Cloning and Sequence of a cDNA Encoding Phenylalanine Ammonia-Lyase from the Tropical Forage Legume *Stylosanthes humilis*

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PAL (EC 4.3.1.5) catalyzes the deamination of Phe to trans-cinnamic acid in the first step of the phenylpropanoid pathway, a key process for the synthesis of phenolic compounds in plants. In legumes, phenolic compounds have many important roles including flavonoid signal molecules, antibiotic phytoalexins for defense against pathogens, and precursors for the structural and defensive cell wall polymer lignin. In forages, there is a negative correlation between lignin content and digestibility, and this is particularly exacerbated in tropical forages, which generally have higher lignin contents (Minson, 1990). It has been demonstrated in transgenic tobacco that reduction of PAL activity achieved via co-suppression of PAL gene expression leads to a reduction in lignin content (Bate et al., 1994), but as yet this type of experiment has not been undertaken in a forage plant in which effects on digestibility can be tested. We are interested in manipulating lignin content and digestibility in tropical forages using antisense and ribozyme strategies in transgenic plants (McIntyre et al., 1995). In this work we have chosen to study *Stylosanthes humilis* Kunth as a model tropical pasture legume (McIntyre et al., 1995) because of its well-developed transformation system (Manners and Way, 1989) and its inbreeding and diploid genetics. As a starting point in this project we have cloned and sequenced a full-length cDNA encoding PAL from *S. humilis*.

A cDNA library of *S. humilis* (McIntyre et al., 1995) was screened under low-stringency hybridization conditions with a partial cDNA of PAL isolated from French bean (Edwards et al., 1985). The longest hybridizing cDNA clone was subcloned into pBluescript SK+ and sequenced. The cDNA comprises a 2443-bp insert with a 2145-bp open reading frame. There are 5' and 3' untranslated regions of 31 and 267 bp, with the latter including a 15-bp poly(A) 3' terminal region.

### Table 1. Characteristics of PAL cDNA from *S. humilis*

| Organism: | *Stylosanthes humilis* cv Paterson (common name: Townsville stylo). |
| Function: | Encodes a subunit of the homotetrameric enzyme PAL (EC 4.3.1.5), which catalyzes the conversion of L-Phe to trans-cinnamate and NH₃. |
| Source: | A cDNA library constructed in Agt10 using poly(A)+ RNA isolated from stems, petioles, and midribs of mature plants (McIntyre et al., 1995). |
| Techniques: | The cDNA library was screened with a 32P-labeled PAL cDNA isolated from French bean (Edwards et al., 1985). The selected cDNA was subcloned into the EcoR1 site of pBluescript SK+ and sequenced using the dideoxy chain termination method in a combination of manual and automated sequencing systems. Restriction-fragment-based subclones and synthetic oligonucleotide primers were used to ensure that both strands were sequenced completely. |
| Features of cDNA: | The cDNA insert is 2443 bp long and contains a 2145-bp open reading frame. There are 5' and 3' untranslated regions of 31 and 267 bp, with the latter including a 15-bp poly(A) 3' terminal region. |
| Features and Identification of Deduced Protein: | Contains 715 amino acid residues with a predicted Mr of 77,994 and a pl of 6.05. Comparisons of the amino acid sequences to other published PAL sequences showed high homologies, e.g. 87% homology to PAL of alfalfa (Govri et al., 1991), 86% to PAL of pea (Kawamata et al., 1992), 80% to PAL of tomato (Lee et al., 1992), and 79% to PAL of sweet potato (Tanaka et al., 1989). |
| Expression Characteristics: | Expression of a transcript of about 2500 nucleotides in length was detected by northern analysis in total RNA from stems, roots, and young expanding leaves but not in RNA from old, fully expanded leaves. |

PAL genes were present (Curtis et al., 1995). Northern analysis detected expression of PAL mRNAs in stems, young leaves, and roots but not in old leaves. This full-length cDNA clone should now permit the design of anti-
sense, sense, and ribozyme constructs to introduce into transgenic plants to study their effects on lignification and digestibility.

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The EMBL accession number for the sequence reported in this article is L36822.

LITERATURE CITED


Curtis MD, Cameron DF, Manners JM (1995) Molecular evidence that diploid *Stylosanthes humilis* and diploid *Stylosanthes hamata* are progenitors of allotetraploid *Stylosanthes hamata* cv. Verano. Genome (in press)


