

## GENETIC CONTROL OF ANTHOCYANIN METHYLATION IN PETUNIA AND GRAPEVINE

PROVENZANO S.\*, SPELT K.\*\*, BRUGLIERA F.\*\*\*, QUATTROCCHIO F.\*\*, KOES R.\*\*,  
FERRANDINO A.\*, TANAKA Y.\*\*\*\*, SCHUBERT A.\*

\*) Department Colture arboree, University of Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (Italy)

\*\*) Department of Molecular Cell Biology, Vrije Universiteit, University Amsterdam

\*\*\*) Calgene Pacific Pty Ltd, 16 Gipps Street, Collingwood, Victoria 3066 (Australia)

\*\*\*\*) Institute for Plant Science, Suntory Ltd, 1-1-1 Wakayamadai, Shimamoto, Mishima, Osaka 618-8503 (Japan)

*Vitis vinifera*, *Petunia hybrida*, methyltransferase, anthocyanin

Anthocyanins belong to the class of flavonoids and are the most widespread pigments in the plant kingdom. They are involved in a series of biological activities such as protecting against oxidative damage and attracting pollinators, and they can also produce benefits on the human health. The glycosylated anthocyanins can be “decorated” on the ring B through methylation and acylation. The methylation of the 3’ hydroxyl group of the anthocyanin B-ring converts cyanidin into peonidin or delphinidin into petunidin; the methylation of both the 3’ and the 5’ hydroxyl groups, convert delphinidin into malvidin. These modifications increase the stability of anthocyanins and modify their water solubility thus significantly contributing to the accumulation of coloured molecules in petals or fruits. Here we describe the cloning and characterization of two anthocyanin methyltransferases of *Petunia hybrida* and we analyse their function *in vitro* and *in vivo*. Genetic studies had previously shown that in petunia anthocyanin methylation is controlled by the two loci *MT* and *MF*. By sequence analysis of petunia mutants we show that the two genes correspond to the two loci, and we characterize the relative mutations. Expression analysis shows that the *MT* and *MF* genes are controlled by known regulators of anthocyanin biosynthesis in petunia (AN1 and AN2). Methyltransferase activity *in vitro* was demonstrated using MT recombinant protein. The complementation by transient and stable transformation of *mt* and *mf* mutants induced the production of methylated anthocyanins as analyzed by LC-MS. We also transformed the same petunia mutants with a methyltransferase from *Vitis vinifera* already studied *in vitro* (Hugueney *et al.*, 2009, Plant Physiology 150:2057-2070). The results show the presence of methylated compounds in the flower extracts and the preference of this gene for the production of di-methylated anthocyanins. Petunia is a useful model plant to study the *in vivo* function of phenylpropanoid biosynthetic genes. Moreover the possibility to use transient expression via *Agrobacterium* infiltration in petals has proven to be a fast method to test gene function in this species.