Advances in Biological Sciences Research (ABSR), volume 6
2017 2nd International Conference on Biological Sciences and Technology (BST 2017)

Research Progress of Anthocyanin Biosynthesis and Regulation from Purple Pepper (*Capsicum Annuum* L.)

Bo-ya LU[1,a] and Zhen-hui GONG[1,b,*]

[1]College of Horticulture, Northwest A&F University, Yangling, China

[a]berryluboya@gmail.com, [b]gzhh168@sina.com

*Corresponding author

Keywords: Purple pepper, Anthocyanin, Biosynthetic pathway, Transcription factors, Environmental factors.

Abstract. Anthocyanin is a water-soluble pigment, is a class of secondary metabolites, and it belongs to the flavonoid polyphenols. The peel is purple, which caused by the anthocyanin accumulation, so the purple pepper is loved by more and more consumers by high nutritional value and health effects. With the deepening of research about anthocyanin biosynthetic pathways in plants, some research of this aspect are carried out in the purple pepper. This paper mainly discuss the biosynthetic pathway of anthocyanins in purple pepper, and the research progress of the transcription factors that involved in the regulation and the effects of the external environment, which is helpful to have a better understanding of the mechanism of anthocyanin biosynthesis and provide a theoretical reference for the breeding of purple pepper varieties.

Introduction

The current research shows that there are three main types of pigments that affect the color of plants, namely anthocyanins, carotenoids and betaines. The deposition of anthocyanins is the cause of the different tissues and organs of many plants showing red, purple and blue [1, 2, 3, 4]. There are already more than 550 species of anthocyanins, but they all have the basic structural framework C6-C3-C6 [5]. Anthocyanins are non-toxic and harmless, so they can be used safely and play a health role, such as regulating the immune system, prevention of cancer and cardiovascular disease, prevention of mutations and reducing radiation. Additionally, there is another name for anthocyanins, that is "natural Swiss swords", that is because anthocyanins play a role in different ways in undertaking environmental stress and resistance to herbivores and pathogens [6, 7]. At the same time, anthocyanins can be used as antioxidants with nutritional and healthy functional characteristics [7, 8, 9].

At present, China is the largest pepper production and exporting country, therefore, the production demand of pepper gradually increased, and the corresponding breeding work has been paid more attention. Thence, selecting high yield, disease and insect resistance, high quality pepper is the main breeding objectives. It is because the anthocyanin accumulation in the peel, directly determines the purple pepper ornamental, edible and market value. Purple pepper is popular among consumers, adding it have a higher yield with less pests and diseases infection. Therefore, the cultivation of purple pepper is a very important part of breeding work.

Anthocyanin Biosynthetic Pathway in Pepper

Anthocyanin biosynthetic pathway belongs to the flavonoid pathway, which has been studied widely. The pathway regards phenylalanine as precursor to form the

anthocyanin after a series of enzymatic reactions. First of all, phenylalanine is acted to form cinnnamic acid, in addition to coumaroyl CoA, other substances such as lignin and coumarin are synthesized at the same time. Both self-development and environmental factors can affect the phenylalanine ammonia lyase gene expression. Subsequently, there is a reaction between in malonyl-CoA and 4-coumaroyl CoA to produce chalcone with the catalysis of chalcone synthase (CHS). The role of chalcone isomerase (CHI) is to catalyze it to flacanone, which changes from yellow to colorless in this step. If chalcone isomerase activity is reduced, chalcone synthase can no longer be catalyzed, leading to the block in of the flavonoid synthesis pathway. Flavanone hydroxylase (F3H) is a key enzyme in anthocyanin biosynthesis pathway and is located at the branching point. With the role of flavanone hydroxylase, dicyanoflavone alcohol can be generated from flavanone. At the same time, dicyanoflavone alcohol can be catalyzed continuously; the reaction product is a direct precursor of anthocyanin synthesis [10]. Dicyanoflavone alcohol could generate colorless anthocyanin in the role of dicyanoflavone reductase (DFR), such as white delphin, white cyanidin and so on. Dicyanoflavone reductase has the selectivity in the process of catalyzing dicyanoflavone alcohol to form anthocyanin, which is one of the main reasons for determining plant color. Under the action of anthocyanidin synthase (ANS), anthocyanins show a color. Flavonoid 3, 5-glycosyltransferase (UFGT) has an important role on the stability of anthocyanins, which make anthocyanins in a steady state. After the completion of this pathway, anthocyanins will be further modified by glycosylation, acylation and other modifications. The only anthocyanin has been found so far in pepper is delphindins, it is delphinidin- 3-p- coumaroyl-rutinoside-5-glucoside [11, 12].

Regulation of Transcription Factors on Anthocyanin Biosynthesis

The transcription factor MYB, bHLH and WD40 could form regulatory complex MBW to participate in the anthocyanin biosynthesis. There are two ways for the transcriptional activation of anthocyanins. The first one is to form a binary complex with MYB and WD40. There is no bHLH transcription factor involved in this process, only the binary complex is involved in the regulation of the structural genes. The other way is the three members are all participate in the anthocyanin regulation by forming a ternary complex.

At present, there are not many studies on the biosynthesis of anthocyanins in pepper, the existing research mainly focuses on the regulation of MYB on anthocyanin biosynthesis in pepper leaves and fruits. *CaMYB1*, *CaMYB2* and *CaMYB3* were isolated from pepper, which encoding 340, 262 and 345 amino acids, respectively, containing R2R3 structure and R3 domain that MYB interacts with the bHLH proteins [13].Virus induced gene silencing is a commonly used method for studying transcription factors in pepper. Aguilar Barragán [14] silenced *CaMYB* gene in pepper fruits by VIGS, leading to the decrease of anthocyanin content and the decrease expression of the related regulatory genes CHS, CHI, F3’5H, DFR and 3GT (UFGT). Zhang [15] used VIGS to silence *CaMYB* gene in pepper leaves, revealing that the color of pepper leaves are green due to the reduction of anthocyanin content. These cases indicate that *CaMYB* plays an important role in anthocyanin biosynthesis by regulating the structural genes expression in pepper.

Effects of Environmental Factors on Anthocyanin Biosynthesis

Transcription factor regulation is the main factor in the accumulation of anthocyanins

in pepper. However, environmental factors such as light intensity and quality, temperature, sugar substances, drought, metal ions and pH value will affect the anthocyanin biosynthesis. As for light, there are some cis-acting element in the promoters of structural genes which can respond to light signals and is often combined with light-stimulated transcription factors to regulate the expression of structural genes. Gordon J. Lightbourn [12] studied the effect of light intensity on anthocyanin content in pepper. Under strong light stimulation, the content of anthocyanin was higher in mature leaves than in immature leaves, and the structural genes such as CHS, DFR and ANS showed upward trend. Temperature is also an important factor which is affecting anthocyanin biosynthesis. Generally, low temperature can induce the expression of structural genes DFR, CHS, PAL, CHI while the high temperature could inhibit their expression [16]. In addition, other environmental factors such as fruit bagging, hormone, nitrogen, pathogen infection will have impacts on anthocyanin accumulation.

Conclusions

The process of anthocyanin synthesis and accumulation is often related to the development and growth of pepper, which is controlled by internal and external factors. Transcription factors could regulate structural genes expression and environmental factors may also have an effect on contents of anthocyanin. Those two factors play roles in anthocyanin accumulation in pepper fruits and leaves. There are more and more research about anthocyanin accumulation in pepper, but most studies focus on MYB and little research focus on bHLH or WD40. Another problem is that most studies used VIGS technology to verify the function of transcription factors, more research methods should be used in the future study. Purple peppers have an ornamental value due to the anthocyanin accumulation, so the research on the biosynthesis of anthocyanin will help us have a better understanding of the mechanism and it is helpful to cultivate purple pepper varieties.

References

[1] L. Lepiniec, I. Debeaujon, J. Routaboul, A. Baudry, L. Pourcel, N. Nesi, M. Caboche, Genetics and biochemistry of seed flavonoids, Annu Rev Plant Bio. 57 (2006) 405-430.

[2] R. Koes, W. Verweij, F. Quattrocchio, Flavonoids: a colorful model for the regulation and evolution of biochemical pathways, Trends Plant Sci. 10 (2005) 236-242.

[3] A. Allan, R. Hellens, W. Laing, MYB transcription factors that colour our fruit, Trends Plant Sci. 13 (2008) 99-102.

[4] E. Grotewold, The genetics and biochemistry of floral pigments, Annu Rev Plant Bio. 57 (2006) 761-780.

[5] Q. You, B. Wang, F. Chen, Z. Huang, X. Wang, P. Luo, Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars, Food Chem. 125 (2011) 201-208.

[6] Y. Shang, J. Venail, S. Mackay, P. Bailey, K. Schwinn, P. Jameson, C. Martin, K. Davies, The molecular basis for venation patterning of pigmentation and its effect on pollinator attraction in flowers of Antirrhinum, New Phytol. 189 (2010) 602-615.

[7] K. Gould, Functional role of anthocyanins in the leaves of Quintinia serrata A Cunn, J. Exp. Bot. 51 (2000) 1107-1115.

[8] E. Butelli, L. Titta, M. Giorgio, H. Mock, A. Matros, S. Peterek, E. Schijlen, R. Hall, A. Bovy, J. Luo, C. Martin, Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors, Nat. Biotechnol. 26 (2008) 1301-1308.

[9] L. Pourcel, J. Routaboul, V. Cheynier, L. Lepiniec, I. Debeaujon, Flavonoid oxidation in plants: from biochemical properties to physiological functions, Trends Plant Sci. 12 (2007) 29-36.

[10] K. Springob, J. Nakajima, M. Yamazaki, K. Saito, Recent Advances in the Biosynthesis and Accumulation of Anthocyanins, Nat. Prod. Rep. 20 (2003) 288-303.

[11] C. Aza-González, H. Núñez-Palenius, N. Ochoa-Alejo, Molecular biology of chili pepper anthocyanin biosynthesis, J. Mex. Chem. Soc. 56 (2012) 93-98.

[12] G. Lightbourn, J. Stommel, R. Griesbach, Epistatic interactions influencing anthocyanin gene expression in Capsicum annuum, J. Am. Soc. Hortic. Sci. 132 (2007) 824-829.

[13] D. Li, K. Zhao, B. Xie, B. Zhang, K. Luo, Establishment of a highly efficient transformation system for pepper (Capsicum annuum L.), Plant Cell Rep. 21 (2003) 785-788

[14] A. Aguilar-Barragán, N. Ochoa-Alejo, Virus-induced silencing of MYB and WD40 transcription factor genes affects the accumulation of anthocyanins in chilli pepper fruit, Biol. Plantarum 58 (2014) 567-574.

[15] Z. Zhang, D. Li, J. Jin, Y. Yin, H. Zhang, W. Chai, Z. Gong, VIGS approach reveals the modulation of anthocyanin biosynthetic genes by CaMYB in chili pepper leaves, Front. Plant Sci. 6 (2015).

[16] M. Islam, M. Jalaluddin, J. Garner, M. Yoshimoto, O. Yamakawa, Artificial shading and temperature influence on anthocyanin compositions in sweetpotato leaves, HortScience 40 (2005) 176-180.