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How to make our crops red

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Introduction

In the plant kingdom there are three major compounds that confer colours to the plants: anthocyanins, carotenoids and chlorophylls. The concentration of each molecule and their combination are the primary determinant of plant pigmentation. Among all, anthocyanins are of outstanding interest for their well-documented beneficial effects on plant physiological processes and human health. Indeed, anthocyanins are generally accepted the be enhancers of plant reproductive success by facilitating communication between plants and their pollinators and seed-dispersers. More recently, they are reported to be implicated in plant defense mechanisms as well. Furthermore, the human health-promoting benefits of anthocyanins as dietary antioxydant have been documented. High concentrations of such compounds in food were demonstrated to be associated with low level of obesity and blindness. In addition, they may a positive role in anti-inflammatory and anti-tumorigenesis processes. The aim of this review is to summarize recent findings on genes regulating the plant anthocyanin biosynthetic pathway.

MYBs are key transcriptional factors in anthocyanin biosynthetic pathway in plant

The anthocyanins are part of a large secondary ubiquitous metabolites family known as flavonoide. Their biosynthetic pathway is well known in different plant species including *Arabidopsis thaliana* and many *Solanaceae*. It has been demonstrated that anthocyanin accumulation is basically controlled by the regulation of genes encoding the structural enzymes of this pathway. Indeed, different studies converged on the fact that such coordinated expression is tightly controlled at the transcriptional level, usually, by a sub-group of MYB transcription factor (TF). Plant MYBs represent a large gene family whose members have many different functions: metabolic and enzymatic pathways regulation (including the anthocyanin pathway), development, signal transduction and disease resistance. Like many TFs, a structurally conserved DNA-binding domain consisting of up to three perfect or imperfect repeats characterizes them. This domain forms a helix-turn-helix structure of about 53 amino acids. To form a three-dimensional helix-turn-helix structure, three regularly spaced tryptophan residues are present in the protein structure as tryptophan cluster, which is a characteristic of each single MYB repeat[1]. The first MYB gene described was *c-myb*, a DNA-binding transcriptional regulator consisting of three repeats called R1, R2 and R3. Later on, all MYB proteins were classified into three subfamilies depending on the number of adjacent repeats in the MYB domain (one, two or three). We refer to MYB-like proteins with one repeat as "MYB1R" factors, with two as 'R2R3" MYB factors, and with three repeats as "MYB3R" factors. In particular, those associated with the anthocyanin pathway regulation fall in the R2R3-MYB subgroup. In *Arabidopsis*, there are 133 R2R3

MYB type TF genes divided into 24 subgroups. In this model species, it has been demonstrated that anthocyanin accumulation is under the control of *PRODUCTION OF ANTHOCYANIN PIGMENT 1* (*PAP1*, or *At-MYB75*) MYB gene. Its function as a transcription factor was demonstrated by the protein sequence itself, which has a highly conserved amino acid sequence in the DNA-binding domain. Several structural genes in the anthocyanin biosynthtic pathway were found to be under the control of *PAP1* and were up-regulated when *PAP1* was overexpressed in transgenic *Arabidopsis* plants, resulting in an accumulation of anthocyanins that produced purple plants[2]. Moreover, there are evidences on the co-factor role of some basic helix-loop-helix (bHLH) transcription factors in anthocyanin controlled accumulation. It seems that while R2R3 MYB TFs determine the amount of anthocyanin production the bHLH TF genes play an important role regarding the time and the site this production must take place. These findings may lead to new approaches in the modern breeding for the development of fruit with new colour patterns.

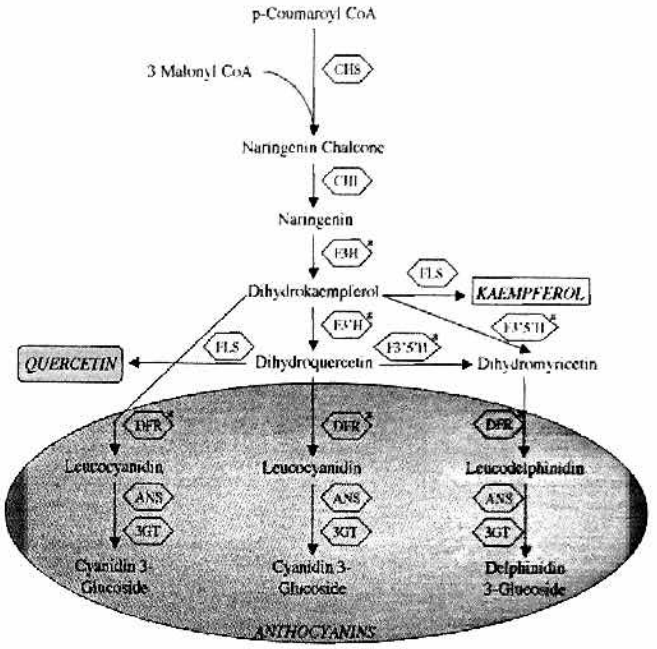

Figure 1.—Schematic diagram of the anthocyanin biosynthetic pathway, starting with the core phenylpropanoid pathway, adapted from André et al[5]. Structural genes discussed are in exagonal boxes and were abbreviated: CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3'H, flavonoid 30 hydroxylase; F3'5'H, flavonoid 3'5' hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol 4-reductase; ANS, anthocyanidin synthase. Enzyme boxes with star represented structurale enzymes under genetic control of AN1, anthocyanin 1 transcription factor.

MYBs control anthocyanin accumulation in Solanum tuberosum

In nature there are many potato species that show differences in tuber color beacause of different tissue-specific anthocyanin accumulation. Pioneering investigations about anthocyanin in potato allowed the identification of three distinct loci - D, R and P - responsible for the tuber skin colors in the tetraploid potato (*Solanum tuberosum* L.). Production of red and purple anthocyanins is known to be controlled by R and P loci, respectively, while D (developer locus) is responsible for the tissue-specific anthocyanin accumulation in tuber skin. Recently, the genome localization of D, R, and P loci was respectively assigned to chromosomes 10, 2, and 11, and their structural and regulatory function in the anthocyanin biosynthetic pathway enlightened. Some fundamental papers[3,4] clarified that R encodes a dihydroflavonol 4-reductase (dfr), P a flavonoid 3',5'-hydroxylase (f3'5'h), and D an R2R3 MYB transcription factor similar to Petunia an2. As in several species, including maize, apple and grape, this potato R2R3 MYB gene (*AN1*) regulates the expression of multiple anthocyanin structural genes. (Figure 1)

Gene expression profile data, mutant and co-segregation analysis showed how much variable and tissue-specific might be the accumulation of anthocyanins in the tubers. This high variability is mostly due to different allelic forms of *AN1* gene as already demonstrated by De Jong et al.[3], but information on anthocyanin pathway regulation in potato remain to be clatified. In order to gain new insights into anthyocians accumulation mechanisms by MYB-TFs, at DiSSPAPA we are currently studying the expression of an1-TF in different cultivated and wild potato species (*S. bulbocastanum* and *S. commersonii*) having different plody leveles (2x and 4x). In particular, by comparing the an1-TF expression levels between diploid and tetraploids genotypes we want to verify whether a complete genome duplication might alter this gene regulation network. Morover we are tring to analyse the unknown gene structure and new possible alleles of an1 in wild potato species.

Finding the way to control and exactly regulate the anthocyanins accumulation in plants might rep-

resent an exciting break towards the production of new potato varieties enriched in antioxydant molecules and, consequently, with great human health-promoting benefits.

References

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