

THE GENETIC CONTROL OF THE TWO BRANCHES OF THE PATHWAY LEADING TO THE BIOSYNTHESIS OF PROANTHOCYANIDINS IN LEGUME LEAVES

F. PAOLOCCI*, M.P. ROBBINS**, L. MADEO*, S. ARCIONI*, F. DAMIANI*

*) Institute of Plant Genetics – CNR, Research Division of Perugia, Via Madonna Alta 130, 06128 Perugia, Italy

***) Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, SY23 3EB, UK

flavonoids, condensed tannins, bHLH, MYB, real time RT-PCR

Proanthocyanidins (PAs), also known as condensed tannins (CTs), are oligomeric and polymeric end products of the flavonoid biosynthetic pathway and are composed primarily of catechin and epicatechin units in higher plant species. They are widespread in nature, occurring in numerous plant species including many important plant-derived food materials. PAs exert beneficial effects on human health, they strongly affects plant quality traits, and the palatability and nutritive value of forage legumes are highly influenced by their concentration and structure (reviewed in Tanner et al. 2004). The understanding of the biosynthesis of the PA starter units (catechin and epicatechin) has progressed significantly over the last few years. The regulatory machine controlling PA biosynthesis has been best characterized in *Arabidopsis* in which a ternary transcriptional complex comprising *TTG1* (encoding a WD-repeat protein), *TT2* (encoding a R2R3 MYB-class protein) and *TT8* (encoding a bHLH-class protein) has been showed to regulate the tissue-specific accumulation of these secondary metabolites (Lepiniec et al. 2006). However, *Arabidopsis* does not offer a complete model system to dissect at full the PA regulatory machine, in that this species lacks either the accumulation of these metabolites in vegetative tissues either the branch pathway leading to the synthesis of catechin. Thus, whether the two branches of the PA pathway are controlled by same or independent regulators has not yet been addressed. To this purpose, we have firstly cloned and characterized *LAR* and *ANR*, two genes encoding enzymes committed to the catechin and epicatechin biosynthesis, respectively in *Lotus corniculatus*. Interestingly, our homology-based gene cloning strategy allowed us to identify two *LAR* gene families in *Lotus*, named *LAR1* and *LAR2*, and a putative conserved duplication of the catechin pathway in distantly related plant species. Afterwards, we gained more insights into the genetic control of leaf PA accumulation by monitoring through real time RT-PCR analysis the steady state levels of the PA-specifically committed genes *LAR1*, *LAR2* and *ANR* and of upstream genes (i.e. *ANR* and *DFR*) in natural PA polymorphic *L.corniculatus* lines and in mutant lines induced by the ectopic expression of *SN*, a maize *bHLH* anthocyanin activator gene, and of *FaMYB1*, a flavonoid repressor *MYB* gene from strawberry (Robbins et al. 2003; Paolocci et al.2005; Aharoni et al. 2001).

Here we report for the first time on the transcriptional regulation of the two branches of the PA pathways in a crop species. Commonalities and dissimilarities between the genetic control of PA biosynthesis in *Arabidopsis* and *Lotus* spp. are also discussed.

References

Aharoni et al (2001) *Plant J* 28: 319-332

Lepiniec L et al (2006) *Annu Rev Plant Biol* 57: 405-430

Paolocci F et al (2005) *J Exp Bot* 56: 1093-1103

Robbins MP et al (2003) *J Exp Bot* 54: 239-248

Tanner GJ (2004). In KM Davies, ed, *Plant pigments and their manipulation*. Annual plant reviews Vol 12. Blackwell Publishing-CRC press, Boca Raton, pp 150-184