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Genome-scale identification and analysis of *LEA* genes in rice (*Oryza sativa* L.)

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Abstract

LEA proteins are late embryonic proteins abundant in higher plant embryos. It has been found that *LEA* genes are a gene family and play important roles in the protection of water stress. In this study, we employed bioinformatics approaches to identify new members of *LEA* gene family in rice. A total of 34 rice *LEA* (*OsLEA*) genes were identified, of which 25 were new. Four *OsLEA* genes were found to have alternative splicing. The *OsLEA* genes are distributed on all rice chromosomes except for chromosomes 10 and 12. Two independent series of gene conversion events were observed. Microarray data and semiquantitative reverse transcription PCR analysis revealed that the expressions of *OsLEA* genes are very diverse, some are consititutive, some are regulated and some appear to be related to stress tolerance. Two conserved motifs CACGTA and CACGCACG were found to be overrepresented in the 1 kb upstream regions of the ABA-induced and drought-induced *LEA* genes.

Keywords: LEA; Late embryogenesis abundant proteins; Gene family; Expression analysis; Rice (*Oryza sativa* L.)

1. Introduction

LEA proteins are late embryonic proteins abundant in higher plant embryos. It is considered that LEA proteins can increase drought tolerance of plant [1]. Links of LEA proteins with desiccation tolerance were found in tomato, wheat and barley [2]. Expression of a barley *LEA* gene confers tolerance to water deficit in transgenic rice and wheat [3,4]. However, precise functions of LEA proteins have not been elucidated clearly.

LEA genes have been identified in many plant species [5,6]. At least six groups of LEA proteins have been categorized by virtue of similarities in their deduced amino acid sequences and the first three groups are the major ones [1,7,8]. Group 1 (e.g. *Gossypium hirsutum* D19, *Triticum aestivum* EM, and *Hordeum vulgare* B19) is characterized by an internal 20-amino-acid signature motif repeated up to four times depending on the species [9] and a high proportion of Gly, Glu, and Gln residues. Group 2, also referred to as dehydrin, is characterized by a highly conserved 15-amino-acid lysine-rich sequence, or called K-segment, with a consensus EKKGIMDKIKEKLPG. Dehydrins are induced by dehydration-related stresses such as low temperature, drought and high salinity [10] and response to wounding [11,12]. Group 3 (e.g. *Hordeum vulgare* HVA1 and *Daucus carota* Dc8) has an 11-amino-acid fragment (TAQAAKEKAGE), which has been intensely analyzed. Group 4 consists of LEA14 and cotton D113. Group 5 (e.g. D34, D29 and DcECP31) also has an 11-mer repeat, in which each amino acid has the chemical properties similar to Group 3, but lacks a high degree of residue specificity at each position. Group 6 (e.g. D95) has not been well defined.

In addition to determine the structure of the LEA proteins, it is also important to investigate the molecular mechanisms that regulate *LEA* genes. Many *LEA* genes are expressed in response to ABA, drought, and salt stress [13,14], and cis- and trans-elements involved in ABA-induced gene expression have been analysed extensively [15]. The best characterized cis-element in the context of drought stress is ABA-responsive element (ABRE), which contains the palindromic motif CACGTC. A 9-bp conserved sequence, TACGACAT, termed dehydration responsive element (DRE) has been reported in promoter regions of cold- and drought-inducible genes [16].

Draft genomic sequences of both *indica* rice and *japonica* rice [17,18] and, more recently, finished sequences of

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chromosomes 1, 4 and 10 and the whole genome of *japonica* rice have been released [19–22]. These data enable genome-wide investigation of genes by bioinformatics approaches in rice. In this study, we searched for rice *LEA* (*OsLEA*) genes throughout the rice genome and performed an evolution analysis of the *OsLEA* gene family. We also investigated the expression patterns of *OsLEA* genes using microarray data and semiquantitative reverse transcription (sqRT)-PCR and analyzed the promoter regions of the expressed *OsLEA* genes.

2. Methods

2.1. Identification of OsLEA genes

A set of known *LEA* gene sequences were searched against the Pfam database (http://www.sanger.ac.uk/Software/Pfam/) using the 'hmmpfam' program supplied in the HMMER package [23]. Unique HMM profiles for LEA protein were classified into seven categories, i.e. LEA_1, LEA_2, LEA_3, LEA_4, LEA_5, Dehydrin and SMP. These profiles were then used to scan pseuduomolecules of the *japonica* genome (release 4) from TIGR (http://www.tigr.org/tdb/e2k1/osa1/pseudomolecules/info.shtml) using program HMMSEARCH. After *OsLEA* genes were identified, their positions in the rice genome were determined by searching the pseudomolecules with their coding sequences. In addition, 32,000 full length cDNA (FL-cDNA) clones of rice were downloaded from the Knowledge-based Oryza Molecular Biological Encyclopedia (KOME; http://cdna01.dna.affrc.go.jp/cDNA/) [24] and possible alternative splicing in *OsLEA* genes were investigated by aligning FL-cDNA with *OsLEA* genes.

2.2. Sequence alignment and gene conversion analysis

A multiple sequence alignment of the predicted *OsLEA* genes was performed using program CLUSTAL X (v1.81)

Table 1
Summary of *OsLEA* family members

No.	Gene name[†]	Chr	Hmm file	Tigr gene name	Intron no.	FL-cDNA Accession no.	Duplicate gene[††]
1	*OsLEA1*	4	LEA_1	LOC_Os04g49980.1	1	001-119-E08	
2	*OsLEA2*	6	LEA_1	LOC_Os06g02040.1	0	002-135-D09	
3	*OsLEA3*	6	LEA_1	LOC_Os06g21910.1	1	001-120-D12	
4	*OsLEA4*	8	LEA_1	LOC_Os08g23870.1	0	002-135-A01	
5	*OsLEA5 (Lea14A)*	1	LEA_2	LOC_Os01g12580.1	0	001-118-H06	
6	*OsLEA6*	1	LEA_2	LOC_Os01g43530.1	1		*OsLEA8*
7	*OsLEA7a*	3	LEA_2	LOC_Os03g62620.1	2	J033071I21	
8	*OsLEA7b*	3	LEA_2	LOC_Os03g62620.2	2	001-035-H09	
9	*OsLEA7c*	3	LEA_2	LOC_Os03g62620.3	2	J023069E08	
10	*OsLEA8a*	5	LEA_2	LOC_Os05g50710.1	1	001-040-B08	*OsLEA6*
11	*OsLEA8b*	5	LEA_2	LOC_Os05g50710.2	1	J013050C18	*OsLEA6*
12	*OsLEA9*	1	LEA_3	LOC_Os01g21250.1	1		*OsLEA12*
13	*OsLEA10*	2	LEA_3	LOC_Os02g35650.1	1		
14	*OsLEA11*	3	LEA_3	LOC_Os03g28260.1	2		
15	*OsLEA12*	5	LEA_3	LOC_Os05g29930.1	1		*OsLEA9*
16	*OsLEA13*	8	LEA_3	LOC_Os08g34990.1	0		
17	*OsLEA14a (WSI18)*	1	LEA_4	LOC_Os01g50910.1	2	001-125-H02	*OsLEA19*
18	*OsLEA14b (WSI18)*	1	LEA_4	LOC_Os01g50910.2	2	J033070H10	
19	*OsLEA15*	2	LEA_4	LOC_Os02g15250.1	0	J013001K10	
20	*OsLEA16*	3	LEA_4	LOC_Os03g07180.1	1	J033036H02	
21	*OsLEA17*	3	LEA_4	LOC_Os03g20680.1	1		
22	*OsLEA18*	4	LEA_4	LOC_Os04g52110.1	1	J033022D23	
23	*OsLEA19a*	5	LEA_4	LOC_Os05g46480.1	2	002-157-F03	*OsLEA14*
24	*OsLEA19b*	5	LEA_4	LOC_Os05g46480.2	0	001-124-D08	*OsLEA14*
25	*OsLEA20*	1	LEA_5	LOC_Os01g06630.1	1	001-125-F04	
26	*OsLEA21* (*EMP1*)	5	LEA_5	LOC_Os05g28210.1	1	001-119-E02	
27	*OsLEA22* (*RAB25*)	1	Dehydrin	LOC_Os01g50700.1	2	001-119-F09	
28	*OsLEA23*	2	Dehydrin	LOC_Os02g44870.1	1	J023042N13	
29	*OsLEA24*	3	Dehydrin	LOC_Os03g45280.1	1	001-023-B08	
30	*OsLEA25*	11	Dehydrin	LOC_Os11g26570.1	1		
31	*OsLEA26* (*RAB16B*)	11	Dehydrin	LOC_Os11g26750.1	1	002-155-A09	
32	*OsLEA27* (*RAB16C*)	11	Dehydrin	LOC_Os11g26760.1	1	J023096D05	*OsLEA26*
33	*OsLEA28* (*RAB16D*)	11	Dehydrin	LOC_Os11g26780.1	1	001-116-H03	*OsLEA26*
34	*OsLEA29* (*RAB21*)	11	Dehydrin	LOC_Os11g26790.1	1	J033107K14	*OsLEA26*
35	*OsLEA30*	3	SMP	LOC_Os03g06360.1	2	002-115-B06	
36	*OsLEA31*	3	SMP	LOC_Os03g53610.1	1		
37	*OsLEA32*	3	SMP	LOC_Os03g53620.1	1	002-135-H02	*OsLEA31*
38	*OsLEA33* (*D34*)	6	SMP	LOC_Os06g23350.1	2	002-131-G03	
39	*OsLEA34*	9	SMP	LOC_Os09g28860.1	1		

[†] Previous names reported are shown in brackets; a, b, and c indicate different forms of a gene resulted from alternative splicing.
[††] Genes being duplicates of those listed in the 'Gene name' column.

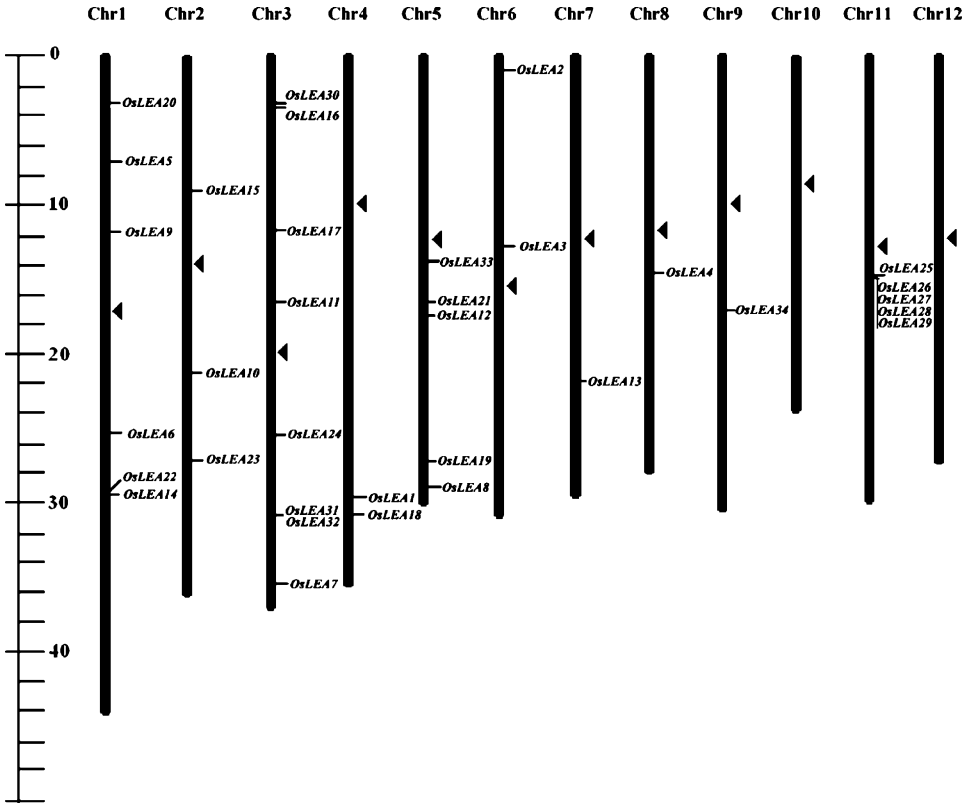

Fig. 1. Physical locations of 34 *OsLEA* genes in rice chromosomes. Arrowheads to the right of physical maps represent the chromosomal positions of centromeres (CEN). The vertical coordinate represents the chromosome position (Mb).

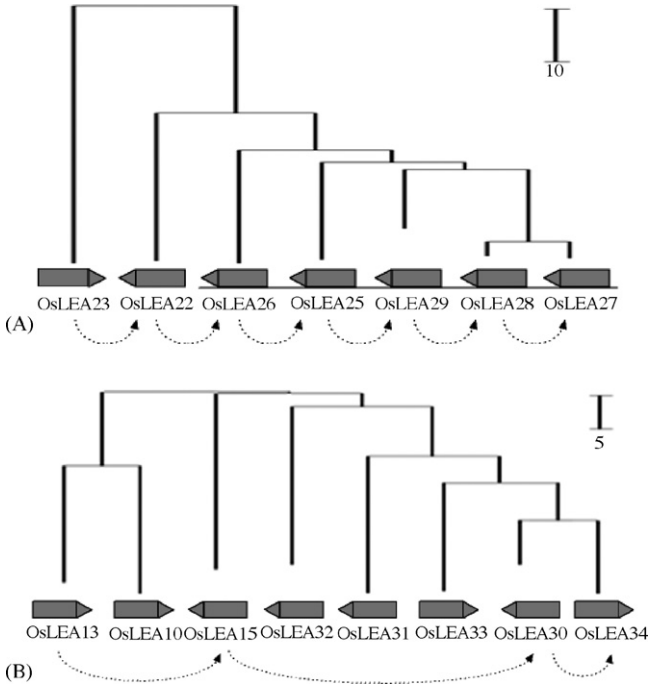

Fig. 2. Two gene conversion events occurred in LEA family. The pentagon represents gene and direction. The dash arrow denotes the gene conversion.

under the default settings [25]. Unconserved regions of the alignment were removed manually. Gene conversion was tested with program GENECONV [26], in which global and pairwise *P* values were calculated to assess the statistical significance of the observed fragment lengths.

2.3. Expression analysis

The expression of *OsLEA* genes were investigated using both bioinformatics and experimental approaches. In the bioinformatics analysis, the FL-cDNA sequences and ESTs of *OsLEA* genes were searched from the TIGR Plant Transcript Assemblies (http://plantta.tigr.org/) using BLASTN [27] with a threshold of 95% identity. In addition, the expression behaviors of *OsLEA* genes were examined in two sets of rice microarray data downloaded from the Gene Expression Omnibus (GEO) at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/geo/). One set was gene series 661 (GSE661) including gene sample 9853 (GSM9853), GSM9854, GSM9855, GSM9856, GSM9857, GSM9858, GSM9859 and GSM9860 under ABA and GA treatments. The other set was GSE2415 under treatments of flooded standing stress (FS), flooded standing stress (FD), drought stress (Dr), osmotic stress (Os), salt and cold at seedling stage.

In the experimental analysis, the expressions of several *OsLEA* genes under ABA or stress treatments were examined using sqRT-PCR. Plants of *japonica* rice variety Nipponbare

were grown by hydroponics in the greenhouse. Seedlings of 40 days old were treated with 15% (v/v) PEG 6000, 0.1 mM ABA and 200 mM NaCl for 16 h, respectively. Untreated seedlings were used as control. Total RNAs of the aerial parts of the control and the treated seedlings were extracted with Trizol (Invetrogen) kit following the manufacturer's instructions and then treated with DNase. First-strand cDNAs were synthesized from the total RNAs and then were used as templates for sqRT-PCR. The sqRT-PCR procedure was: 95 °C 5 min, followed by 32 cycles of 95 °C 30 s, 55 °C 30 s and 72 °C 5 min, and a final 5 min extension at 72 °C. Primers used for amplifying the *OsLEA* genes are listed in Table 2. In addition, *actin* gene was used as internal control to ensure equal amount of cDNA used in each sqRT-PCR reaction. The primers used for *actin* gene were 5′-GGAACTGGTATGGTCAAGGC-3′ and 5′-AGTCT-CATGGATACCCGCAG-3′.

2.4. Promoter motif analysis

Based on the microarray data, *OsLEA* genes were classified into regulated and non-regulated groups in regard to either the ABA treatment or the drought stress. A 1000 bp genomic sequence upstream from the 5′ end of the FL-cDNA clone of each *OsLEA* gene was examined to identify conserved motifs

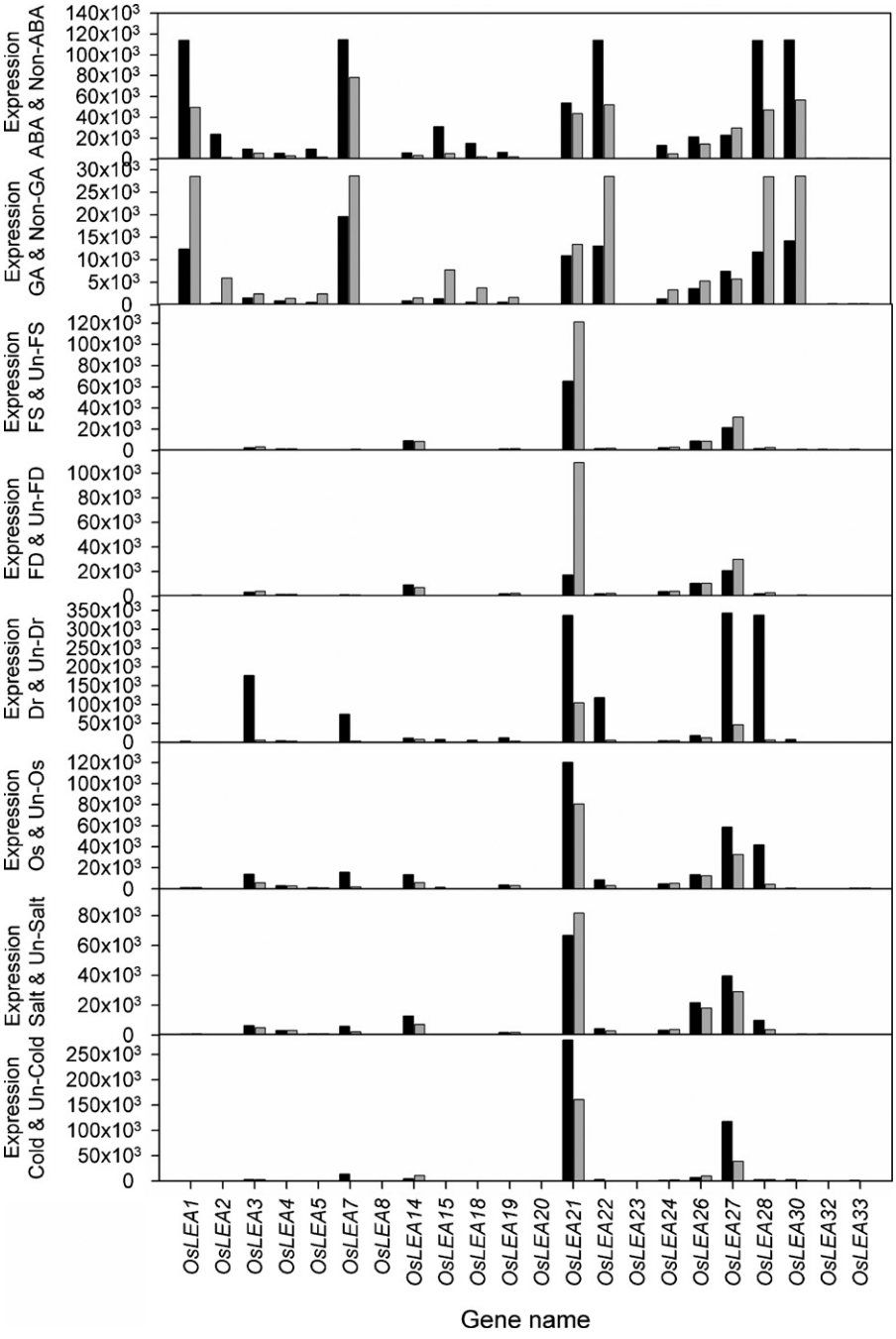

Fig. 3. Gene expression of *OsLEA* based on Microarray data. Black histogram: treated; grey histogram: untreated (CK).

(*cis*-regulatory elements) related to ABA or drought response by comparing the regulated and the non-regulated groups. The program ELPH implementing the Gibbs sampling method (http://www.cbcb.umd.edu/software/ELPH/) was used to characterize conserved motifs with default parameter setting. Identified motifs were plotted according to their positions within the regions and their consensus sequences were graphed using WebLogo [28].

3. Results and discussion

3.1. Number, types and distribution of OsLEA genes

A total of 34 *OsLEA* genes, including 4 of LEA_1, 4 of LEA_2, 5 of LEA_3, 6 of LEA_4, 2 of LEA_5, 8 of Dehydrin and 5 of SMP types, were identified from the genome of *japonica* rice variety Nipponbare, of which 25 have not been described before (Table 1). Four (11.8%) of the *OsLEA* genes were found to have alternative splicing, which results in either two (for *OsLEA8*, *OsLEA14,* and *OsLEA19*) or three (for *OsLEA7*) forms of mature mRNA (Table 1). This frequency of alternative splicing is much lower than the average (27%) occurred in the rice genome [24]. In general, the *OsLEA* genes contain very few introns. Most (22/34 or 64.7%) of them contain only one intron; seven (20.6%) have two introns; and five (14.7%) have no intron (Table 1).

The 34 *OsLEA* genes were distributed in the whole genome except for chromosomes 10 and 12, with chromosomes 1 and 3 containing the most (Fig. 1). Most of the genes are sparsely located, but there are some genes located in adjacent BAC clones, including *OsLEA14_OsLEA22* on chromosome 1 and *OsLEA31_OsLEA32* on chromosome 3. The largest cluster is on the long arm of chromosome 11, which consists of five genes (*OsLEA25, 26*, *27*, *28*, and *29*) all belonging to Dehydrin domain (Fig. 1). Examination on duplicate regions in the rice genome (http://www.tigr.org/tdb/e2k1/osa1/segmental_dup/index.shtml) showed that 13 (38.24%) of the *OsLEA* genes are located on duplicate regions (Table 1). Five genes (*OsLEA6*, *8*, *9*, *12*, *14*, and *19*) and six genes (*OsLEA26*, *27*, *28, 29*, *31*, and *32*) appear to originate from segmental and tandem duplications, respectively.

3.2. Sequence similarity and gene conversion among OsLEA genes

Pair-wise comparisons of full protein sequences showed that the overall identities between the *OsLEA* genes varied from 25%–92%. The highest identity was found between *OsLEA27* and *OsLEA28* (92%), followed by *OsLEA30* and *OsLEA34* (90%), *OsLEA6* and *OsLEA8* (70%), *OsLEA28* and *OsLEA29* (67%), *OsLEA5* and *OsLEA8* (63%), and *OsLEA26* and *OsLEA27* (60%).

Two independent series of gene conversion events were observed (Fig. 2). One series occurred within the Dehydrin subfamily involving seven members beginning from *OsLEA23* and ending at *OsLEA27* (Fig. 2A). The other series involved four *OsLEA* genes beginning from *OsLEA13* and ending at *OsLEA34* (SMP) across different subfamilies (Fig. 2B). The result implies that SMP domain was derived from LEA_4 and the latter was derived from LEA_3 (Table 1). This is consistent with what we have found by searching all LEA domains against the genome sequences retrieved from NCBI databank that LEA_3 exists in both fungi and plant; LEA_4 exists only in seed plant; and SMP exists in angiosperm.

3.3. Expression of OsLEA genes

Twenty-five *OsLEA* genes were found to have FL-cDNA sequences (Table 1) and three genes (*OsLEA6*, *OsLEA13,* and *OsLEA31*) were found to have ESTs in public databases, indicating that these 28 *OsLEA* gene*s* could express in rice. Twenty-two *OsLEA* genes were found in the two sets of microarray data (Fig. 3). Sixteen genes appeared to be up-regulated by ABA but down-regulated by GA; one gene (*OsLEA27*) appeared to be regulated in a converse manner, namely, down-regulated by ABA but up-regulated by GA. Six genes (*OsLEA3*, *7*, *21*, *22*, *27*, and *28*) were up-regulated by drought; the others did not respond to drought. *OsLEA21* was

Table 2
Primers used for amplifying 15 *OsLEA* genes

Gene name	Forward primer (5′ → 3′)	Reverse primer (5′ → 3′)
OsLEA5	AACCCCTACTCCCACTCCAT	CCGACCCTCATCTCGTAGTC
OsLEA6	TCTGCGAGCTCACCTACA	GCTCACGTCCTTGATCAG
OsLEA7	ATCTGCTCCGCGTCAACTAC	AGAGGTGCACGAAGAACCAG
OsLEA10	AGAGAGGAGATCGGCAGGA	TGTGGAGCGTGATGCACT
OsLEA12	AGCTGTGTTGCCCTGCT	AATCCTCTCCTGCACCG
OsLEA15	AGAGACCGTGAGGAATGC	GATGTCGGAGAGGTTGCT
OsLEA16	ACGCGAAAGCGATTATCTGT	CCTCGGTGATCTTCTCCTTG
OsLEA20	ATCTCGCCACGAGGAAATAA	GCTCTTGATGACGGTCTTGC
OsLEA22	GAGAAGACGTTCGCCTAC	CTCCTTTATCTTCTCCTTGATG
OsLEA26	TGTGTGATCGGTGTTTCGAT	CATATACCACACGCGCACTT
OsLEA29	AGCTCGTCTGAGGATGATGG	CCATGATGCCCTTCTTCTTG
OsLEA30	AACACGCTTCGGTGTACGTT	CTCCCACGATCCAATTCAGT
OsLEA31	ACAAGGACGCCGTGACGAT	TCCCGGAGCTTGATCCTGT
OsLEA32	GAGTGCCAGTCGAAGAGGAG	TCAAGCACTCGCTAAATTCG
OsLEA34	GCGGAGAACCTCGTGTT	CGGTTTGCGTCCTACAT

regulated by all the eight treatments, including four up-regulated (ABA, Dr, Os and Cold) and four down-regulated (GA, FS, FD, Salt).

Fifteen *OsLEA* genes were analyzed with sqRT-PCR (Table 2; Fig. 4). Six genes (*OsLEA6*, *15*, *16*, *20*, *28,* and *32*) were not detected under both the normal condition and the treatments. Among them, three (*OsLEA15, 20,* and *32*) was consistent with the results of the microarray experiments, but one (*OsLEA28*) was different, which showed differential expression in the microarray experiments; the residual two genes (*OsLEA6* and *16*) were not found in the microarray data, but the former had EST found in public databases. Two genes (*OsLEA7* and *30*) were strongly and one (*OsLEA32*) was weakly expressed under the normal condition and did not respond to the treatments in both RT-PCR and microarray experiments; one gene (*OsLEA22*) weakly responded to two treatments (ABA and NaCl); one gene (*OsLEA34*) weakly responded to only one treatment (ABA); and three genes (*OsLEA5*, *12,* and *29*) exhibited strong response to all the three treatments. These results suggest that the functions and expressions of *OsLEA* gene family members are highly diverse.

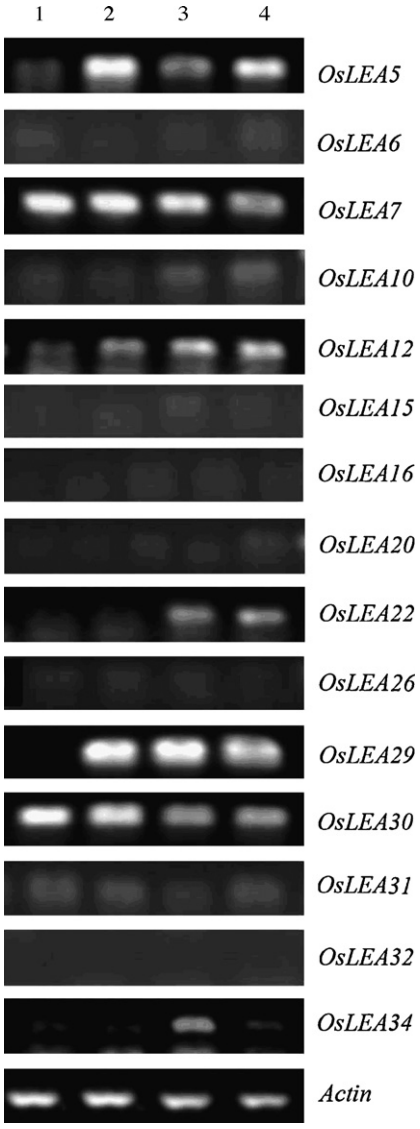

Fig. 4. Expressions of 15 *OsLEA* genes in the aerial part of plants grown under treatments detected by sqRT-PCR in rice. *Actin* gene was used as internal control. Lanes 1: CK (untreated); 2: treated with PEG 6000; 3: treated with ABA; 4: treated with NaCl. See the text for details.

Fig. 5. WebLogo plot of consensus promoter motifs of ABA-induced (A) and drought-induced (B) *OsLEAs* identified using ELPH.

3.4. Conserved motifs in OsLEA promoters

Two conserved motifs, CACGTA and CACGCACG, were found to be overrepresented in the 1 kb upstream regions of the ABA-regulated genes and the drought-regulated genes, respectively (Fig. 5). The motif CACGTA has all the characteristics of ABRE, which was previously shown to have a strong positional preference for the (−250, −50) interval [15].

4. Conclusion

Following the completion of rice genome sequencing project, research focus has been shifted from sequencing to mining and interpreting sequence data. For the most effective functional analysis of gene family, all available information needs to be integrated. To date, more and more availability of entire genome sequences and microarray data provides us with numerous opportunities to study the gene family. In this study, on the basis of known *LEA* genes, we identified 25 new rice *LEA* genes. We found that most of the *OsLEA* genes were originated by segmental and tandem duplications. Based on microarray data and RT-PCR experiments, we revealed that the expression of *OsLEA* genes is highly diverse. We also characterized two *cis*-elements, which might be related to the response of *OsLEA* genes to ABA and drought. These results will enhance our understanding on the functions and diversity of LEA proteins.

References

[1] L. Dure, M. Crouch, J. Harada, T.H.D. Ho, J. Mundy, R. Quatrano, T. Thomas, Z.R. Sung, Common amino acid sequence domains among the LEA proteins of higher plants, Plant Mol. Biol. 12 (1989) 475–486.
[2] J. Ingram, D. Bartels, The molecular basis of dehydration tolerance in plants, Annu. Rev. Plant Physiol. Plant Mol. Biol. 47 (1996) 377–403.

[3] D. Xu, X. Duan, B. Wang, B. Hong, T. Ho, R. Wu, Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice, Plant Physiol. 110 (1996) 249–257.
[4] E. Sivamani, A. Bahieldrin, J.M. Wraith, T. Al-Niemi, W.E. Dyer, T.H.D. Ho, Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressin the barley HVA1 gene, Plant Sci. 155 (2000) 1–9.
[5] E.A. Bray, Molecular responses to water deficit, Plant Physiol. 103 (1993) 1035–1040.
[6] L. Dure, Structural motifs in LEA protein. In: T.J. Close, E.A. Bray (eds). Plant responses to cellular dehydration during environmental stress, Curr. Topics in Plant Physiol. Rockville MD: American Society of Plant Physiologists 10 91–103.
[7] J. Baker, C. Steele, L. Dure, Sequence and characterization of 6 LTA proteins and their genes from cotton, Plant Mol. Biol. 11 (1988) 277–291.
[8] M.J. Wise, LEAping to conclusions: a computational reanalysis of late embryogenesis abundant proteins and their possible roles, BMC Bioinform. 4 (2003) 52–70.
[9] M. Espelund, S. Saeboe-Larssen, D.W. Hughes, G.A. Galau, F. Larsen, K.S. Jakobsen, Late embryogenesis-abundant genes encoding proteins with different numbers of hydrophilic repeats are regulated differentially by abscisic acid and osmotic stress, Plant J. 2 (1992) 241–252.
[10] T.J. Close, Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins, Physiol. Plant 97 (1996) 795–803.
[11] D.T. Rouse, R. Marotta, R.W. Parish, Promoter and expression studies on an *Arabidopsis thaliana* dehydrin gene, FEBS Lett. 381 (1996) 252–256.
[12] S. Richard, M.J. Morency, C. Drevet, L. Jouanin, A. Seguin, Isolation and characterization of a dehydrin gene from white spruce induced upon wounding, drought and cold stresses, Plant Mol. Biol. 43 (2000) 1–10.
[13] E.A. Bray, Plant responses to water deficit, Trends Plant Sci. 2 (1997) 48–54.
[14] K. Shinozaki, K. Yamaguchi-Shinozaki, Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways, Curr. Opin. Plant Biol. 3 (2000) 217–223.
[15] P.M. Chandler, M. Robertson, Gene expression regulated by abscisic acid and its relation to stress tolerance, Annu. Rev. Plant Physiol. Plant Mol. Biol. 45 (1996) 113–141.
[16] H. Wang, R. Datla, F. Georges, et al., Promoters from *kin1* and *cor6.6*, two homologous *Arabidopsis thaliana* genes: transcriptional regulation and gene expression induced by low temperature, ABA, osmoticum and dehydration, Plant Mol. Biol. 28 (1995) 605–617.
[17] J. Yu, S.N. Hu, et al., A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*), Science 296 (2002) 79–92.
[18] S.A. Goff, D. Ricke, et al., A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*), Science 296 (2002) 92–100.
[19] T. Sasaki, T. Matsumoto, et al., The genome sequence and structure of rice chromosome 1, Nature 420 (2002) 312–316.
[20] Q. Feng, Y. Zhang, et al., Sequence and analysis of rice chromosome 4, Nature 420 (2003) 316–320.
[21] The Rice Chromosome 10 Sequencing Consortium, In-depth view of structure, activity, and evolution of rice chromosome 10, Science 300 (2003) 1566–1569.
[22] International Rice Genome Sequencing Project, The map-based sequence of the rice genome, Nature 436 (2005) 793–800.
[23] S.R. Eddy, Profile hidden Markov models, Bioinformatics 14 (1998) 755–763.
[24] S. Kikuchi, K. Satoh, T. Nagata, et al., Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice, Science 301 (2003) 376–379.
[25] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, D.G. Higgins, The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, Nucl. Acids Res. 24 (1997) 4876–4882.
[26] S. Sawyer, Statistical tests for detecting gene conversion, Mol. Biol. Evol. 6 (1989) 526–538.
[27] S.F. Altschul, W. Gish, W. Miller, W.E. Myers, D.J. Lipman, Basic local alignment search tool, J. Mol. Biol. 215 (1990) 403–410.
[28] G.E. Crooks, G. Hon, J.M. Chandonia, S.E. Brenner, WebLogo: a sequence logo generator, Genome Res. 14 (2004) 1188–1190.