

EXPLORING THE MYB-bHLH-WD REGULATORY COMPLEX OF THE FLAVONOID PATHWAY IN *VITIS VINIFERA*: THE bHLH SIDE

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The regulation of the flavonoid pathway has been extensively studied in many plant species. It occurs mostly via the control of the coordinate expression of structural biosynthetic genes by a complex of R2R3 MYB transcription factors, MYC-like basic Helix-Loop-Helix (bHLH), and WD40 proteins. In *Vitis vinifera*, a small set of MYB transcription factors have been characterized and their ability to regulate the synthesis of different flavonoid end-products like anthocyanins or proanthocyanidins have been shown to be strictly dependent on a bHLH partner. Recently, the partial characterization of two grape bHLH factors (VvMYC1 and VvMYCA1), putatively involved in flavonoid regulation in berry has been described.

By exploring the last released grape genome prediction we found another gene that we named VvJAF13, coding for a bHLH protein with high similarity to known bHLH factors regulating flavonoid synthesis in other species. Molecular analysis of VvJAF13 revealed that it displays all the basic features for participating to the MYB-bHLH-WD regulatory complex and to be a candidate regulator of the flavonoid pathway. *VvJAF13* has been isolated from *Vitis vinifera* cv Corvina berry cDNA and over-expressed in the *Petunia hybrida an1* mutant, defective in a bHLH factor needed for anthocyanin production in petals. Moreover, grape *VvMYC1* has been also isolated and over-expressed in the same *Petunia* mutant line. Phenotypic and molecular analysis of transgenic plants showed that only *VvMYC1* was able to complement the *an1* mutation by restoring the wild type flower pigmentation, while *VvJAF13* was not. Interestingly, however, the plants over-expressing *VvJAF13* lost also their residual petal pigmentation and displayed completely white flowers. On the basis of these results the possible role of *VvMYC1* and *VvJAF13* in the regulation of grape flavonoid biosynthesis is inferred.