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Molecular characterization of a cDNA encoding Fructose-biphosphate aldolase from cultured cells of Co donopsis lanceolata

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Object

The gene encoding Fructose biphosphate aldolase (*Cl*FBPA) from the *Codonopsis lanceolata*, which is known to be involved in Glycolysis, Pentose phosphate cycle and Calvin cycle was cloned. And its structure uniqueness, comparative and phylogenic analysis were studied. The expression patterns of *Cl*FBPA were investigated in various whole plant tissues (L, leaf; S, stem; R, root) and in cultured cells (Ca, callus; Ar, adventitious root) by reverse transcriptase-polymerase chain reaction.

Materialsandmethods

Plant materials

We used the *Codonopsis lanceolata* which were grown in field on soil.

Methods ① Total RNA isolation and construction of a cDNA library

② Nucleotide sequencing and sequence analysis

③ Quantitative RT-PCR analysis

ResultsandDiscussion

The EST clones homologous to fructose biphosphate aldolase gene were obtained from a cDNA library constructed with the 4-year root of *Codonopsis lanceolata* and the gene was named a *Cl*FBPA. The cDNA of *Cl*FBPA consists of a 1356-bp fragment with the translational start site of the major open reading frame (ORF) at nucleotide 54 and the TAA stop site at nucleotide 1130. *Cl*FBPA encoding a polypeptide of 358 amino acid residues were the 86,5-48,3% identities to FBPA sequences from other plant species. The highest similarities of a *Cl*FBPA encoding FBP ald were with FBP alds from *Persea americana* (86,5 %), *Mesembryanthemum crystallinum* (84,9%) and *Arabidopsis thaliana* (84,9%). A multiple alignment revealed the several regions of high homology as illustrated . The phylogenic analysis of 12 different plant FBPAs has been carried out using a Clustal X program.

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The expression patterns of *Cl*FBPA were investigated in various whole plant tissues (L, leaf; S, stem; R, root) and in cultured cells (Ca, callus; Ar, adventitious root) by reverse transcriptase-polymerase chain reaction (RT-PCR) (Fig. 1). For RT-PCR, primer which specific for *Cl*FBPA gene was used. The expression analysis showed that there is a considerable variation in the levels of *Cl*FBPA expression in different tissues. *Cl*FBPA was strongly expressed in stems, adventitious roots and callus, while expressed weakly in leaves and roots. In the next stage of our study we will identify the expression patterns of *Cl*FBPA using field grown leaves under anoxygen, salt, oxidative, light and dark stresses.

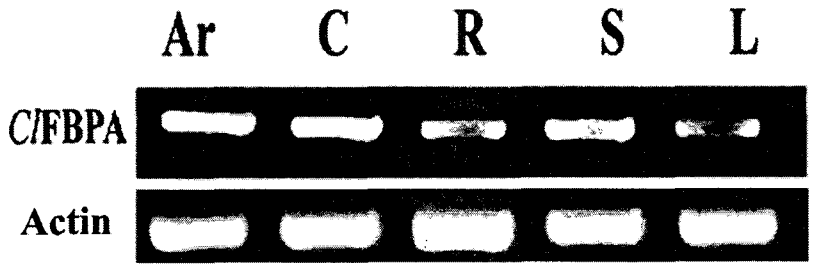

Fig.1.Expression patterns of *Cl*FBPA in various tissues of whole plant and cultured cells of *Codonopsis lanceolata*. Total RNAs were extracted from adventitious root (Ar), callus (C), root (R), stems (S) and leaves (L). Reverse transcription of aliquots (200 ng) of total RNA was carried out, and conventional PCR was performed on the cDNA using gene-specific primers. Actin was used as a control.