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Identification and characterization of MATE family genes involved in anthocyanins transport in grape berries

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Abstract. Anthocyanins are the predominant pigments responsible for the colour of red wine and are stored in the skin of grape berries. In the cells, anthocyanins are synthesised in the cytoplasm and accumulated into the vacuole. However, little is known about the transport of these compounds to the vacuole. Recently, the sequencing of *Vitis vinifera* genome allowed us to identify genes encoding proteins highly homologous to a putative anthocyanin permease MTP77 described in tomato. We named these *Vitis* proteins, anthoMATE since they contain conserved domains of the multidrug and toxic compound extrusion protein family (MATE). The expression of these genes is concomitant with anthocyanin accumulation in the skin of grape berries. Additional results show different gene expression in different *Vitis vinifera* cultivars and in different organs for the genes encoding these *Vitis* proteins. In *Vitis* transformed roots cells, we detected its subcellular localization into the tonoplast membrane, the site where anthocyanin transport occurs. The demonstration of their putative role in anthocyanin transport is currently under validation.

Introduction. Anthocyanins are toxic endogenous chemicals for the cell because of their high chemical reactivity. Therefore they need to be removed from the cytoplasm to the vacuole immediately after their synthesis. Vacuoles offer a large storage space, which is important for flavonoids to reach concentrations high enough to function in the protection against predators and pathogens or as UV light sunscreens [1]. Most of the structural genes encoding for the enzymes that participate in the formation of anthocyanin are well-known [2]. Nevertheless, the storage mechanism has not been functionally characterized yet. The *Arabidopsis thaliana* gene *Transparent Testa 12* encodes a MATE protein required for flavonoid sequestration in vacuoles of the seed coat endothelium [3]. A similar MATE-type transporter, MTP77, has been identified in tomato as a putative anthocyanin permease [4]. MATE (multidrug and toxic extrusion compounds) family are organic cation exporters involved in the detoxification of secondary metabolites [5]. Recently, the sequencing of *Vitis vinifera* genome allowed us to identify genes encoding proteins that contain conserved domains of the MATE family. The identification and molecular characterization of MATE family genes involved in anthocyanins transport in grape berries is described here.

Materials and Methods. *Plant Material* – *V. vinifera*. L berries from different cultivars were harvested during summer 2007 in Domaine de Vassal (SupAgro, Montpellier France).

Real-Time PCR - RNA was quantified and reverse transcription was performed as described by Ageorges et al., 2006 [6].

Hairy roots expressing anthoMATE:GFP protein - Induction and culture of transformed roots lines were performed as described by Torregrosa et Bouquet 1997 [7].

Results and Discussion. The sequencing of *Vitis vinifera* genome allowed us to identify genes with high homology to putative flavonoid MATE permeases [3, 4]. Two of these identified genes were recently cloned and named *anthoMATE1* and *anthoMATE3*. The *anthoMATE* genes encode proteins that are 38% identical to TT12 but are even more like MTP77 (68% identical). These two proteins are highly homologous (86% identical). The expression profile of both genes during berry development stage shows a lack of expression until véraison and an increase during ripening. At a second stage, we evaluated genes expression in flesh, skin and seed of *Syrah* grape berries at 3 stages: pre-véraison, véraison and maturation. *AnthoMATE1* and *anthoMATE3* are significantly more expressed in the skin during maturation than other tissues. In fact, in

grape berries anthocyanins are synthesised in the skin during maturation. A gene expression profile associated with anthocyanin accumulation was obtained for both *anthoMATE* genes. These results and the high homology suggest redundancy in anthocyanin transport between these proteins.

In order to investigate a substrate specificity of these redundant proteins, we used RT-PCR to measure the expression of *anthoMATE1* and *anthoMATE3* in different cultivars and in different organs. It was surprising to find out that *anthoMATE1* was not expressed at detectable levels in roots, leaves and tendrils while it was expressed in berries. Instead, *anthoMATE3* was expressed both in roots and berries. Secondly, the expression of these 2 genes was significantly different in sixteen cultivars showing different anthocyanins profile. The expression of *anthoMATE3* was correlated with methylated anthocyanins (Kendall's tau = 0.7, $P < 0.001$). Indeed, no correlation was statistically found for *anthoMATE1*. Significantly expression of *anthoMATE1* was observed in white cultivars, this suggests functions for this *anthoMATE* protein in addition to or other than anthocyanin transport. An *in vivo* localization of AnthoMATE was performed in stable transformed roots lines of *Vitis*. We detected its presence at the tonoplast of young apex root epidermal cells. Subcellular localization was tested using an *anthoMATE*:GFP protein fusion. Cells were stained with propidium iodide a red fluorescent dye of plasmic wall, green fluorescence was visible in a thin membrane around the nucleus (Fig.1A). Secondly, cells were stained with DAPI a fluorescent stain that binds to DNA. When incubated in presence of 0.3 M sorbitol, the volume of the vacuoles strongly decreased sorting out the tonoplast from the cell wall and from the nucleus confirming the location of the putative transporter (Fig.1B).

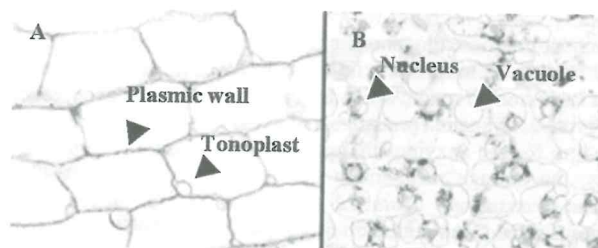


Fig.1. Laser scanning confocal microscopy images of Grapevine root epidermal cell expressing *anthoMATE*:GFP fusion. (A) Stained with 1 μ M propidium iodide in 10 mM PBS. (B) Stained with 1 μ M DAPI in 10 mM PBS + 0.3 M sorbitol.

Studies in order to demonstrate the function of *anthoMATE1* and *anthoMATE3* in anthocyanin transport are in progress. Mutants generation that modulates the expression of *anthoMATE* genes, will allow us to characterize their *in vivo* role through a reverse genetic approach. In addition, *anthoMATE1* and *anthoMATE3* *in vitro* transport activities towards various anthocyanins substrates are currently under validation

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