Characterization of a bZIP transcription factor that regulates the phenylpropanoid pathway in grapevine

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Abstract. In grapevine (*Vitis vinifera* L.) flavonoids accumulate preferentially in the skin and seeds of grapes determining the colour but also the final flavour and astringency of red and white wines. Although the general flavonoid pathway has been genetically and biochemically elucidated in many plant species and most recently also in grapevine, its regulation still remains not completely characterized.

In this study we report the characterization of a grapevine bZIP transcription factor involved in flavonoid biosynthesis regulation. According to the ten-classes classification proposed in Arabidopsis, this VvbZIP belongs to the S-group of bZIP and its expression along fruit development is higher at the flowering stage. The overexpression of *VvbZIP* in tobacco transgenic lines positively correlates with the flower content of quercetin, kampferol, glycosilated cyanidin, and proanthocyanidins. Furthermore, transient expression assays by bombardment of grapevine suspension cultures, have revealed that VvbZIP activates the flavonoid pathway at different sites and through the interaction with other co-factors.

Introduction. Flavonoids are involved in several aspects of plant development and defence: besides the attraction of pollinators and dispersers to fruits and flowers, they also protect against several stresses including pathogen attack, wounding and UV irradiation.

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.

Materials and Methods. A F1 population from the cross Syrah x Pinot Noir was characterized for the content of flavonoids during berry development. In particular, samples from the parental Pinot Noir were collected along six time points (flowering, two and four weeks post-flowering, veraison, 16° brix and 18° brix) and stored at -80°C. Each sample was grinded with liquid nitrogen and used to measure the content of anthocyanins monoglucosides and flavonols after hydrolysis (Mattivi et al., 2006), proanthocyanidins (PAs) (Mattivi et al., 2009), and also for RNA extraction. Total RNA from each sample was used for gene expression quantitation in the Real-time RT-PCR exsperiments. Full-length *VvbZIP* cDNA was cloned and transformed into tobacco using the

pART27 vector under the control of the CaMV 35S promoter (Gleave, 1992). Expression of *VvbZIP* in transformed plants was determined by Real-time RT-PCR. PAs in transgenic tobacco flowers were determined with the DMACA reagent as in Bogs et al. 2005. The construct pART7bZIP was also used for transient functional assay of the activity on the promoters of the structural genes of the flavonoid pathway using particle gun bombardment and Chardonnay suspension culture cells as in Bogs et al., 2005.

Results and Discussion. The transcriptome analysis of the extreme genotypes of the F1 population from the cross Syrah x Pinot Noir segregating for flavonoid content 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 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.

To test the *in-planta* function, *VvbZIP* was stably overexpressed into tobacco plants (*Nicotiana tabacum*). The overexpression of *VvbZIP* in 7 transgenic lines positively correlated with the flower content of quercetin, kaempferol glycosilated cyanidin (Fig.1) and proanthocyanidins (data not shown), suggesting a broad regulatory role of this factor.

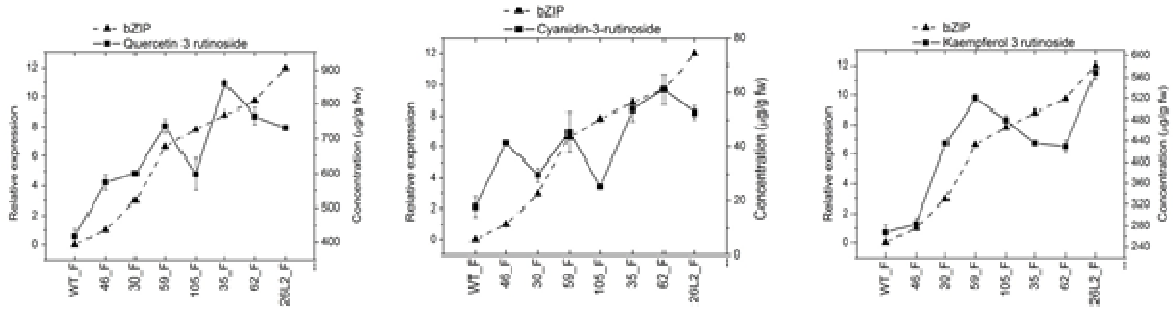

Fig. 1 Ectopic expression of *VvbZIP* and accumulation of quercetin, kaempferol and cyanidin in tobacco transgenic lines. WT: wild type.

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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