The Genetic Components of a Natural Color Palette: A Comprehensive List of Carotenoid Pathway Mutations in Plants

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Carotenoids comprise the most widely distributed natural pigments. In plants, they play indispensable roles in photosynthesis, furnish colors to flowers and fruit and serve as precursor molecules for the synthesis of apocarotenoids, including aroma and scent, phytohormones and other signaling molecules. Dietary carotenoids are vital to human health as a source of provitamin A and antioxidants. Hence, the enormous interest in carotenoids of crop plants. Over the past three decades, the carotenoid biosynthesis pathway has been mainly deciphered due to the characterization of natural and induced mutations that impair this process. Over the year, numerous mutations have been studied in dozens of plant species. Their phenotypes have significantly expanded our understanding of the biochemical and molecular processes underlying carotenoid accumulation in crops. Several of them were employed in the breeding of crops with higher nutritional value. This compendium of all known random and targeted mutants available in the carotenoid metabolic pathway in plants provides a valuable resource for future research on carotenoid biosynthesis in plant species.

Keywords: carotenoid biosynthesis, MEP pathway, mutants, genetic screens, chemical-genetics

INTRODUCTION

Isoprenoids represent the most functional and diverse class of naturally occurring metabolites present in all organisms. Carotenoids, the largest group of natural pigments, belong to a subgroup of isoprenoid-derived compounds (Maoka, 2020). In plants, carotenoids play diverse functions, first and foremost in photosynthesis as accessory light-harvesting pigments and photoprotectants, and as precursors for the hormones abscisic acid (ABA), strigolactones (Ruiz-Sola and Rodríguez-Concepción, 2012), and other growth regulators (Wang et al., 2020c). In addition, carotenoids play secondary roles in providing distinctive hues and colors to flowers and fruits. Carotenoids’ significance is not limited to plants; their contributions to human health as antioxidants and provitamin A make them indispensable in our diet (Rao and Rao, 2007; Meléndez-Martinez et al., 2021).

Isoprenoid biosynthesis in plants occurs in the cytosol and plastids by the mevalonic acid (MVA) and methylerythritol 4-phosphate (MEP) pathways, respectively. The MVA pathway provides the isopentenyl diphosphate (IPP) precursor for synthesizing sterols, terpenoids, and...
brassinosteroids. In contrast, the plastidial MEP pathway supplies IPP and dimethylallyl diphosphate (DMAPP) necessary for synthesizing tocopherols, chlorophylls, carotenoids, gibberellic acids, many other terpenoids.

Carotenoid biosynthesis and metabolism have been extensively studied due to their essential roles in plant development and physiology. In the last three decades, all the essential enzymes and genes of the MEP and carotenoid biosynthetic pathways have been identified (Cunningham and Gantt, 1998; Hirschberg, 2001; Phillips et al., 2008b; Cordoba et al., 2009; Ruiz-Sola and Rodriguez-Concepción, 2012; Nisar et al., 2015; Figure 1). Most of the genes in these pathways have been characterized using mutants. Collections of chemically mutagenized plants and transgenic insertion mutations have been the mainstay to obtain mutations in the carotenoid biosynthesis in plants. Molecular characterization of the genes employed transgenic approaches like gene silencing and over-expression. More recently, gene-specific mutations have been created using CRISPR (Clustered Randomly Interspaced Short Palindromic Repeats) along with Cas editing systems.

Mutations that alter metabolism have been widely used in biological research. The isolation and characterization of mutants with altered biochemical properties enabled the discovery of new enzymes and helped decipher new biosynthetic pathways (Beadle, 1945). Many mutations that alter carotenoids in plants have been described over the years. This review presents an overview of the natural and induced mutants available in the carotenoid biosynthesis metabolic pathways in plants.

**MEP PATHWAY – THE BACKBONE**

In plants, the building blocks for isoprenoid biosynthesis and provide the substrate for carotenoid biosynthesis, IPP and DMAPP, are synthesized by MEP (“non-mevalonate”) pathway (Chappell, 1995). The MEP pathway starts with the condensation of pyruvate and glyceraldehyde 3-phosphate (Rodríguez-Concepción and Boronat, 2002). It comprises seven enzymatic steps starting from pyruvate and D-glyceraldehyde-3-phosphate to produce IPP and DMAPP. The first rate-limiting step in the “non-mevalonate” MEP pathway is catalyzed by the 1-deoxy-D-xylulose-5-phosphate synthase (DXS) to form 1-deoxy-D-xylulose-5-phosphate (DXP) from the condensation of D-glyceraldehyde-3-phosphate and pyruvate (Lichtenthaler, 1999). The first mutant reported was the *clal-1* (cloroplastos alterados) isolated from the T-DNA-generated library of *Arabidopsis thaliana* in the CLA1 gene (Mandel et al., 1996). Later on, it was established that the *CLA1* gene encodes the first step in the MEP pathway, and it is the same as DXS (Estevez et al., 2000). After that there are three more mutations that are allic to *clal-1* are reported in *Arabidopsis* *viv.*, *chs5* (Araki et al., 2000), *ivr111* (Crowell et al., 2003), and *clal-2* (Gutiérrez-Nava et al., 2004). *clal* mutant exhibits lethal albino phenotype, whereas *ivr111* and *chs5* show variegated phenotype and temperature-sensitive chlorotic phenotype, respectively. Like the *clal* mutant, white-lethal-seeding-2297 (wls-2297) was isolated from the T-DNA mutant collection of tomatoes, which also exhibit lethal albino phenotype (García-Alcázar et al., 2017).

The second reaction, which involves the reduction and rearrangement of DXP into 2-C-Methyl-D-erythritol 4-phosphate (MEP), is catalyzed by DXP reductoisomerase (DXR) enzyme. Four T-DNA insertion mutant lines are available for the *DXR* gene, two each in Arabidopsis and rice, all displaying the albino phenotype (Budziszewski et al., 2001; Jung et al., 2008; Xing et al., 2010). In the third step of the MEP pathway, 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase (CMS or IspD), converts MEP into 4-diphosphocytidyl-2-C-Methyl-D-erythritol (CDP-ME) by adding CTP to it. Two T-DNA insertion mutants, *ispD-1* and *ispD-2*, were isolated from Arabidopsis with the albino phenotype (Hsieh et al., 2008). However, two weak alleles, *isp1-1* and *isp 1–2*, have also been isolated from EMS population of Arabidopsis, exhibiting high chlorophyll fluorescence phenotype (Hojo et al., 2005).

The fourth enzyme in the MEP pathway leads to the formation of 4-diphosphocytidyl-2-C-methyl- D-erythritol 2-phosphate (CDP-MEP) from CDP-ME in an ATP-dependent manner. This reaction is catalyzed by 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (CMK or IspE), encoded by the gene IspE. *IspE-1*, a T-DNA insertion mutant in Arabidopsis, and *green revertible yellow leaf* (gyr340), an EMS mutant in rice, display albino and virescent phenotype, respectively, at the early stages of plant development (Hsieh et al., 2008; Chen et al., 2018b). The enzyme 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (ME-cpp) synthase (MCS or IspF), catalyzes the cyclization of CDP-MEP into 2-C-methyl-d-erythritol 2,4-cyclodiphosphate (ME-cpp), is the fifth step in the MEP pathway. In Arabidopsis, two T-DNA null mutant lines are available, *ispF-1* and *GT-0946*, displaying the lethal albino phenotype. By contrast, in rice, an EMS mutant in this gene, 505ys is reported exhibiting yellow-green leaf phenotype throughout plant development (Budziszewski et al., 2001; Hsieh and Goodman, 2006; Huang et al., 2018a).

The penultimate step of the MEP pathway is carried out by 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase (HDS or IspG), which converts ME-cpp to 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMBPP). In Arabidopsis, there are three allelic mutations in HDS- *clb1-1* (chloroplast biogenesis), a T-DNA insertion line, and *clb1-2 and csb3* (constitutive *subtilisin*) isolated from EMS population (Gutiérrez-Nava et al., 2004; Gil et al., 2005). The *clb* null mutants show albino phenotypes, whereas *csb* is a partial loss-of-function mutation. Seed carotenoid deficient (scd-1 and scd-2), *lemon white* (*lw*), *viviparous12* (*vp12*) belong to the category of spontaneous/natural mutations reported in the *HDS* gene in maize all show a characteristic of albino plants with pale-yellow seeds (Zhang et al., 2019a).

The last step in the MEP pathway is 1-hydroxy-2-methyl-butenyl 4-diphosphate reductase (HDR or IspH), which converts HMBPP into both isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) at a ratio of 6:1 (Rohdich et al., 2006; Tritzsch et al., 2010). The null T-DNA insertion mutant in Arabidopsis, *IspH-1*, and the EMS-induced mutant, *clb6-1*, showed albino phenotypes (Guevara-García et al., 2005; Hsieh and Goodman, 2005), whereas an EMS mutant in maize, *zebra7*, displayed transverse yellow/green striped leaves at the
Schematic representation of MEP and carotenoid biosynthesis pathway. Name of the enzymes are highlighted in bold. G3P, Glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-Methyl-D-erythritol 4-phosphate; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-MEP, 4-diphosphocytidyl-2-C-Methyl-D-erythritol 2-phosphate; ME-cPP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl diphosphate; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, DXP reductoisomerase; CMS, 4-diphosphocytidyl-2-C-methyl-D-erythritol synthase; CMK, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; MCS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (ME-cPP) synthase; HDS, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase; HDR, 1-hydroxy-2-methyl-butenyl 4-diphosphate reductase; IDI, isopentenyl diphosphate isomerase; GGPPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; ZISO, ζ-carotene isomerase; ZDS, ζ-carotene desaturase; CRTISO, carotenoid isomerase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CYP97A/C, cytochrome P450 enzymes; CHY1/2, carotene hydroxylase; VDE, violaxanthin deepoxidase; ZEP, zeaxanthin epoxidase; NXS, neoxanthin synthase; CCS, capsanthin-capsorubin synthase; NCED, 9-cis-epoxycarotenoid dioxygenase; XISO, xanthophyll isomerase.
early stages of development (Lu et al., 2012). The first-ever report of CRISPR/Cas editing in the MEP pathway was reported in the *ispH* gene of *N. benthamiana*, where newly developed leaves showed photobleached phenotype (Yin et al., 2015). Although the MEP pathway leads to the synthesis of both IPP and DMAPP, the 6:1 ratio of these compounds may limit carotenoid biosynthesis (Pankratov et al., 2016). The interconversion of IPP and DMAPP is mediated by isopentenyl diphosphate isomerase (IDI). Plants have two IDI enzymes, IDI1 and IDI2, targeted to different cell compartments. T-DNA single null mutants in Arabidopsis (idi1-1, idi1-2, idi2-1, *Atipi1*, and *Atipi2*) either in IDI1 or IDI2 do not exhibit an apparent phenotype while the double mutants are either not viable or show severe pleiotropic phenotype (Okada et al., 2008; Phillips et al., 2008a). In tomato, four allelic mutants (three EMS-generated and one spontaneous) of the plastidial enzyme IDI1 exists—*fcd1-1, fcd1-2, fcd1-3* and *fcdm* (fruit carotenoid deficient). Contrary to the Arabidopsis *IDI1* mutants, *fcd1* mutants showed a reduced concentration of carotenoids in cotyledon, fruits, and flowers (Pankratov et al., 2016).

Synthesis of Geranylgeranyl diphosphate (GGPP) from IPP and DMAPP, is a three-step head-to-tail condensation process catalyzed by GGPP synthase (GGPPS). GGPP serves as the central precursor for carotenoids and terpenoids, tocopherols, chlorophyll side chains, giberellic, and plastoquinones. There are 11 isoforms of GGPPS in Arabidopsis, from which T-DNA mutants of *ggpps2, ggpps6, ggpps7, ggpps8*, and *ggpps10* did not show any developmental defect (Ruiz-Sola et al., 2016), T-DNA mutants in *GGPPS11* (*ggpps11-2, ggpps11-3, ggpps11-4*) showed albino-lethal phenotype highlighting its importance in plant development. There are two more weak allelic mutations, namely, *ggpps11-1 and ggpps11-5*, which showed variegated and paler leaf phenotype (Ruppel et al., 2013). Like Arabidopsis, multiple isoforms of GGPPS exist in tomato and CRISPR mutants for plastidial *GGPPS2* and *GGPPS3* were generated (Barja et al., 2021). Mutants impaired in *GGPPS3* (*slg3-1 and slg3-2*) but not in *GGPPS2* (*slg2-1 and slg2-2*) showed lower levels of photosynthetic pigments, whereas double mutants were not viable. The list of mutants described in the MEP pathway is presented in Table 1.

**CAROTENOID BIOSYNTHESIS – THE CENTRAL PATHWAY**

### Phytoene Synthesis – The Bottleneck

The first committed step in C40 carotenoid biosynthesis is the head-to-head condensation of two GGPP molecules by the key regulatory enzyme phytoene synthase (PSY) to form 15-*cis* phytoene. While many plant species have a single *PSY* gene, some species contain three isoforms. In tomato, for example, *PSY1* functions in chloroplasts, *PSY2* in chloroplasts, and *PSY3* in root plastids. Defects in the functional copy of *PSY1* lead to yellow fruit lacking carotenoids. Several loss-of-function mutations in the *PSY1* gene of tomato (genetic locus *r*) have been isolated (*r2997, r3756, r4, yf2*, P114490; Fray and Grierson, 1993; Yuan et al., 2008; Gady et al., 2012; Kachanovsky et al., 2012; Chen et al., 2019). Likewise, CRISPR mutants have also been generated for *PSY1* in tomato and maize, *PSY* in wheat, and both *PSY1* and *PSY2* in carrots (Zhu et al., 2016; Dahan-Meir et al., 2018; D’Ambrosio et al., 2018; Oleszkiewicz et al., 2021; Zhang et al., 2021). Reduced accumulation of carotenoids characterize CRISPR mutants in wheat, while the albino plants and white seeds are observed in maize (Zhu et al., 2016; Zhang et al., 2021). In carrots, *PSY1* and *PSY2* mutants display pale orange to yellow pigmentation in callus, with *PSY2* is critical for carotenogenesis in roots (Oleszkiewicz et al., 2021). Spontaneous loss-of-function mutant lines of *PSY* exist in pepper, maize, loquat, and poppy, leading to reduced levels of carotenoids, while gain-of-function mutant exists in cassava leading to enhanced carotenoid production (Robertson and Anderson, 1961; Buckner et al., 1996; Kim et al., 2010; Welsch et al., 2010; Fu et al., 2014; Jeong et al., 2019; Pollack et al., 2019). Eight missense mutations in *PSY* were reported in melon. However, no phenotype has been recorded in these lines (Vicente-Dólera et al., 2014).

### Desaturation and Isomerization – The Poly-Cis Pathway

Conversion of 15-*cis*-phytoene to all-*trans* lycopene entails four double bond desaturations (dehydrogenations) and three *cis*-trans isomerizations. Intermediate carotenoids in this pathway are all *cis*-configured (Isaacscon et al., 2004). Phytoene desaturation is catalyzed by phytoene desaturase (PDS) in a two-step process leading to the production of phytofluene followed by 9,15-*ζ*-tri-*cis*-carotene. Loss-of-function mutations in *PDS* resulted in albinism and dwarf phenotypes in the Arabidopsis T-DNA insertion mutant *pds3*, reduced carotenoid content in petals of *B. napus* in the *ywf* (*yellow-white flower*) mutant, white seed, and premature seed germination in the maize *vp5* (*viviparous5*) mutant and the lethal albino phenotype in *phs1* (*pre-harvest sprouting1*) in rice (Hable et al., 1998; Qin et al., 2007; Fang et al., 2008; Zhao et al., 2021). *PDS* has been used as a reporter gene in transient gene silencing studies due to the bleached leaves phenotype visible to the naked eye (Kumagai et al., 1995; Ratcliff et al., 2001). It should be noted that PDS, the first gene identified in the carotenoid biosynthesis pathway in plants, was initially detected due to mutations that conferred resistance to the “bleaching herbicide” norflurazon (Chamovitz et al., 1990). Several missense mutations in the cyanobacterial PDS that alter conserved amino acid residues of the enzyme in plants lower the binding affinity of norflurazon and thus confer herbicide resistance (Chamovitz et al., 1993; Wagner et al., 2002). Additional mutations in PDS from algae and plants that confer resistance to “bleaching herbicides” have been reported (Michel et al., 2004; Suarez et al., 2014; Dang et al., 2018; Taparia et al., 2019).

The first isomerization step by *ζ*-carotene isomerase (ZISO) converts 9,15-*ζ*-tri-*cis*-carotene to 9,9′-*di-cis*-ζ-carotene. In photosynthetically active chloroplasts, this isomerization can be mediated by light. However, ZISO is critical in chloroplasts and root plastids. The tillering mutants *t20* and *hild12* in rice, which are impaired in ZISO, display a delayed greening phenotype.
similar to the T-DNA insertion and EMS mutants in ZISO of Arabidopsis (zie; Chen et al., 2010; Liu et al., 2020a; Zhou et al., 2021). Other mutants of ZISO in tomato (Zeta, c2803), Arabidopsis (ziso-155), maize (y9), and orange (pinalate), accumulates high levels of phytoene, phytofluene, and ζ-carotene in fruits and seeds (Li et al., 2007; Kachanovsky et al., 2012; Rodrigo et al., 2019; Cazzonelli et al., 2020; Gupta et al., 2021).

Zeta-carotene desaturase (ZDS) catalyzes the final desaturation steps, converting 9,9′-di-cis-ζ-carotene to 9,9′,11,11′-tetra-cis lycopene ("prolycopene"). Several ZDS mutants have been reported and characterized in plants. The strong-mutant alleles spontaneous cell death (spec-1) and chlorophyll biogenesis (clb5-1, clb5-2) in Arabidopsis display albino-lethal phenotype while weak alleles spec-1-3 and clb5-3 have mild phenotype (Dong et al., 2007; Avendaño-Vázquez et al., 2014). Null mutants in maize (alb1, vp-wl2, vp9), rice (ale1, phs2) and sunflower (nd-1) also display albino-lethal phenotypes (Conti et al., 2004; Fang et al., 2008, 2019; Chen et al., 2017; Wang et al., 2020b).

In the second isomerization reaction, tetra-cis lycopene is converted to all-trans lycopene by the enzyme carotenoid isomerase (CRTISO). CRTISO was first characterized in tomato tangerine (t3183, t3184) and Arabidopsis ccr2 mutants, which accumulate tetra-cis lycopene in fruits and seedlings, imparting the orange-yellow color to the fruits and seedlings, respectively (Isaacson et al., 2002; Park et al., 2002). Later on, CRTISO mutants were identified in rice (phs3, zebra2, mit3), melon (yofi), orange flower calendula, and orange color Chinese cabbage, marked with an accumulation of tetra-cis lycopene and reduced lutein content (Fang et al., 2008; Kishimoto and Ohmiya, 2012; Galpaz et al., 2013; Zhao et al., 2014; Su et al., 2015; Liu et al., 2018). CRISPR mutants of CRTISO were also reported in Chinese kale and tomato with similar phenotypes.

### Table 1: List of mutants identified in MEP pathway.

| Gene | Insertional mutagenesis | CRISPR/Cas | Induced mutagenesis | Spontaneous/natural mutation |
|------|-------------------------|------------|---------------------|-----------------------------|
| DXS  | cla1-1 (At4g15560; Arabidopsis); Mandel et al., 1996; Estezev et al., 2003 | | chs5, luv111 (EMS), cle1-2 (At4g15560; Ethylenimine; Arabidopsis) | |
| DXS  | wls-2297 (Soyloc01g067890) (Tomato; Garcia-Alcázar et al., 2017) | | (Araki et al., 2000; Crowell et al., 2003; Gutiérrez-Nava et al., 2004) |
| DXR  | Gk_215C01, 4036 (At5g62790; Arabidopsis; Burdziszewski et al., 2001; Xing et al., 2010); 1A-14224 and 1C-03301 (Os01g01710) (Rice) (Jung et al., 2008) | | |
| CMS/ispD | ispD-1, ispD-2 (At2g02500; Arabidopsis) (Hsieh et al., 2008) | | isp1-1, isp1-2 (At2g02500; EMS; Arabidopsis) (Hojo et al., 2005) |
| CMK/ispE | ispE-1 (At2g26930; Arabidopsis; Hsieh et al., 2008) | | gry340 (Os01g58790; EMS; Rice; Chen et al., 2018b) |
| MDS/ispF | ispF-1, GT0946 (At1g63970; Arabidopsis; Burdziszewski et al., 2001; Hsieh and Goodman, 2006) | | 5′06y (Os02g46600; EMS; Rice; Huang et al., 2018a) |
| HDS/ispG | clb4-2 (At5g60600; Arabidopsis; Gutiérrez-Nava et al., 2004) | | cld-4, cld3 (At5g60600; EMS; Arabidopsis; Gutiérrez-Nava et al., 2004; Gil et al., 2005) |
| HDR/ispH | ispH-1 (At4g34350; Arabidopsis; Hsieh and Goodman, 2005) | | cldb-61 (At4g34350; EMS; Arabidopsis; Guevara-García et al., 2005) |
| IDI  | id1-1, id1-2, Atipa1 (At5g16440) id2-1, Atipa2 (At3g02780; Arabidopsis) (Okada et al., 2008; Phillips et al., 2008a) | | cfd-1–1, cfd-1–2, cfd-1–3 (Soyloc04g056390; EMS; Tomato; Pankratov et al., 2016) |
| GGPPS | ggpps2 (At2g18620), ggpps6 (At3g14530), ggpps7 (At3g14550), ggpps8 (At3g20160), ggpps10 (At3g32040), ggpps11–3, ggpps11–4, ggpps11–5 (At4g36810; Arabidopsis) (Ruppe et al., 2013; Ruiz-Sola et al., 2016) | | ggps11–1–1 (At4g36810; EMS; Arabidopsis; Ruppe et al., 2013) |

TABLE 1 | List of mutants identified in MEP pathway.
Lycopene Cyclization – The Branching Point

Cyclization of lycopene bifurcates the pathway into two branches, the β-β branch leading to violaxanthin and neoxanthin and the e-β branch leading to lutein. In the β-β branch, two β-rings are formed at both ends of the lycopene molecule by the enzyme lycopene β-cyclase (LCYB, CRT-L) to produce β-carotene. In contrast, in the e-β branch, lycopene is first cyclized by lycopene epsilon-cyclase (LCYE) and then by LCYB to produce α-carotene. Some plants have several lycopene beta-cyclase paralogos, which are differentially expressed. The lycopene beta-cyclases expressed in chloroplast-containing tissues (designated as LCYB) are indispensable for plant growth, whereas beta-cyclases that predominantly function in chromoplasts-containing tissues like fruits and flowers (designated as CYCB) solely affect the colors. In tomato, two types of mutations exist in the CYCB, Beta (B). A dominant mutation that leads to high β-carotene in fruit, and old-gold (B^0) and beta-crimson (B^c), which are recessive loss-of-function mutants with increased lycopene and reduced β-carotene in fruits (Ronen et al., 2000). Similar mutations in lcyb of papaya, maize (lcyb-m2.1), and rice (phs4) accumulate lycopene leading to red-fleshed papaya fruit, slightly pink kernels in maize, and pink-embryo seeds in rice (Fang et al., 2008; Bai et al., 2009; Devitt et al., 2010).

The Arabidopsis suppressor of zeaxanthin-less1 (ssl1) mutant, which has a point mutation in LCYB, accumulates more lutein and small amounts of xanthophyll-cycle pigments (Li et al., 2009). A loss-of-function mutation in LCYE in the Arabidopsis mutant lut2 eliminated lutein and increased the concentration of β-carotene along with xanthophyll-cycle pigments (Li et al., 1996). A dominant mutation, DEL, in the gene LCY-E of tomato increases the expression of lycopene e-cyclase in the fruits, which accumulate δ-carotene (Ronen et al., 1999). Multiplex CRISPR/Cas9-based genome editing has also been done in LCYB1, LCYB2, CYCB, LCYE, and SGR1 genes to achieve lycopene enriched tomatoes (Li et al., 2018b). CRISPR and EMS mutants of LCYE in banana and wheat accumulated β-carotene in fruits and leaves, respectively (Richaud et al., 2018; Kaur et al., 2020).

Hydroxylation – The Primary Route for Xanthophyll Biosynthesis

The biosynthesis of xanthophylls from α-carotene and β-carotene requires ring-specific hydroxylations. The e- and β-ring hydroxylation of α-carotene are catalyzed by heme-containing cytochrome P450 enzymes (CYP97A and CYP97C), yielding lutein. The non-heme β-carotene hydroxylases BCH1 and BCH2, catalyze the β-ring hydroxylation of β-carotene to produce zeaxanthin. BCH1/2 mutations have been reported in tomato, Arabidopsis, rice, pepper, and maize. The mutations white flower (wf) in tomato and E172-3 in pepper in the genes Crtr-b2/ BCH2 abolish xanthophyll accumulation in flowers of tomato and pepper fruits (Galpaz et al., 2006; Borovsky et al., 2013).

Similarly, dsm2 mutants in rice and crtRB1 mutants in maize reduce the accumulation of zeaxanthin and increase β-carotene content (Du et al., 2010; Yan et al., 2010). However, single T-DNA mutants of BCH1 (b1) and BCH2 (b2) in Arabidopsis do not have a significant impact on carotenoid composition in leaves, and the double mutant b1b2 only show a partial reduction in β-carotene derived xanthophylls (Tian et al., 2003).

Mutations in CYP97A in Arabidopsis (lut5), rice (cyp94a-4), and orange carrots increase the level of α-carotene and reduce lutein concentrations (Kim and DellaPenna, 2006; Lv et al., 2012; Arango et al., 2014). The Arabidopsis lut1 mutation in CYP97C is marked by the absence of lutein, the accumulation of zeinoxanthin, and xanthophyll-cycle pigments (Pogson et al., 1996; Tian et al., 2004). Double, triple, and quadruple carotene hydroxylase mutations exist in Arabidopsis. One such mutant, nox (no xanthophyll), obtained by combining all the four hydroxylase mutants, is devoid of xanthophylls and predominantly accumulates α- and β-carotene (Dall’Osto et al., 2013).

The last steps in the β-branch of xanthophyll biosynthesis convert zeaxanthin to violaxanthin by the zeaxanthin epoxidase (ZEP) followed by neoxanthin synthesis through an unknown reaction. These xanthophylls, together with lutein, are components of (LHCs). Zeaxanthin plays an essential role in excess energy dissipation that protects the light-harvesting complexes (LHCs). However, since it is not a constituent of the LHC, it is rapidly synthesized under light conditions by deepoxidation of violaxanthin catalyzed by the violaxanthin deepoxidase (VDE) with antheraxanthin (A) as an intermediate. The interconversion of zeaxanthin to violaxanthin and vice versa is known as the xanthophyll (or violaxanthin) cycle. Mutations in ZEP in N. plumaginifolia (aba2), Arabidopsis (aba1 and npq2), and rice (Osaba1) show accumulation of zeaxanthin and absence of violaxanthin and neoxanthin in mutant leaves (Duckham et al., 1991; Rock and Zeevaart, 1991; Marin et al., 1996; Niyogi et al., 1998; Agrawal et al., 2001; Gonzalez-Jorge et al., 2016). However, ZEP mutants in tomato (hp3), pepper, and B. napus display differential accumulation of carotenoids in fruits, flowers, and leaves (Galpaz et al., 2008; Liu et al., 2020b; Lee et al., 2021). The hp3 mutation increases total carotenoids in fruits accompanied by an atypical accumulation of zeaxanthin and eliminates violaxanthin and neoxanthin in leaves (Galpaz et al., 2008). In pepper and B. napus, mutations in ZEP increase the level of zeaxanthin at the expense of violaxanthin in fruits and flowers, with no change in leaf carotenoids. The only mutation known in VDE is the Arabidopsis npq1, where the mutant cannot convert violaxanthin to antheraxanthin and zeaxanthin in excessive light, thus resulting in high-light sensitive plants (Niyogi et al., 1998). The last step in the carotenoid biosynthetic pathway is the synthesis of neoxanthin from violaxanthin, which takes place in a poorly understood enzymatic reaction. Two mutants that lack neoxanthin in tomato (neoxanthin-deficient1, nxd1) and Arabidopsis (ABA-deficient4, aba4) were identified. However, the exact role of NXD1 and ABA4 proteins remained unknown as their exact enzymatic activities have not been established (North et al., 2007; Neuman et al., 2014; Perreau et al., 2020). Since NXD1 exists in the cytoplasm while ABA4 is found within plastids, it is likely
that the latter is involved in this reaction (Perreau et al., 2020). An additional enzyme in peppers (Capsicum species), capsanthin-capsorubin synthase (CCS), converts antheraxanthin and violaxanthin to capsanthin and capsorubin, respectively (Bouvier et al., 1994). There are many allelic variations present in the ccs gene in pepper which includes structural variation in both promoter and coding region, early translational termination, frame-shift mutations, and missense mutations imparting different hues to fruit color in non-red pepper accessions (Lefebvre et al., 1998; Popovske and Paran, 2000; Ha et al., 2007; Guzman et al., 2010; Li et al., 2013b; Jeong et al., 2019). The mutants available in the carotenoid biosynthetic pathway are listed in Table 2.

### Carotenoid Cleavage – The Apocarotenoids

Carotenoid molecules can be cleaved at different double bonds by distinct dioxygenases divided into two categories, namely, 9-cis-epoxyapocarotenoid dioxygenases (NCEDs) and carotenoid cleavage dioxygenases (CCDs). The NCEDs are related to the abscisic acid (ABA) production from 9-cis-epoxyapocarotenoids, while CCDs have broader substrate specificities. CCD7/8 are involved in the synthesis of strigolactones, and CCD1/4 generate many apocarotenoids with diverse functions. NCED cleaves 9-cis-violaxanthin and 9-cis-neoxanthin to form xanthoxin, the first committed step in ABA biosynthesis. NCEDs in plants are encoded by many genes having differential expression and roles in different tissues. Mutants have been generated in different NCED genes in maize (sp14), Arabidopsis (nced2, nced3, nced5, nced6, and nced9), tomato (not), rice (nced3 and nced5), lettuce (nced4), and wheat (nced1; Burbidge et al., 1999; luchi et al., 2001; Lefebvre et al., 2006; Wan and Li, 2006; Frey et al., 2012; Gonzalez-Jorge et al., 2013; Bertier et al., 2018; Huang et al., 2018b, 2019; Schwartz et al., 2018; Zhang et al., 2019b). All the nced mutants, irrespective of the same gene mutation, display reduced levels of ABA and hypersensitivity to water stress.

Strigolactones that control shoot branching are generated by the action of CCD7 and CCD8, which act sequentially on 9-cis-β-carotene as substrate. Several CCD7/8 mutants have been isolated and characterized in Arabidopsis (ccd7/max3 and ccd8/max4), petunia (dad1 and dad3), tomato and rice (htd1 and d10), all exhibiting dwarf phenotype and excessive shoot branching (Booker et al., 2004; Snowden et al., 2005; Auldridge et al., 2006; Zou et al., 2006; Arite et al., 2007; Drummond et al., 2009; Hasegawa et al., 2018). Likewise, the CRISPR mutants of CCD7/8 in rice, tomato, tobacco, and grapevine also show a similar phenotype (Yang et al., 2017; Gao et al., 2018; Bari et al., 2019; Ren et al., 2020). CCD4 in flowering plants is mainly active in chromoplasts, where it determines coloration in petals and fruits by degrading carotenoid pigments. Knockout mutants of CCD4 in ipomea, chrysanthemum, and brassica caused white petals to turn pale-yellow/yellow, and in peach, they caused a change from white to yellow-fleshed fruits (Adami et al., 2013; Ma et al., 2014; Zhang et al., 2015; Jo et al., 2016; Watanabe et al., 2018; Wen et al., 2020). In Arabidopsis, ccd4 and ccd1 mutants increased seed carotenoid levels, mainly in lutein, neoxanthin, and violaxanthin, with a more pronounced effect in ccd1 mutants (Gonzalez-Jorge et al., 2013). The list of mutants for carotenoid cleavage is presented in Table 3.

### Regulation of Carotenoid Biosynthesis – The Or Perspective

The regulation of carotenoid biosynthesis is complex and depends on many different factors, including the type of tissue and developmental and environmental signals (Sun and Li, 2020). Carotenoid biosynthesis is enhanced following plastid differentiation to chromoplasts, which accumulate large amounts of carotenoids. The tomato mutations HIGH-PIGMENT1 (hp1), HIGH-PIGMENT2 (hp2), and HIGH-PIGMENT3 (hp3) increase chromoplast number and size and thus elevate carotenoid concentration in the fruits (Mistilli et al., 1999; Cookson et al., 2003; Levin et al., 2006; Galpaz et al., 2008; Wang et al., 2008). However, this regulation of carotenoids is indirect. An R2R3-MYB transcription factor was implicated in the regulation of carotenoid biosynthesis in Mimulus lewisiis flowers based on the analysis of the mutation Reduced Carotenoid Pigmentation 1 (RCP1; Sagawa et al., 2016). Since the transcription of all carotenoid genes was decreased in the rep1 flowers, and overexpressing RCP1 decreased anthocyanin production, the effects of R2R3-MYB on the carotenoid pathway are likely indirect. Several other transcriptional and post-translational regulators in different plant species have been proposed as potential regulators of the carotenoid biosynthesis pathway. However, lack of mechanistic aspects of these regulators leaves many gaps in the understanding of their mode of action that do not enable to substantiate their direct effects on specific carotenoid genes or enzymes. The gene ORANGE (OR), is an exceptional case. The role of ORANGE protein as a post-translational regulator of carotenoids accumulation has been well established (Chayut et al., 2017; Kim et al., 2018; Welsch et al., 2018; Osorio, 2019). Therefore, we have included the mutations in the Or gene in this review. OR was first identified as a dominant spontaneous mutation in Brassica oleracea (BrOr), having a retrotransposon insertion in the gene of a plastidial DnaJ, a cysteine-rich domain-containing protein, leading to orange color of inflorescence (Li et al., 2001; Lu et al., 2006). Genome edited lines in rice (OsOr) displayed carotenoid accumulation in rice callus (Endo et al., 2019). In melon, a natural gain-of-function mutation ("golden SNP"), Or<sup>Al</sup>, changes green to orange-fleshed melon fruits (Tzuri et al., 2015). An EMS-induced nonsense mutation in the gene reduced beta-carotene levels in melon fruit (Chayut et al., 2017). OR regulates PSY post-translationally, promotes chromoplast biogenesis, and affects plastid number (Zhou et al., 2015; Sun et al., 2020b). The mutations in the Or gene are listed in Table 4.

### CONCLUSION AND PERSPECTIVES

This review aims to assemble all known natural and induced mutants available in the carotenoid metabolic pathway in plants

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**Table 3**

| Mutant | Description |
|--------|-------------|
| hp1    | Tomato mutation |
| hp2    | Tomato mutation |
| hp3    | Tomato mutation |
| ccd1   | Arabidopsis mutants |
| ccd4   | Arabidopsis mutants |
| rep1   | Tomato mutation |
| Or     | Rice mutation |

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### TABLE 2 | List of mutants identified in carotenoid biosynthetic pathway.

| Gene | Insertional mutagen | CRISPR | Induced mutagen | Spontaneous/natural mutation |
|------|----------------------|---------|-----------------|-----------------------------|
| **PSY** | | | | |
| | Psy1 (Solyc03g031860; Tomato; Dahan-Meir et al., 2018; D’Ambrosio et al., 2018) | | | |
| | Maize (GRMZM2G300348; Zhu et al., 2016) | | | |
| | Wheat (Zhang et al., 2021) | | | |
| | Carrot | | | |
| | psy1 (GeneBank Gene ID: 108227339), psy2 (GeneBank Gene ID: 108214656) (Oleszkiewicz et al., 2021) | | | |
| **PDS** | | | | |
| | pds3 (At4g14210; Arabidopsis; Qin et al., 2007) | | | |
| | phs1 (Os03g08570; Rice; Fang et al., 2008) | | | |
| | Tomato (Solyc03g123760) (Pan et al., 2016; Li et al., 2018a) | | | |
| | Rice OsPDS (Os03g08670) OoPDS1 (Os03g0184000) (Shan et al., 2013; Zhang et al., 2014; Ishizaki, 2016; Banakar et al., 2019) | | | |
| | Tobacco (Nekrasov et al., 2013; Li et al., 2013a; Ali et al., 2015, 2018; Gao et al., 2015; Yin et al., 2015; Endo et al., 2016; Chen et al., 2018a; Ren et al., 2019a, 2021; Schmitz et al., 2020) | | | |
| | Arabidopsis pds3 (At4g14210) (Li et al., 2013a; Totsuki and Higashiyama, 2017; Ali et al., 2018; Wolabu et al., 2020) | | | |
| | Brassica | | | |
| | BoPDS (Bo016089) | | | |
| | BoPDS1 (Bo010982) | | | |
| | BaPDS1 (GenBank Accession No. KX426039), BaPDS2 (GenBank Accession No. KX426040) (Murovec et al., 2018; Sun et al., 2018; Ma et al., 2019a, b; Lee et al., 2020) | | | |
| | Banana | | | |
| | PDS (GenBank Accession JQ762260) | | | |
| | MaPDS (Ma08_g16510) (Hu et al., 2017; Kaur et al., 2018; Naim et al., 2018; Ntui et al., 2020; Wu et al., 2020) | | | |
| | Citrus (Jia and Nian, 2014; Jia and Wang, 2014; Jia et al., 2017, 2019; Zhang et al., 2017; Zhu et al., 2019; Dutt et al., 2020) | | | |
| | Grape (Nakajima et al., 2017; Ren et al., 2019a, b, 2021) | | | |
| | Hop | | | |
| | HPDS (NCBI accession number: MT089893) (Awasthi et al., 2021) | | | |
| | | | | |

(Continued)
TABLE 2 | Continued

| Gene                | Insertional mutagenesis | CRISPR                     | Induced mutagen | Spontaneous/natural mutation |
|---------------------|-------------------------|-----------------------------|-----------------|-----------------------------|
| Apple               |                         |                             |                 |                             |
| MdPDS (MD04G0021400) |                         |                             |                 |                             |
| (Nishitani et al., 2016; Osakabe et al., 2018; Charrier et al., 2019) | | | | |
| Cassava             |                         |                             |                 |                             |
| (Manes.05G1903700)  |                         |                             |                 |                             |
| (Odipio et al., 2017) |                         |                             |                 |                             |
| Melon               |                         |                             |                 |                             |
| CmPDS (MELG01772.2) |                         |                             |                 |                             |
| (Tian et al., 2017; Hooghvorst et al., 2019) | | | | |
| Strawberry          |                         |                             |                 |                             |
| FvPDS (LG4-gene12690) |                         |                             |                 |                             |
| (Wilson et al., 2019) |                         |                             |                 |                             |
| Hieracium           |                         |                             |                 |                             |
| (Henderson et al., 2023) |                         |                             |                 |                             |
| Populus             |                         |                             |                 |                             |
| (Fan et al., 2015; Liu et al., 2015; Wang et al., 2020a) | | | | |
| Chicory             |                         |                             |                 |                             |
| OPDS (GenBank accession MK455771) | |                             |                 |                             |
| (Bernard et al., 2019) |                         |                             |                 |                             |
| Yam                 |                         |                             |                 |                             |
| (Syombua et al., 2021) |                         |                             |                 |                             |
| Carrot              |                         |                             |                 |                             |
| (Genbank accession no. XM_017396654.1) | |                             |                 |                             |
| (Xu et al., 2019) |                         |                             |                 |                             |
| Rehmannia           |                         |                             |                 |                             |
| (Li et al., 2021) |                         |                             |                 |                             |
| Witloof             |                         |                             |                 |                             |
| (De Bruyn et al., 2020) |                         |                             |                 |                             |
| Sorghum             |                         |                             |                 |                             |
| (Liu et al., 2019) |                         |                             |                 |                             |
| Soybean             |                         |                             |                 |                             |
| GmPDS11 (Glyma.11G253000), GmPDS18 (Glyma.18G003900) | |                             |                 |                             |
| (Du et al., 2016) |                         |                             |                 |                             |
| Tragopogon          |                         |                             |                 |                             |
| (Shan et al., 2016) |                         |                             |                 |                             |
| Medicago            |                         |                             |                 |                             |
| (Meng et al., 2017; Wolabu et al., 2020) | |                             |                 |                             |
| Potato              |                         |                             |                 |                             |
| (Wang et al., 2015; Bánfalvi et al., 2020) | |                             |                 |                             |
| Hevea               |                         |                             |                 |                             |
| (Dai et al., 2021) |                         |                             |                 |                             |
| Wheat               |                         |                             |                 |                             |
| (Upadhyay et al., 2013; Howells et al., 2018) | |                             |                 |                             |
| Lilium              |                         |                             |                 |                             |
| (Yan et al., 2019) |                         |                             |                 |                             |
| Kwaifruit           |                         |                             |                 |                             |

(Continued)
### TABLE 2 | Continued

| Gene     | Insertional mutagenesis | CRISPR                        | Induced mutagen | Spontaneous/natural mutation |
|----------|-------------------------|-------------------------------|-----------------|------------------------------|
| ZISO     | zic 1–3, zic 1–4, zic 1–5, zic 1–6 (At1g10830) (Arabidopsis) (Chen et al., 2010) | AcPDS (Ach19g199631) (Wang et al., 2018) Petunia PhPDS (GenBank ID: KP677483) (Zhang et al., 2016) t20-2, t20-3 (Os12g21710; Rice) (Liu et al., 2020a) | htc12 (EMS), t20 (Co-60 radiation; Os12g21710; Rice) (Liu et al., 2020a; Zhou et al., 2021) zic1-1, zic 1–2, zso-155 (At1g10830) (EMS; Arabidopsis) (Chen et al., 2010; Cazzonelli et al., 2020) e2803, ZISO (Soly12g098710; EMS; Tomato) (Kachanovsky et al., 2012, Gupta et al., 2021) | Pinalate (Orange) (Rodrigo et al., 2019) pale yellow9 (Maize) (Li et al., 2007) |
| ZDS      | cib5-3, spc1-1, spc 1-2, spc1-3 (At3g04870; Arabidopsis) (Dong et al., 2007; Avendaño-Vázquez et al., 2014) ab1, vp-w2, vp9-8113, vp9-99-2226-1, vp9-R (GRMZM2G454952; Maize) (Chen et al., 2017; Wang et al., 2020b) phs2-1, phs2-2 (Os07g10490; Rice) (Fang et al., 2008) | Chinese kale (Sun et al., 2020a) Tomato (Soly10g081650) (Dahan-Meir et al., 2018; Lakshmi Jayaraj et al., 2021) | t3002, t3406, t9776 (EMS; Soly10g081650; tomato) (Isaacson et al., 2002; Kachanovsky et al., 2012) cib5-1 (EMS), cib 5–2 (fast-neutron; At3g04870; Arabidopsis) (Avendaño-Vázquez et al., 2014) ccr2-1 (EMS), ccr2-5 (At1g06820; fast-neutron; Arabidopsis) (Park et al., 2002) | ndt-1 HaZDS (GenBank accession no AJ514406; sunflower) (Conti et al., 2004) |
| CRTISO   | phs3-1, phs3-2 (Os11g36440; Rice) (Fang et al., 2008) ccr2-3 (At1g06820; Arabidopsis) (Park et al., 2002) | Chinese kale (Sun et al., 2020a) | t3002, t4388, t3406, t5776 (EMS; Soly10g081650; tomato) (Isaacson et al., 2002; Kachanovsky et al., 2012) ccr2-1 (EMS), ccr2-5 (At1g06820; fast-neutron; Arabidopsis) (Park et al., 2002) yool (EMS; Genbank accession number JX491406) (Melon) (Galpaz et al., 2013) zb2, mtd3-1, mtd3-2, mtd 3–3, mtd3-4 (EMS; Os11g06440; Rice) (Zhao et al., 2014; Liu et al., 2018) | phs3-3 (Os11g06440; Rice) (Fang et al., 2008) Orange flowered calendula (Kishimoto and Ohmiya, 2012) t3183, LA0351, LA3002, LA3128 (Soly10g081650; tomato) (Isaacson et al., 2002; Yoo et al., 2017) Chinese cabbage (Bra031539) (Su et al., 2015) |

(Continued)
TABLE 2 | Continued

| Gene | Insertional mutagenesis | CRISPR | Induced mutagen | Spontaneous/natural mutation |
|------|-------------------------|--------|-----------------|-----------------------------|
| **LCYB** |                         |        |                 |                             |
| lcyB-m2.1 (Maize) |                         | Tomato | melon (EMS; Cucurbita pepo) | B, B⁺, B' (Solvyc06g074240; Tomato) |
| (Bai et al., 2009) |                         | LcyB1 (Solvyc04g040190), LcyB2 (Solvyc10g079480) | (Vicente-Dolera et al., 2014) | (Ronen et al., 2000) |
| phs4-1, phs4-2 (Rice) |                         | (Li et al., 2018b) | szT1 (Ar1g32640; EMS; Arabidopsis) | Red papaya cultivars (Devitt et al., 2013) |
| (Fang et al., 2008) |                         |        | (Li et al., 2009) | Capsicum (CA06g00080) (Jeong et al., 2019) |
| **LCYE** |                         | Tomato | Wheat (EMS) LCYE-A (Genbank: EU649785), LCYE-B (Genbank: EU649786) | Delta (Solvyc12g008980; Tomato; Ronen et al., 1999) |
| phs1 (Rice) |                         | (Li et al., 2018b) | (Richtaud et al., 2018) | |
| (Fang et al., 2008) |                         | Banana | lut2-1, lut2-2 (EMS; At5g57030; Arabidopsis) | |
| **CHY/CYP** |                         | Tomato | wlf1-1 (X-ray), wlf1-2 (EMS), wlf1-3 (Solvyc03g007960; fast-neutron; Tomato) | Orange-rooted carrots (Arango et al., 2014) |
| dsm2, cyp97a4-1, cyp97a4-2, cyp97a4-3 (Os04g48880; Rice) |                         | CtrR-b2 (Solvyc03g007960) | (Galpaz et al., 2006) | Maize (Yan et al., 2013) |
| (Du et al., 2010; Lv et al., 2012) |                         | (O’Ambrosio et al., 2018) | lut1-1, lut1-2, (At3g53130) | Capsicum (CA03g25820) (Jeong et al., 2019) |
| lut1-4, lut1-3, (At3g53130), lut5-1 (At1g31800), b1 (At4g25700), b2 (At5g52570), nox (Arabidopsis) |                         |         | (Pogson et al., 1996; Kim and DellaPenna, 2008) | |
| (Tian et al., 2003, 2004; Kim and DellaPenna, 2008; Dall’Osto et al., 2013) |                         |         | E172-3 (EMS; Pepper) | |
| **ZEP** |                         | Bna409.ZEP, BnaC09.ZEP (B. napus) | npq1-1, npq1-2 (EMS; At1g08550; Arabidopsis) | Capsicum (CA02g10990) (Jeong et al., 2019; Lee et al., 2021) |
| aba2 (N. plumbaginifolia) |                         | (Li et al., 2020b) | (Galpaz et al., 2008) | |
| (Marin et al., 1996) |                         |         | aba-1, aba-3, aba-4, npq2-1, npq2-2, aba1-6 (EMS; At5g67030; Arabidopsis) | |
| Osaba1 (Rice) |                         |         | (Duckham et al., 1991; Rock and Zeevaart, 1991; Niyogi et al., 1998; Gonzalez-Jorge et al., 2016) | |
| (Agrawal et al., 2001) |                         |         | npq1-1, npq1-2 (EMS; At1g08550; Arabidopsis) | |
| aba1-7 (At1g67030; Arabidopsis) |                         |         | (Niyogi et al., 1998) | |
| (Gonzalez-Jorge et al., 2016) |                         |         |         | |
| **VDE** |                         |         |         | Pepper (CA06g22860; Lefebvre et al., 1998; Popovsky and Paran, 2000; Ha et al., 2007; Guzman et al., 2010; Li et al., 2013b; Jeong et al., 2019; | |
| **CCS** |                         |         |         | |
| **ABA4/NXS?** |                         |         |         | |
| aba4-1, aba4-2, aba4-3 |                         |         |         | |
| (North et al., 2007) |                         |         |         | |
| nxd1-1, nxd1-2 (EMS; Solvyc12g041880; Tomato) |                         |         | (Neuman et al., 2014) | |

in one place to facilitate comparisons between different plants and assist researchers who seek to study this pathway. Since the founding of the field of biochemical genetic eight decades ago (Beadle, 1945), mutations have been a central tool for researchers to discover biosynthetic pathways and study their regulation. This has also been true in the case of carotenoid biosynthesis in plants, where the genetic approach paved the way for discovering genes and enzymes. Additional utility for mutant isolation and characterization has been known for the physiological and developmental studies of carotenoids in plant life, photosynthesis,
TABLE 3 | List of mutants identified in carotenoid degradation pathway.

| Gene | Insertional mutagenesis | CRISPR | Induced mutagen | Spontaneous/natural mutation |
|------|-------------------------|--------|----------------|----------------------------|
| NCED | T5004, 129808, nced3-2 (At3g14440), nced5-2, nced3-5, nced5-4 (At1g30100), nced6-1 (At3g24220), nced9-1, nced9-2 (At1g78390), nced2-3 (At4g18350); Arabidopsis | nced5-1, nced5-2, nced3-3, nced3-4, nced3-9 (Rice) | not (X-ray), NCED1 (EMS; Solyco7g066570); Burbridge et al., 1999; Gupta et al., 2017) |
|      |                         |        |                |                            |
|      |                         |        |                |                            |

TABLE 4 | List of mutants identified in orange gene.

| Gene | Insertional mutagenesis | CRISPR | Induced mutagen | Spontaneous/natural mutation |
|------|-------------------------|--------|----------------|----------------------------|
| Orange | or-1 (At5g61670; Arabidopsis) | OsOr (Os02g0651300; Rice; Endo et al., 2019) | CmOr (low-β; MELOC005449; Melon; EMS; Chayut et al., 2017) | CmOr (MELOC005449; Melon; Tzuri et al., 2015) |

Regarding the CRISPR/Cas genome editing systems and genomic information of plant species opens new avenues for gene-targeted mutagenesis to generate both leaky and null mutants in those genes and mutation where null mutants are not viable. Genome editing technologies are not restricted to generating mutants and can also be used for biofortification of carotenoids in crop plants. In the future, we will soon reach saturated mutagenesis in model plants and other plant species to better understand the genes' functions and thus help improve crop plants for a better future.

**AUTHOR CONTRIBUTIONS**

PG and JH have contributed equally in preparing the article.

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