

Genetic manipulation of condensed tannin biosynthesis in *Lotus* spp.

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Condensed tannins (CTs), also known as proanthocyanidins (PAs), are plant secondary metabolites that share most of their biosynthetic pathway with anthocyanins. CTs are polymeric flavonoids composed primarily of epicatechin and/or catechin units. They act as antioxidants with beneficial effects on human and animal health. *In planta*, CTs act as protective agents against pathogens, pests and diseases and control seed permeability and dormancy. These compounds strongly affect plant quality traits: the palatability and nutritive value of forage legumes are highly influenced by their concentration and structure. High concentrations of CTs can decrease the palatability and digestibility of plants. Conversely, moderate quantities of CTs (2-4% dry matter) in forage prevent proteolysis during ensiling and rumen fermentation, thereby protecting ruminants against pasture bloat (Tanner, 2004). Unfortunately, CTs are not accumulated in leaves of the most valuable forage species such as alfalfa and clovers. The genus *Lotus* offers a wide range of options for studying the regulation of CTs as it includes species which accumulate CTs only in flowers and stems (*L. japonicus*, *L. tenuis*) or in flower, stems and leaves (*L. corniculatus*). Equally interesting is that, as opposed to *Arabidopsis* that has yielded critical information regarding the transcriptional control of genes involved in CT biosynthesis in seed coats, in *L. corniculatus* and *L. japonicus* CT polymers are synthesised from both epicatechin and catechin starter units and not only from epicatechin. We aimed to understand the genetic and environmental determinants controlling leaf CT accumulation in *L. corniculatus*. To this purpose, either partial or full length cDNAs from the structural genes of the CT pathways (*PAL*, *CHS*, *DFR*, *ANS*, *ANR*, *LAR1* and *LAR2*) were cloned. Their expression patterns were studied under different growth conditions and in different genetic backgrounds resulting from the transformation of wild type genotypes, polymorphic for the levels of leaf CTs, with exogenous regulators of anthocyanins belonging either to the *bHLH* or *MYB* gene families. More specifically, here we show that in *L. corniculatus* it is possible to specifically up- and down-regulate leaf CT biosynthesis using heterologous activator/repressor genes, without inducing significant alteration on levels of other flavonoid end products. We also show that the epicatechin (*via ANR*) and catechin (*via LAR*) branches of the CT pathways are subjected to a coordinate transcriptional regulation (Paolocci *et al.*, 2007). Strategies to switch on the CT pathway in legume species that don't synthesize these polymers in leaves are also discussed.

References

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