are not involved in direct interaction with the biosynthetic machinery. These pathways may be overlapping at the level of induction in response to various stimuli and hence the common factors may be the ones which act upstream to the actual biosynthetic pathway.

Solanaceae family provides a colorful palette encompassing both carotenoid and anthocyanin-based pigments, which need to be studied in depth to reveal the mechanisms that determine the dominance of a particular pigment in the fruits of a species. Its members, being moderately divergent from each other and showing similar effects of domestication on their genome, offer a uniform genetic background against which this hugely varying trait can be studied and useful conclusions can be drawn. This article provides the background information on the factors regulating different color pathways either directly or indirectly. It highlights the major differences between the TF-mediated regulation of two important pigment producing pathways responsible for generating the variety of fruit color seen within the Solanaceae family. Because of these regulatory mechanisms, Solanaceae plants manifest a wide range of fruit colors due to which Solanaceae is
acknowledged as ‘color diversity hub’. In the anthocyanin pathway, the reasons of color variation are largely qualitative. The regulation is hence aimed at controlling the type as well as amount of pigments finally produced and the physiological conditions (like pH) at which they are finally stored. Consequently, this pathway is regulated by a set of identical TFs that either decide which branch of the pathway would be activated or influence the physiological conditions of the cell. In the carotenoid pathway, the color diversity depends on the quantity of pigment produced as well as on the point where the pathway is arrested. The TFs in this case are accordingly found to influence the sequestration and degradation of these pigments, which determines their final concentration in the tissue.

TFs also regulate important rate-determining steps that decide the direction in which the pathway shall proceed and the point at which it will be terminated. In the absence of a common pattern of TF-mediated regulation among different plants and absence of TFs that are found to specifically affect the biosynthetic pathway, it is suggested that the carotenoid pathway is influenced by other regulatory methods like miRNA-mediated PTGS, which needs to be explored.

Key points
- Solanaceae presents a tremendous variety of fruit color, which is mainly attributed to pigments belonging to two major classes—carotenoids and anthocyanins. These pigments are synthesized via two independent pathways that do not merge at any point. The variation in fruit color within Solanaceae can be explained by studying the regulation of these two respective pathways.
- The anthocyanin pathway is known to be regulated at transcriptional level by TFs that have been clearly identified in many other plants as well.
- The carotenoid pathway shows lower dependence on specialized TFs for its regulation. This pathway is mainly regulated by various post-transcriptional regulatory mechanisms.

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References
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87. Toledo−Ortiz G, Huq E, Rodrı ´guez−Concepcio´n M. Direct regulation of phytoene synthase gene expression


