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Plant Gene Register

Isolation and Characterization of a Drought-Induced Soybean cDNA Encoding a D95 Family Late-Embryogenesis-Abundant Protein[1]

Niranjan Maitra and John C. Cushman*

Department of Biochemistry and Molecular Biology, 350 Noble Research Center, Oklahoma State University, Stillwater, Oklahoma 74078

LEA proteins represent several families of proteins that accumulate to high levels in maturing seeds. The expression of these proteins is under developmental control, but they may also be expressed in dissected embryos exposed to exogenous ABA (Hughes and Galau, 1991). Many *Lea* genes are also expressed in vegetative tissues exposed to exogenous ABA as well as water, osmotic, and low-temperature stress (Bray, 1993). The majority of LEA proteins are hydrophilic, display a preponderance of specific amino acids, and are localized to the cytoplasm (Dure, 1993b) and the nucleus (Goday et al., 1994). LEA proteins have been proposed to participate in various protective roles during times of severe desiccation stress. Their presence in young wheat seedlings has also been correlated with desiccation tolerance (Reid and Walker-Simmons, 1993). Several families of LEA proteins (D19 and D113) are predicted to bind water. This water-binding capacity is thought to prevent complete desiccation of tissues and thereby preserve protein and membrane integrity (Dure, 1993b). Another diverse LEA protein family (D11) may function to preserve protein structure through the renaturation of unfolded proteins (Dure, 1993a). Other LEA protein families (D7 and D29) may be involved in sequestering ions that accumulate in desiccating tissues (Dure, 1993b).

Here we report the isolation and expression of a conserved cDNA clone encoding a LEA protein from water-stressed soybean leaves belonging to the D95 family. The predicted soybean *Lea* gene product shares 78.8 and 68.2% amino acid identity with the predicted cotton *Lea14* (D95) (Galau et al., 1993) and resurrection plant pcC27–45 gene products, respectively (Piatkowski et al., 1990). This family of proteins shares no amino acid sequence similarity with other LEA proteins. In contrast to other LEA proteins, which are very hydrophilic, D95 LEA proteins display a slightly hydropathic character throughout the length of the protein (Galau et al., 1993). The N-terminal 40 amino acids of the soybean LEA14 homolog form a predicted mildly hydrophilic α helix with the remainder of the protein evenly divided between hydrophobic and hydrophilic regions (Table I). Helical wheel analysis of the first 36 amino acid residues further suggests that the N-terminal α helix is amphipathic in character. The predicted secondary structure of the D95 LEA protein family is vaguely reminiscent of the D113 LEA family, members of which are characterized by a conserved, N-terminal α helix of 60 to 80 amino acids, with the remainder of the protein in a random coil (Dure, 1993b).

The expression characteristics of the soybean D95 *Lea* cDNA described here resemble those described for other D95 family members. The soybean cDNA hybridizes with a 0.8-kb transcript that is up-regulated by water stress in both leaves and roots of soybean. Interestingly, the transcript was found to be more abundant in roots than in leaves. Similarly, the cotton *Lea14* gene (D95) was found to cross-hybridize to an abundant 0.8-kb message in cultured embryos and seeds of soybean and tobacco, respectively (Jakobson et al., 1994). The cotton *Lea14* transcript was also highly induced in mature leaves of water-stressed cotton (Galau et al., 1993). The related gene (pcC27–45) from the resurrection plant, *Craterostigma plantagineum,* is also highly expressed in desiccated leaf and root tissue (Piatkowski et al., 1990). In transgenic tobacco, the pcC27–45 5′ flanking region drove reporter gene expression in naturally occurring desiccation-tolerant tissues such as mature embryos and pollen (Michel et al., 1993). The expression of the pcC27–45 cDNA in transgenic tobacco under the control of a triplicated cauliflower mosaic virus 35S promoter did not have any significant effect on desiccation-related physiological functions as measured by an ion leakage test or on photosynthesis and transpiration rates when stressed transgenic tobacco plants were compared to untransformed plants (Iturriaga et al., 1992). It is possible that desiccation-protective effects of this conserved D95 LEA family will be evident only when expressed at very high levels, in conjunction with other LEA proteins, or in conjunction with osmotic adjustment strategies such as increased levels of compatible solutes. Until more definitive experiments are performed, the function of this water stress-induced LEA family in stress tolerance will remain speculative.

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* Corresponding author; fax 1–405–744–7799.

Abbreviation: LEA, late-embryogenesis-abundant.

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Table I. *Characteristics of a cDNA clone encoding a D95 family LEA protein from soybean*

Organism:
Glycine max L. cv Essex.
Location:
Nuclear genome.
Function:
Probable desiccation-protectant protein.
Cloning and Sequencing Techniques:
cDNA library in λ-UNIZAP XR (Stratagene) prepared from polyadenylated mRNA isolated after 24 h of PEG-8000 stress from leaf tissue of *G. max* cv Essex (N. Maitra and J.C. Cushman, unpublished data). The cDNA library was screened at low stringency with a ^{32}P-labeled insert from a cDNA encoding cotton *Lea14* (D95) (gift from G.A. Galau). The complete nucleotide sequences of both strands were determined on an ABI 373A automated DNA using the Prism Ready Reaction Dyedeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).
Sequence Identification:
Nucleotide and amino acid sequence comparisons with GenBank/EMBL data bases were conducted using the EMBL FASTA E-Mail server and retrieved using the NCBI RETRIEVE E-mail server.
Features of the Transcript:
The cDNA clone is 596 nucleotides in length with a 20-nucleotide 5′ untranslated leader sequence and a 121-nucleotide 3′ untranslated trailer sequence including a poly(A) tail of 19 residues. Two polyadenylation signals having the AATAAA consensus sequence are located 72 and 64 nucleotides upstream of the start of the poly(A) tail.
Features of the Deduced Amino Acid Sequence:
The *Lea* open reading frame of 152 codons predicts a protein of 16,476 D with an isoelectric point of 4.8. Based on the secondary structural prediction of the University of Wisconsin Genetics Computer Group programs PEPTIDESTRUCTURE and HELICALWHEEL, a putative amphiphilic α-helical structure comprises the N-terminal 36 amino acids with the remainder of the protein assuming a hydropathic, random coil.
Cell-Type/Expression:
The soybean *Lea* cDNA clone detects a 0.8-kb transcript on northern blots that is expressed in leaf and root tissue in a drought-inducible manner. Expression is enhanced 3-fold in both leaves and root tissue upon exposure to −1.2 MPa water potential imposed by adding PEG-8000 to the hydroponic medium. Maximal transcript accumulation occurs in leaves 6 to 12 h after exposure to water stress and in roots 24 h after exposure to water stress. Transcripts are about 3-fold more abundant in roots than in leaves.
Cellular Localization:
Cytoplasm.

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The GenBank accession number for the sequence reported in this article is U08108.

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