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Poster Communication Abstract – 4.08

INDUCTION OF ANTHOCYANIN BIOSYNTHESIS TRIGGERED BY PATHOGEN ATTACK IS ASSOCIATED WITH DFR AND RUBY PROMOTER METHYLATION IN BLOOD ORANGE FRUIT [CITRUS SINENSIS L. (OSBECK)]

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The anthocyanins present in the red oranges have received great attention due to their contribution to the organoleptic qualities of fruits as well as to the beneficial health effects on either humans or animals. Several abiotic environmental factors influence the pigmentation of fruits, such as light, nutritional status, xenobiotic or hormone treatments and low temperature. Much less is known about the effect of biotic stress on anthocyanin production in sweet orange, although in other species, anthocyanins are often indicated as “defense molecules” produced in response to specific pathogen attack. Epigenetic factors such as DNA methylation have emerged as relevant modulators of plant responses to the surrounding environment. Most of the research works have been focused on the role of DNA methylation in determining plant phenotype in response to abiotic stress, whereas only a few studies have tried to shed the light upon how biotic factors might affect DNA methylation configuration. A deeper understanding of the host-pathogen interactions is essential to clarify the molecular mechanisms underlying the infection and eventually to develop new methods for the storage, transport, and post-harvest marketing of citrus fruits. For this reason, in this work, we evaluated the effect of *Penicillium digitatum* inoculation on the anthocyanin content and the expression of genes involved in their biosynthesis pathway 3 and 5 days post inoculation (DPI) using RT-real time PCR. Moreover, the level of the *dfr* and *ruby* promoter DNA methylation was monitored by McrBC digestion followed in real-time, to combine the gene expression results with the DNA methylation dynamics during fungal infection. In this respect, the expression level of DNA de-methylases involved in DNA methylation

rearrangements was also measured. The results clearly indicate that anthocyanin content sharply increases in the inoculated fruits and this rise occurs by activating the expression of several genes in the biosynthetic pathway. The induction of gene expression is accompanied by maintenance of high levels of methylation at the DFR and RUBY promoters in the inoculated fruits, thus suggesting that DNA methylation is not a repressive mark of anthocyanin related gene expression in sweet orange subjected to biotic stress. Finally, by measuring the expression levels of the *Citrus* DNA demethylase genes, we found that none of them is up-regulated in response to fungal infection, this result being in accordance with the observed maintenance of high-level DFR and Ruby promoter methylation. In conclusion, this is the first report that correlates fungal infection with anthocyanin biosynthesis induction in blood oranges. Our study lays the foundation for future work aimed to unravel the role of anthocyanin in protecting sweet orange from disease.