16. Cloning and Characterization of Caffeic acid O-methyltransferase cDNA from Orchardgrass

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오차드그래스로부터 *comt* 유전자의 클로닝과 특성해명

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Key words : Caffeic acid *O*-methyltransferase (*comt*), Cell wall, Lignin, Orchardgrass, RNAi vector.

<Objective>

Lignin is an important chemical component of forage cell walls. It is essentially undigestible and inhibits rumen fermentation of forage cell wall polysaccharides. The composition of lignin is an important factor that influences cell wall degradability of forages. The inhibitor effects of lignin on forage digestibility depend on lignin monomer composition, lignin content and the extent of cross-linking to the cell wall polysaccharides. To improve digestibility of orchardgrass (*Dactylis glomerata* L.) by down-regulating a lignin biosynthetic enzyme through antisense suppression, a cDNA for caffeic acid O-methyltransferase (*comt*) was isolated and characterized.

<Materials and Methods>

- Material : Orchardgrass (*Dactylis glomerata* L.) - cv. Potomac
- Methods : cDNA encoding *comt* gene was amplified by RACE-PCR

  Southern and Northern blot analyses

  RNAi vector for *comt* down-regulation was introduced into the genome of orchardgrass.

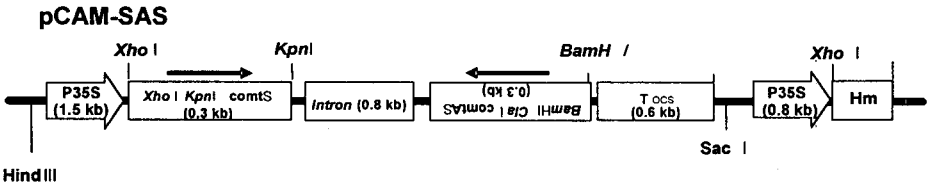

Fig. 1. Identification of RNAi *comt-1* transgene in transgenic plants.

<Results and Discussion>

The cDNA of caffeic acid O-methyltransfrase gene of orchardgrass was cloned by RACE-PCR. A full-length cDNA was isolated and shown to encode a caffeic acid O-methyltransferase (*OgComt-1*) deduced by its predicted amino acid sequence (Fig. 2). The amino acid sequence of *OgComt-1* was highly similar with those of *comt* cDNAs isolated from other plants. Southern blot analysis using *DgComt-1* cDNA as a probe indicated that *OgComt-1* is comprised of small gene family (Fig. 3). Northern hybridization analysis

revealed that the *Ogcomt-1* transcripts were accumulated in root, stem and leaf tissues (Fig. 4). Generation of transgenic plants containing RNAi expression vector (Fig. 1) for *Ogcomt-1* suppression is now in progress.

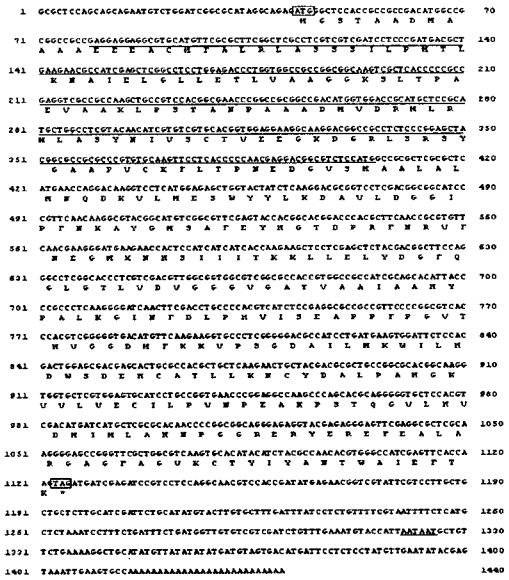

Fig. 2. Nucleotide and deduced amino acid sequence of cDNA for the COMT of orchardgrass plant. Boxes indicate the translation initiation termination codons. The site of polyadenylation signal and DNA fragment used for RNAi vector are underlined.

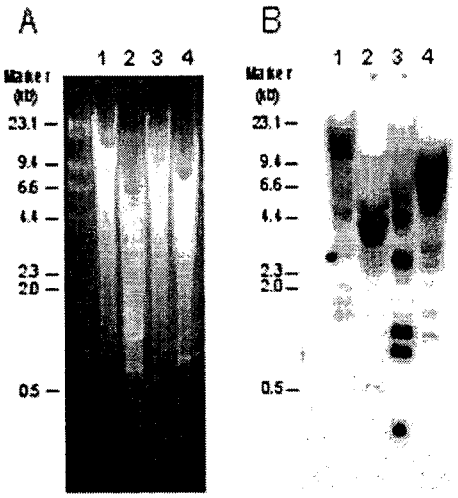

Fig. 3. Southern blot analysis of genomic DNA of orchardgrass. Genomic DNA (10 μg) was digested with *Eco*RI (lane 1), *Hin*dIII (lane 2), *Bam*HI (lane 3) or *Kpn*I (lane 4) and separated on an 0.8 % agarose gel. A, EtBr-stained gel after agarose gel electrophoresis. B, Southern blot. Full-length *Dgcomt-1* cDNA was used as a hybridization probe.

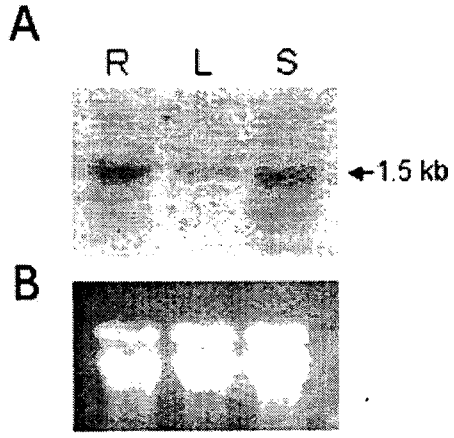

Fig. 4. Expression of the *Ogcomt-1* gene in different tissues of orchardgrass. A, Northern blot. B, EtBr-stained gel after agarose gel electrophoresis. R, root; L, leaf; S, stem.