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Sergio Arcioni

Consiglio Nazionale delle Ricerche, Italy

F. Damiani

Consiglio Nazionale delle Ricerche, Italy

F. Paolocci

Consiglio Nazionale delle Ricerche, Italy

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Exogenous transcription factors alter the patterns of condensed tannin accumulation in *Lotus corniculatus*

Arcioni S., Damiani F., Paolucci F.

Consiglio Nazionale delle Ricerche-Istituto Genetica Vegetale, via Madonna Alta 130, 06128 Perugia, Italy, E-mail: sergio.arcioni@igv.cnr.it

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Introduction Condensed tannins (CT) are secondary metabolites useful in forage legumes to prevent protein degradation and ruminal bloating . The synthesis and accumulation of such compound is limited to seed coat in alfalfa and clover . Conversely , accumulation of CT in leaves occurs in some *Lotus* species and few other minor legumes . Understanding the genetic mechanism of CT synthesis and accumulation is the prerequisite to transfer the trait into the most important forage legumes . To this aim *L. corniculatus* plants polymorphic for CT accumulation in leaves were transformed with an anthocyanin activator *bHLH* transcription factor from maize (*Sn*) and a flavonoid repressor *MYB* transcription factor from *Fragaria vesca* (*FaMYB1*) . Here we report phenotypical effect of transformation and alteration in the expression of late genes of the pathway (DFR , LAR , ANR) previously characterised in our lab (Paolucci et al . , 1999 ; 2007) .

Materials and methods S50 and S41 plants , showing low and high leaf CT levels , respectively , were isolated from cv Leo and used as recipient genotypes for *Agrobacterium rhizogenes*-mediated transformation . Each transcription factor was driven by the constitutive promoter CAMV35S . Transformation and regeneration protocols and leaf-CT staining were performed according to Paolucci et al . (1999) , gene expression analysis by real time RT-PCR as described by Paolucci et al . (2007) .

Results and discussion Transformation modified CT content in leaves as reported in Table 1 . Transformation deeply modify CT levels . S41 depletes CT level after transformation with the negative regulator . S50 transformed with *Sn* increases CT . The effects of transformation is more evident in contrasting genotypes , i.e . *Sn* does not increase CT in S41 and *FaMYB1* poorly depletes CT levels in S50 (not shown) .

Table 1 CT levels in transformed and control plants .

	S50	S50 Sn	S41	S41FaMYB1
%CT cells	0.50	0.91	0.99	0.22

In Figure 1 it is reported the expression levels of key genes of the CT pathway . It is worth of mention that *Sn* enhances , whereas , *FaMYB1* reduces , the expression of the structural genes .

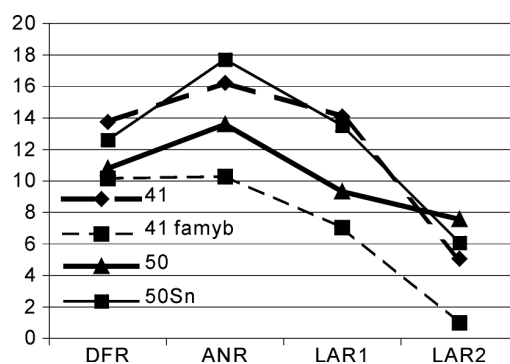


Figure 1 Gene expression .

Conclusion Results suggest the possibility of inducing CT accumulation in alfalfa by altering the expression levels of endogenous activator or repressor genes .

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