CHARACTERIZATION OF A bZIP FACTOR THAT REGULATES THE FLAVONOID PATHWAY IN GRAPEVINE

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Flavonoids compose one of the most abundant and important subgroups of secondary metabolites detected so far in higher plants. They are involved in several aspects of plant development and defence. Besides the attraction of pollinators and dispersers to fruits and flowers, flavonoids also protect against a plethora of stresses including pathogen attack, wounding and UV irradiation. Flavonoid content and composition of fruits and plant products have been associated with fruit quality including taste, colour and health-promoting effects (Czemmel et al., 2012).

In grapevine (*Vitis vinifera* L.) flavonoids accumulate preferentially in the skin and seeds of grapes (Downey et al., 2004) determining the colour, but also the final flavour and astringency of red and white wines. Furthermore, flavonoids extracted from grape berries and seeds exhibit strong nutraceutical potential with a broad spectrum of pharmacological and therapeutic effects (Nassiri-Asl and Hosseinzadeh, 2009).

The synthesis of these compounds is mainly regulated at transcriptional level. Flavonoids accumulate at specific stages and in specific tissues during flower and berry development, as a consequence of the timely expression of the genes necessary for their synthesis. Although the general flavonoid pathway has been genetically and biochemically elucidated also in grapevine, its regulation still remains not completely characterized (Hichri et al., 2011). Similarly knowledge is still lacking about the molecular basis of the varietal variability in terms of quality and quantity of flavonoids.

To shed light on this issue a mapping population derived from the cross Syrah x Pinot Noir segregating for flavonoid content was characterized at the metabolic and transcriptional level. The transcriptome analysis of the extreme genotypes of the population has allowed the identification of a set of differentially expressed genes. Among them, a basic leucin zipper transcription factor (*VvbZIP*) has been selected and further characterized to elucidate its role in regulating flavonoid biosynthesis.

A phylogenetic analysis of all the 55 predicted grapevine bZIP factors was performed. According to the ten-classes classification proposed in Arabidopsis, *VvbZIP* belongs to the S-group of the family.

The analysis of the different classes of flavonoids and of *VvbZIP* expression in Pinot Noir samples collected from flowering to technological maturity (18°Brix), showed a peak of *VvbZIP* expression as well as of the flavonols quercetin and kaemperol at flowering time.

Further evidence of the role of this gene in flavonols regulation came from the analysis of Chardonnay plants exposed to UV-light and monitored over a 3-d time period. The light treatment caused an induction of the *VvbZIP, VvFLS1* and *VvMYBF1* transcripts within the first 10 h, followed by the accumulation of flavonols at 24 h post treatment.

To test the *in-planta* function, *VvbZIP* was stably overexpressed into tobacco plants (*Nicotiana tabacum*). The overexpression of *VvbZIP* observed in 7 transgenic lines positively correlated with the flower content of quercetin, kaempferol and cyanidin in the glycosylated form suggesting a broader regulatory role of this factor (Fig.1).

Luciferase reporter-assays carried out by bombardment of Chardonnay suspension cultures (Czemmel et al., 2009), revealed that VvbZIP binds to the promoters of *VvCHS, VvFLS1, VvANR* genes activating their transcription, likely through the interaction with other co-factors such as MYBF1 and MYBPA1.

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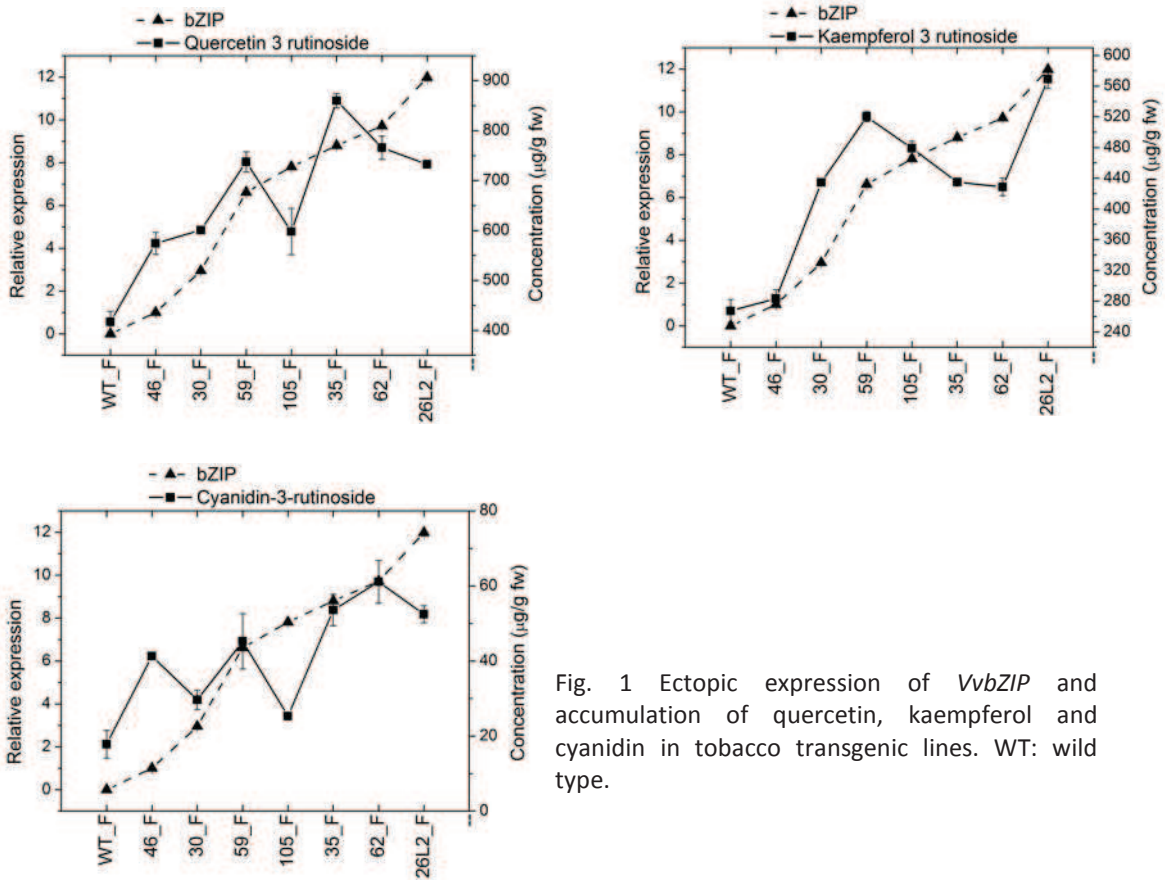

Fig. 1 Ectopic expression of *VvbZIP* and accumulation of quercetin, kaempferol and cyanidin in tobacco transgenic lines. WT: wild type.

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