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INTERFERENCE OF *LAR* GENES DEPLETES THE SYNTHESIS OF PROANTHOCYANIDINS IN *LOTUS CORNICULATUS* LEAVES

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Proanthocyanidins, RNAi, LAR, Lotus corniculatus

Lotus corniculatus is a useful model system for studying the synthesis of proanthocyanidins (PAs), also known as condensed tannins (Paolocci *et al*., 1999). These flavonoids are relevant to many industrial applications, but they also harbour properties that are of prominent interest to human and animal nutrition and, in general, health. Indeed, PAs help preventing bloating in ruminants when these animals are fed with fresh forage legumes because they increase the rate of rumen bypass proteins, ultimately leading to higher protein assimilation and to better animal performances (Barry and McNabb, 1999)

In a recent study, we cloned the structural genes coding for leucocyanidin reductase (LAR) and for anthocyanidin reductase (ANR). These are the enzymes that lead to the synthesis of catechin and epicatechin, respectively, from the legume species *Lotus corniculatus* (Paolocci *et al*., 2007). Catechins and epicatechins are the building blocks for the synthesis of PA polymers in many crop species. Two *LAR* gene families, *LAR1* and *LAR2*, with an overall nucleotide homology of 60% were recovered. However, *in vitro* evidence showing the possibility of reducing anthocyanidins to catechins has been output only for *LAR1*. Differently from *LAR1*, *LAR2* genes are also very poorly expressed in PA accumulating organs (i.e leaves and stems). To test for the specific relevance of *LAR1* and *LAR2* genes on PA synthesis, *LAR1*- and *LAR2*- based RNAi constructs have been prepared and utilised to transform two genotypes of *L. corniculatus* accumulating different levels of leaf PAs. Preliminary colorimetric and spectrophotometric results show that leaf PA synthesis is severely compromised by both *LAR1* and *LAR2* interfering constructs, regardless of the recipient genotype used. Of note, the down regulation of *LAR1* genes affected also the expression of *LAR2* ones and vice versa, likely because of the partial sequence homology between these gene families. Preliminary qRT-PCR data also suggest that the genes of the flavonoid pathways upstream *LAR1* and *LAR2* are also down-regulated. Together, these data let us to postulate that the down-regulation of the late genes of the PA pathway induces a negative feed-back mechanism on early genes. Detailed metabolic evaluation of all these lines is an ongoing effort in our lab as well as the molecular and metabolic characterization of *Lotus corniculatus* lines expressing *ANR*-based RNAi constructs. This approach should definitely clarify whether a cross-talk between the catechin and epicatechin branches of the PA pathways does exist.