An Investigation of the Role of the Regulatory Gene *VvMYBA1* in Colour, Flavour and Aroma Metabolism Using Transgenic Grapevines

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ABSTRACT

Anthocyanins are flavonoid compounds responsible for most of the red, purple and blue colours of leaves, fruit and flowers of many plant species. They are produced through the anthocyanin biosynthesis pathway and in grapevine the VvMYBA1 and VvMYBA2 transcription factors are responsible for the transcriptional activation of genes encoding enzymes required for their synthesis. White grapevine cultivars contain inactive versions of the *VvMYBA1* and *VvMYBA2* genes and hence cannot produce anthocyanins in berries. While much is now known about anthocyanin biosynthesis in grapevine, there are still some genes involved in anthocyanin modification and transport which have not yet been identified. In several other plant species recent research has established a link between anthocyanin biosynthesis and the synthesis of volatile aroma compounds.

In this research project, the aim was to further characterise VvMYBA and its role in anthocyanin and flavour metabolism. To do this, transgenic and natural mutant grapevines in which berry colour has been altered due to differential expression of *VvMYBA* genes were used. Two different approaches were taken to investigate the effect of *VvMYBA* gene expression on the transcriptome and flavour metabolism in berries, with the aim of linking transcriptomic changes to metabolomic changes. Microarray analysis was performed to identify differences in global transcription levels in berries differing in their *VvMYBA* gene expression. Microscale wines were also made from both whole berries and free run juice and volatile wine flavour/aroma compounds were analysed using HS-SPME-GC/MS.

This research has shown that the presence of VvMYBA in berries does have an effect on the abundance of volatile flavour/aroma compounds in wines; however this was often in a cultivar specific manner. One conserved difference was that red wines, made from berries expressing *VvMYBA*, contained less linalool compared to white wines, made from berries not expressing *VvMYBA*. Light exclusion studies and transcript analysis of genes associated with linalool metabolism have suggested that the accumulation of anthocyanins in red grapes may cause a shading effect which down-regulates linalool synthesis.

From microarray studies, two putative acyltransferase genes were identified, one belonging to the BAHD protein family and the other to the serine carboxypeptidase-like (SCPL) family. At the commencement of this study, no anthocyanin acyltransferases had been identified in grapevine and it was hypothesised that one or both of these genes could have this function.

Acylation of anthocyanins has been shown to change the hue of the pigment in the fruit and flowers of various plant species, and to increase their stability in products such as wine. Gene expression studies, bioinformatics analyses and *in vitro* and *in planta* functional assays were used to characterised these two genes. Through these studies the first *Vitis vinifera* anthocyanin acyltransferase gene (*VvAnAT*) was identified. VvAnAT belongs to the BAHD acyltransferase protein family and recombinant enzyme kinetic studies show that it can utilise a range of CoA thioester acyl donors and shows a preference towards monoglucoside anthocyanins as the acyl acceptor substrate. Using promoter activation assays the ability of the VvMYBA1 transcription factor to activate the transcription of the *VvAnAT* gene was shown. The putative SCPL gene did not function as an anthocyanin acyltransferase in *in planta* experiments; further studies are required to understand the function of this gene.

The outcomes of this PhD project have added to the current understanding of anthocyanin synthesis and its regulation in grapevine. Knowledge and identification of a grapevine anthocyanin acyltransferase gene can be used in breeding programs aiming to improve grapevine cultivars that cannot currently produce acylated anthocyanins, and hence increase their potential wine colour stability properties.