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Isolation and characterization of 4-coumarate:CoA ligase (4CL) from *Zea mays* L. genomic DNA

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Objectives

We are interested in the regulation of lignin biosynthesis, and are using maize as a model plant to assess strategies for altering lignin quantity and quality to yield low lignin contents of forage crop. Lignin precursors (cinnamyl alcohols) are derived from phenylpropanoid metabolism. We have been investigating the last enzyme, 4-coumarate:CoA ligase (4CL), in the general phenylpropanoid pathway. Using antisense and RNAi vector, forage crop with low lignin contents will be produced using gene-delivery system as final goal. As first step, we finished the characterization of 4CL gene at genomic level.

Materials and Methods

Efficient PCR protocols havs been developed for identifying lignin biosynthesis genes (4CL) directly from genomic DNAs. For isolating 4CL gene from genomic DNAs, long PCR condition with DMSO were employed and amplified. This PCR product was cloned into T-vector (Promega) and characterized including intron and exon.

Results and Discussion

Long PCR with DMSO produced expected size of genes from genomic DNA with 4 different maize species and others (tobacco and tomato). Therefore, we recommend long PCR with addition of DMSO for amplification of specific genes using genomic DNA. After cloning into T-vector, DNA sequences were analyzed. According sequence data, full size of 4CL was 3.0 kb and consisted of over 2 introns. While there were reports about mRNA sequences about 4CL, full genomic sequences of 4CL genes from maize did not report so far. Therefore, this report is the first time full sequence of 4CL at genomic DNA. We are now working antisense and RNAi vector constructions. Currently, 1st exon region about 300 bases and 5′ and 3′ end DNA sequences (about 21 bases) are using for constructing vectors for down-regulating lignin biosynthesis.

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