THE EXPRESSION OF *VvMYBPA1* IN TOBACCO REMODULATES THE PHENYLPROPANOID PATHWAY AND DIVERTS THE SYNTHESIS OF ANTHOCYANINS INTO CONDENSED TANNINS IN FLOWERS

PASSERI V.**, DAMIANI F.*, MARTENS S.**, CARVALHO E.**, PAOLOCCI F.*

- *) Institute of Plant Genetics CNR, Research Division Perugia
- **) Fondazione Edmund Mach, Istituto Agrario San Michele all'Adige IASMA, Centro Ricerca e Innovazione, Department of Food Quality and Nutrition, Via E. Mach 1, 38010 San Michele all'Adige (Italy)
- [#] Present address: Vrije Universiteit Institute for Molecular and Cellular Biology Amsterdam

Nicotiana tabacum, secondary metabolites, Pathway engineering, MYB family, HPLC MS

Condensed tannins (CTs) have an important role in several nutritive processes and, although high levels of CTs may deplete the taste of food and feed, the presence of a moderate amount of these secondary metabolites may improve the food and feed quality. In the last decades, a number of studies in different plant species have been produced in order to identify the genes and the environmental conditions that induce the synthesis of such compounds in specific tissues and organs of the plant. Indeed, the structural genes, mostly common with the anthocyanin pathway, coding for the enzymes that regulate the synthesis of phenylalanine down to catechin and epicatechin, the monomers that are the base elements of CTs, have been cloned in both model and crop species. Likewise, the transcription factors responsible for the synthesis of anthocyanins and CTs have been characterized in several species. More specifically, genes of the R2R3MYB family are known as the responsible of the tissue-specific synthesis and compartimentalization of anthocyanins and CTs. Nevertheless, engineering in crop species the CTs pathway by overexpressing MYB genes has been thus far problematic.

As a model study, in this work we investigated the effects in tobacco of the over-expression of *MYBPA1*, a *R2R3MYB* gene that controls the synthesis of CTs in grape. SR1 primary transgenic tobacco plants (T₀), and their progeny (T₁ and T₂) were produced. Large variability for anthocyanin pigmentation was reported, inversely related with CT accumulation. In fact control plants with higher red pigmentation do not accumulate CT while increased transgene expression is paralleled by reduced red pigmentation and increased accumulation of CTs. Targeted qRT-PCR analyses proved a large modulation in the expression of both early and late genes of the flavonoid pathway in both floral limbs and tubes. Three selected T₂ plants, featured by a different level of *MYBPA1* expression, along with a control line, were also analysed by HPLC/ESI MS for polyphenols in floral limbs and tubes, separately.

A trend between transgene expression and the accumulation of several compounds was observed. Besides CTs, *MYBPA1* likely increases, directly or indirectly, the accumulation of chlorogenic acid, dihydro benzoic acid, dihydrochalcones and stilbenes both in the floral limbs and tubes. Expression analyses are going to identify the structural genes of the above mentioned pathways targeted by the transgene.