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Molecular and functional characterization of *CaLEA6*, the gene for a hydrophobic LEA protein from *Capsicum annuum*

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Abstract

We used differential screening to isolate a full-length dehydration-responsive cDNA clone encoding a hydrophobic late embryogenesis abundant (LEA)-like protein from PEG-treated hot pepper leaves. Named *CaLEA6* (for *Capsicum annuum LEA*), this gene belongs to the atypical hydrophobic LEA Group 6. The full-length *CaLEA6* is 709 bp long with an open reading frame encoding 164 amino acids. It is predicted to produce a highly hydrophobic, but cytoplasmic, protein. The putative M_r of *Ca*LEA6 protein is 18 kDa, with a theoretical p*I* of 4.63. Based on our Southern blot analysis, *CaLEA6* appears to exist as a small gene family. *CaLEA6* was not expressed prior to any treatment, but its transcript was rapidly and greatly increased following trials with PEG, ABA, and NaCl. Chilling also induced its rapid induction, but to a much lesser extent. Accumulation of *Ca*LEA6 protein occurred soon after NaCl applications, but considerably delayed after treatment with PEG. Tobacco plants that overexpressed *CaLEA6* showed enhanced tolerance to dehydration and NaCl but not to chilling, as defined by their leaf fresh weights, Chl contents, and the general health status of the leaves. Therefore, we suggest that *Ca*LEA6 protein plays a potentially protective role when water deficit is induced by dehydration and high salinity, but not low temperature.

Keywords: Abiotic stresses; LEA proteins; Salinity; Transgenic plants

1. Introduction

Abiotic stresses, such as water deficit, increased soil salinity, and extreme temperature, can limit plant growth and productivity (Yancey et al., 1982). Therefore, plants have developed various strategies for coping with unfavorable conditions. These early events of adaptation include the sensing and subsequent signal transduction to initiate various metabolic activities through the induction of stress-responsive genes (Bray, 1997). Studies on molecular responses to specific stresses have involved two-dimensional PAGE and differential screening of cDNA libraries. These methods have led to the identification of several newly synthesized molecules and multiple changes in gene expression as well as the isolation of many induced genes under those conditions (Riccardi et al., 1998).

Water deficit caused by dehydration is the most common abiotic stress to which land plants are exposed, affecting growth and development by altering their metabolism and gene expression (Bray, 1997; Ingram and Bartels, 1996). Therefore, during such periods, plants

Abbreviations: ABA, abscisic acid; bp, base pairs; cDNA, DNA complementary to RNA; Chl, chlorophyll; DW, distilled water; GST, glutathione-*S*-transferase; h, hour; kb, kilobase(s); kDa, kilodalton; LEA, late embryogenesis-abundant; min, minute; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; PEG, polyethylene glycol; pfu, plaque-forming units; p*I*, isoelectric point; poly(A), polyadenylation; rRNA, ribosomal RNA; UTR, untranslated region.

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undergo many physiological changes and induce a large number of genes for adaptation (Ingram and Bartels, 1996). Water deficit is also triggered by osmotic stress due to high salinity or chilling. This deficit often leads to increased cellular levels of ABA (Zeevaart and Creelman, 1988). Consequently, many dehydration-responsive genes can be induced by high salinity, chilling, or ABA applications, although some are mediated independently (Gilmour and Thomashow, 1991). Various dehydration-induced genes have been identified in a wide range of plant species; their functions have been predicted from their deduced amino acid sequences. Nevertheless, the exact functions of some genes are still unknown. Because the molecular basis for plant tolerance to water stress is not completely understood, functional characterization of the genes induced during dehydration is a next logical step in addressing the plant's molecular mechanism for responding to water stress.

Under water-deficit conditions, a typical change in gene expression is the induction of genes involved in the synthesis of various osmolytes and low-molecular-weight proteins, e.g., dehydrins and LEA proteins (Ingram and Bartels, 1996; Ramanjulu and Bartels, 2002). Among these genes, those that encode LEA proteins are of special interest because they presumably play a protective role under such conditions (Baker et al., 1988; Ingram and Bartels, 1996; Xu et al., 1996). LEA proteins are a large group of plant proteins that are heavily synthesized and stored during seed maturation (Bray, 1997; Ingram and Bartels, 1996). First identified during the desiccation phases of seed development, they reportedly protect specific cellular structures or ameliorate the effect of drought stress by sequestering ions and maintaining minimum cellular-water requirements (Baker et al., 1988; Dure et al., 1989).

LEA proteins are classified into seven subgroups, based on their amino acid sequence homology and specific motifs, which presumably undertake different functions during periods of water deficit (Dure, 1993; Ramanjulu and Bartels, 2002). For example, wheat Em, a Group 1 LEA protein, induces enhanced tolerance against osmotic stress in yeast (Swire-Clark and Marcotte, 1999). Group 2 LEA proteins from tomato and Group 3 LEA proteins from wheat seedlings are also correlated with dehydration tolerance (Ried and Walker-Simmons, 1993; Zhang et al., 2000). Likewise, transgenic rice that overexpresses *HVA1*, a Group 3 LEA gene from barley, shows increased tolerance against both water and salt stresses (Xu et al., 1996). A Group 4 LEA gene, tomato *le-25*, also confers increased tolerance to yeast during salt and chilling stresses (Imai et al., 1996). Most of these LEA proteins are cytosolic and hydrophilic, and contain random coil or α-helices (Soulages et al., 2002; Swire-Clark and Marcotte, 1999; Zhang et al., 2000). However, some atypical LEA proteins, e.g., soybean D95-4, cotton Lea14-A, tomato ER5, tomato Lemmi9, and pcP27-45 from the resurrection plant, are hydrophobic (Galau et al., 1993; Maitra and Cushman, 1994; Piatkowski et al., 1990; Van der Eycken et al., 1996; Zegzouti et al., 1997). As such, they are likely to function differently from those that are hydrophilic, despite their involvement in the dehydration response (Zegzouti et al., 1997). Nevertheless, their exact roles are still unknown.

In this report, we present the molecular and functional characterization of *CaLEA6*, a new hot pepper gene that encodes a hydrophobic LEA protein. We also demonstrate here that *CaLEA6*, which specifically expressed in the leaves, responds to various dehydration-related abiotic stresses. Furthermore, we examined the level of tolerance to drought and salt stresses in transgenic tobacco plants that overexpress *CaLEA6* to determine whether *CaLEA6* might act as a stress protectant under those dehydration conditions or not.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of hot pepper (*Capsicum annuum* L.) and tobacco (*Nicotiana tabacum* L. var SR1) were sterilized for 30 min in 1% (v/v) sodium hypochlorite solution. They were then placed in pots filled with soil (Bioplug #2, Hungnong Seeds, Korea) and imbibed overnight in DW to promote germination. The resultant seedlings were raised for 4–5 weeks in a growth chamber maintained at 25±1 °C, under a 16-h photoperiod. Light was provided from four banks of Truelite II fluorescent lamps (Durotest, USA) at an intensity of 150 μmol m^{-2} s^{-1}.

2.2. Dehydration, chilling, salt, and ABA treatments

After the young pepper plants were carefully taken from their pots and washed in running water to remove the soil, the dehydration treatment was conducted by immersing their roots for up to 48 h in 10% (w/v) PEG-6000 solution, which solution is chemically inert and is known to lower leaf water potential in a time-dependent manner (Michel, 1970; Lee et al., 2004). For our salt treatment, the roots of the pepper plants were immersed in a 400 mM NaCl solution for up to 60 h. For the chilling treatment, plants were placed in a dark chamber maintained at 4 °C under high humidity to prevent wilting. Finally, the ABA treatment was applied by immersing the petioles of detached leaves in a 100 μM ABA solution. After each type of treatment, the leaves were immediately harvested and frozen in liquid nitrogen. Samples were stored at −80 °C until further use.

In another set of experiments, dehydration of whole plants was administered by either withholding water to the pots or immersing their roots in a Hoagland solution containing 10% (w/v) PEG-6000. Meanwhile, dehydration of their detached leaves is accomplished by air-drying in the