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Isolation and characterization of ferulate 5-hydroxylase (F5H) from *Zea mays* L. genomic DNA

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Objectives

Ferulate 5-hydroxylase (F5H) is a cytochrome P450-dependent monooxygenase that catalyses the hydroxylation of ferulic acid, coniferaldehyde and coniferyl alcohol in the pathways leading to sinapic acid and syringyl lignin biosynthesis. 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 (Ferulate 5- hydroxylase: F5H) are derived from phenylpropanoid metabolism. As final goal, using antisense and RNAi vector, forage crop with low lignin contents will be produced using gene-delivery system. As first step, we finish the characterization of F5H gene at genomic level.

Materials and Methods

Efficient PCR protocols have been developed for identifying lignin biosynthesis genes (F5H) directly from genomic DNAs. Long PCR protocol addition with DMSO was employed and used for amplifying genomic DNA. This PCR product was cloned into T-vector (Promega) and characterized including intron and exon.

Results and Discussion

In our protocol, long PCR with DMSO showed excellent amplification from genomic DNA. With over 10 different primer sets, F5H gene could be amplified. Interestingly, two bands (about 2kb and 2.4 kb) appeared at the same time. Even though, we applied other maize species, result showed the same. Currently, we successfully cloned and analyzed one of them. According to DNA sequence data, full size was 2.0 Kb and over 1 intron. We are currently working on characterizing other F5H genes which is a little longer size. While there were reports about mRNA sequences about F5H gene, full genomic sequences did not report so far. This is the first report about full sequences of F5H at genomic level. We are currently 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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