

# Artificially induced autopolyploidy increases genome-wide methylation in grapevine

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Epigenetic processes are reversible chemical modifications acting on two genetic information-containing targets: DNA, and histones. They promote changes in chromatin structure, affecting genome stability, and gene expression, which lead to phenotypic changes without modifications in the genome sequence. Spontaneous or induced polyploidization is hypothesized to require extensive structural and functional reprogramming of the plant genomes, likely associated to the regulation of the activity of transposable elements (TEs), and redundant genes. In the current work, we have compared the amount of global CpG methylation in wild type (2n) and autotetraploid (4n) grapevines. Shoot apical meristems of *Vitis vinifera* 'Crimson Seedless' and *Vitis* hybrids 'BRS Clara', 'Paulsen P1103', 'IAC 572 Jales' and 'IAC 313 Tropical' were cultivated in liquid medium supplemented with 80  $\mu$ M oryzalin for 24 hours at 16°C. Subsequently, apices were regenerated in vitro, and fully developed plants, rooted and submitted to cytogenetics and flow cytometry analyses. Control diploid plants were mock cultivated, and regenerated from isolated shoot apices. Cultivar parental genome information was obtained from genealogy analyses. The effects of ploidy and global methylation variation were investigated by correlation analyses and Generalized Linear Mixed Models (GLMMs). Viable autotetraploid plants were obtained for all investigated cultivars. Antimitotic agent concentration, treatment time, and regeneration system affect the effectiveness of polyploidy induction. Recovery of non-chimerical tetraploid plants ranged from 8.6 to 30%, depending on the cultivar. Light microscopy analyses of epidermal structure morphology revealed a positive correlation between chromosome number and linear measures of stomata length and width, and chloroplast density per guard cell area. Stomata density per area was negatively correlated to the ploidy level. Global genome methylation levels were higher in autotetraploid plants, in comparison to wild-type and diploid in vitro regenerated plants. Methylated CpG levels were higher in autotetraploids from hybrids, in comparison to *V. vinifera*. Further transcription analyses may help to elucidate the predicted role of methylation as a "genome shock" response in artificially induced grapevine polyploids.

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