Structure, organization and expression of two closely related novel \textit{Lea} (late-embryogenesis-abundant) genes in \textit{Arabidopsis thaliana}

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Abstract

We have isolated and sequenced a 9.5 kb genomic region from \textit{A. thaliana}, located on chromosome 2, which contains two tandemly arranged closely related genes (AtM10 and AtM17) coding for a new family of LEA proteins. The deduced proteins have a molecular mass of 11 and 29 kDa, respectively, are extremely hydrophilic except at their N-termini and share 70% amino acid (aa) identity. A 47 aa motif containing a 6-cysteine domain is present once in AtM10 and four times in AtM17. The short intergenic region, the identical position of the intron and the overall sequence homology suggest that these two genes evolved through a duplication event. This conclusion is supported by the presence of two homologous strictosidine synthase-like (pseudo)genes downstream from AtM17 and AtM10. Expression studies, using AtM10 and AtM17 cDNAs, revealed that both transcripts accumulate exclusively in seeds from late embryogenesis until two days after imbibition. Expression of both genes in young seedlings is repressed during ABA, salt or drought treatment, whereas a cold stress induces the expression of AtM17 only. \textit{In situ} hybridization revealed that AtM10 transcripts are detected throughout the embryo while those of AtM17 are more localized to cotyledon cells.

Introduction

In most species of higher plants, seed development can be divided into three main stages: the first is characterized by a series of mitotic divisions of the zygote and cell differentiation leading to pattern formation. Only a few genes have been described that are specifically expressed during this early phase of embryogenesis (Thomas, 1993). The maturation stage corresponds to extensive cell and organ expansion. The axis and cotyledon(s) reach their maximum size while an abundant accumulation of reserves such as carbohydrates, proteins and oils occurs in most of the embryonic cells to be used later by the new seedling. The genes coding for storage proteins, trypsin inhibitors or lectins have been widely studied in numerous plant species. They are known to be expressed abundantly and exclusively in developing seed during the maturation phase and have been designated with the abbreviation MAT (Hughes and Galau, 1989; Parcy \textit{et al.}, 1994). The final developmental stage, late embryogenesis, is marked not only by water loss (as much as 90%), but also by a great change in protein synthesis. In many plants, the embryo then becomes dormant with an extremely reduced metabolism. This desiccated state allows survival of the seeds under unfavourable conditions and their wide dispersion. At this stage, the MAT mRNAs completely disappear whereas a group of late-embryogenesis-abundant (LEA) mRNAs continues to accumulate in drying seed and persists until germination. This class of genes was first found in cotton

\textsuperscript{The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AF076979.}
Materials and methods

Plant material and growth conditions

Arabidopsis thaliana L. (ecotype Columbia) plants were grown on a mixture of sterile soil and vermiculite under controlled environment at 20 °C, 50% relative humidity and with a photoperiod of 16 h light/8 h dark. For germination assays, seeds were placed in Petri dishes containing filter paper moistened with sterile water and maintained at room temperature under continuous illumination. After 5 days of imbibition (T0), seedlings were incubated at 4 °C (for cold treatment) or transferred to a dry filter (for drought treatment) or to a filter soaked with 60 μM ABA or 0.4 M NaCl. Control plantlets were kept under initial conditions for 9 days and irrigated as needed. Seedlings were collected at various intervals. All tissue was flash-frozen in liquid nitrogen then stored at −70 °C until used.

RNA isolation and northern analysis

RNA extractions were performed according to Kay et al. (1987). Ten μg of total RNA was size-fractionated on 1% agarose/formaldehyde gels and transferred onto nylon membranes (Hybond-N, Amersham) by capillary blotting with 20× SSC solution. Hybridizations were carried out at 42 °C in 50% formamide, 5× SSC, 2× Denhardt’s solution, 0.5% SDS and 100 μg/ml denatured calf thymus DNA. The filters were washed twice with 2× SSC for 15 min at room temperature and twice with 1× SSC, 0.5% SDS for 30 min at 65 °C. After exposure to X-rays films, the probe was stripped from the filters for subsequent hybridization with a radish 18S rRNA probe, pRG3 (Grellet et al., 1989), to control the relative amounts of rRNA. [α-32P]-dCTP-labelled probes were prepared from gel-purified cDNA inserts using the Megaprime DNA labelling system (Amersham).

DNA isolation and Southern blot analysis

Genomic DNA was prepared by grinding young leaves in liquid nitrogen and extracting according to the CTAB procedure described by Doyle and Doyle (1990). Five μg of total DNA was digested with different restriction endonucleases and the resulting fragments were fractionated on 0.8% agarose gels, transferred onto Hybond-N membranes and hybridized according to the manufacturer’s instructions. The conditions of washing were the same as described above.