Epigenome of *Malus domestica*

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Introduction

The aim of this study was to examine the epigenomes of two diploid apple scion cultivars from three distinct environments: 1) *in vitro* shoots maintained for 16 years in tissue culture; 2) *in vivo* mother trees (20 years old); 3) acclimatized *in vitro* plants (one year after acclimatization). Using whole-genome bisulfite sequencing (WGBS), the level of DNA methylation was measured in these three biological samples to determine whether an epigenetic footprint was left within the epigenome of apple due to different environments (*in vivo* mother tree vs. *in vitro*) or a change in the environment (*in vitro* culture to acclimatized stage). The study further assessed if the DNA methylation pattern of acclimatized plants mirrored that of the original parental material (i.e., *in vivo* mother plant vs. one year after acclimatization).

Materials and methods

Plant material was collected from three sources. The first source was *in vitro* leaves from four-week-old *in vitro* shoot cultures of *Malus x domestica* Borkh. scion cultivars 'McIntosh' and 'Húsvéti rozmaring'. Shoot cultures were maintained for 16 years and subcultured monthly on basal medium (Magyar-Tábori et al., 2009). The second source was *in vivo* leaves collected from *in vivo* mother trees of both cultivars from which *in vitro* cultures had been originally established 16 years earlier. The third source was leaves collected from rooted *in vitro* apple shoots one year after acclimatization (Magyar-Tábori *et al*., 2009). DNA was extracted and purified from all three samples of each cultivar with a NucleoSpin plant II DNA extraction kit (Macherey-Nagel, Düren, Germany), following the manufacturer's instructions. Bisulfite treatment was applied with the Pico MethylSeq Library Prep kit (Zymo Research, Irvine, CA, USA) based on the user manual. WGBS was performed on a Illumina HiSeq 2500 (Illumina, San Diego, CA, USA). Differential methylation, statistical analysis, DNA methylation distribution plots and gene clustering were performed with SeqMonk v1.41.0 (https://github.com/s-andrews/SeqMonk). Genes that displayed significant differences in DNA methylation in either their promoter or coding regions according to the $\chi 2$ test were classified as differentially methylated genes (DMGs). All assembled DMGs (based on the $\chi 2$ test) were considered for functional mapping of biological processes, molecular functions and cellular compounds. The functions of all DMGs in the three environments were determined, focusing on biological processes, molecular functions and cellular components of green plants (*Viridiplantae*), as these might have important roles during *in vitro* culture by Gene Ontology (GO) annotation with Blast2GO v5.1.12 (Conesa and Götz, 2008).

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Results and discussion

In the present study on apple, when studying the level of global DNA methylation, no significant differences were found in the degree of methylated cytosine positions between apple scion cultivars. Analysis of DNA methylation at the level of the entire genome showed significant differences in C methylation between some genes in either their promoters or coding regions. A total of 45,116 genes, including their promoters and coding regions, were studied. Significant differences in DNA methylation were identified in 586 genes, i.e. DMGs, specifically 334, 201, and 131 in CpG, CHG and CHH contexts. The level of methylation in DMGs decreased after acclimatization. *In vitro* tissue culture had the highest level of methylated DMGs. Some DMGs that participate in oxidation-reduction processes, metabolism and biosynthesis. Methylation patterns of the two scion cultivars differed, indicating cultivar-specific regulation of the epigenome during the adaptation of apple to various environments. The level of DNA methylation of DMGs was lower in 'McIntosh' than in 'Húsvéti rozmaring' , which might indicate differences in adapting to an *in vitro* environment. According to GO annotation of all DMGs, a total of 235, 310 and 189 DMGs play important roles in biological processes, molecular functions and cellular components, respectively.

Conclusions

Our experiments show that the levels of global genomic DNA methylation in apple were steady, independent of the cultivar or growth environment. However, analyses of the methylation pattern in the entire genome confirmed that individual genes display constantly changing levels of methylation. The dynamic changes in their methylation levels might regulate responses and adaptation to a changing environment (*in vitro* environment or recovery to the *in vivo* environment). After acclimatization, the pattern of DNA methylation in the two apple cultivars were similar to the methylation pattern of the mother plant.

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References

Conesa, A. & Götz, S. (2008): Blast2GO: A comprehensive suite for functional analysis in plant genomics. International Journal of Plant Genomics 2008, 619832 . https://doi.org/10.1155/2008/619832

Magyar-Tábori, K., Dobránszki, J., Hudák, I. (2009): Factors affecting the rooting ability of *in vitro* apple shoots. Hungarian Agricultural Research 18.10, 4. https://doi.org/10.1016/j.scienta.2011.05.011