Biochemical and Biophysical Research Communications 460 (2015) 386–391

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Expression of the moss *PpLEA4-20* gene in rice enhances membrane protection and client proteins stability

Li Li [a, b, c], Dandan Deng [c], Xi Chen [c], Baomei Wu [c], Ke Hu [c], Tianhang Qiu [c], Suxia Cui [c, *], Fang Huang [a, *]

[a] Key Laboratory of Photobiology, Institute of Botany, The Chinese Academy of Sciences, Beijing 100093, China
[b] University of the Chinese Academy of Sciences, Beijing 100049, China
[c] College of Life Sciences, Capital Normal University, Beijing 100048, China

ARTICLE INFO

Article history:
Received 4 March 2015
Available online 17 March 2015

Keywords:
PpLEA4-20
Transformation
Rice
2-DE
Membrane protection
Protein targets

ABSTRACT

Green vegetative tissues of the moss *Physcomitrella patens* possess a powerful ability to tolerate severe drought stress. Proteomics analysis have revealed that a large number of late embryogenesis abundant (LEA) proteins were key players in the drought tolerance of the photosynthetic tissues. PpLEA4-20, a member of the moss LEA protein family, was selected for further function study using an ectopic expression method in rice. Through molecular identification via PCR, southern blotting and TAIL-PCR, we demonstrated that the *PpLEA4-20* gene was transformed and inserted into a non-encoded region in chromosome 4 of rice and expressed stably in transgenic rice. Unexpectedly, PpLEA4-20 protein emerged as two high-expressed spots on 2-D gels generated from transgenic rice, suggesting that PpLEA4-20 proteins are complete compatible and might be modified in rice. Both growth and physiological analysis showed that seedlings of transgenic *PpLEA4-20* rice displayed altered phenotypes and tolerance to salt. In addition, electrolyte leakage was reduced in transgenic *PpLEA4-20* compared to wild type under stress conditions. Anti-aggregation analysis found that the PpLEA4-20 protein expressed in rice remained soluble at high temperature and in addition to some native proteins from transgenic *PpLEA4-20* rice. Based on Nano LC MS/MS analysis, we identified several proteins from transgenic *PpLEA4-20* rice of increased heat-stability. Our results provide evidence for a role of PpLEA4-20 in salt tolerance and stabilization of client proteins.

1. Introduction

During the late stage of plant embryo development, seeds produce late embryogenesis abundant (LEA) proteins prior to dehydration, which are thought to be associated with the establishment of desiccation tolerance in seeds [1]. Subsequently, LEA proteins are found accumulated in vegetative organs, especially when subjected to various stresses such as cold, drought or high salinity [1,2]. Although numerous studies support the viewpoint that LEA proteins have a broad impact in the abiotic stress response in plants [3,4], their precise biological functions are elusive.

According to data from LEAPdb [5], approximately 700 LEA proteins have been discovered in various plant species [6], and in desiccation tolerant bacteria and invertebrates [7]. In general, *LEA* genes are highly represented in plant genomes, including 51 genes in Arabidopsis [4,8]; 35 genes in rice, 33 genes in poplar, 10 genes in green algae [8] and 77 genes in moss *Physcomitrella patens* [9]. Using Pfam nomenclature based on sequence motifs, Hundertmark and Hincha [8] developed a new system for the classification of LEA proteins, and the 51 *LEA* genes from Arabidopsis were classified into nine distinct groups. The encoded LEA proteins have experimentally been localized to various subcellular compartments including the cytosol, nucleus, plastids, mitochondria, endoplasmic reticulum, vacuolar and pexophagosome, in addition to being secreted [4]. The diversity and compartmentalization of LEA proteins in plant tissues further raises questions about their physiological function and modes of action. Although LEA proteins might function as protectants of biomolecules and membranes, in addition to

Abbreviations: IPG, immobilized pH gradient; LEA, late embryogenesis abundance; LC, liquid chromatograph; MS, mass spectrometer; MALDI, matrix-assisted laser desorption/ionization; PEG, polyethylene glycol; TOF, time of flight.

* Corresponding authors.
E-mail addresses: sxcui@cnu.edu.cn (S. Cui), fhuang@ibcas.ac.cn (F. Huang).

http://dx.doi.org/10.1016/j.bbrc.2015.03.043
0006-291X/

sequestering ions and reactive oxygen species (ROS) [3], their accurate function is still necessary to be characterized one by one.

Physcomitrella patens is a moss with high tolerance to extreme environments such as cold, salt and drought [10]. With the release of its genome sequence, the moss is positioned to become an excellent model system for the study of various stress processes [11,12]. Using a proteomic approach, we previously observed that a number of LEA proteins (n = 11) were strongly expressed and accumulated in green vegetative tissues of the moss during periods of dehydration [12]. This might imply an essential role for LEA proteins in mediating drought tolerance in vegetative tissues, especially in photosynthetic cells. Thus, it would be of great interest to identify the biomolecules protected by moss LEA proteins and probe the molecular mechanisms evolved in photosynthetic cells to cope with drought stress.

In the present study, we investigated the function of PpLEA4-20, a member of the moss LEA protein family, using an ectopic expression approach in rice, in which transgenic lines were generated and subjected to different stress conditions. Using two-dimensional (2-DE) electrophoresis analysis, expression of PpLEA4-20 protein was detected in transgenic rice leaves. Notably, we found that *PpLEA4-20* expression in rice conferred salt tolerance and we identified a group of rice proteins with increased stabilization in transgenic *PpLEA4-20* rice. To our knowledge, this is the first report identifying putative protein targets stabilized by LEA proteins.

2. Materials and methods

2.1. Plant materials and stress treatment

Leafy-shoots of 20 day old *Physcomitrella patens* were obtained as described previously [12]. To recovery more RNA transcripts of PpLEA4-20 for full-length cDNA cloning, leafy-shoots were subjected to dehydration stress for 3 days.

Transgenic seedlings of rice (*Oryza sativa* L. Japonica) were cultured in hydroponic condition with Hoagland solution [13] and placed a growth chamber at 28 ± 1 °C with a 16-h light/8-h dark cycle for two weeks. Hoagland solution was renewed every 2 days for nutrition. To avoid damage caused by rapid stress, a progressive osmotic stress treatment was performed. Rice seedlings were first transferred into a low concentration of PEG-8000 (15% w/v) or NaCl (100 mM) for 2 days, and then a high concentration of PEG-8000 (30%) or NaCl (200 mM) for 10 days. For control experiments, wild type rice was used. After 6 days of recovery, plant survival rates were calculated.

2.2. Gene cloning, vector construction, and rice transformation

Full-length cDNA encoding PpLEA4-20 was cloned from dried leafy-shoots of *Physcomitrella patens* using the primers 5'-GGGGTACCCCTTCCCACTAAATACCGT-3' and 5'-GGACTAGTTCCAGAGCCAATGCTTG-3'. Using pCAMBIA1390 vector (Cambia, Queensland, Australia) containing the maize ubiquitin promoter and hygromycin B resistance gene, *PpLEA4-20* (829 bp) was inserted at BamHI and SpeI sites (Fig. 1). The *Ubi::PpLEA4-20* construct was introduced into rice callus derived from mature embryos, by *Agrobacterium tumefaciens* (LBA4404)-mediated transformation [14]. Transgenic *PpLEA4-20* rice candidates were selected for analysis based on hygromycin resistance (30 mg/L).

2.3. Detection of PpLEA4-20 transgene in rice

Genomic DNA was extracted from transgenic rice leaf tissues by the CTAB method [15]. The inserted moss *PpLEA4-20* fragment was amplified using a forward primer: 5'-CCTTCCCACTAAATACCGT-3' and a reverse primer: 5'-TCCAGAGCCAATGCTTG-3' in T1, T2 and T3 generation of rice plants. PCR analysis was performed using the following cycling parameters: denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min; an initial denaturation step of 5 min and a final extension time of 10 min; 30 cycles. The PCR product was analyzed on a 0.8% agarose gel.

Insertion number of the *PpLEA4-20* in transgenic rice was analyzed by southern blotting according to the standard procedures. The probe for hygromycin was amplified using the primers: 5'- GCGCTTCTGCGGGCGATTTG-3' and 5'- CGCGCTACTTCGAGCGGAGG-3' and the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science, Germany) by PCR. Genomic DNA from transgenic rice and wild type was extracted and digested with HindIII. Digested DNA was separated on 0.8% agarose gel and transferred to nylon membrane (Hybond-N+, Millipore). The membrane was hybridized with DIG-labeled probes according to the manufacturer's instruction. For signal detection, the Image Quant LAS 4000 mini system (GE Healthcare, USA) was used.

Localization of *PpLEA4-20* in the genome of transgenic rice was analyzed by thermal asymmetric interlaced (TAIL) – PCR [16]. Three special primers (sp) based on hygromycin and one arbitrary degenerate primer (ad) were used for primary, secondary, and tertiary TAIL-PCR reactions. These primers were sp1: 5'-GTTGGCTTGTATGGAGCAGCAG-3', sp2: 5'- CGTCCGAGGGCAAAGAAATAGAG-3', sp3: 5'- AATAATGTGTGAGTAGTTCCCAGAT-3' and ad: 5'- NTCGASTWTSGWGTT-3'. Products of each reaction were analyzed on a 1% agarose gel and distinct DNA bands were recovered. Bands were sequenced and resulting data was aligned to the rice genome released to NCBI.

2.4. Protein expression analysis

Two-dimensional electrophoresis (2-DE) was used to detect PpLEA4-20 expression in transgenic plants. Total proteins were extracted from rice leaves using fractionation method [12] and was measured using a modified Bradford method [17]. Protein extracts from wild type rice and transgenic rice were loaded on separate 24 cm IPG pH4-7 strips (GE healthcare, Uppsala) for isoelectric focusing (IEF) and resolved by SDS-PAGE. Gel images were analyzed using the PDQuest software v8.0 (Bio-Rad, USA). Spots of interest were excised and subjected to MALDI TOF/TOF MS identification using an established method [12].

2.5. Anti-aggregation assay

Soluble fraction of proteins was extracted from rice leaves according to established protocol [18]. Fractions of soluble proteins were heated individually at different temperatures (25 °C, 55 °C, 65 °C and 75 °C) for 10 min. Insoluble pellets were removed by centrifugation at 15000 rpm for 10 min to recover heat-soluble proteins in the supernatant. Heat-soluble proteins were separated by SDS-PAGE on 12.5% (w/v) acrylamide gels and stained with Coomassie Brilliant Blue. The bands of interest were excised and subjected to in-gel trypsin digestion followed by Nano LC MS/MS analysis.

2.6. Nano LC-MS/MS

Both desalting and MS identification of digested peptides was carried out as previously described [19]. In brief, desalted peptides were analyzed using a TripleTOF 5600^{+} mass spectrometer (AB Sciex, Canada) coupled to an Ekspert™ nanoLC 425 system (Eksigent, OH). Five microliters of each sample was loaded onto a trap column (ChromXp C18, 350 μm × 0.5 mm, 5 μm, 120 Å, Eksigent,

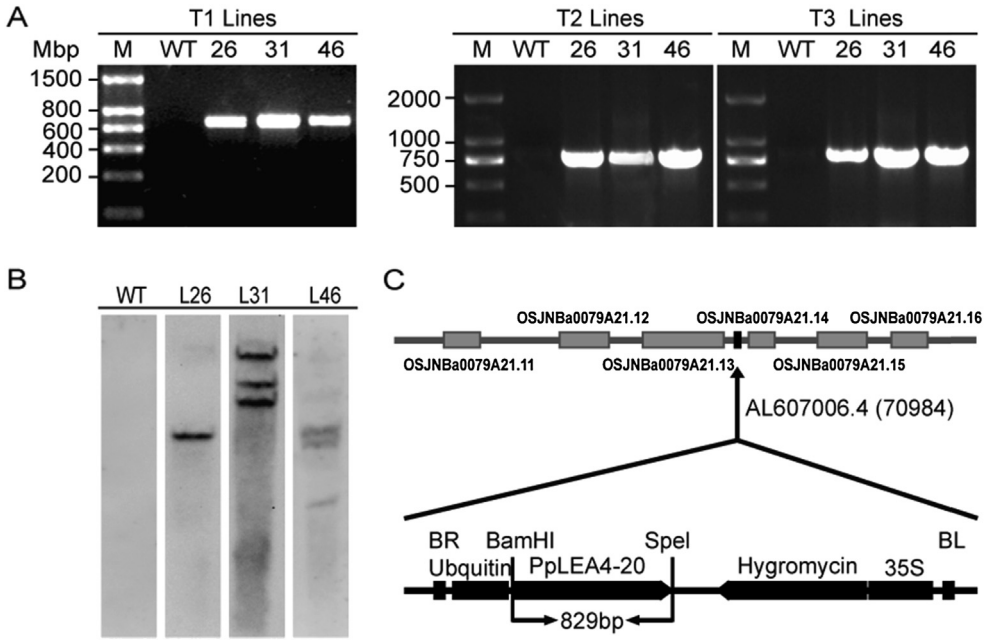

Fig. 1. Mapping of the *PpLEA4-20* gene in transgenic rice. (**A**) Transgene integration was confirmed in the T1-T3 generations of three transgenic rice lines: L26, L31 and L46. A DNA fragment of 829 bp was amplified by PCR and sequenced. (**B**) Southern blot analysis for gene insertion events from three transgenic rice plants. In L26, one insertion event was detected and in L31 and L46, three insertion inserts were detected. (**C**) The location of the transgene in L26 rice genome was detected using TAIL-PCR. The *PpLEA4-20-HYP* transgene was located in a noncoding sequence of rice chromosome 4.

USA). Peptide separation was carried out on a C18 column (ChromXp C18, 15 cm × 75 μm × 3 μm, 120 Å, Eksigent). Peptides were separated using a linear solvent gradient: mobile phase A − 1% formic acid and mobile phase B − 1% formic acid in acetonitrile (5% A to 35% B) for 60 min. The mass spectrometer was operated in positive ionization and high sensitivity mode. The MS survey spectrum was accumulated from a mass range of 400–1250 m/z in 250 ms. For information dependent acquisition (IDA) MS/MS experiments, the first 20 features above 150 counts threshold and having a charge state of +2 to +5 were fragmented using rolling collision energy +/−5%, with 100 ms spectra accumulation/experiment. Each MS/MS experiment put the precursor m/z on a 15 s dynamic exclusion list. Auto calibration was performed every 2 samples to assure high mass accuracy in both MS and MS/MS acquisition.

2.7. Protein identification

TripleTOF 5600^{+} raw data were processed using MS Data Converter (ABSciex) to generate.mgf files. All Mascot searches were performed against current NCBI-nr databases of green plants (17893860 sequences) using the following settings: trypsin with up to two missed cleavages, carbamidomethylation fixed at Cys, variable oxidation at Met, variable protein N-terminal acetylation and variable deamidation at Asn and Gln. The mass error tolerance for precursor ions was set to 10 ppm. A decoy reverse database search was also performed to estimate the false positive rate of protein identification. For unambiguous identification of proteins, the false discovery rate (FDR) was set to 1% and a matching of at least 2 peptides, each with an ion score of greater than 40 was considered adequate.

2.8. Water retention and membrane permeability studies

Water retention capability of transgenic *PpLEA4-20* rice leaves was analyzed according to a previous method [20] with modification. Fully expanded leaves with sheath were collected from 10 plants and inserted into water for 3 h to reach saturation in a controlled-environment chamber at 25 °C and 50% relative humidity. After saturation, leaves were maintained in the chamber and weighed every hour for 12 h, and then oven dried. To evaluate role of PpLEA4-20 in membrane protection, electrolyte leakage was measured as previously described [21]. Three replicates were done for the assays.

3. Results

3.1. Generating transgenic PpLEA4-20 rice

PpLEA4-20 protein from *Physcomitrella patens* is composed of 208 amino acids and has a predicted molecular weight of 20.3 kDa. Recently, PpLEA4-20 has been characterized as a drought responsive protein expressed in green vegetative tissues [12]. Sequence analysis showed that PpLEA4-20 has a low level of similarity (≦44%) compared with homologs of other plant species. To investigate the function of PpLEA4-20 in stress tolerance of green vegetative tissues, transgenic rice expressing the *PpLEA4-20* gene were generated by *Agrobacterium*-mediated transformation. Transgene integration was confirmed in generations T1 to T3 plants by PCR analysis of genomic DNA. Three independent lines: L26, L31 and L46 expressing PPLEA4-20 were selected and studied (Fig. 1A).

3.2. Transgene insertion number and localization

As shown in Fig. 1A, *PpLEA4-20* gene was inserted into the rice genome, and maintained stability in subsequent generations. Insertion number of *PpLEA4-20* was assayed by southern blotting. A single insertion event occurred in rice line L26, whereas, three insertions were detected in rice lines L31 and L46 (Fig. 1B). Using TAIL-PCR, it was determined that *PpLEA4-20* gene was inserted into a noncoding sequence of rice chromosome 4 in rice line 26 (Fig. 1C).

3.3. Expression of PpLEA4-20

Using 2-DE analysis, leaf proteins from wild type rice and the three transgenic lines L26, L31 and L46 were separated. Compared to wild type, no significant changes in protein abundance was detected except for two high-abundance spots that were specific to maps generated from transgenic *PpLEA4-20* lines (Fig. 2A and B). Using MALDI TOF/TOF MS analysis, the two spots were identified as PpLEA4-20 protein (Table S1). Evidently, *PpLEA4-20* insertion into the rice genome produced two protein products with different isoelectric points (Fig. 2), suggesting that an unknown modification occurred. In addition, we noticed that the level of PpLEA4-20 in abundance was not proportional to number of insertion events. Clearly, the abundance of PpLEA4-20 in the single gene insertion line L26 was intermediate to lines L31 and L46 with three gene insertion events (Fig. 2C).

3.4. Stress tolerance

At the seedling stage, transgenic *PpLEA4-20* rice displayed altered phenotypes compared to wild type rice under non-stress conditions. The single gene insertion line L26 had shorter and thicker roots, as well as taller shoots (Fig. 3A, C and D). However, water retention of leaf tissues showed no significant change between the transgenic line and wild type (Fig. 3B). To examine the role of PpLEA4-20 under osmotic stress conditions, 2-week old seedlings were subjected to PEG or NaCl treatment. We detected a reduced transgenic rice shoot height of 41.7% and 37.5% in response to PEG and NaCl treatment, respectively (Fig. 3A and C). Compared to wild type, the reduced shoot height was 22.2% and 27.8% in response to corresponding treatments. In contrast to shoots, both wild type and transgenic rice roots decreased in length and increased in mass under osmotic stress conditions (Fig. 3A and D). Compared to unstressed rice, root weight increased 1.4-fold and 3.2-fold in PEG-treated transgenic rice and wild type rice, respectively. In our survival assay, transgenic rice were more tolerant compared to wild type in response to NaCl treatment, but no improvement was detected under PEG treatment (Fig. 3E). Overall these results suggested that *PpLEA4-20* integration into the rice genome improved seedling resistance to NaCl stress.

Considering that osmotic stress can result in electrolyte leakage because of membrane damaged [22], we examined the effects of osmotic stress on electrolyte leakage in transgenic and wild type plants. We found that electrolyte leakage of cell membrane decreased in transgenic rice. Compared to wild type, electrolyte leakage of transgenic rice decreased at a rate of 33.7%, 36.6% and 11.8% under normal, PEG- and NaCl-treatment, respectively (Fig. 3F). Our finding suggestively implied that PpLEA4-20 expression in rice might protect membranes against damage caused by osmotic stress.

3.5. Anti-aggregation analysis

Since most LEA proteins are highly hydrophilic and generally have thermostability [3], we extracted soluble fractions of leaf proteins from the transgenic line L26 and wild type plants, respectively, for an anti-aggregation assay. As shown in Fig. 4, most rice proteins were aggregated due to heat denaturation at elevated temperatures. However, electrophoretic profiles of heating-soluble proteins were altered greatly in the transgenic line L26, especially at high temperature (65 °C and 75 °C), compared to wild type (Fig. 4). Of these, a ~25 kDa protein band (B3) showed almost no change in abundance in response to temperture increase in the transgenic line. The B3 band and enhanced bands that accumulated in response to 75 °C treatment were excised and subjected to Nano LC MS/MS analysis. As expected, PpLEA4-20 was one of major components in B3 band (Fig. 4). Other proteins identified were marked, including a dihydrolipoyl dehydrogenase, methyl-CpG binding domain containing protein and mucin-22-like in B1; sedoheptulose-1,7-bisphosphatase in B2; thiamine biosynthesis protein and ribosome-recycling factor in B3 and ferritin, photosystem II extrinsic subunit and a unknown protein in B4 (Fig. 4). The MS/MS data set for all proteins are provided in Supplementary Data Table S2. The results provide a clear evidence that PpLEA4-20 is heat-stable, and *PpLEA4-20* expression in rice stabilized a group of rice proteins against heat denaturation. The anti-aggregated rice proteins might be potential targets protected by the LEA protein.

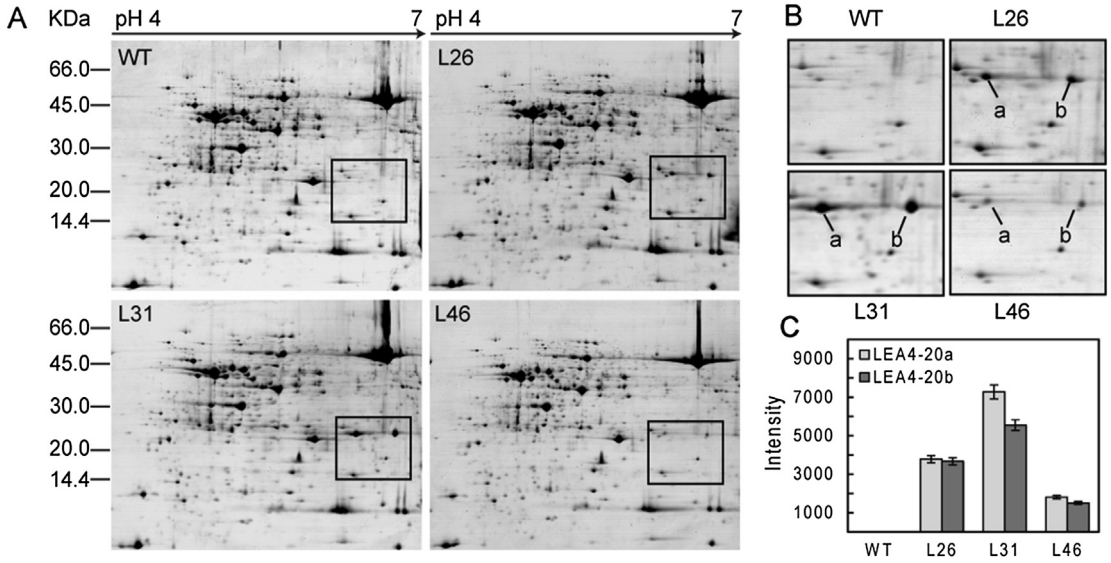

Fig. 2. Expression of PpLEA4-20 protein transgenic rice leaves. (**A**) Leaf proteins from three transgenic rice lines: L26, L31 and L46 in addition to wild type rice were separated by 2-DE. In the first dimension for IEF, 800 μg of protein was loaded on a 24 cm IPG strip with a linear gradient of pH 4–7. In the second dimension, 12.5% SDS-PAGE gels were used. Proteins were visualized using CBB R-250. (**B**) The enlarged diagram corresponds to boxes from (A). Two protein spots corresponding to PpLEA4-20 are labeled. (**C**) PpLEA4-20 protein accumulation in WT (wild type), L26, L31 and L46.

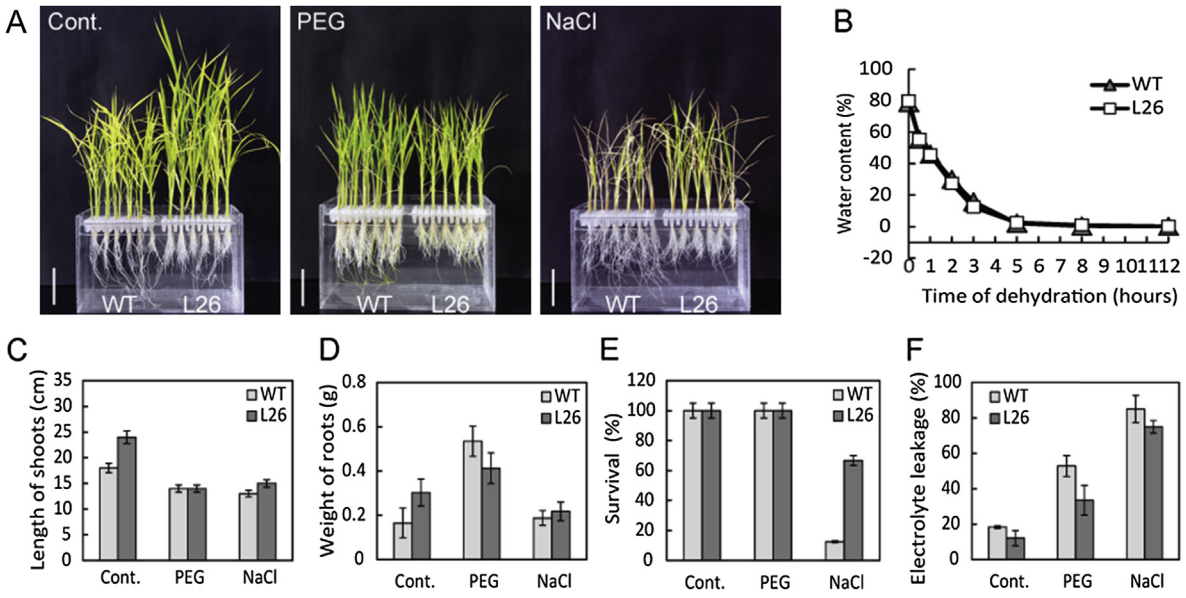

Fig. 3. Changes in phenotype and physiology of transgenic rice line L26 expressing PpLEA4-20 in response to PEG and NaCl Treatment. (**A**) Two-week old rice seedlings were subjected to progressive stress treatments for 12 days. The photos were taken at 6 days after rehydration. Bar = 6 cm. (**B**) Change in water retention of leaves. (**C** and **D**). Changes in shoot length and root weight of plants. (**E**). Survival analysis. (**F**) Electrolyte leakage assay.

4. Discussion

Drought tolerance in green vegetative tissues was acquired during early stages of plant colonization but degenerated or lost in the process of evolution [11]. Using a comprehensive proteomic approach, a large number of LEA proteins were identified that might play a potential role in drought tolerance of green photosynthetic tissues in primitive moss plants [12]. In the present study, PpLEA4-20, a member of the moss LEA family that accumulates in moss green tissues, was investigated using a ectopic expression approach in rice. We demonstrated that the *PpLEA4-20* was inserted stably into the rice genome and was expressed efficiently (Figs. 1 and 2). Seedlings of transgenic *PpLEA4-20* rice displayed altered phenotypes and tolerance to salt (Fig. 3). By anti-aggregation assay, we identified several protein candidates that could be protected by PpLEA4-20 (Fig. 4).

To data, main roles of LEA proteins might be as protectants of both biomolecules and membranes by chaperone-type effects or molecular shield function; they also could sequester ions and reactive oxygen species, and contribute to bioglass formation in the presence of nonreducing sugars [3]. Thus, high abundance of LEA proteins is necessary for its physiological function [23]. In this study, the expressive level of PpLEA4-20 in transgenic rices was clearly presented on 2D gels (Fig. 2). Compared to the amount of protein in dried moss plants [12], the abundance of PpLEA4-20 had doubled in the single gene insertion transgenic line L26. Therefore, the transgenic rice line L26 is very appropriate material for in vivo function study of PpLEA4-20 protein.

From our study, it was evident that PpLEA4-20 protein expressed in rice improved NaCl tolerance but not PEG tolerance in transgenic seedlings (Fig. 3). A similar finding of improved NaCl tolerance conferred by tomato LEA expression in yeast has been reported [24]. The results support the putative function of PpLEA4-20 in ion-sequestration [3]. We also found that transgenic line L26 sufferred less electrolyte leakage from cells (Fig. 3), suggesting that PpLEA4-20 protected cell membranes from injury under stress

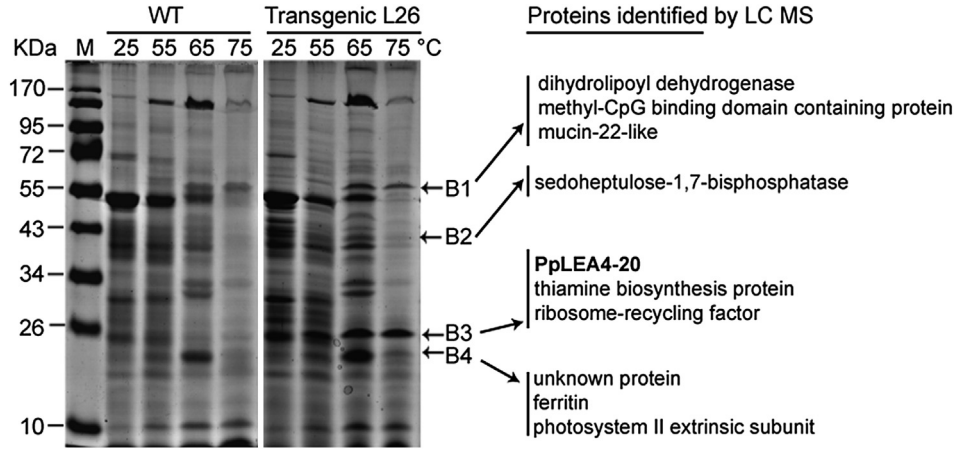

Fig. 4. Electrophoretic profiles of heating-stable proteins of rice leaves. Soluble fractions of leaf proteins from transgenic rice line L26 and wild type plants were heated at 25 °C, 55 °C, 65 °C and 75 °C for 10 min, respectively. Thirty μg of heating-soluble proteins were separated by 12.5% SDS-PAGE and gels were stained with coomassie brilliant blue. Arrows indicate the position of altered heat-solubility proteins in transgenic L26 rice. Heating-stable proteins were identified by Nano LC MS/MS analysis and marked on the right.

conditions. In particular, an increase of non-aggregated rice proteins in response to elevated temperatures was observed in transgenic line L26 and identfied by MS (Fig. 4 and Table S2). The finding provided evidence for a role of PpLEA4-20 stabilization of client proteins.

In conclusion, PpLEA4-20 protein is complete compatible and high expressive in transgenic rice. The function of PpLEA4-20 is associated with ion-sequestration, membrane and proteins protection. Several potential target proteins of PpLEA4-20 have been identified. To our knowledge, this is the first report describing targets of LEA proteins in vivo protection effect. Given drought tolerance was acquired based on accumulation of multiple LEA proteins species [12] and their distinction in subcellular localication [4], it is reasonable for the viewpoint that each LEA protein play a featured role in drought tolerance of plants. Thus, our study provide a usable system for characterizing function of individual LEA protein in vivo.

Conflict of interest

None.

Acknowledgments

We would like to thank the Biotechnology Research Institute in Chinese Academy of Agricultural Sciences for plant transformation assistance. This work was funded by the National Nature Science Foundation of China (31370816) and Major Scientific Research Projects (2011ZX08001-003) of the Ministry of Agriculture of China.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.043.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.043.

References

[1] J. Ingram, D. Bartels, The molecular basis of dehydration tolerance in plants, Annu Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 377–403.
[2] M.F. Thomashow, Plant cold acclimation: freezing tolerance genes and regulatory mechanisms, Annu. Rev. Plant Physiology Plant Mol. Biol. 50 (1999) 571–599.
[3] X. Sun, E.H. Rikkerink, W.T. Jones, V.N. Uversky, Multifarious roles of intrinsic disorder in proteins illustrate its broad impact on plant biology, Plant Cell. 25 (2013) 38–55.
[4] A. Candat, G. Paszkiewicz, M. Neveu, R. Gautier, D.C. Logan, M.H. Avelange-Macherel, D. Macherel, The ubiquitous distribution of late embryogenesis abundant proteins across cell compartments in Arabidopsis offers tailored protection against abiotic stress, Plant Cell. 26 (2014) 3148–3166.
[5] G. Hunault, E. Jaspard, LEAPdb: a database for the late embryogenesis abundant proteins, BMC Genomics 11 (2010) 221.
[6] M.D. Shih, F.A. Hoekstra, Y.I.C. Hsing, Late embryogenesis abundant proteins, Adv. Bot. Res. 48 (2008) 211–255.
[7] S.C. Hand, M.A. Menze, M. Toner, L. Boswell, D. Moore, LEA proteins during water stress: not just for plants anymore, Annu Rev. Physiol. 73 (2011) 115–134.
[8] M. Hundertmark, D.K. Hincha, LEA (late embryogenesis abundant) proteins and their encoding genes in Arabidopsis thaliana, BMC Genomics 9 (2008) 118.
[9] K. Komatsu, N. Suzuki, M. Kuwamura, Y. Nishikawa, M. Nakatani, H. Ohtawa, D. Takezawa, M. Seki, M. Tanaka, T. Taji, T. Hayashi, Y. Sakata, Group A PP2Cs evolved in land plants as key regulators of intrinsic desiccation tolerance, Nat. Commun. 4 (2013) 2219.
[10] A.C. Cuming, S.H. Cho, Y. Kamisugi, H. Graham, R.S. Quatrano, Microarray analysis of transcriptional responses to abscisic acid and osmotic, salt, and drought stress in the moss, Physcomitrella patens, New. Phytol. 176 (2007) 275–287.
[11] S.A. Rensing, D. Lang, A.D. Zimmer, A. Terry, A. Salamov, H. Shapiro, T. Nishiyama, P.F. Perroud, E.A. Lindquist, Y. Kamisugi, T. Tanahashi, K. Sakakibara, T. Fujita, K. Oishi, I.T. Shin, Y. Kuroki, A. Toyoda, Y. Suzuki, S. Hashimoto, K. Yamaguchi, S. Sugano, Y. Kohara, A. Fujiyama, A. Anterola, S. Aoki, N. Ashton, W.B. Barbazuk, E. Barker, J.L. Bennetzen, R. Blankenship, S.H. Cho, S.K. Dutcher, M. Estelle, J.A. Fawcett, H. Gundlach, K. Hanada, A. Heyl, K.A. Hicks, J. Hughes, M. Lohr, K. Mayer, A. Melkozernov, T. Murata, D.R. Nelson, B. Pils, M. Prigge, B. Reiss, T. Renner, S. Rombauts, P.J. Rushton, A. Sanderfoot, G. Schween, S.H. Shiu, K. Stueber, F.L. Theodoulou, H. Tu, Y. Van de Peer, P.J. Verrier, E. Waters, A. Wood, L. Yang, D. Cove, A.C. Cuming, M. Hasebe, S. Lucas, B.D. Mishler, R. Reski, I.V. Grigoriev, R.S. Quatrano, J.L. Boore, The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants, Science 319 (2008) 64–69.
[12] S. Cui, J. Hu, S. Guo, J. Wang, Y. Cheng, X. Dang, L. Wu, Y. He, Proteome analysis of Physcomitrella patens exposed to progressive dehydration and rehydration, J. Exp. Bot. 63 (2012) 711–726.
[13] E.J. Hewitt, T.A. Smith, Plant Mineral Nutrition, English Universities Press, London, 1975.
[14] Y. Hiei, T. Komari, Agrobacterium-mediated transformation of rice using immature embryos or calli induced from mature seed, Nat. Protoc. 3 (2008) 824–834.
[15] F.M. Ausubel, Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, fifth ed., Wiley, New York, 2002.
[16] C. Zheng, L. Zhang, Z. Tang, C. Zhao, S. Yuan, TAIL-PCR and its cloning in plant gene, Genomics Appl. Biol. 28 (2009) 544–548.
[17] S. Cui, J. Hu, B. Yang, L. Shi, F. Huang, S.N. Tsai, S.M. Ngai, Y. He, J. Zhang, Proteomic characterization of Phragmites communis in ecotypes of swamp and desert dune, Proteomics 9 (2009) 3950–3967.
[18] S. Cui, F. Huang, J. Wang, X. Ma, Y. Cheng, J. Liu, A proteomic analysis of cold stress responses in rice seedlings, Proteomics 5 (2005) 3162–3172.
[19] H. Wang, B. Gau, W.O. Slade, M. Juergens, P. Li, L.M. Hicks, The global phosphoproteome of Chlamydomonas reinhardtii reveals complex organellar phosphorylation in the flagella and thylakoid membrane, Mol. Cell. Proteomics 13 (2014) 2337–2353.
[20] J.M. Clarke, T.N. McCAIG, Excised-leaf water retention capability as an indicator of drought resistance of triticum genotypes, Can. J. Plant Sci. 62 (1982) 571–578.
[21] A.C. Leopold, M.E. Musgrave, K.M. Williams, Solute leakage resulting from leaf desiccation, Plant Physiol. 68 (1981) 1222–1225.
[22] M. Bajji, J.M. Kinet, S. Lutts, The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat, Plant Growth Regul. 36 (2002) 61–70.
[23] N.X. Dang, A.V. Popova, M. Hundertmark, D.K. Hincha, Functional characterization of selected LEA proteins from Arabidopsis thaliana in yeast and in vitro, Planta 240 (2014) 325–336.
[24] R. Imai, L. Chang, A. Ohta, E.A. Bray, M. Takagi, A lea-class gene of tomato confers salt and freezing tolerance when expressed in Saccharomyces cerevisiae, Gene 170 (1996) 243–248.