Identification of Putative *At*TT2 R2R3-MYB Transcription Factor Orthologues in Tanniferous Tissues of *L. Corniculatus* Var. *Japonicus* Cv *Gifu*

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Introduction R2R3-MYB plant transcription factors are sequence–specific DNA-binding proteins, which regulate the expression of specific gene(s) following the R2R3 DNA-binding domain interacting with the corresponding promoter sequence(s). The biosynthetic pathway leading to the production of anthocyanins has been demonstrated to be under MYB transcriptional regulatory control (Cone *et al*., 1986), while the accumulation of proanthocyanidins (PAs) in *Arabidopsis* seed coats is determined by the R2R3-MYB *At*TT2 (Nesi *et al*., 2001). Using an informatics approach, partial sequences of putative *AtTT2* orthologues have been identified and cloned from the forage legume *Lotus corniculatus* var. *japonicus* cv *Gifu*.

Materials and methods Total RNA and cDNA were prepared from flower, stem and leaf tissue harvested from *Lotus corniculatus* var. *japonicus* cv Gifu grown under glass. 180 bp fragments were amplified using degenerate PCR primers designed to consensus sequences within the MYB DNA-binding domain (Romero *et al*., 1998). The subsequent PCR products were cloned into *E. coli* via pGEMT easy prior to preparation for sequencing and analysis via DNA for windows and ClustalX.

Results We isolated and cloned candidate sequences *Lj*MYB38 and *L*jMYB72 from cDNA derived from stem and flower tissues. Multiple amino acid sequence alignment of the DNA binding domain of *Lj*MYB38 and *Lj*MYB72 revealed 81% and 70% identity and 87% and 88% respective similarity to *At*TT2 Within the amino acid sequence of the *Arabidopsis* basic helix-loop-helix interaction motif, spanning helices 1 & 2 of the R3 domain, the essential residue at position 20 was Asp-20 while *Lotus* sequences differed with Lys-20 (Figure 1).

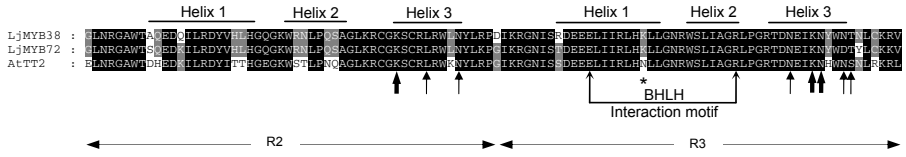

Figure 1 Multiple amino acid sequence alignment of the R2R3-MYB DNA-binding domain of *Arabidopsis* AtTT2 with R2R3-MYBs cloned from *Lotus*. Homologous regions are highlighted in black, while grey shading represents amino acids with similar physico-chemical properties. Arrows represent amino acids that interact with DNA with arrow thickness denoting stronger interaction. An essential difference in the amino acid sequence of the basic helix-loop-helix (BHLH) interaction motif is indicated by *.

Conclusions These data indicate that two putative orthologues to *At*TT2, the R2R3-MYB transcription factor required for PA biosynthesis in *Arabidopsis*, are expressed in the tanniferous stem and flower tissue of *L. corniculatus*. The presence of Lys-20 in the R3 domain of *Lj*MYB38 and *Lj*MYB72, as opposed to Asp-20 in *At*TT2 represents a significant alteration in the amino acid sequence of BHLH interaction motif (Zimmermann *et al*., 2004). Thus, the BHLH protein with which *Lj*MYB38 and *Lj*MYB72 could interact, may be distinct from the corresponding BHLH, *At*TT8 (Nesi *et al*., 2000), in *Arabidopsis* and might contribute to the differential tissue specific biosynthesis of PAs between these species.

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