

DNA methylation dynamics influence pigmentation in sweet orange fruit [*Citrus sinensis* L. (Osbeck)] under cold stress

Sicilia A., Scialò E., Lo Piero A.R.

rlopiero@unict.it

Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Santa Sofia 98, 95123 Catania, Italy

The blood red color of pigmented orange fruit varieties [*Citrus sinensis* L. (Osbeck)] is due to the presence of anthocyanin pigments that largely contribute to determine the high organoleptic qualities and the nutritional properties of the fruits. The content of pigments in sweet orange depends primarily on genetic factors and on environmental conditions. In particular, it has been extensively shown that cold temperature induces an increase of anthocyanin content that is achieved by the induction of the related gene expression. The purpose of our work is to understand the mechanism underlying the color variegation occurring inside the blood oranges during the cold induction of anthocyanin biosynthesis, despite the fact that the entire fruit is genotypically programmed to produce pigments. Therefore, the amount of anthocyanin and the expression of both structural and regulatory genes have been monitored in either high-pigmented (HP) or not/low pigmented (NP) segments of the same fruit during the storage at 4 °C for a total experimental period of 25 days. Our results clearly indicate that the anthocyanin content is directly correlated with the levels of gene transcription, with higher pigmented areas showing higher enhancement of gene expression. Furthermore, we analyzed the reshaping of the DNA methylation status at the promoter regions of genes related to anthocyanin biosynthetic pathway, such as *DFR* and *Ruby*. Our results unequivocally demonstrate that in the promoter regions of both *DFR* and *Ruby*, the amount of cytosine methylation strongly decreases along the cold storage in the HP areas, whereas it increases in the NP areas of the same fruit, probably causing a partial block of the gene transcription. Finally, by measuring the changes in the expression levels of the Citrus DNA demethylases, we found that DML1 might play a crucial role in determining the observed demethylation of *DFR* and *Ruby* promoters, with its expression induced by cold in the HP areas of the fruits. This is the first report in which different levels of gene expression implicated in anthocyanin production in blood orange fruit is correlated with an epigenetic control mechanism such as promoter methylation.

Keywords: *Citrus sinensis*, sweet orange, anthocyanin, pigment variegation, DNA methylation, cold stress.