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Update section

Short communication

Sequence analysis of a cDNA encoding a Group 3 LEA mRNA inducible by ABA or dehydration stress in wheat

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Abstract

A cDNA clone (pMA2005) of a Group 3 LEA (late embryogenesis abundant) protein has been sequenced from wheat. The wheat cDNA clone codes for a protein with ten tandem repeats of an 11 amino acid sequence and has homology to other Group 3 LEAs reported in barley, carrot, cotton and rape (L. Dure *et al.*, Plant Mol Biol 12: 475–486, 1989). The deduced amino acid sequence indicates that the wheat protein has a molecular weight of 23000 and is a basic, hydrophilic protein. Northern analysis with the cDNA clone shows that dehydration of wheat shoot tissue results in increased transcript levels that correlate with increases in endogenous ABA.

Introduction

LEA mRNAs and proteins accumulate in embryonic tissue during seed maturation [4]. LEA mRNAs and proteins can also be induced by the application of abscisic acid (ABA) or by tissue dehydration. A protective role for these proteins during plant tissue dehydration that could be mediated by ABA has been proposed [2, 5]. LEA proteins deduced from several higher plant cDNA and/or genomic DNA sequences have been compared and fall into three groups based on sequence homologies [5]. In wheat a cDNA clone encoding a Group 1 LEA protein, Em, has been characterized [7]. We report here the sequence analysis of cDNA clone pMA2005 that encodes a Group 3 LEA mRNA from wheat and induction of the mRNA in dehydration-stressed shoot tissue. The deduced protein sequence of the pMA2005 clone contains tandemly repeated 11

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*The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X56882.

amino acid stretches similar to that reported for other Group 3 LEA proteins from barley, carrot, cotton and rape [5].

Materials and methods

A wheat embryonic axes cDNA library, constructed in Lambda Zap (Stratagene, La Jolla, CA) was screened and the cDNA clone pMA2005 selected by differential screening as previously described [8]. Both strands of the pMA2005 cDNA were sequenced in their entirety using a deletion series (Erase-a Base System, Promega) and dideoxy chain termination (Sequenase Version 2.0 DNA Sequencing Kit, United States Biochemical Corporation) of double-stranded DNA templates. The sequencing data were analyzed using the UWGCG Sequence Analysis software package (version 6.2) [3] with the assistance of the VADMS Center, Washington State University. Poly(A)$^+$ RNA was obtained from isolated wheat embryonic axes or from shoot tissue [1]. Northern analysis was con-

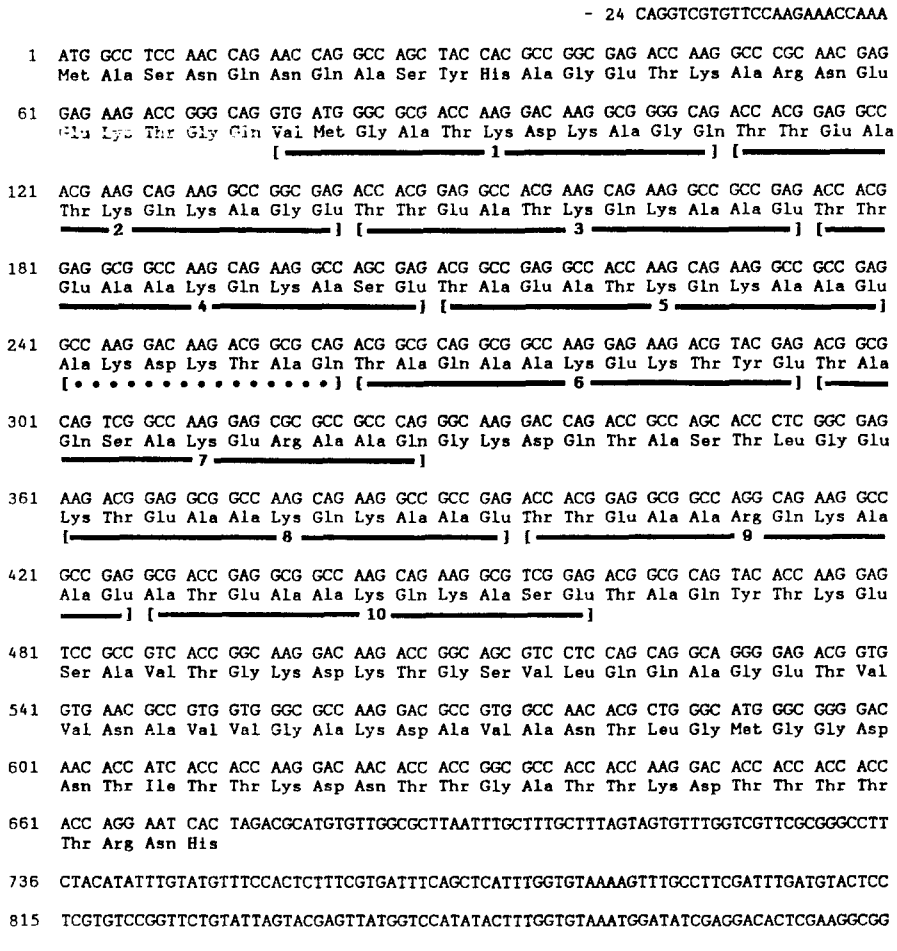

Fig. 1. Nucleotide sequence of pMA2005. Dideoxy sequencing data of a 934 bp cDNA encoding a Group 3 LEA protein in wheat is shown along with the deduced amino acid sequence. The ten tandem repeats of 11 amino acid tracts are bracketed, underlined and numbered. The truncated version of the repeat is shown underdotted and the unrelated 11 amino acid stretch is located between repeats 7 and 8. A probable polyadenylation signal, AATAAA, is underlined, though a poly(A) tail was not present in the clone.

ducted as previously described [8]. ABA was extracted from shoot tissue and measured by immunoassay using a monoclonal antibody to (+)ABA as previously described [9].

Results and discussion

The cDNA clone pMA2005 was obtained from a cDNA library prepared from ABA-treated embryonic axes of wheat (*Triticum aestivum*) cv. Brevor [8]. The cDNA clone was selected by differentially screening with cDNA prepared from dormant and nondormant axes [8]. In Fig. 1 the nucleotide sequence and deduced amino acid sequence of pMA2005 are shown. The sequenced cDNA has a length of 934 bp, which includes an open reading frame encoding a protein of 224 amino acids plus 5' and 3' flanking regions. The deduced protein sequence of the wheat clone shows homology to the Group 3 LEA proteins. Highest homology was found to the barley clone pHVA1 [6] (91% at the nucleotide level, 95% at the amino acid level). The repeating 11 amino acid tract, typical of Group 3 LEA proteins, is repeated 10 times in the wheat clone (Fig. 1) and 9 times in barley [6]. Both the wheat (Fig. 1) and barley [6] clones contain an identical truncated version of the repeating unit that consists of only 7 of the 11 amino acids. Further, for both the wheat and barley clones the repeating sequences are interrupted by one 11 amino acid stretch that has no homology with the repeating sequence. This unrelated stretch in the wheat clone (Fig. 1) is identical to that of barley [6] except for two amino acid substitutions. Analysis of the entire amino acid sequence shows a hydrophilic, basic polypeptide with a molecular weight of 23000.

Northern analysis with clone pMA2005 has shown that gene expression is high in embryos from mature wheat seed and that this message can be enhanced by incubation of embryos in ABA [8]. To test whether the message accumulates in shoot tissue when ABA levels are elevated by water stress, we measured transcript levels in dehydrated shoot tissue. As shown in Fig. 2A, dehydration of wheat shoots also induces high

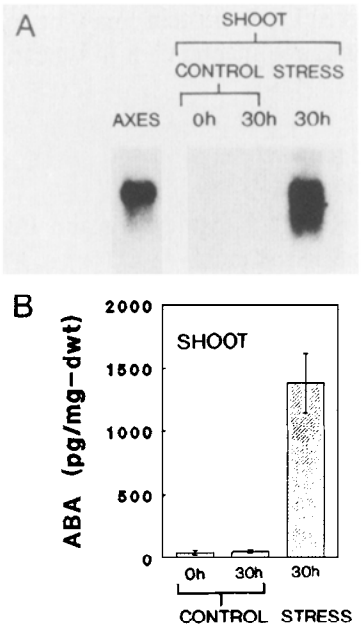

Fig. 2. Northern analysis of the wheat Group 3 LEA transcript and ABA measurement in wheat. A. Two-day old wheat seedlings were slowly dehydrated by placement in open petri dishes over 25% glycerol (v/v) in a desiccator and incubated at 22 °C for 30 hours. Control seedlings were placed on moistened blotter paper and incubated under the same conditions. Poly(A)$^+$ RNA (0.5μg) obtained from isolated mature wheat embryonic axes (non-stressed) or from the shoot tissue was separated by formaldehyde-agarose gel electrophoresis and transferred to a nylon membrane. Northern analysis conducted with the pMA2005 cDNA revealed transcripts of approximately 1 kb. B. ABA levels in control tissue at 0 and 30 hours and in shoot tissue dehydrated for 30 hours. ABA was measured by immunoassay [9].

levels of pMA2005 mRNA compared to hydrated controls. Induction of the wheat clone transcript correlates with endogenous increases in ABA in dehydrated shoot tissue (Fig. 2B). An antibody has been produced against a fusion protein developed from the cDNA sequence of pMA2005. Upon dehydration a protein that is strongly cross-reactive with the antibody accumulates in wheat shoots. The protein is not present in unstressed shoots (J.L. Ried and M. Walker-Simmons, unpublished data). While the function of the pMA2005 protein product is not yet certain, the induction of gene expression for this highly hydrophilic protein in dehydrated tissue suggests that

this Group 3 LEA protein may be part of a drought tolerance mechanism in wheat.

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