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Overexpression of *Late Embryogenesis Abundant 14* enhances *Arabidopsis* salt stress tolerance

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ABSTRACT

Late embryogenesis abundant (LEA) proteins are implicated in various abiotic stresses in higher plants. In this study, we identified a LEA protein from *Arabidopsis thaliana*, AtLEA14, which was ubiquitously expressed in different tissues and remarkably induced with increased duration of salt treatment. Subcellular distribution analysis demonstrated that AtLEA14 was mainly localized in the cytoplasm. Transgenic *Arabidopsis* and yeast overexpressing *AtLEA14* all exhibited enhanced tolerance to high salinity. The transcripts of salt stress-responsive marker genes (*COR15a*, *KIN1*, *RD29B* and *ERD10*) were overactivated in *AtLEA14* overexpressing lines compared with those in wild type plants under normal or salt stress conditions. In vivo and in vitro analysis showed that AtLEA14 could effectively stabilize AtPP2-B11, an important E3 ligase. These results suggested that AtLEA14 had important protective functions under salt stress conditions in *Arabidopsis*.

1. Introduction

Soil salinity, one of the serious abiotic stresses, affects approximately 20% of the world's arable land and 40% of the irrigated land at different degrees [1]. To survive, plants have evolved diverse and elaborate mechanisms to protect themselves from salt stress through a series of physiological and morphological changes, such as the Salt Overly Sensitive (SOS) regulatory pathway, the mitogen-activated protein kinase (MAPK) cascade, the different categories of ion channels and the biosynthesis of products that can alleviate salt stress responses including antioxidants, chaperones, and late embryogenesis abundant (LEA) proteins [2–5].

Diverse groups of LEA proteins usually accumulate to high levels during the late stage of embryo development, which are also the universal products involved in the response to environmental stresses by stabilizing proteins, nucleic acids, cell membranes, and redox balance [6–8]. Consistent with their functions, the expression of most LEA genes can be apparently induced by various abiotic stresses, and the biological functions of plant LEA proteins have been well illustrated. Heterologous expression of *HVA1*, a LEA protein gene from barley, can enhance the drought and salt stress tolerance of transgenic wheat and rice [9,10]. Tomato *LE25* increases the salt and chilling stress tolerance when overexpressed in yeast [11]. Transgenic *Arabidopsis* plants overexpressing *NtLEA7-3* are much more resistant to cold, drought, and salt stresses [12]. In addition, other LEA proteins, such as PsLEAM from pea, IbLEA14 from sweet potato, D-19 from cotton and Em from wheat, also enhance abiotic stress tolerance [6,13–15].

In *Arabidopsis* genome, 51 LEA proteins have been identified and classified into nine distinct groups, namely, dehydrin, LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, PvLEA18, SMP, and AtM [16]. Among the 51 genes, 22 members (43%) showed high expression levels in the non-seed organs, and most *LEAs* were induced under stress or hormone treatment, such as drought, high salinity, cold, and ABA [16]. The functions of different *Arabidopsis* LEA proteins have been studied. For example, it has been reported that Early Responsive to Dehydration (ERD) 10 and ERD14 of *Arabidopsis*, members of the dehydrin family, may function as chaperones under abiotic stresses [17]. *AtLEA4-5* is induced by various abiotic stresses, and its overexpressing lines show higher tolerance to severe drought compared with wild type plants [18,19]. The *Arabidopsis* LEA protein AtEM6, which is subgrouped to LEA_5, is required for normal seed development, and loss of *AtEM6* resulted in the premature

Abbreviations: LEA14, late embryogenesis abundant 14; GUS, β-glucuronidase; GFP, green fluorescent protein.

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dehydration of the distal end of siliques [20]. Constitutive expression of the cold-regulated gene *COR15a* enhances the freezing tolerance of chloroplast and protoplast [21]. However, the functional details of other *Arabidopsis LEA* members remain unclear.

In the current study, we explored *LEA14*, which belongs to the *Arabidopsis* LEA_2 subgroup. Compared with other dynamically disordered LEAs, this subgroup, particularly, AtLEA14, displays a stable three-dimensional structure [22]. Additionally, AtLEA14 contains significantly high numbers of hydrophobic residues, and it is likely to function differently from other LEA proteins that are highly hydrophilic [16]. However, the biological function of AtLEA14 remains unknown. Our results showed that *AtLEA14* could be dramatically induced by salt stress, and its overexpression enhanced the salt stress tolerance of transgenic *Arabidopsis* and yeast. Accumulation of AtLEA14 also upregulated the expression of abiotic stress-responsive genes, and stabilized the protein level of AtPP2-B11. These data suggested that *AtLEA14* was a valuable candidate for plant genetic improvement in the future.

2. Materials and methods

2.1. Plant materials and growth conditions

Arabidopsis seeds of each genotype from Columbia (Col-0) background were harvested at the same time from plants grown under the same condition. The sterile seeds were plated on 1/2 Murashige and Skoog (MS) medium containing 1% (w/v) sucrose. The seed-dotted plates were maintained in the dark at 4 °C for 3 d and then transferred to a growth chamber with 16-h-light/8-h-dark cycles at 22 °C.

2.2. Vector construction and plant transformation

To construct *35S::AtLEA14*, the *AtLEA14* coding sequence was amplified using Col-0 cDNA by PCR with gene specific primers (Table S1). The PCR products were cloned into pBI121 under the control of a cauliflower mosaic virus 35S promoter. A 1000 bp sequence upstream of ATG was amplified from genomic DNA to construct *pLEA14::GUS*. The PCR fragment was cloned into the *Hind*III and *Bam*HI sites of pBI121.

The transformation of *Arabidopsis* plants was performed by floral dip using *Agrobacterium tumefaciens* strain GV3101. Homozygous T3 lines were used for phenotypic analysis.

2.3. Histochemical GUS staining and GUS activity analysis

The *pLEA14::GUS* transgenic lines were incubated overnight at 37 °C in solution containing 1 mg/mL 5-bromo-4-chloro-3-indolyl-glucuronic acid, 5 mM potassium ferrocyanide, 0.03% Triton X-100 and 0.1 M sodium phosphate buffer (pH7.0). Then the plants were cleaned with 70% ethanol and pictures were taken by stereoscope. Plant protein extraction and analysis for GUS activity were performed as previously described [23]. GUS activity was obtained from at least five independent transformants, and each assay was repeated three times.

2.4. Seed germination assay

Plants of different genotypes were grown in the same conditions, and mature seeds were collected at the same time. Seeds were planted on the same plate containing 1/2 MS medium with different concentrations of NaCl (150 mM and 200 mM). Germination was defined as an obvious emergence of the radicle through the seed coat. Germination assays were carried out with three replicates of 100 seeds.

2.5. RNA extraction and real time RT-PCR analysis

Total RNA was isolated from *Arabidopsis thaliana* seedlings using a universal plant total RNA extraction kit (BioTeke, Beijing, China). cDNA was synthesized using PrimeScript reverse transcriptase with oligo dT primer using the PrimeScript RT master mix kit (Takara). A SYBR green real-time PCR master mix (Takara) and a Chromo 4 real-time PCR detector (Bio-Rad) were used. Real time

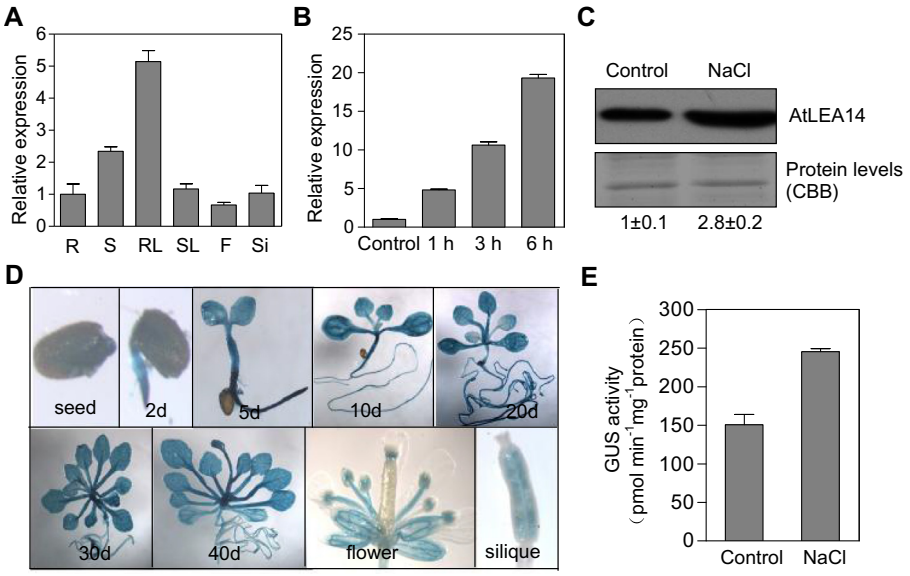

Fig. 1. The expression patterns of *AtLEA14*. (A) Transcript accumulation patterns of *AtLEA14* in different tissues. R: root; S: stem; RL: rosette leaf; SL: stem leaf; F: flower; Si: silique. AtLEA14 transcript (B) and protein (C) accumulation levels upon salt stress. Total RNA or protein from the 2-week-old plants were extracted and analyzed by real time RT-PCR (200 mM NaCl, 0 h, 1 h, 3 h and 6 h) and Western blot (200 mM NaCl, 3 h). Mean values of real time RT-PCR from three biological replicates were normalized to the levels of an internal control, *GAPDH*. Error bar indicates SD ($n = 3$). (D) GUS staining of the *pAtLEA14::GUS* transgenic lines at different developmental stages. (E) GUS activity of the leaves in 4-week-old *pAtLEA14::GUS* transgenic lines treated with or without 200 mM NaCl for 6 h.