Scientific Research Timelines Journal (2025), 3(1):5-9
Online Bimonthly Publication Frequency
https://scientificrtj.com/

Review Paper **Open Access**

OVERVIEW OF FLAVONOID 3',5'-HYDROXYLASE ENZYME AND GENE, INCLUDING A NOVEL DISCOVERY IN BARLEY

Wei Zhao[1] , Xinghuai Jing[2,3], Tao Yang[3*], Yawen Zeng[3*]

[1]*Pu`er University, Pu`er, Yunnan Province 665000, China*
[2]*School of Resource Plant Research, Yunnan University, Kunming, Yunnan Province 650504, China*
[3]*Biotechnology and Genetic Germplasm Institute, Yunnan Academy of Agricultural Sciences, Kunming 650205, China*
[*]**Corresponding authors e-mail:** yt52279076@163.com ; zengyw1967@126.com

ABSTRACT Flavonoid-3'5'-hydroxylase (F3'5'H) is a key enzyme in the anthocyanin metabolic pathway, capable of catalyzing the hydroxylation at the 3' and 5' positions of the B-ring of dihydroquercetin to produce dihydromyricetin, which ultimately forms delphinidin, playing a significant role in plant flower color expression. Barley is a crop with strong tolerance and a wide range of adaptability, and it is rich in active substances such as anthocyanins, making it a representative of functional crops. The F3'5'H gene is one of the least studied structural genes in the barley anthocyanin synthesis pathway. Introducing genes into crops through genetic engineering and other means to express them and thus endow crops with corresponding functional substances is a common method for the production of functional crops. This article reviews the discovery of the gene, its role in the synthesis pathway, gene structure and evolutionary analysis, commercial applications, and current research on barley, aiming to provide some insights for the development and application of this gene in functional crops.

Keywords: *Flavonoid; Anthocyanin; Barley; Crop; Genetics; Gene*

INTRODUCTION Flavonoid-3'5'-hydroxylase (F3'5'H) is a key enzyme in the anthocyanin metabolic pathway, belonging to the CYP75A subfamily of the cytochrome P450 family (Renault et al., 2014). In the Asteraceae family, the F3'5'H gene is classified as the CYP75B subfamily, which is a special type of F3'5'H gene (Seitz et al., 2006). It can catalyze the hydroxylation of the 3 and 5 positions of the B-ring of dihydrokaempferol to produce dihydromyricetin, ultimately forming delphinidin and its derivatives (Jeong et al., 2006). These anthocyanins are the main reason why some plants exhibit colors such as blue, purple, and black. Anthocyanins give these plants rich colors to attract animals for pollination, and for humans, the functions of anthocyanins in anti-inflammatory, anti-cancer, preventing diabetes, preventing liver damage, and antioxidant stress have greatly attracted human interest (Wei et al., 2024). Among the many plants rich in anthocyanins, barley has received widespread attention due to its excellent functionality, wide adaptability, and good tolerance. However, in the anthocyanin synthesis pathway of barley, the structural genes F3'H and F3'5'H are among the least studied (Vikhorev et al., 2019).

Overview of F3'5'H

The activity of F3'5'H was first confirmed in the microsomal fraction of verbena flowers, and subsequently in the microsomal fractions of Chinese water lily, red algae, and petunia flowers. Holton et al. (1993) were the first to isolate the petunia F3'5'H (CYP75A1 and A3) genes, which are regulated by two loci, Hf1 and Hf2, with Hf1 playing a dominant role (Tanaka, 2006). In subsequent studies, F3'5'H genes have been isolated from various plants. Currently, at least 58 F3'5'H genes have been published on NCBI, involving at least 38 species. A significant amount of research has focused on the role of F3'5'H genes in changing the flower color of ornamental plants, and F3'5'H genes encoding enzymes with activity have been isolated from plants such as petunia, gentianaceae, Catharanthus, Torenia, Campanula, Asteraceae, Primula, and tomato(Chen et al., 2012).

The role of F3'5'H in anthocyanin synthesis

F3'5'H mainly catalyzes the hydroxylation of the B-ring at the 3' and 5' positions of dihydrokaempferol, which is an upstream intermediate in the anthocyanin synthesis pathway, to produce dihydromyricetin (Jeong et al., 2006). Subsequently, dihydromyricetin is converted into delphinidin glycosides under the action of dihydroflavonol 4-reductase (DFR), anthocyanidin synthase/cyanidin 3-O-glucosyltransferase (ANS/LDOX), and flavonoid 3-O-glucosyltransferase/o-methyltransferase (UFGT/OMT). Through modification by methyltransferases, malvidin glycosides and petunidin glycosides are produced. Finally, various factors such as different pH values and inorganic ions cause the plant to exhibit different colors (Long et al., 2023).The activity of F3'5'H is regulated by cytochrome b5, which, like F3'5'H, belongs to the cytochrome P450 family encoded by difF. Research findings clearly indicate that the difF gene enhances the 3'5' substitution of anthocyanins (precursors),

which is essential for the full activity of F3'5'H. Plants with the difF gene inactivated reversibly by insertion of the dTph1 transposon produce variegated flowers, where the accumulation of 3'5' substituted anthocyanins is reduced by 25-50%. This is evidently due to the decreased activity of the Cyt P450 enzyme F3'5'H. These findings suggest that the Cyt b5 encoded by difF in vivo enhances the activity of the F3'5'H isoenzymes encoded by the hf1 and hf2 loci (de Vetten et al., 1999).

Research on the F3'5'H Gene

Since Holton et al. first isolated the F3'5'H gene from petunia in 1993, at least 38 species' F3'5'H genes have been isolated (Tanaka, 2006, Chen et al., 2012). As these genes continue to be extracted, research on them has become increasingly in-depth. Researchers have analyzed the flavonoid-3'5'-hydroxylase genes (F3'5'H) of various higher plants published in GenBank using bioinformatics methods and found that all of their start codons are ATG, stop codons are TAA, TAG, or TGA, and all contain three conserved sequences "PPGP", "AGTDT", and "FGAGRRICAG". These three conserved domains are responsible for the anchoring site of the membrane connection and the spherical part of the enzyme protein, the formation of the oxygen molecule binding domain, and the C-terminal heme binding region (Yang et al., 2021). Phylogenetic analysis of known F3'H and F3'5'H sequences by researchers indicates that F3'5'H was recruited from F3'H before the differentiation of angiosperms and gymnosperms (Seitz et al., 2006). In subsequent studies, scholars analyzed the phylogeny of F3'H and F3'5'H genes by examining the complete coding sequences of identified genes in the genomes of angiosperms. The results of the phylogenetic tree show that F3'H and F3'5'H genes are the result of a single CYP75 gene duplication and neofunctionalization in the common ancestor genome of monocots and dicots, an event that occurred approximately 129 million years ago (MYA), just before the differentiation of monocots and dicots (estimated time is 110-116 MYA) (Vikhorev et al., 2019). In the Asteraceae, there are special F3'5'H genes that, unlike other plants' F3'5'H genes, belong to the CYP75B family rather than the CYP75A family. This result indicates that the Asteraceae lost the CYP75A-type F3'5'H gene during evolution and then reacquired the F3'5'H gene through duplication and neofunctionalization of the CYP75B gene (Tanaka and Brugliera, 2014). The special evolutionary process of the F3'5'H gene in Asteraceae plants may have been formed during the long-term evolution of pollination needs. Studies suggest that there is a correlation between the type of pollinator and the degree of B-ring hydroxylation of anthocyanins produced, that is, flowers pollinated by insects usually contain derivatives of delphinidin with 3',4,'5'-hydroxylation, while flowers pollinated by birds tend to accumulate derivatives of pelargonidin and 3', 4'-hydroxylated cyanidin (Seitz et al., 2006, Harborne, 2014).

According to some studies, the ancestors of Asteraceae relied on bird pollination, that is, the ancestors of Asteraceae may have only contained the F3'H gene, and in the subsequent evolution process, in order to meet the needs of insect pollination, the F3'5'H gene belonging to the CYP75B family evolved from the F3'H gene (Seitz et al., 2006, Funk et al., 1995).

The expression of F3'5'H gene in plant improvement and some special crop varieties

The F3'5'H gene is mainly used commercially for the regulation of flower color in some cut flowers. The color of flowers comes from flavonoids, carotenoids, betalains, and other pigments, with flavonoids being the main component of flower color (Tanaka, 2006, Ainsworth, 2006). Therefore, controlling the expression of flavonoids (anthocyanins) can achieve control over flower color.

There are many examples of using the F3'5'H gene to regulate the color of cut flowers: in petunias, researchers achieved a shift from light pink to red-purple by increasing the expression of the F3'5'H gene; by introducing the F3'5'H gene from gypsophila, the flower color changed from pink to magenta (Holton et al., 1993, Shimada et al., 1999); by introducing the F3'5'H gene into a specific carnation variety lacking the DFR gene and overexpressing it, blue carnations were produced (Holton, 1996); early rose breeders tried to breed blue roses through hybridization but never succeeded because roses lack the F3'5'H gene, but by introducing the F3'5'H gene from pansies into roses, blue roses were successfully bred, filling the regret of previous rose breeders (Tanaka, 2006, Holton and Tanaka, 1994, Brugliera et al., 2013). In the butterfly pea, the downregulation of the F3'5'H gene causes a shift from blue to light pink (Suzuki et al., 2000). Researchers have attempted to transfer the Phalaenopsis F3'5'H gene and Hyacinthus DFR gene into lilies, resulting in darker corolla colors and the observation of some blue cells (Qi et al., 2013). Cyclamen naturally possesses the F3'5'H gene, and researchers have turned its flower color from purple to red/pink by suppressing the expression of its F3'5'H gene (Boase et al., 2010).

Chrysanthemums are a special type of flower that have lost the F3'5'H gene of the CYP75A family during evolution and only possess the F3'5'H gene of the CYP75B family (Tanaka and Brugliera, 2014). As a result, chrysanthemums lack delphinidin-based anthocyanins, leading to the absence of blue pigments in many chrysanthemum varieties (Sun et al., 2010, Nakayama et al., 1997). Compared to roses and carnations, the molecular breeding of blue chrysanthemums is still in its infancy, although molecular genetic techniques have been widely used to improve other aspects of chrysanthemum varieties (Teixeira da Silva and Culture, 2004). In related studies, the Senecio F3'5'H gene was introduced into white, red, pink, and purple chrysanthemum varieties for overexpression, and the results showed that the overexpression of the F3'5'H gene did not cause phenotypic changes in chrysanthemums. Based on this, they proposed two explanations: one possibility is the competition between endogenous CmF3'H and exogenous F3'5'H, and the other possibility is that some chrysanthemum species that produce delphinidin-based anthocyanins contain CYP75B- rather than CYP75A-type F3'5'H, indicating that the chrysanthemum family may have lost its original F3'5'H function during evolution (He et al., 2013).

A similar problem was encountered during the development of blue roses, where the expression of F3'5'H genes

from petunia, gentian, or butterfly pea in roses resulted in little or no accumulation of delphinidin in the petals of the transgenic plants, even though these genes are functional in petunia, carnation, or yeast. In contrast, the expression of the pansy F3'5'H gene resulted in a significant accumulation of delphinidin-derived anthocyanins in the petals of transgenic rose plants (Brugliera et al., 2013). Through the analysis of similar problems encountered during the development of blue roses, they believe that the inability of chrysanthemums to accumulate delphinidin is due to the substrate specificity of the DFR in chrysanthemums, a situation also found in carnations. Therefore, during the breeding process, carnation varieties lacking specific DFR genes are chosen as breeding materials (Holton and Tanaka, 1994).

In response to this, they proposed two strategies for the breeding of blue chrysanthemums: (1) Select a more effective F3'5'H, other species' F3'5'H should be converted into F3'Hi strains to produce DHMs; (2) Inhibit the internal DFR of chrysanthemums and introduce ScDFR to produce delphinidin in chrysanthemums (He et al., 2013). In subsequent breeding processes, researchers successfully obtained blue transgenic chrysanthemums by using the TGSII vector system to introduce the F3'5'H genes of Campanula, Delphinium, and Clitoria into a pink chrysanthemum variety (Lin, 2021).

In the pigment synthesis pathway, the F3'5'H enzyme must compete with at least five other enzymes for substrates. Whether the exogenous F3'5'H gene-formed enzyme can achieve an effective balance with the endogenous enzymes of the host plant directly affects the synthesis of delphinidin pigment. Therefore, species differences are also an issue that needs to be avoided in the molecular breeding research of blue flowers (Meng and Dai, 2005). Thus, the expansion of the F3'5'H gene is also an important task in flower breeding. Gazania is a chrysanthemum variety with a blue gene. Researchers designed degenerate primers based on the conserved amino acid sequence of F3'5'H and obtained the conserved sequence of the Gazania F3'5'H gene through RACE method. After designing full-length primers, they isolated the homologous gene of the Gazania F3'5'H gene (Meng and Dai, 2005). Aconitum vilmorinianum, a Chinese endemic medicinal herb with blue-purple flowers of certain ornamental value, researchers isolated the F3'5'H gene from its flowers through RT-PCR, expanding the basic content of the F3'5'H gene research for this type of species (Ma et al., 2015). Researchers utilized the F3'5'H genes from Gazania, petunia, snapdragon, gentian, and potato through RT-PCR technology and isolated the F3'5'H gene from the petals of African violet (Pei et al., 2012).

In addition to changing flower color, anthocyanins have also attracted attention for their nutritional value, medicinal value, and ability to enhance plant stress resistance. The F3'5'H genes in many anthocyanin-rich plants have become a research hotspot.In recent years, people have been continuously trying to cultivate edible plants rich in anthocyanins to increase the nutritional and economic value of the plants. Therefore, the F3'5'H gene also plays an important role in breeding work, but at present, research on this gene in most edible plants, especially food crops, is still at a relatively basic stage.Researchers obtained the F3'5'H gene of Fagopyrum tataricum through transcriptome sequencing and conducted a more in-depth analysis, proposing that it is possible to intentionally increase the expression of the F3'5'H gene to enhance its antioxidant capacity (Li et al., 2014). The "ZhuanXinWu" is a local specialty potato variety grown in the high-altitude and cold regions at the border of Xuanwei City in northeastern Yunnan Province and Guizhou Province. It has a cultivation history of over 130 years and is a variety rich in nutritional value. Through RT-PCR technology, researchers have obtained its F3'5'H gene, providing a theoretical basis for the regulatory function in its anthocyanin synthesis pathway and the coloration mechanism of the tubers (Xiao et al., 2015, Mushtaq et al., 2024, Li et al., 2024, Khan et al., 2024, Ali et al., 2024, Ahmed et al., 2024, Khan et al., 2023, Ullah et al., 2022).

F3'5'H in Barley

In this era where people are increasingly focusing on health, the demand for food is no longer just about filling the stomach, but more about the nutritional and medicinal value of food. Barley, as a crop with strong tolerance and wide adaptability, is widely cultivated around the world and is currently the fourth largest grain crop in the world. Barley contains various active ingredients, including anthocyanins, which are representatives of functional crops and are widely used in health foods and the pharmaceutical field (Liu et al., 2024, Mushtaq et al., 2024, Li et al., 2024, Khan et al., 2024). Most of the key genes in the barley anthocyanin synthesis pathway have been isolated and studied, while the structural genes F3'H and F3'5'H in barley have only been isolated in recent years and are among the least studied (Vikhorev et al., 2019). Researchers searched databases based on the F3'5'H gene sequences of dicot plants: grape, goji berry, soybean, black currant, balloon flower, and cyclamen.

Through cross-validation, they discovered the F3'5'H gene located on the 4HL chromosome of the barley genome, which has specific expression in the aleurone layer. They also speculated that there is another F3'5'H gene in the barley genome because the accumulation of delphinidin derivatives is not only in the aleurone layer but also in the pericarp and stem (Strygina et al., 2017, Zeng et al., 2024, Saeed et al., 2024, Rashid et al., 2024). In subsequent studies, researchers successfully identified four F3′5′H gene copies located on the 4HL, 6HL, 6HS, and 7HS chromosomes of the barley genome, and analyzed the gene structure organization. They found that one of the F3′5′H genes had lost multiple functional domains, including the heme-binding domain and oxygen-binding motif. In terms of gene expression, apart from the gene expressed in the aleurone layer, the others are expressed in the pericarp and stem, which is consistent with the previous researchers' speculation (Vikhorev et al., 2019).

CONCLUSION AND PROSPECTS Anthocyanins endow plants with a rich palette of colors, and beyond their aesthetic appeal, they possess significant nutritional and medicinal value, playing a crucial role in human health. The F3'5'H gene, a key player in the anthocyanin synthesis pathway, has been extensively studied and applied in ornamental plants

and has also yielded numerous research findings in some food and economic crops. In particular, in certain food crops, regulating the expression of these genes can lead to the development of varieties rich in anthocyanins, enhancing the functionality of the food and its adaptability to adverse environments. As people increasingly focus on dietary health, there is a substantial market for crops with high nutritional value and special functions, often commanding higher prices than ordinary grains. Therefore, the development of these colored grains is of great significance for improving both public health and farmers' income.

Funding: *This work was supported by the National Natural Science Foundation of China (Grant No. 32160453)*

REFERENCES

Ahmed, H. G. M.-D., Zeng, Y., Yang, X., Faisal, A., Fatima, N., Ullah, A., Hussain, G. S., Iftikhar, M. & Anwar, M. R. 2024. Heritability and Genotypic Association Among Seedling Attribute Against Salinity Stress Tolerance in Wheat Genotypes for Sustainable Food Security. Journal of Crop Health, 76, 519-531.

Ainsworth, C. C. 2006. Flowering and its Manipulation, Wiley Online Library.

Ali, Z., Naeem, M. & Ahmed, H. G. M.-D. 2024. Determination of salinity tolerance in pigmented rice genotypes at seedling stage. Journal of Crop Health, 76, 297-308.

Boase, M. R., Lewis, D. H., Davies, K. M., Marshall, G. B., Patel, D., Schwinn, K. E. & Deroles, S. C. J. B. P. B. 2010. Isolation and antisense suppression of flavonoid 3', 5'-hydroxylase modifies flower pigments and colour in cyclamen. 10, 1-12.

Brugliera, F., Tanaka, Y. & Mason, J. 2013. Flavonoid 3′, 5′ hydroxylase gene sequences and uses therefor. Google Patents.

Chen, X., Gao, L., Zhao, L., Liu, Y., Wang, Y. & Xia, T. 2012. Expression and optimization of flavonoid 3', 5'hydroxylase of tea plant [Camellia sinensis (L.) O. Kuntze] in Escherichia coli. 39, 707-713.

De Vetten, N., Ter Horst, J., Van Schaik, H.-P., De Boer, A., Mol, J. & Koes, R. J. P. O. T. N. A. O. S. 1999. A cytochrome b 5 is required for full activity of flavonoid 3′, 5′-hydroxylase, a cytochrome P450 involved in the formation of blue flower colors. 96, 778-783.

Funk, V., Robinson, H., Mckee, G. S., Pruski, J. F. J. B. & Forests, C. O. N. M. 1995. Neotropical montane Compositae with an emphasis on the Andes. 451-471.

Harborne, J. B. 2014. Introduction to ecological biochemistry, Academic press.

He, H., Ke, H., Keting, H., Qiaoyan, X. & Silan, D. J. P. O. 2013. Flower colour modification of chrysanthemum by suppression of F3'H and overexpression of the exogenous Senecio cruentus F3'5'H gene. 8, e74395.

Holton, A. J. P. A. N. P. A. 1996. Transgenic plants exhibiting altered flower color.

Holton, T. A., Brugliera, F., Lester, D. R., Tanaka, Y., Hyland, C. D., Menting, J. G., Lu, C.-Y., Farcy, E., Stevenson, T. W. & Cornish, E. C. J. N. 1993. Cloning and expression of cytochrome P450 genes controlling flower colour. 366, 276-279.

Holton, T. A. & Tanaka, Y. 1994. Blue roses-a pigment of our imagination.

Jeong, S. T., Goto-Yamamoto, N., Hashizume, K. & Esaka, M. J. P. S. 2006. Expression of the flavonoid 3′-hydroxylase and flavonoid 3′, 5′-hydroxylase genes and flavonoid composition in grape (Vitis vinifera). 170, 61-69.

Khan, M. A., Waseem Akram, M., Iqbal, M., Ghulam Muhu-Din Ahmed, H., Rehman, A., Arslan Iqbal, H. S. M. & Alam, B. 2023. Multivariate and association analyses of quantitative attributes reveal drought tolerance potential of wheat (Triticum aestivum L.) genotypes. Communications in Soil Science and Plant Analysis, 54, 178-195.

Khan, M. A., Yousaf, M. W., Ahmed, H. G. M.-D., Fatima, N. & Alam, B. 2024. Assessing genetic diversity for some Pakistani bread wheat (Triticum aestivum L.) genotypes under drought stress based on yield traits. Genetic Resources and Crop Evolution, 1-11.

Li, G., Yi , S. & Chen, Q.-F. 2014. Sequences Analysis of F 3'5'H from Fagopyrum cymosum. 33, 6-10.

Li, X., Yang, X., Yang, L. E., Muhu-Din Ahmed, H. G., Yao, C., Yang, J., Wang, L., Yang, T., Pu, X. & Zeng, Y. 2024. Evolution and association analysis of SSIIIa in rice landraces of Yunnan Province. Biologia, 1-9.

Lin, J. 2021. Generation of Blue Chrysanthemums via Co-Transformation of F3'5'H Gene, Glycosylation and Acylation Modification Genes. Master of Agriculture.

Liu, X., Liu, Y., Ding, Y. & Pan, Z. 2024. The Process from Barley Newsletters to Barley and Cereal Sciences—Commemorating the 40th Anniver sary of its Founding. 41, 78-80.

Long, C., Liu, L., Zhao, Y., Yang, N., Zhao, C. & Huang, Y. 2023. Key Enzymes and their Transcription Factors of Anthocyanins Biosynthesis in Fruits. 1-12.

Ma, L.-L., Wang, X.-N., Jia, W.-J., Duan , Q., Cui, G.-F., Du , W.-W. & Wang , J.-H. 2015. Cloning and Expression Pattern Analysis of Av-F3'5'H Gene from Aconitum vilmorinianum. 28, 2438-2443.

Meng, L. & Dai, S. 2005. Cloning, Sequencing and Prokaryotic Expression of F3′5'H cDNA from Per-icallis cruentia (L.) Herit., 28-34.

Mushtaq, M. A., Ahmed, H. G. M.-D. & Zeng, Y. 2024. Applications of Artificial Intelligence in Wheat Breeding for Sustainable Food Security. Sustainability, 16, 5688.

Nakayama, M., Koshioka, M., Shibata, M., Hiradate, S., Sugie, H., Yamaguchi, M.-A. J. B., Biotechnology, & Biochemistry 1997. Identification of cyanidin 3-O-(3 ″, 6 ″-O-dimalonyl-β-glucopyranoside) as a flower pigment of Chrysanthemum (Dendranthema grandiflorum). 61, 1607-1608.

Pei, R.-J., Chen, X.-Q., Sun, N., Zhang, N.-N., Liu, Y., Liu, F.-H. & Zhang, L. 2012. Petal Cell pH Value Determination

and F3'5'HGene Fragment Cloningof Saintpqulia ionantha. 19, 11-14.

Rashid, M. A. R., Pan, Z., Wang, Y., Shaheen, T. & Ahmed, H. G. M.-D. 2024. Biofortification of potatoes to reduce malnutrition. Biofortification of Grain and Vegetable Crops. Elsevier.

Renault, H., Bassard, J.-E., Hamberger, B. & Werck-Reichhart, D. J. C. O. I. P. B. 2014. Cytochrome P450-mediated metabolic engineering: current progress and future challenges. 19, 27-34.

Saeed, A., Ahmed, H. G. M.-D., Zeng, Y., Fatima, N., Hussain, G. S., Akram, M. I., Sattar, M. M., Khan, M. A. & Mushtaq, M. A. 2024. Genetic Evaluation and Breeding Strategies under Water Deficit Environment to Develop the Drought Tolerant Wheat Germplasm. Polish Journal of Environmental Studies.

Seitz, C., Eder, C., Deiml, B., Kellner, S., Martens, S. & Forkmann, G. J. P. M. B. 2006. Cloning, functional identification and sequence analysis of flavonoid 3′-hydroxylase and flavonoid 3′, 5′-hydroxylase cDNAs reveals independent evolution of flavonoid 3′, 5′-hydroxylase in the Asteraceae family. 61, 365-381.

Shimada, Y., Nakano-Shimada, R., Ohbayashi, M., Okinaka, Y., Kiyokawa, S. & Kikuchi, Y. J. F. L. 1999. Expression of chimeric P450 genes encoding flavonoid-3′, 5′-hydroxylase in transgenic tobacco and petunia plants 1. 461, 241-245.

Strygina, K. V., Börner, A. & Khlestkina, E. K. J. B. P. B. 2017. Identification and characterization of regulatory network components for anthocyanin synthesis in barley aleurone. 17, 1-9.

Sun, W., Li, C., Wang, L. & Dai, S. J. C. B. O. B. 2010. Analysis of anthocyanins and flavones in different-colored flowers of chrysanthemum. 45, 327.

Suzuki, K.-I., Xue, H.-M., Tanaka, Y., Fukui, Y., Fukuchi-Mizutani, M., Murakami, Y., Katsumoto, Y., Tsuda, S. & Kusumi, T. J. M. B. 2000. Flower color modifications of Torenia hybrida by cosuppression of anthocyanin biosynthesis genes. 6, 239-246.

Tanaka, Y. & Brugliera, F. J. F. Y. O. C. P. R. 2014. Metabolic engineering of flower color pathways using cytochromes P450. 207-229.

Tanaka, Y. J. P. R. 2006. Flower colour and cytochromes P450. 5, 283-291.

Teixeira Da Silva, J. A. J. P. C., Tissue & Culture, O. 2004. Ornamental chrysanthemums: improvement by biotechnology. 79, 1-18.

Ullah, A., Shakeel, A., Ahmed, H. G. M.-D., Naeem, M., Ali, M., Shah, A. N., Wang, L., Jaremko, M., Abdelsalam, N. R. & Ghareeb, R. Y. 2022. Genetic basis and principal component analysis in cotton (Gossypium hirsutum L.) grown under water deficit condition. Frontiers in Plant Science, 13, 981369.

Vikhorev, A. V., Strygina, K. V. & Khlestkina, E. K. J. P. 2019. Duplicated flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase genes in barley genome. 7, e6266.

Wei, Y., Dong, Z., Zhang, X., Luo, L. & Teng, J. 2024. Extraction Technology and Determination of Proanthocyanidins of from Purple Rice Bran. 67-70.

Xiao, J., Yang, X. & Guo, H. 2015. Bioinformatical and Expression Analysis of Flavonoid-3'5'-Hydroxylase Gene from Pigmented Potato Named‘ZhuanXinWu’. 34, 1494-1502.

Yang, X., Chen, Z., Chen, H. & Xie, W. 2021. Bioinformatical Analysis of the cDNAs and Their Putative Amino Acid Sequences of F3′5′Hs in Higher Plants. 40, 24-32.

Zeng, Y., Ahmed, H. G. M.-D., Li, X., Yang, L. E., Pu, X., Yang, X., Yang, T. & Yang, J. 2024. Physiological Mechanisms by Which the Functional Ingredients in Beer Impact Human Health. Molecules, 29, 3110..