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EFFECT OF GRAFTING ON DNA METHYLATION IN APPLE

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Grafting is an agricultural technique that involves the union of the root system (rootstock) of a plant with the aerial part of another (scion), resulting in the connection of the vascular system of two different genotypes and the production of a perfect symbiote relationship. Several benefits in using grafting have been highlighted, like: increased production, resistance to abiotic and biotic stresses, reduction of juvenile period. Grafting also acts as clonal propagation, allowing to maintain the uniformity of cultivars. Apple (*Malus* x *domestica* Borkh.) orchards are cultivated as grafted plants to overcome the high heterozygosity of the genus *Malus*. Two of the most used rootstocks are M.9 and MM.106. M.9 is a dwarfing rootstock used to obtain short but easy-to-harvest trees and to increase the yield. MM.106 is a semi-invigorating rootstock used to produce bigger plants that do not need support during their growth. Although physiological processes involved in grafting are well known, molecular mechanisms coordinating the communication between rootstock and scion are still uncertain. Our work aims at investigating how grafting could influence plant phenotype, and how different apple rootstocks could infer the gene expression of two different apple scions (Florina and Golden Delicious) by inducing changes in their DNA methylation. To perform these analyses, we employed MCSeEd, an NGS-based method for genome-wide investigation of DNA methylation at different contexts (CG, CHG, CHH, 6mA). We produced six grafting combinations: self-grafted Florina and self-grafted Golden Delicious, heterografed M.9/Florina and M.9/Golden Delicious, and heterografted MM.106/Florina and MM.106/Golden Delicious. For each cultivar, self-grafted plants were used as control. Differentially methylated regions (DMRs) were identified for

each context for all the grafting combinations. Specifically, in Golden Delicious we identified 12,973 and 11,155 DMRs when grafted on M.9 and MM.106 compared with self-grafted plants, respectively. The number of DMRs identified accounted to 7,968 and 1,959 when Florina was grafted on M.9 and MM.106 respect to then it was self-grafted. Enrichment analysis revealed a significant increase of DMRs within either regulative (2 kb upstream and downstream gene sequences) or in gene-body regions. Moreover, selected DMRs were used to identify genes differentially methylated (DMGs) due to the rootstock. Specifically, in both M.9 and MM.106 analyses, we identified DMGs involved in tyrosine catabolism, hormone regulation, and cellular division. Specific DMGs from M.9 and MM.106 analyses were also identified. In particular, M.9-DMGs are involved in auxin and abscisic acid regulation, while MM.106-DMGs are involved in epigenetic regulation and brassinosteroid signal transduction. These results suggest that both dwarfing and invigorating rootstocks might regulate the expression of common and specific genes, suggesting a rootstock-induced gene expression.