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# ANALYSIS OF DNA METHYLATION CHANGES INDUCED BY COLD AND 5-AZACYTIDINE TREATMENTS IN BLOOD ORANGE FRUIT [CITRUS SINENSIS L. (OSBECK)]

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Epigenetic changes, including DNA methylation, histone modifications and histone variants and some non-coding RNA (ncRNA) changes, emerged as relevant modulators of plant responses to the environment. In particular, DNA methylation refers to the addition of a methyl group to the cytosine bases of DNA to form 5-methylcytosine that occurs predominantly in the CG, CHG and CHH contexts. The changes of DNA methylation during fruit development and ripening have been investigated in several species, including both climacteric and non-climacteric fleshy fruits. It has been shown that DNA methylation decreased during tomato fruit ripening whereas DNA methylation gradually increases from immature to ripe sweet oranges fruits. These studies suggest that either an increase or a decrease in DNA methylation might be responsible for the normal ripening process. Therefore, the correlation between DNA methylation and gene expression is very variable depending on various factors such as the genomic region, either gene body or promoters, in which the methylation rearrangement occurs. Although the effect of cold stress upon gene expression in sweet orange fruits has been extensively investigated, the analysis of DNA methylation dynamics under abiotic stress is still lacking. Consequently, the aim of this work was to unravel the effect of low temperature (4°C) upon the DNA methylation status in sweet orange fruits during cold storage. Moreover, the effect of the DNA methylase inhibitor 5-azacytidine was also investigated. To reach this goal, blood orange fruits (Tarocco Tapi) were

inoculated either with 5-azacytidine (AZA samples, 50 mM) or with water (H2O inoculated samples) and subsequently stored both at 4°C and room temperature (25 C°) for a total experimental period of 13 days. Genomic DNA was purified from each sample using Plant DNazol and a methylation content sensitive enzyme ddRAD (MCSeEd) profiling system was used to address cytosine methylation at the CG context. Our results indicated that cold storage induces CG-hypomethylation (4°C, H2O samples). 5-azacytidine treatment caused the increase in the methylation status at both temperatures (4°C and 25°C, AZA samples). Moreover, the expression of both methyltransferases and demethylases was measured in order to correlate the DNA methylation status with the transcription of genes involved in DNA methylation rearrangements. We observed that the combined effect of 5-azacytidine and low temperature activate the expression of members of both gene families suggesting that the final DNA methylation status is the result of a balanced regulation of demethylases and methylases expression.