

THE TRANSCRIPTION FACTOR *VvMYBPA1* INDUCES A METABOLIC REPROGRAMMING OF FLAVONOIDS IN TOBACCO FLOWERS

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Proanthocyanidins (PAs) are polyphenolic compounds synthesized using anthocyanidins as precursors through one or more enzyme-catalyzed reactions. PAs are secondary metabolites found in a wide range of plants where they play a chief role in protecting them against herbivores, pathogens and abiotic stresses. Thanks to their beneficial properties, both as antioxidants and anticancer agents, there is an increasing interest in promoting the accumulation of these metabolites in food plants. However, PAs are also relevant to ruminant nutrition and animal health because moderate amounts of these flavonoids in the foliage of the forage legumes prevent ruminal bloating, while reducing greenhouse gas emission by livestock and promoting animal tolerance against intestinal parasites. Yet, de novo engineering of PA biosynthesis in forage and food species has proven difficult. Here we test the hypothesis of taking advantage of the anthocyanin pathway to build on PA biosynthesis. To this end, we ectopically expressed in tobacco the gene *MYBPA1*, that in *Vitis vinifera* specifically activates the structural genes of the PAs pathway (Bogs *et al.*, 2007). T₀ transgenic 35S:*MYBPA1* tobacco lines and their progeny were assayed for flower colour and PAs accumulation. The colour of floral limbs negatively correlated with transgene expression in both primary transformants and their progeny. Spectrophotometric quantification of anthocyanins and PAs as well as qRT-PCR analyses carried out to test the effect of the transgene on endogenous biosynthetic and regulatory genes of flavonoids converge to show that *MYBPA1* prevents anthocyanin formation by channelling all the common precursors toward PA biosynthesis.

To glean deeper insight into the metabolic changes triggered by the transgene, we are currently performing LC-ESI-QTOF-MS/MS analyses aiming to specifically compare the profiles of the secondary metabolites in flowers exhibiting different steady state levels of the transgene and, in turn, different levels of anthocyanins and PAs in floral limbs and tubes.

Transformation of *Medicago sativa* is in progress to test the possibility of diverting the flux from the anthocyanidin pathway to PAs also in this species to produce bloat-safe varieties.