



Regulation and Improvement of Carotenoid Metabolism in Tomato

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ABSTRACT

Carotenoids are essential pigments for human and plant life, but only plants can produce them. Seven hundred fifty carotenoids improve the lives of different organisms. The tomato is used as a model to get complete information on the biosynthesis of carotenoids. Different horticultural crops are used to learn about biochemical and genetic pathways. Some genes and enzymes play a significant role in regulating carotenoids, like SIPSY1 and DXS. Similarly, some pathways, like the MEP pathway, contribute a lot to the biosynthesis of carotenoids in tomatoes. This study presents a thorough overview of carotenoid biosynthesis and emphasizes new developments in genetic pathways that improve tomato carotenoid production, accumulation, and regulation.

Keywords: Carotenoid Metabolism, Biosynthetic Pathways, Lycopene, Plastids

Article History

Article # 23-384

Received: 15-Jun-23

Revised: 01-Jul-23

Accepted: 12-Aug-23

INTRODUCTION

Tomato play a vital role in the human diet, and it benefits human health because it is a rich source of antioxidants and vitamins. Furthermore, it has a tremendous nutritional economic impact (Fu, Meng, et al. 2016). Carotenoids are members of isoprenoids, and they have at least 750 other members distributed among bacteria, plants, fungi, and algae. In a typical carotenoid structure, 40 carbons are present in the polyene backbone and also have conjugated double bonds. Some rings are also present at the end of the structure.

The large conjugated bonds permit carotenoids they absorb light (visible) and, in return, give different colors like yellow, red, and orange. These colors construct the most manifest pigments in plants. Carotenoids are categorized further into two groups that are Xanthophyll and carotene. These are mostly synthesizing different pigments. They are orange, yellow, and red. The isoprenoid biosynthetic pathway is used to produce these pigments in plants, which can be provided in the dark and light periods. These pigments offer in different parts of plants like leaf roots, fruits, maize kernels, flowers, and endosperm. (Liu, Shao et al. 2015) Carotenoids play an essential role in photosynthesis by activating hormone production in plants abscisic acid and strigolactone. Carotenoids act as pigments and play a role as a membrane stabilizer. Thus,

carotenoid is essential for the development of plants and the production of fruit. This carotenoid also responds to stimuli like abiotic stress (Zhang, Li et al. 2018). B-carotene and Lycopene play an essential role in human health, and their catabolite is good for health, and both are carotenoids. Pro-vitamin A is a significant need for our health, and B-carotene acts as a precursor for Pro-vitamin A. Deficiency of pro-vitamin A is a significant health issue. Lycopene acts as an excellent antioxidant that is good for health. (XIE, WEI et al. 2019) So, carotenoids are essential for our life, but here is a problem: humans cannot produce these carotenoids in their bodies. So ultimately, they get these carotenoids from different food sources like fruits and also vegetables. These dietary sources also act as a precursor to get these essential compounds. (Smita, Rajwanshi et al. 2013) Lycopene is essential in decreasing the risk of cancer and cardiovascular diseases. Lycopene acts as a good antioxidant. During metabolism in the body, various harmful by-products, like free-radical produce. Lycopene helps to stop or minimize the production of such hazardous products.

Tomato is an excellent reservoir of Lycopene, and it shows a specific red color that also symbolizes the right concentration of Lycopene. Lycopene is good for health, so using Tomatoes as a food source is also suitable for health. That is the reason why we mainly use Tomato to track the metabolism of Lycopene. (Pandurangiah, Ravishankar, et al. 2016).

Cite this Article as: Rehman I, Nazim R, Meraj A, Ameer A, waqar S, Arshad F and Ali S, 2023. Regulation and Improvement of Carotenoid Metabolism in Tomato. International Journal of Agriculture and Biosciences 2023 12(4): 199-207. <https://doi.org/10.47278/journal.ijab/2023.065>



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Biosynthesis of Carotenoids

Role of Genetic Pathways

Carotenoids are produced mainly in different plastids, but it concentrates in large amounts in the green tissue of chloroplast. In fruits and roots, carotenoids produce in chromoplast.

Different types of studies, like mutation analysis, help detect the biochemical formation of carotenoids, as we can check the biochemical biosynthetic pathways of carotenoids. (Yuan, Zhang, et al. 2015) Over time, much progress has done to know more detailed information about biosynthetic pathways. Genes and genetic modification processes help check out the role of specific genes in regulating pathways.

Similarly, genetic studies show us an elaborate structure of carotenoids and their contents. A recent development in the biosynthesis of carotenoids is the finding and characterization of genes that code enzymes for the carotenoid's biosynthesis pathway. Plants are the primary source of proteins and genes that code our carotenoids and their synthetic path and also help in catabolism.

The experiments were done on different horticulture crops to identify the genes that help in synthesis. Isopentyl diphosphate (IPP) acts as a building block in the formation of carotenoids, and an isomeric form called dimethylallyl diphosphate also takes part in the creation of carotenoids (DMAPP). There are two different routes in plants that are used for the biosynthesis of IPP and DAMPP, these are;

1. Mevalonic acid pathway (MVA).
2. Methylerythritol 4-phosphate (MEP) (Lichtenthaler, 1999) (Eisenreich, Rohdich et al. 2001).

Role of Enzymatic Pathway

The nuclear genome has genes that code the enzyme used in the MEP pathway, and their target is plastid. (Rodríguez-Concepción and Boronat 2002) (Bouvier, Rahier, et al. 2005) Recently, some rules have been formed for the nomenclature of genetic material, like genes, uniquely that encode enzymes for the MEP pathway. (Phillips, León et al. 2008) MEP pathway is catalyzed through DXS. It is a reaction in which DXP is produced due to the condensation of a compound called thiamine. This thiamine is produced by reacting pyruvate with the C1 aldehyde group of glyceraldehyde 3-phosphate. DXP reduces and forms DXR with the help of rearrangement of different molecules that act as intermediate. The conversion of MEP and subsequent production of HMBPP need four enzymatic steps. Then IPP and DMAPP form by the conversion HMBPP. Recently much research has been done; through this, we know about the genes that code enzymes and regulate their expression.

Even have well-known knowledge about the post-transcriptional and post-translational processes. (Cordoba, Salmi et al. 2009) (Hunter, 2007) (Rodríguez-Concepción, 2006) In-plant DXR and HDR act as rate-determining elements that control the MEP pathway reaction. (Vallabhaneni and Wurtzel 2009) (Morris, Ducreux, et al. 2006) (Muñoz-Bertomeu, Arrillaga, et al. 2006) (Mahmoud and Croteau 2001, Flores-Pérez, Pérez-Gil et al. 2008)

DXS in most plants is coded by a gene group containing two classes. (Walter, Hans et al. 2002, Krushkal, Pistilli et al. 2003) Class 1 of DXS isozyme mainly involves synthesizing necessary elements of isoprenoids.

However, class 2 enzyme use in the combination of secondary isoprenoids. There is an expectation that genes that code class 1 enzyme present in the Arabidopsis and soybean plants (Zhang, Li et al. 2009)

and the genes that code the third class of enzyme present in the sorghum and rice plant (Kim, Kim, et al. 2005) These three significant classes of DXS enzymes are essential in the creation of carotenoids. These enzymes work in different parts of plants. So the concentration of carotenoids in a plant depends on the gene expression of class 1 enzyme in the photosynthetic tissues and also in fruits (Khemvong & Suvachittanont, 2005), and class 2 isoform enzyme genes present in the mycorrhizal fungi (Floß, Hause et al. 2008) and even in flower petals (Kishimoto & Ohmiya, 2006) and tissues of seed endosperm contain the class 3 isoform.

Role of Plastids

Plastids are the places where biosynthesis and storage of carotenoids occur. The primary source of plastids is a plant where plastids are present in different forms like chloroplast, amyloplast, and chromoplast. (Lopez-Juez and Pyke 2004) (Jarvis and López-Juez 2013) For different types of plastids, proplastids are the progenitors. In amyloplast, a low amount of carotenoid is present. Most Xanthophyll is present, like zeaxanthin and lutein. (Howitt and Pogson 2006) (Wurtzel, Cuttriss, et al. 2012) It is considered less essential gene activity, and minimum transcription is the primary cause of this less carotenoid in amyloplast. (Bai, Capell et al. 2016) Etioplast also contains a less amount of photosynthetic carotenoid, mainly violaxanthin, because photosynthesis is happening less due to the lack of a precursor called protochlorophyllide, which is a chlorophyll precursor and plays an essential role in photomorphogenesis that occur in illumination. (Rodríguez-Villalón, Gas et al. 2009).

The plastid contains the carotenoid rate-limiting enzyme PSY. It is believed that the primary reason for less amount of carotenoid in the etioplast is the less expression of PSY and its local position in the plastid. Phytochrome-interacting factors (PIF) belong to a transcription factor family known as bHLH that reduces photomorphogenesis in the dark period. This PIF determines as the regulator of the PSY. (Von Lintig, Welsch et al. 1997) PSY promoter contains a G-box element, PIF attaches to that box and reduces the expression of PSY, so ultimately it causes downregulation of protein or carotenoid expression in etioplast. (Toledo-Ortiz, Huq et al. 2010) (Jahns and Holzwarth 2012) (Niyogi and Truong 2013) Different crucial carotenoids like violaxanthin, neoxanthin, and leptin are in the chloroplast in amazingly constant rations. There is a lot of carotenoid accumulation in the chromoplast of different parts of plants, like flowers and fruits. (Egea, Barsan et al. 2010) (Schweiggert and Carle 2017) Chromoplast is not naturally present in some mutant plants like orange cauliflower. However, it evolves in different non-photosynthetic tissues with the help of different kinds of plastids like amyloplast and proplastids (Li, Paolillo, et al. 2001). Some other crops also show this behavior, like carrot roots and papaya (Schweiggert, Steingass, et al. 2011). B-carotene and Lycopene overexpress and concentrate in the form of crystals colored red or orange in a typical chromoplast known as crystalline chromoplast that is primarily present in Tomato. (Jeffery, Holzenburg et al. 2012) (Ruiz-Sola and Rodríguez-Concepción 2012).

A series of events take place for the biosynthesis of carotenoids in plastids. Through the MEP pathway, several precursors are produced for carotenogenesis. (Milborrow and Lee 1998) This pathway also helps in photosynthesis as chlorophyll side chains and other compounds that play a significant role in photosynthesis produce through this pathway. This pathway also produces two compounds, known as isoprene and

diterpene. (Lichtenthaler, Schwender et al. 1997) (Matusova, Rani, et al. 2005) The MEP pathway is primarily found in the plastid, while the MVA pathway is primarily in the cytosol. There needs to be more than a typical shuttle of metabolic reactions for the MEP and MVA pathway that uses more than one shuttle to produce carotenoids. This shuttle moves between the cytosol and the plastid. (Schuhr, Radykewicz et al. 2003) Different intermediates can supply exogenously to MEP and MVA pathways to manipulate the amount of exchange, which is a delicate process. (Hemmerlin, Hoefler et al. 2003) (Heintze, Görlach, et al. 1990, Kasahara, Hanada et al. 2002, Nagata, Suzuki et al. 2002).

Nevertheless, by applying all conditions exchange amount is not so high. (Estévez, Cantero et al. 2001, Flores-Pérez, Pérez-Gil et al. 2010) (Carretero-Paulet, Cairo, et al. 2006) (Sauret-Güeto, Botella-Pavía, et al. 2006) (Hsieh & Goodman, 2005) (Kobayashi, Suzuki et al. 2007) (Crowell, Packard et al. 2003) (Suzuki, Nakagawa et al. 2009) (Budziszewski, Lewis et al. 2001) (Guevara-García et al. et al. 2005) (Page, Hause et al. 2004) There is limited transfer of precursor for the synthesis of isoprenoids in cells naturally. Hence, the MVA pathway provides the primary precursor for producing sterol, sesquiterpene, and brassinosteroid but not on a large scale. The MEP pathway is the primary source of precursors for producing phyloquinone, gibberellin, plastoquinone, isoprene, and side chains of chlorophyll. Different experiments show that not all but primarily precursors for producing carotenoids are taken from the MEP pathway. In an experiment, we mutate Arabidopsis by blocking all the genes of the MEP pathway that produce precursors for carotenoid synthesis, and we conclude that in this condition, some carotenoid synthesizes in the seedling. (Araki, Kusumi, et al. 2000) (Estévez, Cantero, et al. 2000) No effect is shown when we block the MVA pathway in the same plant with mevinolin.

This flow sheet diagram represents the components involved in the biosynthesis of carotenoids. The process starts with Pyruvate + G3P, and several steps are involved in which positive regulation, negative regulation, and several enzymes involved. Like CCD, carotenoid cleavage dioxygenase, DXS 1-deoxy-d-xylulose 5-phosphate synthase, NCED, 9-cis-epoxycarotenoid dioxygenases, RAP2.2, related to ap2 CrtISO, carotene isomerase.

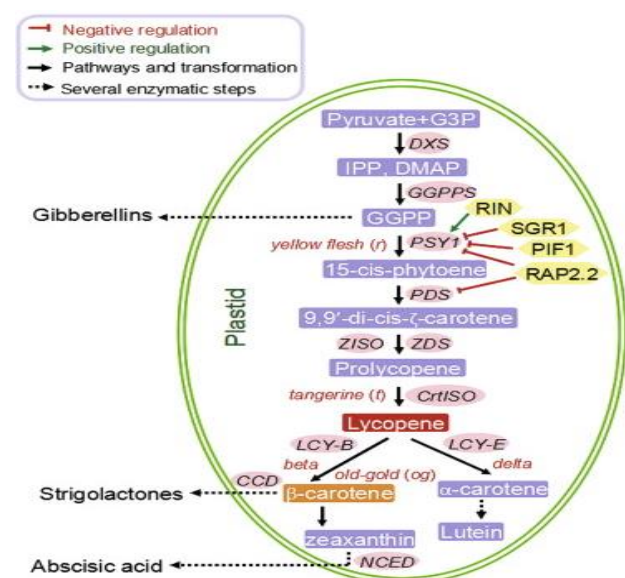


Fig. 1: Process of carotenoid biosynthesis (Liu et al., 2015).

Biosynthesis of Lycopene

Role of Enzymatic Pathways

Geranylgeranyl diphosphate is a mainly studied pathway in Tomato because it synthesizes carotenoids. The nuclear genome encodes the biosynthetic enzymes of carotenoids in plastids. (Davies 2009) 1 deoxy-D-xylulose-5-phosphate synthase (SIDXS), phytoene synthase (SIPSY), alpha-carotene desaturase (SIZDS), geranylgeranyl pyrophosphate synthase (SIGGPPS), phytoene desaturase (SIPDS) and carotene isomerase (CrtISO) are some different enzymes that encode by genes present in Tomato and these genes upregulated and produce a transcript for the formation of these enzymes during the biosynthesis of Lycopene. Furthermore, this whole process occurs during the ripening of Tomato. (Giuliano, Bartley, et al. 1993) (Fraser, Truesdale et al. 1994) (Corona, Aracri et al. 1996) (Pecker, Gabbay et al. 1996)

Role of Genes

On the other hand, some genes performance dramatically decreased during the lycopene formation in tomatoes, like gema cyclase (SILCY-E/CRTL-E) and B-cyclase (SILCY-B/CRTL-E). (Ronen, Cohen et al. 1999) For carotenoid biosynthesis during the early ripening stage in Tomato, the SIDXS gene extensively expresses in the initial regulation. Also, it plays a significant role in the accumulation of Lycopene in tomatoes. (Lois, Rodríguez- Concepción et al. 2000) There are two further isotypes of genes SIDX1 and SIDX2, and they are present in plants. (Walter, Hans, et al. 2002) SIDX1 plays a significant role in forming Lycopene in Tomato, while SIDX2 does not express extensively during fruit ripening. However, SIDX2 is present in the leaf and isolated trichomes in different parts of young tomato plants. (Paetzold, Garms et al. 2010) There is a DXS gene isolated from E. coli upregulated by the promotor, FIBRILLIN (FIB), overexpressed during the formation of carotenoids in the tomatoes. (Enfissi, Fraser et al. 2005).

Bioavailability of Lycopene

Lycopene is a significant isoprenoid and also an essential part of the human diet. (Maiani, Periago Castón et al. 2009) Lycopene has excellent nutritional value; it plays a vital role in preventing prostate cancer (Campbell, Canene-Adams et al. 2004) and helps to stop skin and cardiovascular diseases. (Kohlmeier, Kark et al. 1997).

We get Lycopene from different sources, including tomatoes, but here is a problem, we cannot use that Lycopene from a nutritive source directly because it is present in the Trans-isomer form. Our body needs a cis-isomer form. (Campbell, Engelmann, et al. 2007) This cis-isomer is more bioavailable in the human body than trans-isomer. This cis-isomer has some characteristics, like they are soluble and more easily absorbed in the body than other trans-isomers. (Boileau, Boileau et al. 2002) This division of isomers happens in-vivo. There are different sites of body organs where the cis-isomer form of Lycopene isomerizes. We use heat to convert trans-isomer into cis-isomer of Lycopene. (Liu et al., 2015).

Metabolic Engineering of Lycopene

Lycopene is an integral part of our diet. There are many experiments done to improve the concentration of Lycopene in tomatoes. Lycopene is present mainly in large amount in ripped tomatoes. However, there is a feature of Lycopene as it changes into beta-carotene due to lycopene beta-cyclase. So, we make two constructs called OE and AS. OE construct has high b-cyclase gene expression, while AS shows low expression. These

constructs introduce in the tomato with the help of Agrobacterium-mediated transformation. In this experiment, we use the Pds promoter, as it helps to increase the expression. Ultimately, we conclude that AS has gene expression that shows a high amount of Lycopene and has 50% less expression of B-cyclase. (Rosati, Aquilani et al. 2000).

Modification of Biosynthetic Pathways Manipulation of Genes Codes Lycopene

The first enzyme DXS regulates the activity of the entire MEP pathway by keeping an eye on the finite amount of various isoprenoid precursors, which are essential for the production of carotenoids. So the overproduction of enzymes like DXS, HDR, DXP, and DXP reductoisomerase, and HMBPP by making plant transgenic help in the overproduction of carotenoids in plants like tomato. However, any change in MVA flux did not show any positive results. (Estévez, Cantero et al. 2001) (Botella-Pavía, Besumbes, et al. 2004) (Enfissi, Fraser, et al. 2005).

We identified different alters that mutate form provides new ways and sites for producing carotenoids. Two mutated forms of tomatoes overproduce Lycopene. The first is yellow flush; in this one, locus *r*, a gene *SIPSY1* gets mutated (Fray & Grierson, 1993), and the second one is tangerine; in this one, locus *f*, the *SlCRTISO* gene gets mutated. (Isaacson, Ronen, et al. 2002) Through the first mutant, we now know that after the repression of the *SIPSY1* gene, the level of Lycopene in fruit is down, while much up-regulation of *tSIPSY1* gene overproduces Lycopene. (Fray and Grierson 1993) So yellow color is the symbol of less Lycopene in Tomato because when the *SIPSY1* gene downregulates, its antisense gene upregulate, and due to the expression of the antisense gene, 3% lycopene is present in Tomato, and on maturity, the Tomato get yellow color. (Ray, Moureau et al. 1992) Transgenic Induced local lesion in genome is a technique to identify the knockout mutation in the *SIPSY1* gene. (Gady, Vriezen et al. 2012) So, through this experiment, *SIPSY1* is essential for producing Lycopene in Tomato as its absence gives the yellow color fruit with less or without lycopene/carotenoid.

Another experiment proves that a constitutive promoter for *SIPSY1* for continuous expression also has harmful effects. Due to overexpression, Tomato shows dwarfism, pigmentation in flowers, and leaf changes, and in this situation, we can also get yellow color fruit that does not have carotenoids. The activity of the *SIPSY1* gene reduces or co-suppress just because of the redirecting of GPP that changes the reaction. Instead of the carotenoid formation, it moves toward the gibberellin biosynthesis pathway. (Fray and Grierson 1993).

Bacterial phytoene synthase enzyme (*crtB*) express in the Tomato in a specific manner; this expression over come all the harmful pleiotropic effects in fruit and enhances the production of carotenoids in tomatoes. (Fraser, Romer et al. 2002) (Fraser, Enfissi et al. 2007) Another experiment shows that binding *AtPSY* and *AtPDS* to specific transcription factors like *APETALA2/Ethylene response factor* and another phytochrome interacting factor decreases the carotenoid content in fruit. (Welsch, Maass et al. 2007) (Toledo-Ortiz, Huq, et al. 2010) *SIPSY1* promoter interaction with *MADS-box* and ripening inhibitor (*RIN*) determine their role in regulating carotenoid concentration in tomatoes.

Modification with CRISPR Cas9

Bioactive compounds are ample nutritional constituents primarily found in small amounts in food.

Lycopene is also a bioactive compound, so its proper amount is essential for health. CRISPR Cas9 method is used to modulate genes that code essential enzymes that help in metabolic pathways, so ultimately, its purpose is to enhance the production of Lycopene and improve fruit quality. (Čermák, Bales et al. 2015) (Nonaka, Arai et al. 2017, Li, Li et al. 2018) (Li, Wang et al. 2018) Aluminum-activated-malate transporter 9 (*ALMT9*) is an integral part of malate content in Tomato, and it is identified through CRISPR Cas9. (Ye, Wang et al. 2017) In Tomato, the *locule* plays an indispensable role in the fruit size and contributes a maximum of 50% in the size enlargement of fruit; these *locules* present in the flower petals. Through CRISPR Cas 9, we identified different quantitative trait loci that control the number of loci in fruit. (Li, Qi et al. 2017) In the Cold Spring Harbor Laboratory, some scientists damaged the stem cell system *CLAVATA-WUSCHEL (CLU-WUS)* and produced a large tomato fruit using CRISPR Cas9. (Ma, Nicole et al. 2015) (Wang, Zhang, et al., 2019) To increase the shelf life of unripe Tomato, ripening inhibitors and sometimes DNA demethylase 2 inactivates by using CRISPR. (Lang, Wang et al. 2017) (Ito, Nishizawa-Yokoi, et al. 2015).

Modification in Plastidial Proteins

During the biosynthesis of carotenoid, in the first step, essential regulatory elements catalyze through an enzyme phytoene synthase that plays a vital role in the synthesis of phytoene for GGPP. (Camagna, Grundmann et al. 2019).

Alternatively, it is the protein that influences the differentiation in chromoplast structure in fruit (Rodriguez-Concepcion, Avalos, et al. 2018) (Sun, Yuan et al. 2018) OR is a protein that is DNA-J related in fruit, and it acts as a chaperon protein. (Park, Kim et al. 2016) Alternatively proteins are present in different versions, and they all play a specific role in carotenoid biosynthesis; they enhance *PSY* concentration, while some reduce B-carotene metabolism. (Bai, Rivera et al. 2014) (Chayut, Yuan, et al. 2017) (Kim, Ji et al. 2018) There is an amino acid Arg change with His in the polypeptide chain of OR, and this change causes a mutation known as "golden SNP." This mutation increases the B-carotene amount in some varieties-OR-His form in protein help enhance *PSY* activity.

The other variant OR-Arg also plays an important role, reducing the B-carotene conversion in the other downregulating forms. Both OR-His and OR-Arg versions help to enhance the production and accumulation of carotenoids in tomatoes. (Yazdani, Sun et al. 2019) The concentration of chaperon, specifically heat-shock protein, increases in amount during the ripening of Tomato. (Barsan, Zouine et al. 2012) (Shukla, Upadhyay et al. 2017) (D'Andrea, Simon-Moya et al. 2018) In the ripening process of Tomato, chloroplast converts into chromoplast due to the constitutive expression of some chaperons' proteins, for example (*Hsp21*) that is a small heat shock protein. (Neta-Sharir, Isaacson et al. 2005) *DXS* is an essential protein in the biosynthesis of Lycopene. Still, it sometimes loses its typical structure and change; this change happens due to misfolding in protein standard structure and accumulates in the chloroplast. (Pulido, Toledo-Ortiz et al. 2013) *DXS* in inactive form deliver to *Hsp70* to unfold, and this process is facilitated by the binding of *J20*, which is a DNAJ co-chaperone and acts like an *Hsp70* adapter. Artificial microRNA is used in ripening tomato fruits for the activity of *Clp* protease, and it works against a catalytic core subunit. Ultimately, the amount of *PSY* and *DXS* increased in ripe Tomato. (D'Andrea, Simon-Moya et al. 2018).

Modification of Transcription Pathway

The principal branch of the carotenoid biosynthesis pathway is the cyclization of Lycopene. One path leads to the formation of the ABA and strigolactones because, in this pathway, neoxanthin and zeaxanthin are B-carotene and Violaxanthin enzymes provide precursors for the synthesis. The second route leads to the integration of alpha-carotene and lutein. So, we can control the biosynthesis of carotenoids by regulating these two routes that involve in the cyclization of Lycopene. Two genes known as SILCY-B and SILCY-E regulate these two routes.

Further SILCY-B gene is divided into two genes, known as SICRTL-B and SICYC-B, in flowers and green tissues. The first shows the activity, while the second is in the chromoplast. (Ronen, Carmel-Goren, et al. 2000) Due to the upregulation of SICYC-B, the B-carotene level becomes high in the Tomato. Tomato is a model system in the production of Lycopene. We know the exact pathway and components of the process by determining the transcription regulation factors and signals that activate them. There are different upstreams genes like CRTISO, DXS, PDS, and PSY that upregulate and show increased transcription during the formation of Lycopene in Tomato.

Similarly, there are different downregulated genes like LCYB, LCYE, and CHYB; they suppress their expression during lycopene synthesis. (Isaacson, Ronen, et al. 2002) (Ronen, Cohen, et al. 1999) (Fraser, Truesdale, et al. 1994) An increase in the transcription of LCYE causes the conversion of Lycopene into alpha-carotene. (Liu et al., 2015).

Similarly, B-carotene produces from the conversion of Lycopene due to the transcription of LCYB in transgenic Tomato. (Ronen, Carmel-Goren, et al. 2000) We use different mechanisms to modify carotenoid enzymes, cofactors, protein structure, amino acid sequence, protein-protein interaction, sub-organelles positions, and membrane localization. The most common mechanism we use is the modulation of an amino acid sequence of biosynthetic enzymes, so they either increase or decrease activities. For example, in cassava, an amino acid is changed, and it causes the high production of Lycopene in the yellow cultivar. This amino acid is present in PSY conserved region. (Welsch, Arango et al. 2010) Another critical point in controlling the biosynthesis of carotenoids is carotenoid sequestration and stable storage of carotenoids in plastids of plants. (Vishnevetsky, Ovadis et al. 1999) (Li and Van Eck 2007) For the biosynthesis and extensive accumulation of carotenoids, chromoplast has a specific mechanism that sequesters the carotenoids into lipoprotein-sequestering. (Li, Yang et al. 2012, Li and Yuan 2013).

Conclusion

Human health survival is almost impossible without carotenoids, which are essential nutraceuticals. So, it is necessary to produce many carotenoids like Lycopene. Naturally, carotenoids produce in the body, but they are fewer in number, so different modifications have been done to increase the production of Lycopene in Tomato. We use different horticultural crops to modify the molecular system of Tomato for the high production of Lycopene. However, horticultural crops have yet to be fully discovered. Some information regarded genetic and biochemical advancement that is still hidden. Also, CRISPR can be used more efficiently to permanently get more transcription and translation of our desired genes and knockout suppressor genes. Many crops can produce higher carotenoids than tomatoes; we need to explore their

mechanism at the molecular level, use advanced technologies to establish these mechanisms in our desired plants without any harmful effects, and also make these transgenics able to survive in all environmental conditions.

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