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UNRAVELLING THE ROLE OF THE DNA METHYLATION RESHAPE UPON THE COLD INDUCED ANTHOCYANIN PIGMENTATION IN BLOOD ORANGE (CITRUS SINENSIS L.)

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The 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 (Lo Piero, 2015, J. Agric. Food Chem., 63(16), pp. 4031–4041). The purpose of our work was 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 anthocyanins 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 of anthocyanin biosynthetic genes, with more pigmented areas (HP) showing higher levels of gene expression. Furthermore, we analyzed the reshape of the DNA methylation status at the promoter region of genes related to the 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.

Moreover, by measuring the changes in the expression levels of the DNA demethylases, we found that *Csdml1* might play a crucial role in determining the observed demethylation of DFR and Ruby promoters, as its expression was sharply induced by cold in the HP areas of the fruits. Finally, we also evaluated the effect of 5'-azacytidine inoculation upon the anthocyanin content and the expression of genes related to anthocyanin biosynthesis during fruit cold storage. 5’- Azacytidine is a strong DNA methyltransferase 1 (DNMT1) inhibitor and consistently with our results we observed an increase in both anthocyanin accumulation and gene expression in response to 5'-azacitidine treatment. As far as we know, 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