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Genetic Study of Pigment Synthesis and Related Genes in Dragon Fruit

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Abstract The pigments in dragon fruit, including betalains, carotenoids, and flavonoids, not only enhance the aesthetic appeal of the fruit but also offer various health benefits. Understanding the genetic mechanisms behind pigment synthesis is crucial for developing new varieties of dragon fruit with improved color and nutritional properties. This study explores the genetic mechanisms of pigment synthesis in dragon fruit (*Hylocereus* spp.), focusing on the biosynthetic pathways and key genes involved in the production of betalains, carotenoids, and flavonoids. Through genomic analyses and biochemical characterizations, essential genes such as *CYP76AD1*, *DODA1*, and *MYB* transcription factors were identified as playing critical roles in pigment production, contributing to the vibrant coloration of dragon fruit. The study also examines the impact of environmental factors on pigment synthesis and the potential applications of dragon fruit pigments in the food and pharmaceutical industries. The findings of this study provide a comprehensive understanding of the genetic regulation of pigment synthesis in dragon fruit, offering significant implications for breeding programs and cultivation practices aimed at enhancing the fruit's color intensity and nutritional value.
Keywords Dragon fruit; Pigment synthesis; Betalains; Carotenoids; Flavonoids; Genetic regulation; Biosynthetic pathways

1 Introduction

Dragon fruit (*Hylocereus* spp.), a member of the Cactaceae family, is renowned not only for its unique appearance and nutritional benefits but also for its vibrant pigmentation. The pigment synthesis in dragon fruit involves complex biochemical pathways that produce various pigments, including betalains, anthocyanins, and carotenoids. Pigment synthesis plays a crucial role in determining the color, quality, and nutritional value of dragon fruit. The primary betalain pigments give the fruit a range of colors from yellow to deep red, significantly influencing consumer preferences and market value. Additionally, these pigments are associated with multiple health benefits, including antioxidant, anti-inflammatory, and anticancer properties, making them increasingly favored by the food industry as alternatives to synthetic colorants (Esquivel et al., 2007; Fathordoobady et al., 2021). The commercial value of dragon fruit is significantly influenced by its pigment content, which also plays a crucial role in consumer preference and marketability (Esquivel et al., 2007).

Genetic studies in plant pigmentation have provided insights into the biosynthetic pathways and regulatory mechanisms that control pigment production. In dragon fruit, recent genomic analyses have revealed the presence of genes involved in the betacyanin biosynthetic pathway, which are co-localized on specific chromosomes, suggesting a coordinated regulation of pigment synthesis (Zheng et al., 2021). Additionally, morphological, biochemical, and molecular characterizations have been employed to distinguish different *Hylocereus* species, highlighting the genetic diversity and variation in pigment content among different genotypes (Abirami et al., 2021). These studies are essential for understanding the genetic basis of pigmentation and for the development of breeding strategies aimed at enhancing pigment production.

This study investigates the key genes involved in the pigment synthesis of dragon fruit, elucidating the genetic mechanisms that control pigment production. By identifying and characterizing the genes involved in the betalain biosynthesis pathway, the study analyzes the genetic variation of pigment-related genes across different dragon fruit varieties and their impact on breeding programs aimed at enhancing fruit color and nutritional quality.

Additionally, the study explores the potential industrial applications of dragon fruit pigments as natural colorants and antioxidants. By achieving these objectives, this study will contribute to the understanding of dragon fruit pigment synthesis and promote targeted breeding strategies and biotechnological interventions to improve fruit quality and market value.

2 Classification of Pigments in Dragon Fruit

Dragon fruit (*Hylocereus* spp.) is known for its vibrant colors, which are attributed to the presence of various pigments. These pigments not only contribute to the fruit's aesthetic appeal but also offer numerous health benefits. The primary pigments found in dragon fruit are betalains, carotenoids, and flavonoids. Each of these pigment classes plays a unique role in the fruit's biology and offers various nutritional and therapeutic advantages.

2.1 Betalains

Betalain pigments are a class of water-soluble natural pigments primarily found in plants belonging to the order Caryophyllales, with dragon fruit, a member of the Cactaceae family, being a typical representative. These pigments not only give dragon fruit its vibrant red and purple hues but are also widely distributed in other plants, contributing to a variety of color variations. Betalain pigments are mainly divided into two categories based on their color: betacyanins and betaxanthins. Betacyanins impart red to purple shades to the fruit, while betaxanthins provide yellow to orange hues (Grützner et al., 2021). In dragon fruit, betacyanins are particularly abundant, not only giving the fruit its appealing red or purple color but also being closely associated with various health benefits. Research has shown that betacyanins improve vascular function and have antioxidant, anti-inflammatory, and potential anticancer properties, making dragon fruit a focus of interest in the fields of nutrition and medicine (Zheng et al., 2021; Cheok et al., 2022).

The biosynthesis of betalain pigments is relatively straightforward but is controlled by a complex genetic regulatory network. This process begins with the amino acid tyrosine and, through a series of enzymatic reactions, ultimately produces betacyanins and betaxanthins. In dragon fruit, in particular, MYB transcription factors play a crucial regulatory role in the synthesis of betalain pigments (Zhang et al., 2021). These transcription factors regulate the expression of key genes in the betalain biosynthetic pathway by binding to their promoters, thereby controlling pigment synthesis and accumulation. Additionally, environmental factors such as light, temperature, and nutrient conditions can indirectly influence the accumulation of betalain pigments in dragon fruit by modulating the activity of these transcription factors. As research into the mechanisms of betalain biosynthesis deepens, scientists are expected to further optimize the pigment content in dragon fruit through genetic engineering or cultivation management techniques, enhancing its nutritional value and market competitiveness (Zhao et al., 2022).

2.2 Carotenoids

Carotenoids are a class of fat-soluble pigments widely found in various fruits and vegetables, with colors ranging from yellow to orange to red. These pigments not only give plants their vibrant colors but also play a crucial role in photosynthesis and photoprotection (Sathasivam et al., 2020). Carotenoids are synthesized through the terpenoid pathway, beginning with the production of isopentenyl pyrophosphate (IPP). Through a series of enzymatic reactions, this pathway ultimately leads to the formation of various carotenoid compounds (Metibemu and Ogungbe, 2022).

In dragon fruit, carotenoids such as β-carotene and xanthophylls are present in both the peel and pulp, contributing to the fruit's nutritional value and antioxidant capacity (Liu et al., 2019; Abirami et al., 2021). The enzyme phytoene synthase (PSY) catalyzes the first step in carotenoid biosynthesis, and the regulation of this pathway involves complex interactions with other plant metabolic processes (Liu et al., 2019).

2.3 Flavonoids

Flavonoids are a large class of water-soluble polyphenolic compounds that play a crucial role in plant physiology, participating in pigment formation, flavor, and defense mechanisms. Flavonoids are renowned for their potent antioxidant properties, which help neutralize free radicals and reduce oxidative stress, thereby lowering the risk of chronic diseases such as cardiovascular disease, diabetes, and cancer (Kopustinskiene et al., 2020).

In dragon fruit, flavonoids are primarily found in the peel and contribute to the fruit's antioxidant activity. These compounds are synthesized through the phenylpropanoid pathway and undergo various modifications such as glycosylation and acylation, resulting in a wide range of derivatives (Abirami et al., 2021; Zhao et al., 2022). Studies have shown that flavonoids from dragon fruit peel can be extracted and used in the formulation of health supplements, providing a natural alternative to synthetic antioxidants (Abirami et al., 2021). Additionally, utilizing dragon fruit peel as a source of flavonoids helps reduce agricultural waste and promotes sustainability.

The pigments in dragon fruit—betalains, carotenoids, and flavonoids—each play significant roles in the fruit's coloration and nutritional benefits. Understanding the genetic basis and biosynthetic pathways of these pigments can provide insights into improving the fruit's quality and marketability through targeted breeding and genetic engineering strategies.

3 Synthesis Pathways of Pigments in Dragon Fruit

3.1 Biosynthesis pathways of betalains

Betacyanins are red-violet pigments derived from tyrosine and are a major component of the betalain pigment family. In dragon fruit, the biosynthesis of betacyanins involves several key enzymes, including CYP76AD1, DODA, and cDOPA5GT. These enzymes catalyze the conversion of tyrosine to betalamic acid, which then combines with cyclo-DOPA to form betacyanins (Chang et al., 2021; Morales et al., 2021). Zheng et al. (2021) conducted a comprehensive genomic analysis of white-fleshed dragon fruit (*Hylocereus undatus*), providing important insights into its evolutionary history and genomic characteristics. The study revealed that key genes involved in betacyanin synthesis are co-located on a single chromosome (Figure 1). This co-localization may enhance the efficiency of betalain production, which is significant for the nutritional value and commercial appeal of dragon fruit.

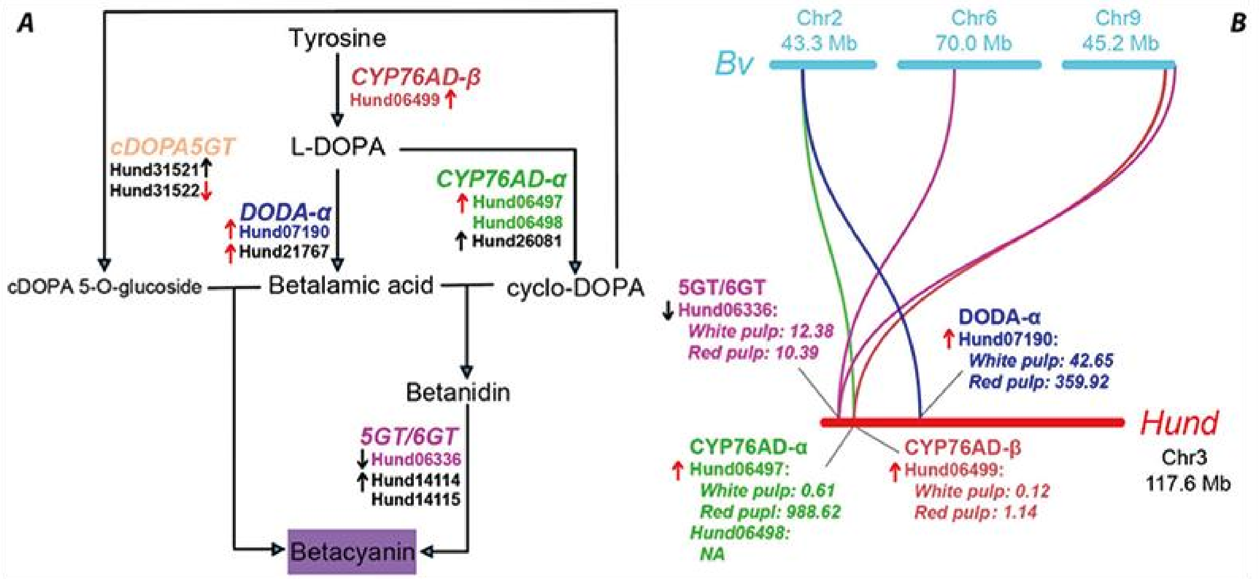

Figure 1 The betacyanin biosynthetic pathway in *H. undatus* and *B. vulgaris* (Adopted from Zheng et al., 2021)

Image caption: A: The positions of key enzyme genes related to betacyanin synthesis in the dragon fruit genome and their expression changes during pulp development; B: The co-localization of these genes on the dragon fruit chromosomes, particularly within a 12 Mb region of chromosome 3, which may enhance the efficiency of betacyanin synthesis. The results reveal the chromosomal co-localization of betacyanin genes in dragon fruit, confirming their potential significance in antioxidant production (Adapted from Zheng et al., 2021)

Betaxanthins are yellow pigments also derived from tyrosine. The biosynthesis pathway of betaxanthins involves the same initial steps as betacyanins, up to the formation of betalamic acid. Betalamic acid then combines with amino acids or amines to produce various betaxanthins (Chang et al., 2021; Morales et al., 2021). The presence of both betacyanins and betaxanthins in dragon fruit contributes to its vibrant coloration and antioxidant properties (Sarker and Oba, 2020).

The regulation of betalain synthesis in dragon fruit is primarily controlled by MYB transcription factors. These factors regulate the expression of key enzymes involved in the betalain biosynthesis pathway (Zhao et al., 2022). The co-localization of betalain biosynthetic genes on a single chromosome may also play a role in the coordinated regulation of these genes, enhancing the efficiency of pigment production (Zheng et al., 2021).

3.2 Biosynthesis pathways of carotenoids

Carotenoids are fat-soluble pigments that give dragon fruit its yellow, orange, and red colors. Lycopene is a red carotenoid pigment synthesized through the isoprenoid pathway and serves as a key intermediate in the carotenoid biosynthesis pathway. Lycopene is a red carotenoid pigment synthesized through the isoprenoid pathway. The biosynthesis of lycopene involves the conversion of geranylgeranyl pyrophosphate (GGPP) to phytoene, which is then desaturated to form lycopene. This pathway is regulated by several enzymes, including phytoene synthase and phytoene desaturase (Rodriguez-Amaya, 2019; Zhou et al., 2020).

Beta-carotene is an orange carotenoid pigment and a precursor to vitamin A. It is synthesized from lycopene through the action of lycopene beta-cyclase, which adds beta-ionone rings to both ends of the lycopene molecule (Rodriguez-Amaya, 2019). Beta-carotene is an important antioxidant and contributes to the nutritional value of dragon fruit (Sarker and Oba, 2020).

3.3 Biosynthesis pathways of flavonoids

Anthocyanins are flavonoid pigments derived from the phenylpropanoid pathway. The biosynthesis of anthocyanins involves several enzymatic steps, including the conversion of phenylalanine to cinnamic acid, followed by a series of hydroxylation, methylation, and glycosylation reactions to produce various anthocyanin derivatives (Zhao et al., 2022). These pigments are responsible for the red, purple, and blue colors in many fruits and vegetables. Flavonols are another class of flavonoid pigments synthesized through the phenylpropanoid pathway. The biosynthesis of flavonols involves the conversion of dihydroflavonols to flavonols by the enzyme flavonol synthase. Flavonols contribute to the UV protection and antioxidant properties of plants (Zhao et al., 2022).

The biosynthesis of flavonoids has been extensively studied in various plants, but the specific pathways in dragon fruit are of particular interest due to its unique metabolic processes and potential applications in food and medicine. Transcriptome analysis has shown that wounding stress can induce the activation of metabolic pathways related to the biosynthesis of phenolic and flavonoid compounds in dragon fruit. This includes the phenylpropanoid pathway, the flavonoid pathway, and other related metabolic processes such as glycolysis and the shikimate pathway (Li et al., 2021a). Additionally, the application of trypsin as a preservative has been shown to regulate the biosynthesis of lignin, chlorogenic acid, and flavonoids in *Hylocereus undatus*, suggesting complex interactions between different metabolic pathways (Li et al., 2021b). By understanding these biosynthetic pathways and regulatory mechanisms, researchers can develop strategies to enhance the nutritional and aesthetic qualities of dragon fruit.

4 Genetic Regulation of Pigment Synthesis in Dragon Fruit

4.1 Key genes involved in pigment synthesis

The synthesis of pigments in dragon fruit is primarily regulated by the biosynthetic pathways of betalains and anthocyanins. Key genes involved in the biosynthesis of betalains include *CYP76AD1*, *DODA1*, and *DOPA5GT*, which play crucial roles in the conversion of tyrosine into betalains. Hua et al. (2021) conducted a genome-wide identification study, revealing the critical roles of the *HmoCYP76AD1*, *HmoDODA1*, and *HmocDOPA5GT* genes in the biosynthesis of betalains in the genus *Hylocereus*. The study demonstrated that these genes participate in the synthesis of betacyanins and betaxanthins from L-tyrosine by catalyzing a series of enzymatic reactions. Through gene silencing experiments, the research team found that silencing these genes led to a significant reduction or complete loss of red pigment synthesis in the plants, further confirming their importance in pigment biosynthesis (Figure 2).

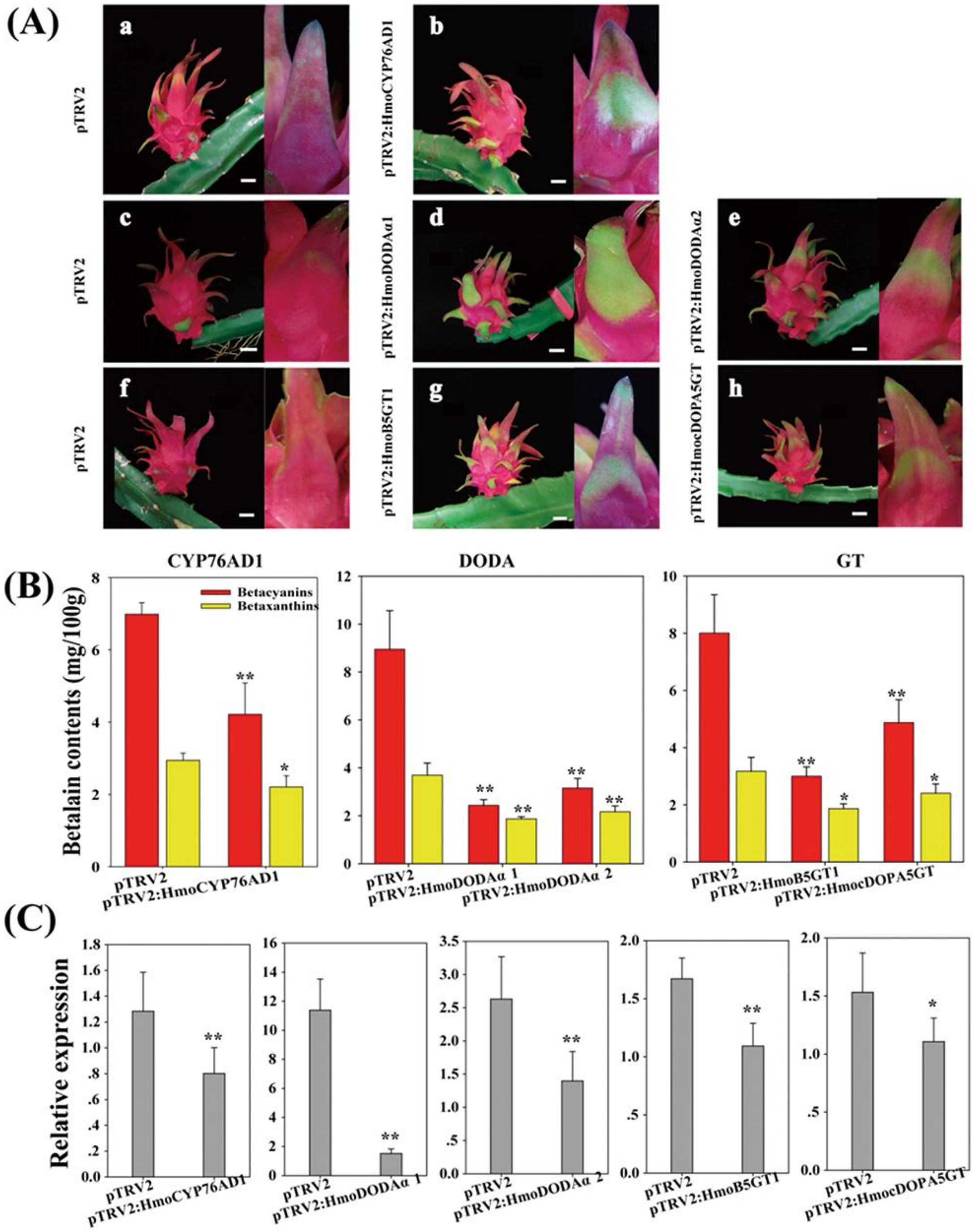

Figure 2 Virus-induced gene silencing (VIGS) phenotypes in pitaya (Adopted from Hua et al., 2021)

Image caption: The figure shows that after silencing the *HmoCYP76AD1*, *HmoDODAα1*, *HmoDODAα2*, and *HmocDOPA5GT* genes, the red pigments in the peel and scales of pitaya were significantly reduced or disappeared, resulting in green scales. Meanwhile, the contents of betacyanins and betaxanthins in the silenced tissues were significantly decreased. The qRT-PCR results confirmed the gene silencing effect. This experiment verified the critical role of these genes in betalain biosynthesis in pitaya (Adapted from Hua et al., 2021)

The study also validated the functions of these genes through transient expression and yeast recombinant expression (Hua et al., 2021). Additionally, WRKY transcription factors, such as WRKY40 and WRKY44, have been identified as important regulatory factors for these biosynthetic genes. These transcription factors can enhance the expression of *CYP76AD1* and other related genes (Zhou et al., 2020; Zhang et al., 2021).

4.2 Gene expression patterns

Gene expression patterns in dragon fruit reveal significant differences between red and white pulp varieties. Transcriptome analyses have shown that genes involved in betalain biosynthesis, such as *CYP76AD1* and *DODA1*, are highly upregulated in red pulp compared to white pulp (Xi et al., 2019). This upregulation is accompanied by increased levels of tyrosine and other intermediates in the betalain pathway (Li et al., 2022).

Furthermore, the expression of anthocyanin biosynthetic genes is also differentially regulated, with red pulp varieties showing higher expression levels of genes like *MYB* and *bHLH* transcription factors, which are known to activate anthocyanin biosynthesis (Fan et al., 2020). These patterns suggest a coordinated regulation of pigment synthesis pathways to achieve the desired coloration in dragon fruits.

4.3 Genetic manipulation for enhanced pigmentation

Genetic manipulation strategies to enhance pigmentation in dragon fruit focus on overexpressing key regulatory genes and biosynthetic enzymes. For instance, overexpression of WRKY transcription factors such as *WRKY40* has been shown to significantly increase betalain content in pitaya by upregulating the expression of *CYP76AD1* (Zhang et al., 2021). Similarly, silencing of these transcription factors results in reduced pigment levels, demonstrating their pivotal role in pigment biosynthesis (Zhang et al., 2021).

Additionally, co-expression of *CYP76AD1*, *DODA1*, and *DOPA5GT* in model organisms like *Nicotiana benthamiana* has been used to validate their roles and enhance betalain production (Hua et al., 2021). These genetic engineering approaches provide valuable insights and tools for breeding dragon varieties with enhanced and novel pigmentation, thereby improving their commercial appeal and nutritional value (Zhou et al., 2020; Hua et al., 2021). By understanding and manipulating the genetic regulation of pigment synthesis, researchers can develop dragon fruits with improved and diverse coloration, meeting consumer preferences and market demands.

5 Environmental Influence on Pigment Synthesis in Dragon Fruit

5.1 Light and temperature effects

Light and temperature are critical environmental factors influencing pigment synthesis in dragon fruit. Studies have shown that temperature variations significantly affect the drying process and the stability of betacyanin pigments. For instance, drying red-fleshed dragon fruit at higher temperatures (up to 80 °C) resulted in shorter drying times without significantly affecting the total betacyanin content and antioxidant capacities of the dried products. This suggests that higher temperatures can be used to efficiently process dragon fruit while preserving its pigment content (Mahayothee et al., 2018). Additionally, the ultrasound-assisted extraction method demonstrated that ultrasonic temperature (ranging from 30 °C-70 °C) plays a significant role in the extraction efficiency of betacyanin, with optimal conditions found at 60 °C (Raj and Dash, 2020).

5.2 Soil and nutrient impacts

The soil composition and nutrient availability also play a vital role in the synthesis of pigments in dragon fruit. The biochemical characterization of different dragon fruit species revealed significant variations in phenol and flavonoid content, which are crucial for antioxidant potential. These variations are likely influenced by the soil and nutrient conditions in which the plants are grown.

For example, higher phenol and flavonoid contents were observed in the peels compared to the pulp, indicating that nutrient-rich soils may enhance the antioxidant properties of the fruit (Abirami et al., 2021). Furthermore, the use of chemical activators like KOH in the thermal carbonization process of dragon fruit peels has shown to produce activated carbon with high surface area, which can be used for various applications, including dye adsorption (Jawad et al., 2020).

5.3 Interaction between environment and genetics

The interaction between environmental factors and genetic makeup is crucial for the efficient synthesis of pigments in dragon fruit. The draft genome of *Hylocereus undatus* revealed the co-localization of betacyanin biosynthetic genes on a specific chromosome region, suggesting a genetic predisposition for efficient pigment synthesis. This genetic arrangement may enhance the plant's ability to produce betacyanin under favorable environmental conditions (Zheng et al., 2021). Additionally, the morphological and molecular characterization of different dragon fruit species indicated considerable genetic variation, which could influence how these plants respond to environmental factors such as light, temperature, and soil nutrients (Abirami et al., 2021). The systematic review of betalain-rich cacti, including dragon fruit, highlighted the potential physiological effects of these pigments on vascular health, further emphasizing the importance of both genetic and environmental factors in pigment synthesis (Cheok et al., 2020).

By understanding the complex interplay between environmental conditions and genetic factors, researchers can optimize cultivation practices to enhance pigment synthesis in dragon fruit, thereby improving its nutritional and commercial value.

6 Analytical Techniques for Studying Pigment Synthesis in Dragon Fruit

6.1 Chromatography techniques

Chromatography techniques are pivotal in the analysis of pigment synthesis in dragon fruit. Liquid chromatography, particularly when coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS), has been effectively utilized to identify and quantify the principal pigments in red dragon fruit (*Hylocereus polyrhizus*). This method allows for the detection of both betacyanins and betaxanthins, with specific UV absorbance maxima and daughter ions aiding in their identification.

For instance, betanin/isobetanin, phyllocactin/isophyllocactin, and apiosyl-betanin/apiosyl-isobetanin were among the main betacyanins identified using this technique (Thu and Quang, 2019). Additionally, high-performance countercurrent chromatography (HPCCC) has been employed to fractionate and profile polar metabolites, including betalains, in fruits. This method, combined with electrospray mass spectrometry, enables the visualization of molecular weight elution profiles and the structural characterization of metabolites (Tran et al., 2019).

6.2 Mass spectrometry applications

Mass spectrometry (MS) is a crucial tool in the study of pigment synthesis in dragon fruit. The application of electrospray ionization mass spectrometry (ESI-MS) allows for the detailed profiling of betalains and their degradation products. For example, in the study of Vietnamese red dragon fruits, ESI-MS/MS was used to identify various betacyanins and betaxanthins, as well as their degradation products such as decarboxy-betanin and neobetanin (Thu and Quang, 2019).

This technique provides high sensitivity and specificity, enabling the detection of trace amounts of pigments and their derivatives. Additionally, off-line ESI-MS fraction monitoring has been used to determine peak elution windows and to identify potentially novel fruit metabolites based on unknown molecular weights and MS/MS fragmentations (Tran et al., 2019).

6.3 Molecular biology tools

Molecular biology tools are essential for understanding the genetic basis of pigment synthesis in dragon fruit. Techniques such as polymerase chain reaction (PCR) and gene sequencing can be used to identify and characterize genes involved in the biosynthesis of betalains. These tools allow researchers to study the expression patterns of these genes and to investigate how they are regulated under different environmental conditions. Furthermore, the use of chemometric tools in conjunction with molecular biology techniques can optimize the extraction and analysis of betacyanins. For instance, the Box–Behnken Design (BBD) and response surface methodology (RSM) have been successfully applied to evaluate and optimize variables affecting the ultrasound-assisted extraction of betacyanins from red dragon fruit, ensuring accurate determination of these pigments (Carrera et al., 2021).

7 Case Studies

7.1 Betalain synthesis in red-fleshed dragon fruit

Betalain pigments, including betacyanins and betaxanthins, are the primary pigments responsible for the red color of red-fleshed dragon fruit. The synthesis of betalains in dragon fruit involves multiple key genes. Zhou et al. (2020) found that the formation of red flesh is mainly due to the upregulation of the key gene *CYP76ADs* in the betacyanin pathway, which is regulated by the WRKY44 transcription factor. Using transcriptome (RNA-Seq) and metabolome (UPLC-MS/MS) analysis, the study conducted an in-depth investigation of three different-colored dragon fruit types. The results showed that the formation of red flesh largely depends on the increased tyrosine content, which further drives the downstream steps of the betacyanin pathway (Figure 3).

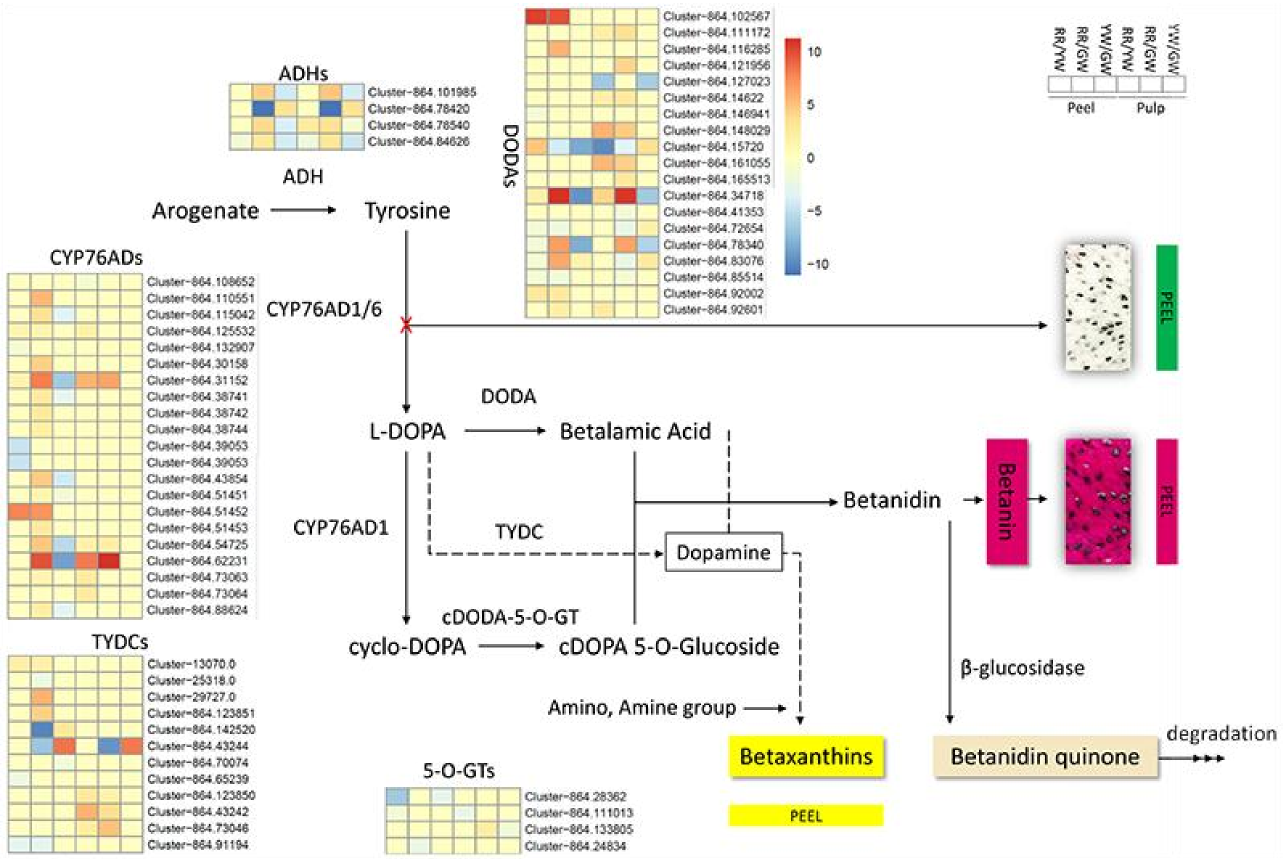

Figure 3 Betalain biosynthesis pathway (and tyrosine biosynthesis from arogenate) (Adopted from Zhou et al., 2020)

Image caption: The figure illustrates the gene regulatory network of the betalain synthesis pathway in pitaya, covering the steps from tyrosine to the formation of red pigments. The different colored boxes represent the peel and pulp colors of different types of pitaya (red, green, yellow). The figure details the differential expression of key enzymes such as CYP76ADs, TYDCs, and DODAs, with arrows indicating the direction of the metabolic pathway. This figure reveals that the upregulation of the *CYP76ADs* gene is crucial for the formation of red peel and pulp, further supporting the conclusion that red pigment synthesis is primarily regulated by the betalain pathway (Adapted from Zhou et al., 2020)

The colocalization of betacyanin biosynthesis genes in specific chromosomal regions in dragon fruit suggests a higher efficiency of betacyanin biosynthesis, contributing to the formation of the vivid red color of the fruit (Zheng et al., 2021). Moreover, in certain dragon varieties, the coexistence of anthocyanins and betalains indicates a complex interaction between these pigment pathways, although the exact mechanisms and regulatory factors require further research (Zhou et al., 2020; Pucker et al., 2021).

7.2 Carotenoid accumulation in yellow-fleshed dragon fruit

Carotenoids are pigments that play a crucial role in the coloration of many fruits. However, recent studies suggest that carotenoids may not be the primary pigments responsible for the coloration of dragon fruit. A comprehensive study combining transcriptomic and metabolomic analyses revealed that the yellow skin color of dragon fruit is not caused by differential regulation of key genes in the carotenoid pathway. The study found no significant differences in the expression of chalcone synthase genes between yellow and green-skinned varieties, and naringenin chalcone, a key compound, was also not detected in the metabolomic analysis. This further supports the conclusion that carotenoids are not involved in the formation of the yellow skin color in dragon fruit (Zhou et al., 2020).

The findings suggest that the yellow color in yellow-fleshed dragon fruit may be caused by other factors rather than simply by the accumulation of carotenoids. Therefore, further research is needed to identify the exact genetic and metabolic pathways responsible for carotenoid accumulation in yellow-fleshed dragon fruit. This discovery is significant because it shifts the focus of research from carotenoids to other pigment pathways, such as betalains and anthocyanins, to better understand the mechanisms behind dragon fruit coloration.

7.3 Anthocyanin enhancement through genetic engineering

Anthocyanins are flavonoid pigments derived from the phenylpropanoid pathway and are regulated by the MBW complex. Genetic engineering has been employed to enhance anthocyanin production in various plants, including dragon fruit. For instance, the overexpression of genes encoding key enzymes in the anthocyanin biosynthesis pathway, such as UDP-glucose flavonoid-3-O-glycosyltransferase (UFGT), has been shown to significantly increase anthocyanin content (Ni et al., 2018).

In ornamental plants like lisianthus, the introduction of betalain biosynthetic genes alongside anthocyanin pathways has resulted in novel flower color varieties, demonstrating the potential of genetic engineering to manipulate pigment synthesis (Tomizawa et al., 2021). In dragon fruit, similar approaches could be used to enhance anthocyanin content, potentially leading to new varieties with improved aesthetic and nutritional qualities. However, the mutual exclusion paradigm between betalains and anthocyanins in certain species, such as *Hylocereus*, poses a challenge that needs to be addressed through further research (Pucker et al., 2021).

8 Applications and Implications

8.1 Health benefits of dragon fruit pigments

Dragon fruit pigments, particularly betalains, have been shown to offer significant health benefits. Betalains, which are abundant in red-fleshed dragon fruit, possess strong antioxidant properties that can help in reducing oxidative stress and inflammation in the body (Zheng et al., 2021; Cheok et al., 2022). Studies have demonstrated that the consumption of betalain-rich dragon fruit can improve vascular function, including enhanced endothelial function and reduced arterial stiffness, which are critical for cardiovascular health (Cheok et al., 2022). Additionally, natural pigments like carotenoids and flavonoids found in dragon fruit have been associated with various health benefits, including improved lipid profiles, reduced risk of chronic diseases such as cardiovascular disease, and potential anti-cancer properties (Lu et al., 2021; Blumfield et al., 2022).

8.2 Industrial applications of pigment genes

The genes responsible for pigment synthesis in dragon fruit have promising industrial applications. For instance, the betacyanin pigment can be used as a natural dye in the food and cosmetic industries due to its vibrant color and antioxidant properties (Asra et al., 2021; Zheng et al., 2021). The encapsulation of betacyanins in alginate microbeads has been explored to enhance their stability and bioavailability, making them suitable for use as food colorants and in oral delivery systems (Fathordoobady et al., 2021). Furthermore, the genetic understanding of pigment biosynthesis pathways can facilitate the development of nutraceutical products aimed at addressing vitamin deficiencies and promoting overall health (Abirami et al., 2021). The use of dragon fruit pigments as natural alternatives to synthetic dyes in histological studies also highlights their potential in biomedical applications (Asra et al., 2021).

8.3 Future research directions in pigment genetics

Future research in the genetics of pigment synthesis in dragon fruit should focus on several key areas. For example, experimental validation of the co-localization of betacyanin biosynthetic genes on specific chromosomes could provide insights into the regulation and efficiency of pigment production (Zheng et al., 2021). Additionally, exploring the genetic correlation between different pigment biosynthesis pathways, such as those for chlorophylls and carotenoids, could reveal new targets for genetic manipulation to enhance pigment content and diversity (Li et al., 2018; Jang et al., 2022).

Another promising direction is the investigation of the health effects of consuming a variety of color-associated pigments, which could lead to the development of dietary recommendations and functional foods aimed at improving public health (Blumfield et al., 2022). Finally, leveraging advanced genomic tools and techniques, such as CRISPR/Cas9, to edit pigment-related genes could pave the way for the creation of dragon fruit varieties with optimized pigment profiles for both health benefits and industrial applications.

9 Concluding Remarks

This study on the genetic synthesis of pigments and related genes in dragon fruit has elucidated the intricate pathways and regulatory mechanisms responsible for the vibrant pigmentation observed in this fruit. Key findings include the identification of essential genes involved in the biosynthesis of betalains, carotenoids, and flavonoids, such as *CYP76AD1*, *DODA*, *PSY* etc. The expression patterns of these genes have been shown to vary significantly with environmental conditions, highlighting the dynamic interplay between genetic and external factors in pigment production. Moreover, advanced analytical techniques like HPLC, MS, and molecular biology tools have been instrumental in characterizing these pigments and their biosynthetic pathways.

Understanding the genetic basis of pigment synthesis enables the development of targeted breeding programs aimed at enhancing specific pigment traits, such as color intensity and nutritional content. By selecting for high-expression alleles of key biosynthetic genes, breeders can produce dragon fruit varieties with superior aesthetic and health-promoting qualities. Additionally, the manipulation of environmental conditions, informed by the gene-environment interactions identified in this study, can optimize pigment production during cultivation. Practices such as controlled lighting, temperature regulation, and precise nutrient management can significantly enhance the quality of the fruit.

The findings from this study not only contribute to the fundamental understanding of plant pigmentation but also offer tangible benefits for agriculture and industry. As we continue to explore and manipulate these genetic pathways, the potential for developing new and improved varieties of dragon fruit becomes increasingly feasible. These advancements will undoubtedly contribute to the economic viability and health benefits of dragon fruit, making it a more attractive option for consumers and growers alike.

In conclusion, the genetic study of pigment synthesis in dragon fruit paves the way for innovative breeding strategies and improved cultivation practices. By leveraging the genetic and environmental insights gained, we can enhance the quality, marketability, and nutritional value of dragon fruit, ensuring its continued success and popularity in the global market.

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Conflict of Interest Disclosure
The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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