

VU Research Portal

Physiological and genetic control of anthocyanin pigmentation in different species

Povero, G.

2011

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Povero, G. (2011). *Physiological and genetic control of anthocyanin pigmentation in different species*.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

Summary and General Discussion

Plant and animals display an enormous morphological variation that arose by mutations and selection during evolution. The molecular basis for this divergence is however, poorly understood. Does it rely primarily on the repeated appearance of new genes, or smaller modifications in the encoded proteins or in regulatory gene sequences? As part of this morphological divergence, animals as well as plants display widely different pigmentation patterns. Because pigmentation is a relatively simple trait, as compared to “shape, and because differences in pigmentation patterns are so easy to detect, it provides an excellent model system to study the evolution of morphological features. Plants are an attractive system for such analyses, because they acquired highly divergent pigmentation patterns in relatively short time, and because a range of plants species are amenable to genetic approaches.

The pigments that color most flowers and fruits are anthocyanins, which belong to a large family of secondary metabolites known as flavonoids. The aim of this study is to gain insight in the regulation of anthocyanin biosynthesis in different species to understand how the different pigmentation patterns arose during evolution.

In the first part of this work (chapters 1 and 2), we review the many different functions of these compounds in the plant life, which include attraction of pollinators and seed dispersers, protection action against both biotic and abiotic stress and signaling to symbionts. In addition we also emphasized the importance of anthocyanins for human health for their antiallergic, anti-inflammatory, antiviral, and antioxidant properties, which explains the growing interest for these compounds as components of the diet and of cosmetics and additives.

Although distinct plant species accumulate anthocyanins in different patterns, the genes that are involved in the biosynthesis of these pigments are very conserved even among very different species. This holds for the structural genes of the pathway, which encode for the enzymes catalyzing the different reaction that lead to the final product, as well as for the regulators that control transcription of the structural genes.

The regulatory proteins responsible for the activation of the anthocyanin pathway and for the tissue-specificity of pigmentation, can be swapped among distantly related plant species and activate anthocyanin biosynthesis in the heterologous host. Therefore, the differences in pigmentation patterns that are seen in the plant kingdom are likely to originate from alterations in the regulatory mechanisms acting on the expression of the regulators, or even upstream of those. Anthocyanin synthesis is not only controlled by strictly tissue-specific factors that control the identity of cells and tissues, but also by external stimuli such as temperature, light, water stress and by plant hormones. Consequently variation in pigmentation can also be observed among genetically identical individuals of the same species when these grow under different conditions.

In chapter 3 we investigated the interactions between sugars and hormones in the regulation of anthocyanin biosynthesis. We confirmed the synergistic effect of these factors by analyses of mRNA levels for different anthocyanin genes after treatment of the plants with different levels of hormones and measure of the endogenous level of sugars. Overall, the results in chapter 3 support a cross-talk between sucrose and hormones in the regulation of anthocyanin biosynthesis in *Arabidopsis thaliana* and suggest a role for sucrose as a consensus-signaling molecule for the induction of this pathway.

The fruits of cultivated tomatoes do not accumulate anthocyanins, although several closely related wild species do. There are a handful of tomato cultivars that show high anthocyanin content in the fruits and in chapter 4 we present a study of the effect of two loci (*Aft* and *atv*) known to be responsible for the anthocyanin phenotype in the homonymous cultivars. A dominant *Aft* allele triggers limited anthocyanin accumulation (“anthocyanin spots”) in fruit upon stimulation by light, while a recessive *atv* allele promotes anthocyanin pigmentation in the entire tomato plant,

particularly in vegetative tissues. Lines that harbour both the dominant *Aft* and the recessive *atv* alleles are known as “*Sunblack*” lines, and have intensely pigmented fruits. Hence, the two loci are expected to both have effect on the activation of anthocyanin biosynthetic genes.

Comparison of the transcript profiles of red (*aft Atv*) tomatoes, *Aft* and *atv* revealed sets of genes that are differentially regulated in these lines and indicate a synergistic effect of the *Aft* and *atv* alleles. While *Aft* mainly affects the expression of genes that are directly involved in anthocyanin synthesis, *atv* prevalently influences the expression of some of the genes that are involved in the biosynthesis of flavonoid precursors. In *Sunblack* fruit peel, besides anthocyanin production, also other metabolic processes are altered. Because these lines have different genetic background, it is not entirely clear whether these differences are due to *atv*, or other (unknown) genes in the genetic background.

In chapter 5 we describe the characterization of a small MYB subfamily in tomato, which consists of three genes, *SIANT1* (*ANTHOCYANIN1*) and *SIAN2* (*ANTHOCYANIN2*), and *SIANT1-like*, that are located on a small genomic fragment on chromosome 10 where the *Aft* locus has been mapped. The last gene is never expressed in tomato and therefore we think does not contribute to pigmentation. The proteins encoded by each of the other two genes can induce anthocyanin expression in nearly any part of the plant (although *SIAN2* has a stronger effect) when ectopically expressed in cultivated tomato plants. This result, together with the finding of polymorphisms in the promoter regions of these two genes which co-segregate with the *Aft* locus, suggest that changes in the regulation of expression of one (or both) *SIAN2* or *SIANT1*, rather than changes in the proteins, are responsible for the *Aft* phenotype (and therefore that the *Aft* locus encodes either *SIAN2* or *SIANT1*). We assessed the contribution of each of these genes to the *Anthocyanin fruit* phenotype by transforming the genomic fragment containing *SIANT1* and *SIAN2* from the *Aft* line in control tomato plants. The transgenic plants harboring the *SIAN2^{Aft}* transgene showed high induction of the expression of the endogenous *SIANT1*, of the tomato homologue of the petunia bHLH anthocyanin regulator *AN1* and of all anthocyanin structural genes. These transgenic plants carried fruits with an “*Aft-like*” phenotype.

Taken together, these results strongly support that *SIAN2* represent the *Aft* locus, and that the difference between the *SIAN2^{AC}* and *SIAN2^{Aft}* alleles is in the regulation of their expression due to sequence changes in the promoter region, rather than in the protein functionality.

SIANT1 and *SIAN2* are homologous to *PhAN4* (*ANTHOCYANIN4*) and *PhAN2* (*ANTHOCYANIN2*), which color anthers and petals in petunia flowers.

In order to address the question of how regulatory genes of the anthocyanin biosynthesis are involved in generating variation among different species, we first studied in detail their involvement in the pigmentation of different plant parts within one species. In particular, in chapter 6, we report the function of different members of a small family of MYB genes responsible in petunia for the pigmentation of different plant parts. We describe here AN4, a MYB protein responsible for the pigmentation of anthers. We show that ectopic expression of members of this protein clade alone induces accumulation of anthocyanin pathway, also in tissues normally not pigmented. This finding supports a function of these genes as “master regulators” of the anthocyanin pathway as they are able to induce transcription also of other regulators (like the bHLH transcription factor *AN1*) which gene products are going to interact in one “anthocyanin inducing” transcription complex.

In conclusion, the data presented in these two chapters show that alterations in the expression pattern of the MYB regulators are sufficient to alter the expression pattern of its bHLH partner and thereby the pigmentation pattern produced in the plant. The results presented here show that such alterations in expression are most probably the result of DNA changes in the regulatory regions of the MYB genes.

If these type of DNA changes are at the basis of the generation of different patterns within the same species (see tomato and petunia), are similar genetic changes also responsible for the variation of pigmentation patterns between species?

The preliminary results of an experimental approach to answer this question are reported in chapter 7. In these experiments gene “swaps” of the promoters of anthocyanin MYB regulators were performed among *Arabidopsis*, tomato, and petunia, to assess whether changes in cis-regulatory elements of these “master genes”, are responsible for the distinct pigmentation patterns of these species, or in the upstream regulatory network. These swaps were carried out using genomic fragments encoding anthocyanin MYB regulators belonging to the PhAN2 clade and the activity of the transgenes was then monitored through the GUS reporter gene contained in the constructs. If such gene constructs maintain the expression pattern from their original host, also in a heterologous host, that would indicate that these MYBs acquired distinct expression patterns by changes in the upstream regulatory network. If on the other hand the MYB gene maintains its original expression pattern also in a heterologous host, it indicates that the divergence of cis-elements in these MYB genes caused the distinct pigmentation patterns

In case of the MYB genes that we studied here, the preliminary results acquired at the time this thesis was written, suggest that the major cause for the divergence of their expression patterns are alterations in the upstream regulatory network.

Finally, this thesis approached the study of the specification of pigmentation patterns in plants, through the acquisition of new knowledge about this process in petunia and tomato and the identification of a role of “master genes” for MYB anthocyanin regulators in a cascade of regulatory events that lead to the activation of the biosynthetic pathway. A first attempt to understand the mechanism by which these “master genes” have guided evolution of patterns in different species suggests a possible existence of an higher level of regulation which will require further studies to be identified.