Polyphenolic phenomena: transgenic analysis of some of the factors that regulate the cell-specific accumulation of condensed tannins (proanthocyanidins) in forage crops

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Introduction Condensed tannins biosynthesised within crops are a well-established mechanism for protecting plant protein in the rumen of grazing livestock. Protein protection mediated by these polymeric flavonoid molecules has been characterised in *Lotus* spp. and offers an interesting contrast to the polyphenol oxidase (PPO) system that confers protein protection in red clover.

Materials and methods Clonal genotypes derived from *Lotus corniculatus* cv. Leo were supplied by Dr K.J. Webb and colleagues at IGER. Transgenic lines harbouring *R2R3-MYB* class transcription factors were produced at IGER while lines harbouring *Sn*, a maize *bHLH* gene originate from Dr F. Damiani at CNR, Perugia. Further details and initial characterisation of *Sn* constructs in three recipient genotypes are outlined in Robbins *et al*. (2003).

Results Transgenic plants were grown under containment conditions and leaves were scored (Table 1) for the presence of cells containing condensed tannins using dimethylaminocinnamaldehyde (Li *et al*., 1996).

Table 1 Presence of CT-containing cells in leaves of transgenic and recipient genotypes of *Lotus corniculatus*

	Low CT genotype S33, S50	High CT genotype S41	*Sn* lines S50	*MybPh2* lines S50
Vascular mesophyll	+	+	+	+++
Pallisade mesophyll	-	+	+	-
Spongy mesophyll	-	+	+	-

+, presence of CT cells; -, CT cells not detected

Other phenotypes resulting from the introduction and expression of *Sn* included enhancement of anthocyanin accumulation in selected cell types; ie. subepidermal cell layers of leaf midrib, leaf base and petiole tissues. Increases in trichome numbers were noted in genotype S33 in selected transgenic lines and this correlated with high level expression of the *Sn* transgene.

Conclusions Results of this study indicate that the ectopic expression of plant transcription factors in *Lotus corniculatus* can modulate the biosynthesis of natural products. Data from *Sn* plants is consistent with studies on *Arabidopsis* which demonstrate the interactive role of *bHLH* and *R2R3-MYB* class genes in the hierarchical control of plant development in higher plants (Zhang *et al*., 2003)

References

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