F203

Expression Patterns and cDNA cloning of Aspartae Aminotransferase 2 from *Canavalia lineata*

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As a molecular biological study on the ammonia assimilation in the root nodules of *C. lineata,* expression patterns and cDNA cloning of aspartate aminotransferase 2 (AAT2) were investigated. Zymogram, immunoblot, and nortern analyses showed that AAT2 gene is expressed in the nodule-enhanced, developmentally regulated manner in the root nodules of *C. lineata.* A complete clone, pCLAAT2, was obtained by screening the cDNA library with 600 bp one-way PCR product as a pobe. DNA sequence and deduced amino acid sequence of 1736 bp cDNA in the clone shows 81~82.7% and 80~88% homology with legume plastid, respectively. Starting from ATG codon at 45 nt, this clone has one open reading frame encoding 466 amino acids of 51 kD protein with pI 8.8. The amino acid residues involved in the catalysis and cofactor binding are also conserved. The 5′ and 3′ untranslated region could form a stable stem-loop structure. In conclusion, The expression of AAT2 gene in the root nodule of *C. lineata* is thought to be regulated by putative secondary structure of 5′ and 3′ unontranslated region at mRNA stability and translational levels.

F204

Cloning of Aspartate Aminotransferase2 Gene from *Canavalia lineata*

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To determine the number of clones necessary for constructing a genomic library, genome size of *C. lineata* was estimated by measuring the nuclear volume. The DAPI-staining nuclear volume was 44.67 um^3/2C and genome size was estimated to be 1.34 x 10^9 bp/C DNA. A genomic library consisted of 3 x 10^5 original recombiants was constructed by inserting *Sau*3A1-digested 40 kb DNA into the pWE15 cosmid vector. Screening the the library with CLAAT2 as a probe, one genomic clone was selected. Among the *Eco*RI fragments of the clone, 3 kb and 1.5 kb *Eco*RI fragments that hybridized with the probe were subcloned into a plasmid vector and sequenced. DNA sequence of 4593 bp AAT2 gene is consisted of 818 bp of 5′ upstream region, 1398 bp of 11 AAT2 exons that is the same as in CLAAT2, 1966 bp of 10 itrons and 546 bp of 3′ flanking region. The sizes of exons and introns were diverse ranging from 39 bp to 265 bp, and from 73 bp to 681 bp, respectively. In the upstream region of the ATG start condon, CAAT box and TAAT box, essential element in transcription of eukaryotic gene, were found. Two consensus sequence motifs in several legume nodule-enhanced genes were found CTCTT once and AAGAT four times.