EXPRESSION AND ISOLATION OF LEA GENE IN DESICCATION-TOLERANT MICROSPORE-DERIVED EMBRYOS IN *BRASSICA NAPUS* L. AND *BRASSICA CAMPESTRIS* L.

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ABSTRACT

Expression of LEA gene in *B. napus* and *B. campestris* microspore-derived embryos which were induced desiccation tolerance by abscisic acid (ABA) was examined. Northern analysis indicated that LEA gene expressed in the desiccation tolerant embryos, but not the desiccation sensitive ones. LEA transcripts started to accumulate within 12h of ABA treatment. The DNA sequences of cDNA of LEA genes isolated from microsore-derived embryos were determined. The predicted amino acid sequence of a clone of *B. napus* had 95% of homology with Lea 76, and that of *B. campestris* had 70% with Lea 76.

INTRODUCTION

Microspore-derived embryos of *Brassica* species (*B. napus*, *B. oleracea* and *B. campestris*) which are desiccated level equivalent to true seeds have been reported to be induced desiccation tolerance, when they were treated with abscisic acid (ABA) (Takahata et al. 1992; 1993, Brown et al. 1993, Wakui et al. 1994). These phenomena were found in several other plants such as alfalfa (Senaratna et al. 1989; 1990) and carrot (Iida et al. 1992). However, the mechanism by which ABA induced desiccation tolerance is not clear.

LEA (late embryogenesis abundant) gene(s) are suggested to play an important role in induction of desiccation tolerance of seeds (Dure et al. 1989, Harada et al. 1989). It is reported that LEA mRNAs increase at the beginning of zygotic embryo desiccation and are regulated by ABA.

In the present study, we describe the LEA gene expression and nucleotide sequence of LEA cDNA clone on *B. napus* and *B. campestris* microspore-derived embryos induced desiccation tolerance.

EXPERIMENTAL

LEA gene expression on microspore-derived embryos

To examine LEA gene expression on microspore-derived embryos, total RNA was isolated from the embryos which were treated with ABA (desiccation tolerant) and without ABA (desiccation sensitive) for one week. Northern bolt analysis was carried out using *B. napus* Lea 76 clone as a probe. Figure 1 shows that accumulation of LEA mRNA is detected in desiccation tolerant embryos, but not in desiccation sensitive ones. LEA mRNA expressed in the embryos of *B. campestris* as well as those of *B. napus*.

Though exposure of one week to ABA was needed to induced complete desiccation tolerance, only 24h exposure were found to be sufficient to induce some desiccation tolerance (Brown et al. 1993, Takahata et al. 1993). We examined LEA mRNA accumulation during one week ABA treatment. The mRNA accumulation was detected within 12h and reached maximum level after 48h (Figure 2). These results were similar to those of zygotic embryos that LEA mRNA was detected after 24h of ABA treatment (Harada et al. 1989).

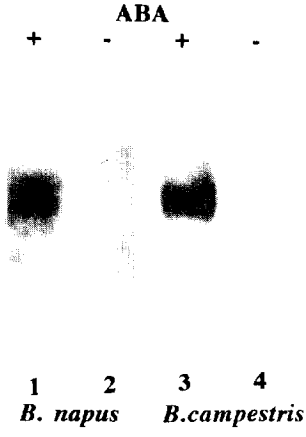

Figure 1. Northern blot analysis of total RNA from microspore-derived embryos of *B. napus* (1, 2) and *B. campestris* (3, 4) probed with Lea 76. +, Treated with 100μM (*B. napus*) and 10μM ABA (*B. campestris*) for 7 days; -, Treated without ABA.

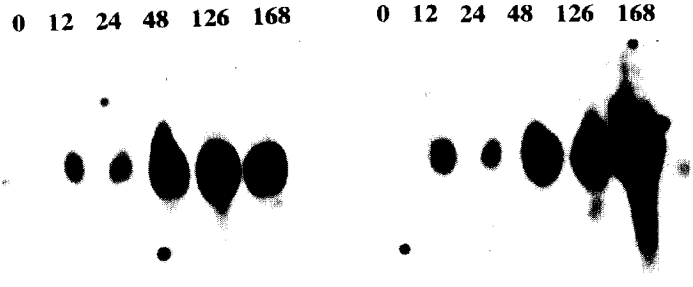

Figure 2. Northern blot analysis of total RNA from microspore-derived embryos of *B. napus* (left) and *B.campestris* (right) probed with Lea 76. Microspore-derived embryos of *B. napus* and *B. campestris* were treated with 100μM and 10μM ABA, respectively. 0-168 indicate hours of ABA treatment.

Isolation and nucleotide sequence of LEA cDNA clone

A cDNA library was constructed in λgt10 using cDNA derived from poly(A)$^+$ RNA from desiccation tolerant microspore-derived embryos of *B. napus* and *B. campestris*. Screening of the library using Lea 76 DNA clone resulted in each 3 clones in the two species. Nucleotide sequence was determined on each one of the clones of *B. napus* and *B. campestris*. The nucleotide sequence of 1098bp cDNA insert of *B. napus* and of 672bp cDNA of *B. campestris* included the poly (A) tail, however, the first ATG beginning codon presented in only that of *B. napus*. The cloned cDNA of *B. napus* contained a complete open reading frame of 846bp. The predicted amino acid sequence of this clone has 95% of homology with Lea 76 gene. The presence of two residue repeats and largely hydrophilic region within polypeptide was similar to that of Lea 76.

The cloned cDNA of *B. campestris* contained an open reading frame of 570bp, which lacked a part of an open reading frame. The deduced amino acid sequence of this clone was 70% and 67% homologous with Lea 76 and the clone of *B. napus*, respectively.

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