

UNDERSTANDING THE ROLES OF VvMYB5a AND VvMYB5b IN THE REGULATION OF THE FLAVONOID BIOSYNTHETIC PATHWAY IN GRAPE

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Flavonoids belong to a class of phenylpropanoid secondary metabolites that are important for grape and wine quality. The pathway, synthesizing the three major compounds, anthocyanins, tannins and flavonols, is highly regulated in order that different flavonoids are produced in different organs and in various stages of development. Although the branches of flavonoid biosynthetic pathway have been well studied in grapes, little is known about the transcriptional mechanism that regulates it. Two MYB transcription factors, VvMYB5a and VvMYB5b, have been recently identified as regulators of the early flavonoid structural genes at different stages of berry development.

In order to gain information about their specific roles in the regulatory network, we performed functional complementation analyses of some well characterized petunia anthocyanin/pH regulatory mutants demonstrating that VvMYB5a and VvMYB5b are involved in the activation of the flavonoid pathway and in the regulation of the vacuolar acidification in the epidermal cells of petals in petunia.

However, the use of heterologous systems to study the gene function may give results that do not reflect the true role of these MYB TF in grapevine. For this reason, *V. vinifera* cv Corvina was transformed to obtain *VvMYB5a* and *VvMYB5b* over-expressing hairy roots lines. A transcriptomic analysis using a 90 K *Vitis vinifera* oligoarray revealed that they are involved in the regulation of numerous processes including phenylpropanoid biosynthesis.

Stable transformation of *Vitis vinifera* cv Shiraz has been performed to silence *VvMYB5a* and *VvMYB5b* simultaneously. The transgenic plants present stunted growth, curly leaves accumulating anthocyanins and abnormal veins. The down-regulation of genes encoding enzymes for tannin synthesis, such as LAR (leucoanthocyanidin reductase) and ANR (anthocyanidin reductase) in the silenced lines may explain the accumulation of pigmentation in leaves.

In addition, to verify if they could also exhibit different functions, each gene has been over-expressed independently. The over-expression of *VvMYB5b* leads to an accumulation of anthocyanins in leaves, which is not visible in plants over-expressing *VvMYB5a*. Taken together, these preliminary results suggest that VvMYB5b may be involved in the regulation of the anthocyanin synthesis, while VvMYB5a could be responsible for tannin production.

Transcriptomic and metabolomic analyses of the transgenic plants will provide more conclusive information about the specific roles of VvMYB5a and VvMYB5b and will help to clarify the regulatory network of the flavonoid biosynthetic pathway.