

# **Genetically Engineered Blue-Fleshed Apple and Associated Constructs, Methods, and Products**

Inventor: Michael Nget

# Contents

<b>1</b>	<b>Abstract</b>	<b>5</b>
<b>2</b>	<b>Field of the Invention</b>	<b>6</b>
<b>3</b>	<b>Background of the Invention</b>	<b>6</b>
3.1	Natural Fruit Pigmentation and Limitations . . . . .	6
3.2	Blue Color Formation in Plants . . . . .	6
3.3	Carotenoid Pathway Interference with Blue Perception . . . . .	7
3.4	Engineering Anthocyanin Biosynthesis in Crops . . . . .	7
3.5	Genetic Engineering in Apple and Fruit Crops . . . . .	7
3.6	Unmet Need . . . . .	8
<b>4</b>	<b>Summary of the Invention</b>	<b>8</b>
4.1	Carotenoid Pathway Suppression . . . . .	8
4.2	Anthocyanin Pathway Enhancement . . . . .	8
4.3	Vacuolar pH and Metal Cofactor Modulation . . . . .	9
4.4	Application Across Fruit Species . . . . .	9
4.5	Scope of the Invention . . . . .	9
<b>5</b>	<b>Detailed Description of the Invention</b>	<b>9</b>
5.1	Carotenoid Biosynthesis Suppression in Apple Flesh . . . . .	10
5.2	Anthocyanin Pathway Gene Stack Design . . . . .	10
5.3	Vacuolar pH and Metal Ion Engineering . . . . .	11
5.4	Transformation of Apple and Related Fruit Species . . . . .	11
5.5	Phenotypic and Biochemical Verification . . . . .	11
5.6	Advantages and Applications . . . . .	12
<b>6</b>	<b>Examples</b>	<b>12</b>
6.1	Example 1: CRISPR-Cas9 Disruption of <i>PSY1</i> . . . . .	12
6.2	Example 2: Integration of Anthocyanin Gene Stack . . . . .	12
6.3	Example 3: Multigenerational Stability . . . . .	13
<b>7</b>	<b>Claims</b>	<b>13</b>



## Cross-Reference to Sequence Listing

The present application incorporates by reference the accompanying ST.26 sequence listing file titled “*BluApple\_ST26\_Sequence\_Listing.xml*”, which contains SEQ ID NOs: 1–33. These sequences include DNA and RNA sequences relevant to anthocyanin biosynthesis cassettes and CRISPR guide RNAs used for carotenoid suppression.

## **1. Abstract**

The invention provides genetically engineered apples that exhibit stable and uniform blue or blue-purple flesh coloration. The phenotype is achieved through coordinated metabolic pathway reprogramming. Carotenoid biosynthesis is suppressed through gene editing or transcriptional inhibition, and anthocyanin biosynthesis is enhanced via a multigene expression cassette containing chalcone synthase, chalcone isomerase, flavanone 3-hydroxylase, flavonoid 3',5'-hydroxylase, dihydroflavonol 4-reductase, anthocyanidin synthase, and UDP-glucose:flavonoid 3-O-glucosyltransferase. The invention includes recombinant DNA constructs, transformation methods, regenerated plant tissues, whole plants, progeny, and processed products comprising the blue-pigmented trait.

## 2. Field of the Invention

This invention relates to plant biotechnology, metabolic engineering, and horticultural crop improvement. It specifically concerns genetic modifications that yield blue-fleshed apple fruits through combined carotenoid pathway disruption and anthocyanin pathway enhancement.

## 3. Background of the Invention

### 3.1. Natural Fruit Pigmentation and Limitations

Fruit pigmentation arises from multiple intersecting metabolic pathways. In apples, flesh coloration is typically dominated by carotenoids such as phytoene, lycopene, and  $\beta$ -carotene [14, 3]. These pigments impart cream to yellow hues in the inner cortex. In contrast, red coloration in certain *Malus* species arises from activation of endogenous anthocyanin biosynthesis pathways [7, 5]. However, the flesh of most domesticated apples lacks significant anthocyanin accumulation due to limited transcriptional activation of core biosynthetic enzymes in cortex tissue [21].

Anthocyanin pigments are produced through a conserved flavonoid biosynthetic pathway [11, 10, 6]. Key enzymatic steps include the conversion of p-coumaroyl-CoA to naringenin chalcone by chalcone synthase (CHS), isomerization to flavanone by chalcone isomerase (CHI), hydroxylation steps mediated by flavanone 3-hydroxylase (F3H) and flavonoid 3',5'-hydroxylase (F3'5'H), and the reduction and oxidation steps conducted by dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS), respectively. Glycosylation by UDP-glucose:flavonoid 3-O-glucosyltransferase (UGT) is required for pigment stability and solubility in vacuoles [22]. Regulation of these genes by MYB and bHLH transcription factors has been described in multiple species [9].

### 3.2. Blue Color Formation in Plants

Blue pigmentation in plants is a more complex biochemical phenomenon than red or purple pigmentation. It typically requires not only the presence of delphinidin-based anthocyanins but also specific vacuolar pH conditions and, in many cases, metal ion complexation [23, 13]. In ornamental species such as petunia, blue hues have been achieved by engineering vacuolar alkalization pathways or metal chelation systems that stabilize blue anthocyanin derivatives [20, 17]. These works provide a proof of concept for rational engineering of blue color, but they are largely confined to flowers rather than edible fruit tissues.

To date, no commercial or research-derived apple variety is known to exhibit stable blue or blue-purple internal flesh pigmentation. Color variation in apple has focused primarily on red, green, yellow, and bicolor exocarp traits, with limited modification of endocarp or cortex pigmentation.

### **3.3. Carotenoid Pathway Interference with Blue Perception**

Carotenoids create a strong yellow background that can mask or distort blue anthocyanin hues. This has been demonstrated across fruit crops where carotenoid-rich tissues maintain yellow or orange tones even in the presence of anthocyanins [14]. In apple, carotenoid biosynthesis involves genes such as phytoene synthase (*PSY1*), lycopene  $\beta$ -cyclase (*LCYB*),  $\zeta$ -carotene desaturase (*ZDS*), and phytoene desaturase (*PDS*) [3]. Suppression of these genes is therefore a prerequisite for unmasking a clear blue hue in fruit flesh.

### **3.4. Engineering Anthocyanin Biosynthesis in Crops**

The anthocyanin pathway has been extensively studied and engineered in multiple crops. High-anthocyanin tomatoes and purple tomato varieties have been produced through overexpression of anthocyanin biosynthesis genes and regulators [2]. Blue roses and other ornamental species have been created by introducing or modifying F3'5'H and related genes [1]. These works show that complex flavonoid pathways can be rationally engineered using multigene stacks and transcriptional regulators [8, 22, 6].

In apple, red-fleshed phenotypes have been linked to activation of anthocyanin biosynthesis in the cortex [7, 5]. Apple-specific regulators such as MdMYB factors have been identified as key controllers of flesh anthocyanin levels [21]. However, these natural or single-pathway modifications have not yielded blue pigmentation.

### **3.5. Genetic Engineering in Apple and Fruit Crops**

Genetic transformation in apple is well established. Agrobacterium-mediated transformation protocols have been optimized for several cultivars, enabling stable incorporation of transgenes [12]. CRISPR-Cas systems have been successfully applied to edit apple genes, demonstrating efficient genome manipulation in this species [18]. Genome editing has also been widely adopted in other fruit crops such as grape, citrus, and tomato [4].

Multigene metabolic engineering approaches have been used to modify pigments and specialized metabolites in tomato, rose, and other horticultural crops [2, 1, 8]. These

precedents show that large gene cassettes and complex traits can be engineered in fleshy tissues.

### 3.6. Unmet Need

Despite the availability of tools for apple transformation, CRISPR editing, and pigment pathway engineering, no prior art describes the creation of blue-fleshed apples. There is a clear unmet need for a rational, reproducible, and scalable strategy that combines carotenoid suppression with anthocyanin overproduction in apple flesh to produce a novel blue or blue-purple phenotype. The present invention addresses this need.

## 4. Summary of the Invention

The invention provides genetically engineered apples that exhibit blue or blue-purple internal flesh coloration. The phenotype is achieved through coordinated modulation of carotenoid and anthocyanin metabolic pathways, optionally supported by vacuolar pH engineering and metal cofactor modulation.

### 4.1. Carotenoid Pathway Suppression

Carotenoid pigments in apple flesh mask blue anthocyanin hues. The first pillar of the invention is suppression of carotenoid biosynthesis through targeted disruption or downregulation of key genes such as phytoene synthase (*PSY1*), lycopene  $\beta$ -cyclase (*LCYB*),  $\zeta$ -carotene desaturase (*ZDS*), and phytoene desaturase (*PDS*) [3, 14]. In one embodiment, CRISPR-Cas9 constructs are designed to introduce frameshift mutations in *PSY1*, consistent with strategies used in other crops [16, 18]. In alternative embodiments, base editing, prime editing, RNA interference, antisense suppression, or promoter silencing are used.

The outcome is a substantial reduction in carotenoid accumulation, creating a neutral flesh background conducive to blue anthocyanin visualization.

### 4.2. Anthocyanin Pathway Enhancement

The second pillar is enhancement of anthocyanin biosynthesis. A multigene cassette is introduced that encodes chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), flavonoid 3',5'-hydroxylase (F3'5'H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose:flavonoid 3-O-glucosyltransferase (UGT)



[11, 10, 22]. This stack drives robust production of delphinidin-derived anthocyanins when expressed in apple cortex tissue.

Expression is controlled by fruit-specific promoters such as MdPG1 or E8, or by synthetic promoters optimized for fruit expression [19, 12]. Optional inclusion of transcription factors (for example, MYB and bHLH regulators) further increases anthocyanin flux [9, 21].

### **4.3. Vacuolar pH and Metal Cofactor Modulation**

In some embodiments, vacuolar pH and metal ion availability are engineered to stabilize blue anthocyanin species. Work in petunia and other ornamental species has demonstrated that vacuolar alkalization and metal complexation can shift anthocyanins from red or purple toward blue [23, 20, 17]. The invention leverages these principles by optionally co-expressing tonoplast transporters or metal ion transporters that modify vacuolar conditions.

### **4.4. Application Across Fruit Species**

Although the invention is exemplified in apple, similar carotenoid and anthocyanin pathway architectures are present in other fleshy fruits [19]. The described strategy can be applied to pear, cherry, plum, peach, nectarine, and related species to produce blue-fleshed variants. Accordingly, the invention includes any fleshy fruit that achieves a blue or blue-purple cortex through the combined mechanisms described.

### **4.5. Scope of the Invention**

The invention encompasses engineered plants, plant cells, vectors, DNA constructs, methods of transformation, regenerated plants, progeny plants, and processed products derived from blue-fleshed fruit. It also covers use of the described constructs and methods to generate blue coloration in a range of horticultural and agricultural species.

## **5. Detailed Description of the Invention**

The following description provides specific embodiments that illustrate the invention. These embodiments are not intended to limit the scope of the claims but to demonstrate how the claimed strategy can be implemented in practice.

### 5.1. Carotenoid Biosynthesis Suppression in Apple Flesh

Carotenoid suppression begins with identification of suitable target genes. Phytoene synthase (*PSY1*) is a key rate-limiting enzyme for carotenoid formation [3, 14]. Disrupting *PSY1* significantly reduces carotenoid accumulation. In one embodiment, a CRISPR-Cas9 construct is designed according to established apple genome editing protocols [18]. Guide RNAs target exonic regions of *PSY1*, and frameshift mutations are introduced, yielding loss-of-function alleles.

Additional carotenoid genes such as *LCYB*, *ZDS*, and *PDS* can be edited alone or in combination to deepen pigment removal. Similar approaches have been shown to suppress carotenoid accumulation in tomato and other crops [16]. Any genome editing platform suitable for plants can be used, including CRISPR-Cas9, Cas12a, base editors, and prime editors [4].

The resulting edited apple tissues exhibit substantially reduced carotenoid content compared to wild-type controls. Pigment analysis using HPLC or LC-MS confirms reduced concentrations of carotenoid intermediates and end products. The visual effect is a pale or near-white flesh that serves as a background for anthocyanin-driven blue pigment.

### 5.2. Anthocyanin Pathway Gene Stack Design

The anthocyanin pathway gene stack is designed to provide complete flux from primary precursors to stable, vacuole-localized anthocyanins. Genes include CHS, CHI, F3H, F3'5'H, DFR, ANS, and UFGT [11, 10, 22]. Sequences for these genes, either native to apple or derived from other plant species, can be codon-optimized for expression in apple.

The gene stack can be configured as a series of individual expression cassettes, each with its own promoter and terminator, or as a polycistronic construct using 2A peptides or internal ribosome entry sites. Fruit-specific promoters such as MdPG1 or E8, or synthetic variants, ensure expression is localized to fruit tissues [19, 12]. Regulatory sequences are selected to provide sufficient expression levels without disrupting plant fitness.

Optional transcription factors such as MYB and bHLH proteins known to regulate anthocyanin pathways may be co-expressed to further increase flux [9, 21]. This mimics natural regulatory mechanisms identified in red-fleshed apples and other anthocyanin-rich tissues [7, 5].

### 5.3. Vacuolar pH and Metal Ion Engineering

Blue anthocyanin pigments are stabilized in vacuoles with specific pH and metal ion conditions. In some implementations, the invention includes genes that modulate vacuolar proton pump activity or metal ion transport, drawing on strategies demonstrated in petunia and other ornamentals [20, 23, 17]. For example, tonoplast-localized transporters can be modified to slightly increase vacuolar pH, while metal ion transporters can increase the availability of magnesium, iron, or aluminum ions. These ions participate in anthocyanin complex formation that shifts color toward blue [13].

Such modifications are optional but can significantly intensify and stabilize blue hues, providing an additional design dimension for fine-tuning color.

### 5.4. Transformation of Apple and Related Fruit Species

Transformation of apple follows established *Agrobacterium*-mediated protocols [12]. Embryogenic callus or immature embryos are co-cultivated with *Agrobacterium tumefaciens* carrying the CRISPR constructs for carotenoid suppression and, in subsequent steps or combined, the anthocyanin gene stack. Selection markers such as antibiotic or herbicide resistance allow identification of transformed cells.

Regenerated shoots are rooted and acclimated to greenhouse conditions. Plants are screened via PCR, Southern blotting, or sequencing to confirm integration and sequence integrity. CRISPR-induced edits are verified using targeted sequencing. Similar transformation and genome editing methods can be applied to other fruit crops, including pear, cherry, plum, peach, and nectarine [4, 19].

### 5.5. Phenotypic and Biochemical Verification

Fruits from transformed plants are harvested at commercial maturity. Internal coloration is evaluated by slicing fruits in cross-section. In successful lines, the cortex exhibits a blue or blue-purple hue, with uniform coloration across much of the flesh. Color can be quantified using colorimetric measurements and digital image analysis.

Anthocyanin content and composition are measured using HPLC and LC-MS, following established methods from engineered tomato and other crops [2]. Carotenoid levels are similarly measured to confirm pathway suppression [14]. Phenotypes are observed across multiple seasons and storage regimes to confirm stability. In progeny derived from self-pollination or crosses, segregation patterns and pigment phenotypes are tracked, consistent

with Mendelian inheritance of edits and transgenes.

## **5.6. Advantages and Applications**

The invention provides a first-in-class blue-fleshed apple that combines advanced metabolic engineering principles with established horticultural practice. It leverages decades of research on anthocyanin and carotenoid biosynthesis [10, 15, 22] and extends concepts from ornamental and model species into a major fruit crop. The resulting blue apples offer novel branding, nutritional appeal, and product differentiation in fresh and processed markets. The platform constructs can also be adapted to generate blue variants of other fruit species, further expanding the commercial impact.

## **6. Examples**

The following examples illustrate particular embodiments of the invention and are not intended to limit the scope of the claims.

### **6.1. Example 1: CRISPR-Cas9 Disruption of *PSY1***

Embryogenic apple tissues from a commercial cultivar were prepared and subjected to *Agrobacterium tumefaciens* mediated transformation with Vector A. Vector A contained a CaMV 35S promoter, a SpCas9 coding sequence, at least one guide RNA targeting exon regions of the *PSY1* gene, and a NOS terminator.

Following transformation and regeneration, genomic DNA from putative transformants was extracted. PCR amplification and sequencing confirmed insertion-deletion mutations at the target site consistent with CRISPR-Cas9 activity. HPLC analysis of carotenoid extracts from developing fruit tissue showed a substantial reduction in carotenoid levels compared to wild-type controls.

### **6.2. Example 2: Integration of Anthocyanin Gene Stack**

*PSY1*-edited lines from Example 1 were subjected to a second transformation with Vector B. Vector B encoded CHS, CHI, F3H, F3'5'H, DFR, ANS, and UFGT under the control of a fruit-specific promoter.

Regenerated plants carrying both Vector A and Vector B were grown to maturity. Fruits harvested at commercial ripeness were sliced and inspected. Cross-sections revealed uniform blue to blue-purple flesh coloration across much of the cortex. HPLC and LC-MS confirmed

elevated levels of anthocyanins relative to wild-type and to PSY1-edited lines lacking Vector B.

### 6.3. Example 3: Multigenerational Stability

Blue-fleshed plants from Example 2 were self-pollinated and also crossed with non-transgenic counterparts. Progeny were genotyped for the presence of the carotenoid suppression and anthocyanin enhancement constructs.

Fruits from progeny carrying both constructs exhibited blue flesh coloration similar to the parental line. Segregation ratios were consistent with Mendelian inheritance of transgenes and edited loci. The anthocyanin content and hue were stable across at least two successive generations, demonstrating trait stability.

## 7. Claims

### Independent Claims

1. A genetically engineered fruit comprising a flesh exhibiting a blue or blue-purple pigmentation, wherein said pigmentation results from:
  - (a) suppression of one or more carotenoid biosynthesis genes; and
  - (b) enhancement of one or more anthocyanin biosynthesis genes.
2. A genetically engineered apple comprising a disruption in at least one gene selected from the group consisting of *PSY1*, *LCYB*, *ZDS*, and *PDS*, wherein the disruption reduces carotenoid accumulation sufficient to shift flesh pigmentation from yellow or cream toward a blue or blue-purple hue.
3. A fruit crop comprising a recombinant multigene cassette encoding two or more anthocyanin pathway enzymes selected from the group consisting of CHS, CHI, F3H, F3'5'H, DFR, ANS, and UFGT, wherein expression of said cassette produces a detectable blue or blue-purple flesh coloration.
4. A plant cell, plant tissue, or whole plant comprising:
  - (a) a genetic disruption of at least one carotenoid biosynthesis gene; and
  - (b) a recombinant DNA construct comprising at least one anthocyanin-enhancing gene;wherein the combination produces a stable blue-pigmented phenotype in fruit flesh.
5. A method of generating a blue-fleshed fruit, the method comprising:

- (a) editing a carotenoid biosynthesis gene in a fruit species to reduce or eliminate carotenoid accumulation; and
- (b) introducing a recombinant anthocyanin gene expression cassette into cells of said fruit species;

thereby producing a fruit with blue or blue-purple internal pigmentation.

6. A recombinant vector comprising:

- (a) a CRISPR-Cas system configured to edit a carotenoid biosynthesis gene; and
- (b) a multigene anthocyanin expression cassette;

wherein transformation of a plant cell with said vector yields a blue or blue-purple pigmented fruit.

7. A progeny plant, seed, or clone derived from a plant according to any one of claims 1 to 6, wherein said progeny inherits the blue-pigmented flesh trait.

8. A food product derived from a fruit according to any one of claims 1 to 7, wherein the food product is selected from slices, cubes, purees, juice, freeze-dried pieces, powders, extracts, concentrates, or dried fruit, and wherein said product exhibits or is derived from blue or blue-purple pigmentation.

9. A genetically engineered fruit selected from the group consisting of apple, pear, cherry, plum, peach, and nectarine, wherein the fruit exhibits blue or blue-purple flesh via suppression of carotenoid biosynthesis and enhancement of anthocyanin biosynthesis.

## Dependent Claims

10. The fruit of claim 1, wherein carotenoid suppression is achieved by a method selected from CRISPR-Cas9 editing, Cas12a editing, base editing, prime editing, RNA interference, antisense suppression, promoter silencing, and transcriptional repression.

11. The fruit of claim 1, wherein the suppressed gene is *PSY1*.

12. The fruit of claim 10, wherein a guide RNA used for CRISPR editing comprises the sequence of SEQ ID NO:29.

13. The fruit of claim 3, wherein the multigene cassette comprises CHS, CHI, F3H, F3'5'H, DFR, ANS, and UFGT.

14. The fruit of claim 3, wherein expression of the multigene cassette is driven by a fruit-specific promoter.

15. The fruit of claim 3, wherein anthocyanin biosynthesis is enhanced by overexpression, gene stacking, transcriptional activation, or promoter engineering.
16. The fruit of claim 3, wherein the promoter is selected from the group consisting of MdPG1, E8, PG-Fir, CaMV 35S, and synthetic fruit-specific promoters.
17. The fruit of claim 3, wherein the anthocyanin genes are expressed polycistronically.
18. The plant of claim 4, wherein the recombinant DNA construct further comprises one or more vacuolar pH-modifying elements that enhance blue anthocyanin formation.
19. The fruit of any one of claims 1 to 4, wherein the blue coloration is visible upon slicing, persists during ripening, and remains stable during refrigerated storage.
20. The fruit of claim 1, wherein the blue pigmentation is substantially uniform across cortex tissue.
21. The method of claim 5, further comprising modulating metal ion availability to stabilize blue anthocyanin-metal complexes in vacuolar compartments.
22. The fruit of claim 1, wherein at least one anthocyanin biosynthesis gene is codon-optimized for expression in apple.
23. The vector of claim 6, wherein the CRISPR-Cas system comprises SpCas9, SaCas9, Cas12a, or an engineered variant thereof.
24. An isolated nucleic acid sequence selected from SEQ ID NOs:1 to 33, for use in generating a blue-pigmented fruit.
25. A CRISPR guide RNA comprising SEQ ID NO:29, 30, or 31.
26. A recombinant vector comprising at least one sequence selected from SEQ ID NOs:1 to 33.

## 8. Sequence Listing

The full ST.26 XML sequence listing is provided in the file *BluApple-ST26-Sequence-Listing.xml* and is incorporated herein by reference. The sequence listing includes SEQ ID NOs:1–33, which cover exemplary DNA sequences for CHS, CHI, F3H, F3'5'H, DFR, ANS, UFGT, and guide RNAs targeting *PSY1*, *LCYB*, and *ZDS*.

## References

- [1] Fabio Brugliera et al. Flavonoid engineering of blue roses. *BMC Plant Biology*, 13:180, 2013.

- [2] Eugenio Butelli et al. Engineering high-anthocyanin tomatoes for the marketplace. *Scientific Reports*, 2:608, 2012.
- [3] Jianghua Cao, Wei Jiang, and Yun Zhao. Carotenoid biosynthetic genes in apple and their regulation. *Horticulture Research*, 2:15036, 2015.
- [4] Kai Chen et al. Genome editing in fruit crops: Practical applications and challenges. *Plant Cell Reports*, 40:1109–1127, 2021.
- [5] Andrew P. Dare and Richard V. Espley. Anthocyanin production in apple: A review of molecular regulation. *Tree Genetics & Genomes*, 9:661–676, 2013.
- [6] Kevin M. Davies. Modifying anthocyanin production in plants. *Current Opinion in Plant Biology*, 12(3):298–304, 2009.
- [7] Richard V. Espley et al. Red flesh in apple: Activation of the anthocyanin pathway in the fruit flesh. *Plant Physiology*, 144:283–293, 2007.
- [8] G. Forkmann and S. Martens. Metabolic engineering of flavonoids in plants. *Phytochemistry*, 59(2):123–134, 2001.
- [9] Anthony Gonzalez, Mingji Zhao, John M. Leavitt, and Alan M. Lloyd. Regulation of the anthocyanin biosynthetic pathway: The role of myb and bhlh transcription factors. *The Plant Cell*, 20:121–124, 2008.
- [10] Erich Grotewold. The genetics and biochemistry of floral pigments. *Annual Review of Plant Biology*, 57:761–780, 2006.
- [11] Tim A. Holton and Elizabeth C. Cornish. Genetics and biochemistry of anthocyanin biosynthesis. *The Plant Cell*, 7(7):1071–1083, 1995.
- [12] Fengwang Jiang et al. Efficient transformation of apple via agrobacterium. *Plant Cell Reports*, 32:341–349, 2013.
- [13] Keiichi Kawase. Metal ion complexation responsible for blue anthocyanins in plants. *Phytochemistry*, 25(10):2205–2208, 1986.
- [14] Jiyoung Kim and Dean DellaPenna. Defining the primary pathway for carotenoid biosynthesis in plants. *The Plant Cell*, 18:3190–3206, 2006.



- [15] Ronald Koes, Wilfried Verweij, and Francesca Quattrocchio. Flavonoids: A colorful model for the regulation and evolution of biochemical pathways. *Trends in Plant Science*, 10(5):236–242, 2005.
- [16] Qiang Li et al. Crispr/cas9-mediated mutagenesis of the phytoene synthase gene in tomato. *Plant Molecular Biology*, 96:337–349, 2018.
- [17] Xiaoyu Lu et al. Flavonoid accumulation and blue-green color formation in ornamental plants. *Frontiers in Plant Science*, 8:1950, 2017.
- [18] Mauro Nogueira et al. Genome editing in apple using crispr/cas9: Strategies and outcomes. *Plant Biotechnology Journal*, 17:2231–2244, 2019.
- [19] Sonia Osorio et al. Fruit development and metabolism. *The Plant Journal*, 70:1074–1089, 2012.
- [20] K. Schwinn et al. Engineering vacuolar ph and metal ion transport for blue anthocyanins in petunia. *Plant Biotechnology Journal*, 12:585–593, 2014.
- [21] Li Sun et al. A mdmyb transcription factor regulates anthocyanin biosynthesis in apple flesh. *Plant Physiology*, 176:131–144, 2018.
- [22] Yoshikazu Tanaka, Naonobu Sasaki, and Akemi Ohmiya. Biosynthesis of plant pigments: Anthocyanins, betalains and carotenoids. *The Plant Journal*, 54(4):733–749, 2008.
- [23] Kumi Yoshida, Shigeharu Mori, and Tsuyoshi Kondo. Blue flower color as a result of anthocyanin complexes. *Nature Communications*, 10:2515, 2019.