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Full Length Research Paper

Eukaryotic translation initiation factor 5A of wheat: Identification, phylogeny and expression

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In the present study, we report on the characterization of a full-length cDNA clone (*TaeIF5A-1*) and as well as two genomic sequences (*TaeIF5A-2* and *TaeIF5A-3*) encoding eIF5A in wheat (*Triticum aestivum*). In addition, 9 partial DNA sequences of eIF5A gene were also isolated from different species of triticeae tribe. Phylogenetic analysis of eIF5A coding genes in general revealed that *TaeIF5A-1* represents ancestral types of the gene family in plant. Chromosome location analysis shows that *TaeIF5A-2* was located on the chromosome 2BL. The mRNA level of *TaeIF5A-1* has spatial and temporal difference, indicating it mainly plays important role in leaf development.

Key words: eIF5A, *Triticum aestivum*, physical mapping, phylogenetic tree, gene expression.

INTRODUCTION

Eukaryotic translation initiation factor eIF5A, formerly called eIF-4D, is a small (18 kDa) abundant protein that is highly conserved in eukaryotes (Gordon et al., 1987; Bartig et al., 1992). The eIF5A is the only known cellular protein that undergoes an unusual post-translational modification on a specific lysine residue to form hypusine [N^{ε}-(4-amino-2-hydroxybutyl)-lysine] (Cooper et al., 1983) through the sequential actions of deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DHH) (Park et al., 1993, 1997, 2006). As a candidate of the translation factor, eIF5A was originally isolated from a polyribosome-bound fraction and was suggested to be involved in the formation of the first peptide bond (Kemper et al., 1976; Benne et al., 1978). However, the recent *in vitro* and *in vivo* data have demonstrated that eIF-5A is not a conventional translation initiation factor. Inactivation of eIF-5A in yeast leads to the reduction in the rate of translation by 30%, but is not immediately abolished (Kang and Hershey, 1994; Zuk and Jacobson, 1998). Disruption of the eIF-5A in yeast induces a lethal phenotype (Kang and Hershey, 1994; Zuk and Jacobson, 1998; Schnier et al., 1991). Inhibition of deoxyhypusine synthase activity in mammalian cells causes growth arrest (Jaukes et al., 1993; Park et al., 1994), cell death (Tome et al., 1997) and tumor differentiation (Chen et al., 1996).

In plants, eIF5A has been so far isolated from *Arabidopsis thaliana* (Wang et al., 2003), alfalfa (Pay et al., 1991), tobacco (Chamot and Kuhlemeier, 1992), maize (Dresselhaus et al., 1999), tomato (Wang et al., 2001) and rice (Chou et al., 2004). The available literature indicated that the expression of eIF5A was temporal and spatial difference and suppressing eIF5A activation causes pleiotropic effects. Transcript analysis reveals that two tobacco eIF5A genes *NeIF-5A1* and *NeIF-5A2* are expressed differently. *NeIF-5A1* is preferentially expressed in photosynthetic tissues, while *NeIF-5A2* is constitutively expressed in all plant tissues examined (Chamot and Kuhlemeier, 1992). Two rice eIF5A genes, *OseIF5A-1* and *OseIF5A-2* are expressed in rice leaves and panicles and high relatively amounts of both genes were detected in senescent leaves. In addition, both *OseIF5A-1* and *OseIF5A-2* were spatially regulated during rice leaf development (Chou et al., 2004). More recently, a correlation has been found between expression of eIF5A and programmed cell death in tomato tissue (Wang et al., 2001). Reducing endogenous DHS protein and, accordingly, levels of activated eIF-5A in *Arabidopsis* by constitutively expressing an antisense DHS transgene has been shown to have dramatic effects on growth and development such as delayed leaf sense-

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cence, increased tolerance to drought stress, and enhanced seed yield (Wang et al., 2003). Thompson et al. (2004) thought that senescence was regulated by eIF5A with implications for plant growth and development.

However, identification of eIF5A gene has not yet been reported in wheat and the any other species of triticeae tribe. To better understand the function and evolution relationship of eIF5A in plant, we have isolated and characterized eIF5A from wheat (*Triticum aestivum* cv. Chinese Spring) and have constructed the phylogenetic trees based on the amino acid variation of eIF5A sequences and the second *intron* of eIF5A nucleotide sequence. Physical locating analysis and the transcriptional levels of *TaeIF5A-1* gene in different wheat tissues by semi-quantitative RT-PCR were also carried out.

MATERIALS AND METHODS

Plant materials

Ten species of the Triticeace tribe were used in this study. The species of genome composition, accession number and origin were listed in Table 1.

RNA isolation and 5', 3'- RACE

Total RNA was extracted from the young leaf, root, pollen, young spike, young embryo and flag leaf of *T.aestivum* cv.Chinese Spring by using TRIZOL Reagent (*In vitrogen*, USA). Based on the alignment of some eukaryotic translation initiation factor 5A (eIF5A) gene sequences, two specific primers P1: 5'-CTGCGTCTC GTCTCCACCAC-3' (sense), P2: 5'-ACCCAACAGATATCTTGATGCCT-3' (antisense), and two degenerate primers P3: 5'-TCAA (G/A)(G/A)(C/A/G) CCG (T/C/A) CCCTGCAAG-3' (sense), P4: 5'-ATCTG(T/C) TC (T/C) TCACC CAT (A/T/G/C) GC -3' (antisense) were designed to amplify wheat eIF5A gene.

To amplify full-length cDNA of wheat eIF5A, 5' and 3'-Rapid amplification of cDNA ends (RACE) were performed by using GeneRacer^{TM} kit (*In vitrogen*, USA), for total RNA (1 µg) from the young leaf. The procedures were carried out according to the user manual of GeneRacer^{TM} kit.

DNA isolation and PCR amplification

Genomic DNA was extracted from leaves of seeding about 2 weeks with a modified CTAB protocol as described in (Saghai-Maroof et al. (1984). Primers P1 and P2 were used to amplify the complete ORF of wheat eIF5A gene. To investigate the second *in tron* of eIF5A, nested PCR was performed with P1 and P2 as first amplification primers and P3, P4 as nested-primers. PCR amplification was performed with an icycler thermalcyler (Bio-RAD Laboratories) in 50 µL volume, which consisted of 200-300 ng of genomic DNA (or PCR amplification product), 200 µM of each dNTPs, 4 µM of each primer, 1U *Taq* polymerase, 1.5 mM Mg^{2+} and 1×PCR buffer. The cycling parameters were 94°C for 3 min to pre-denature, followed by 40 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 7 min.

Sequence analysis

Amplification products were separated in 1.0% agarose gels. The desired DNA (or cDNA) were recovered from gels using DNA purification kit (Shenerg Biocolor, China) and ligated to the pMD18-T vector plasmid (Takara, Japan). The positive clones were screened and the sequence was carried out under DNAMAN 6.0. The phylogenetic tree was constructed using multiple sequence alignment software MEGA 3.1.

Semi-quantitative RT-PCR

Total RNA (1 µg) from the different tissues of *T. aestivum* were reverse transcribed to the first-strand cDNA by using the first-strand cDNA synthesis kit (Takara, Japan) following the protocol given by the manufacturer. Primers P1, P2 were used for *TaeIF5A* amplification by PCR. Primers P5: 5'-CGCATCAACGTGTACTACAA-3' (sense), P6: 5'-TACGAGCTGGTGGACTGAGA-3' (antisense) were used for the internal control β-tubulin amplification. The PCR reaction mixture consisted of 2 µL first-strand cDNA, 200 µM of each dNTPs, 4 µM of each primer, 1 U *Taq* polymerase, 1.5 mM Mg^{2+} and 1×PCR buffer in a final volume of 25 µL. PCR amplification was performed as follows: pre-denaturation for 3 min at 94°C, then thirty cycles of PCR were carried out. Each cycle of PCR included 1 min of denaturation at 94°C , 1 min of primer annealing at 60°C , 2 min of extension at 72°C and a final extension at 72°C for 7 min. The PCR products were separated on 1.0% agarose gels, and the density of each PCR product was determined using a gel image system (Bio-RAD Laboratories). The procedure was repeated three times.

RESULTS

Isolation and sequence analyses of the wheat eIF5A cDNA

A full-length cDNA encoding *TaeIF5A-1* (GenBank accession number DQ167203) was isolated by 5' and 3'-Rapid Amplification of cDNA ends (RACE) from *T. aestivum* cv.Chinese Spring. The cDNA contains 768 base pairs, including a 96-base pair 5'-nocoding sequence and a 189-base pair 3'-nocoding sequence, and encodes a 161-amino acid polypeptide with a calculated molecular mass of 17.3 kDa (Figure 1). The derived amino acid sequence of *TaeIF5A-1* has high degree of similarity with LeeIF5A-1 (GenBank accession number AF296083), SteIF5A-1 (GenBank accession number AB004827), OseIF5A-1 (GenBank accession number AF094773), AteIF5A-1 (GenBank accession number AAD39281) and ZmeIF5A-1 (GenBank accession number Y07920) (Figure 2).

Isolation of DNA sequences eIF5A gene from 10 Species of triticeae tribe

Genomic PCR amplification using the primers P1 and P2 was conducted, and two desires DNA band was detected in wheat (Figure 3). Genomic nested PCR were also performed: the first PCR amplification was carried out using the primers P1, P2; the second PCR amplification was performed using the primers P3, P4. A target DNA band was detected from each species besides two bands of *T. aestivum* cv. Chinese Spring (Figure 4). The frag-

Table 1. Twelve DNA or cDNA sequences of eIF5A from ten species of the Triticeace tribe.

No.	Species	Genome	Accession	Origin	Length (bp)	Genebank No.	Molecular Type	Note
1.	*Triticum aestivum*	AABBDD		Sichuan,China	768	DQ167203	cDNA	*TaeIF5A-1*
	cv. ChineseSpring				1910	DQ167201	DNA	*TaeIF5A-2*
					1679	DQ167202	DNA	*TaeIF5A-3*
2.	*Dasypyrum villosum*	VV	SCND 1365	Sichuan,China	1338	Q279900	DNA(partial)	
3.	*Crithopsis delileana*	KK		Denmark	1494	DQ279899	DNA(partial)	
4.	*Psathyrostachys juncea*	NsNs	PI 499558	NPGS	1375	DQ279898	DNA(partial)	
5.	*Secale cereale*	RR		JingZhou, China	1298	DQ279897	DNA(partial)	
6.	*Aegilops tauschii*	DD	PI 542277	NPGS	1282	DQ279896	DNA(partial)	
7.	*Eremopyrum bonaepartis*	FF	PI 222956	NPGS	1334	DQ279895	DNA(partial)	
8.	*Thinopyrum elongatum*	EE	PI 153179	NPGS	1334	DQ279894	DNA(partial)	
9.	*Pseudoroegneria spicata*	StSt	PI 232131	NPGS	1358	DQ279893	DNA(partial)	
10.	*Dasypyrum breviaristatum*	VaVbVaVb	PI546317	NPGS	1344	DQ414518	DNA(partial)	

NPGS: National Plant Germplasm System; SCND: Sichuan Agricultural University.

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1          GAAACTAGCCTCCGGATCCATCCATCCGCCCCTCGCTGCCGCTGCGTCTCGTCTCCACCA

61         CCCCACTTGCCCCCGGACTCAGGAGGATCGTCGGAGATGTCGGACACCGATGAGCGCCAC
1                                                M  S  D  T  D  E  R  H

121        TTCGAGTCCAAGGCCGACTCCGGCGCCTCCAAGACCTACCCGCAGCAGGCCGGCGCCATC
9           F  E  S  K  A  D  S  G  A  S  K  T  Y  P  Q  Q  A  G  A  I

181        CGCAAGGGTGGACACATCGTCATCAAGGCCCGTCCCTGCAAGGTTGTTGAGGTCTCCACC
29          R  K  G  G  H  I  V  I  K  A  R  P  C  K  V  V  E  V  S  T

241        TCCAAGACTGGGAAGCATGGTCACGCAAAGTGTCACTTTGTTGCCATTGACATCTTTAAT
49          S  K  T  G  K  H  G  H  A  K  C  H  F  V  A  I  D  I  F  N

301        GGAAAGAAGCTTGAGGATATCGTTCCTTCATCCCACAACTGTGACGTCCCCCATGTTGAC
69          G  K  K  L  E  D  I  V  P  S  S  H  N  C  D  V  P  H  V  D

361        CGCCAAGATTATCAGCTGATTGACATAACTGATGATGGATATGTCAGCCTTCTCACTGAG
89          R  Q  D  Y  Q  L  I  D  I  T  D  D  G  Y  V  S  L  L  T  E

421        AGTGGTAACACTAAGGATGACCTGAAGCTTCCCACTGATGATGTTCTGCTTGGCCAGATC
109         S  G  N  T  K  D  D  L  K  L  P  T  D  D  V  L  L  G  Q  I

481        AAGACTGGATTTGCTGATGGCAAGGACCTGATCCTGTCTGTGATGTCCGCCATGGGTGAA
129         K  T  G  F  A  D  G  K  D  L  I  L  S  V  M  S  A  M  G  E

541        GAACAGATCTGCGCTGTGAAGGAAATCGGTGGTGGCAAGTAAGCAGCTGTGCGTGGTTGC
149         E  Q  I  C  A  V  K  E  I  G  G  G  K  *

601        CTACCTGCGATACTTGGTATCTAGTGCTTCTGGGTGTTTGAGATACACATACATAGGCAT

661        CAAGATATCTGTTGGGTGAAACAAGTTTGATCCAGATTTGTGTGTTTTTTACACCGAATG

721        CTCGAGGTGCTGCTAGTACTTTGTTATCTATCTATCTATCTCTATTAT
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Figure 1. The complete cDNA sequence of eIF5a and its putative protein sequence.

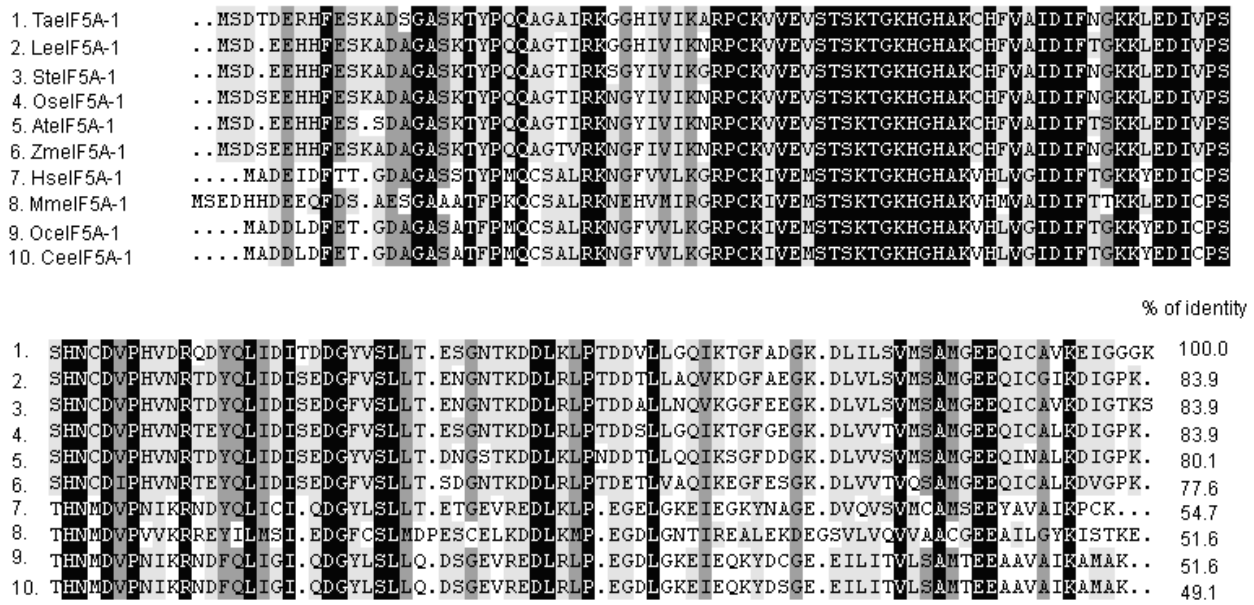

Figure 2. Alignment of the amino acid sequence of eIF5A from wheat, tomato, potato, rice, Arabidopsis, maize, human, mouse, rabbit and C. elegan. The protein sequence shown in the diagrams are listed in the GenBank database under the following accession number: AteIF5A-1 (AAD39281), CeeIF5A-1 (P34563), HseIF5A-1 (AF293386), ZmeIF5A-1 (Y07920), MmeIF5A-1 (BC008093), SteIF5A-1 (AB004827), LeeIF5A5a-1 (AF296083), OseIF5A-1 (AF094773), OceIF5A-1 (P10160), TaeIF5A-1 (DQ167203).

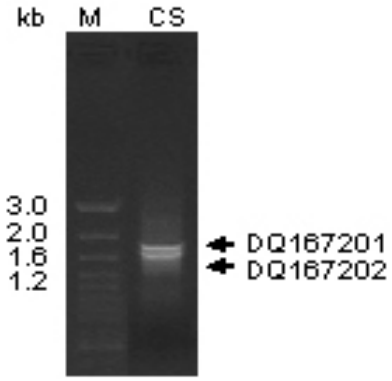

Figure 3. PCR profile of wheat genomic DNA with the primers, P1 and P2. M: Marker, CS: Triticum *aestivum* cv. Chinese Spring, DQ167201and DQ167201: GenBank accession numbers.

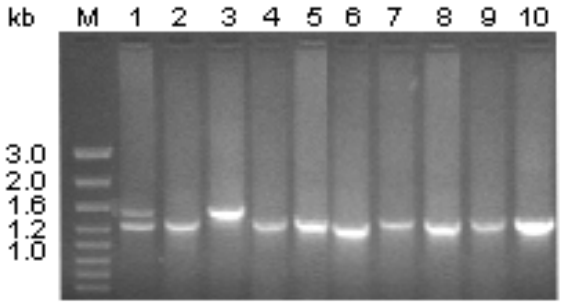

Figure 4. The Genomic nested PCR prouducts of ten species of triticeae tribe with the primers P1, P2, P3 and P4. M: Marker, 1: Triticum *aestivum* cv. Chinese Spring, 2: *Pseudoroegneria spicata*, 3: *Crithopsis delileana*, 4: *Dasypyrum villosum*, 5: *Eremopyrum bonaepartis*, 6: *Aegilops tauschii*, 7: *Psathyrostachys juncea*, 8: *Thinopyrum elongatum*, 9: *Dasypyrum breviaristatum*, 10: *Secale cereale*

ments were cloned into the plasmid vector pMD18-T and sequenced (Takara, Japan). 11 genomic DNA sequences (or partial sequences) encoding eIF5A were obtained from 10 species of the Triticeace tribe (Table 1). They are *T. aestivum* (,GenBank accession number DQ167201 called *TaeIF5A-2* and DQ167202 called *TaeIF5A-3*), *Dasypyrum villosum* (GenBank accession number DQ279900), *Crithopsis delileana* (GenBank accession number DQ279899), *Psathyrostachys juncea* (GenBank accession number DQ279898), *Secale cereale* (GenBank accession number DQ279897), *Aegilops tauschii* (GenBank accession number DQ279896), *Eremopyrum bonaepartis* (GenBank accession number DQ279895), *Thinopyrum elongatum* (GenBank accession number DQ279894), *Pseudoroegneria spicata* (GenBank accession number DQ279893) and *Dasypyrum breviaris-tatum* (GenBank accession number DQ414518).

Phylogenetic analysis of eIF5A

To investigate the evolutionary relationships between the

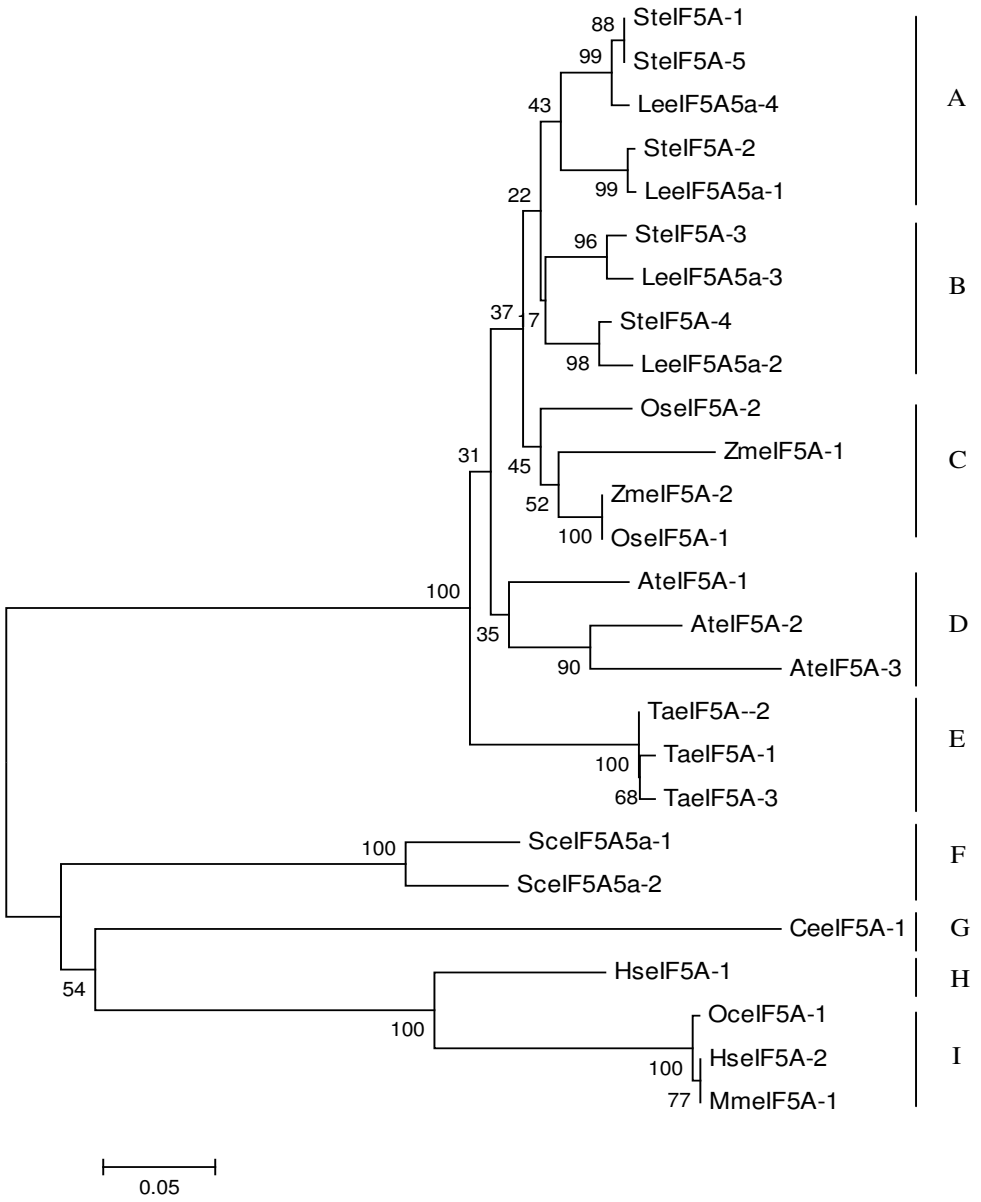

Figure 5. Phylogeny of the eIF5A gene family. The tree was constructed by software of MEGA 3.1 with Neighbor-Joining (NJ) and bootstrap of replications 1000, random seed 64238. The protein sequences shown in the diagrams are listed in the GenBank database under the following accession number: AteIF5A-1 (AAD39281), AteIF5A-2 (AAG52496) , AteIF5A-3 (AAK62643), CeeIF5A-1 (P34563), HseIF5A-1 (AF293386), HseIF5A-2 (P10159), ZmeIF5A-1 (Y07920), ZmeIF5A-2 (BI430734), MmeIF5A-1 (BC008093), StoIF5A-1 (AB004827), StoIF5A-2 (AB004826), StoIF5A-3 (AB004825), StoIF5A-4 (AB004824), StoIF5A-5(AB004823), LeeIF5A5a-1 (AF296083), LeeIF5A5a-2 (AF296084), LeeIF5A5a-3 (AF296085), LeeIF5A5a-4 (AF296086), OseIF5A-1 (AF094773), OseIF5A-2 (AJ312906), SceIF5A5a-1 (P19211), SceIF5A5a-2 (P23301), OceIF5A-1 (P10160), TaeIF5A-1 (DQ167203), TaeIF5A-2 (DQ167201), TaeIF5A-3 (DQ167202).

wheat eIF5A and the representative eIF5As from the database, a neighbeour-joining tree of the eIF5A gene family in eukaryotes was constructed based on the amino sequence (Figure 5). Two major clades of plant and animals were identified and supported by bootstrapping. In plant, the amino sequences of eIF5A were divided into five subgroups. Three potato gene members (StoIF5A-1, StoIF5A-5, and StoIF5A-2) and two tomato gene members (LeeIF5A-1, LeeIF5A-4) were clustered together as group A. Another two potato gene members (StoIF5A-3, StoIF5A-4) and two tomato gene members (LeeIF5A-2, LeeIF5A-3) were clustered together in group B. And four eIF5As of Rice and Maize (OseIF5A-1, OseIF5A-2, and ZmeIF5A-1and ZmeIF5A-2) formed group C. In addition,

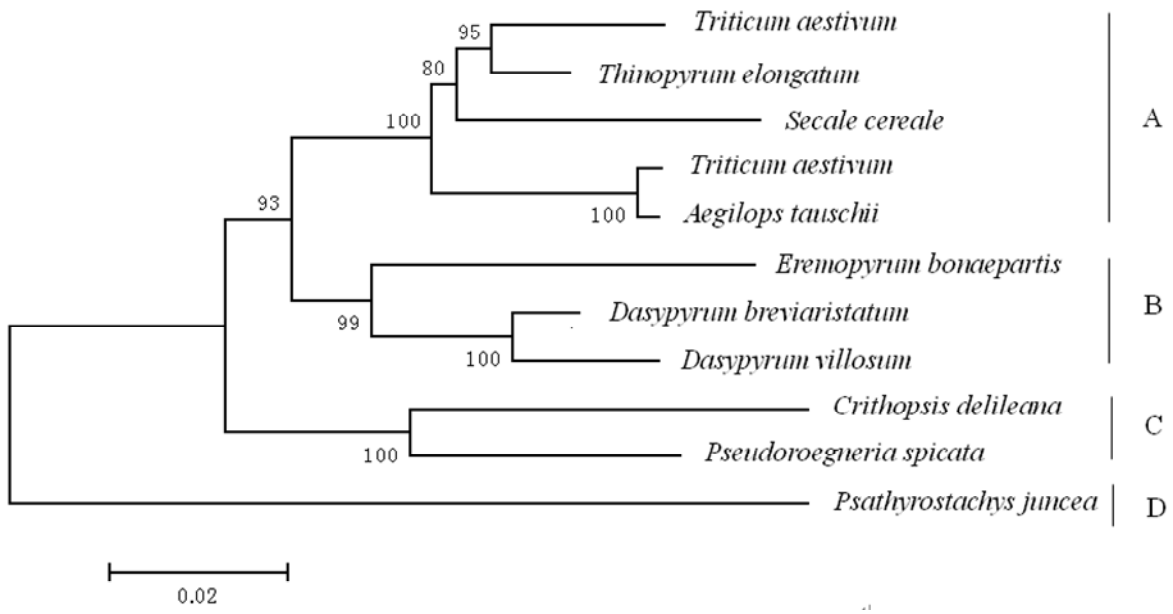

Figure 6. Phylogenetic tree of ten species of triticeae tribe based on the second introns of homoeologous eIF5A. The tree was constructed by software of MEGA 3.1 with Neighbor-Joining (NJ) and bootstrap of replications 1000, random seed 64238. The nucleotide sequences shown in the diagrams are listed in the GenBank database under the following accession number: *Triticum aestivum* (DQ167201 and DQ167202), *Thinopyrum elongatum* (DQ279894), *Secale cereale* (DQ279897), *Aegilops tauschii* (DQ279896), *Eremopyrum bonaepartis* (DQ279895), *Dasypyrum breviaristatum* (DQ414518), *Dasypyrum villosum* (DQ279900), *Crithopsis delileana* (DQ279899),

three *Arabidopsis* eIF5As (AteIF5A-1, AteIF5A-2 and AteIF5A-3) form a group D. Three wheat eIF5As (TaeIF5A-1, TaeIF5A-2 and TaeIF5A-3) were clustered together in group E.

Based on the second intron of eIF5A nucleotide sequence, a neighbour-joining tree of ten species of triticeae tribe was also constructed (Figure 6). *T. aestivum* (*TaeIF5A-2* and *TaeIF5A-3*), *T. elongatum* (DQ279894), *S. cereale* (DQ279897) and *A. tauschii* (DQ279896) were clustered together group A. *Eremopyrum bonaepartis* (DQ279895), *D. breviaristatum* (DQ414518) and *D. villosum* (DQ279900) formed group B. *C. delileana* (DQ279899) and *P. spicata* (DQ279893) were clustered together group C where as *P. juncea* (DQ279898) forms a clade which is basal to other species.

Structure and organization of eIF5A

Comparison of the two eIF5A's genomic sequences of *TaeIF5A-2*, *TaeIF5A-3* and cDNA sequence of *TaeIF5A-1*, as is the genomic sequence of rice eIF5A (GenBank accession number AC08428) showed that *TaeIF5A-2* and *TaeIF5A-3* were comprised 6 exons and 5 introns. The ATG start codon and the TAA stop codon locate on the exon 2 and exon 6 respectively (Figure 7). The identity between genomic sequences of *TaeIF5A-2* and *TaeIF5A-3* was found to be 82.3%. However, they share identical transcriptions of 636 bp, in which have only 6 nucleotides difference. Putative transcriptions of *TaeIF5A-2* and *TaeIF5A-3* are only 6 nucleotides difference with *TaeIF5A-1*. Further studies are needed to determine whether *TaeIF5A-1* is completed by alternative splicing of *TaeIF5A-2* and *TaeIF5A-3*. Moreover, the intron 2 of the two sequences was larger and showed more variation than other introns sequences. The introns 2 of *TaeIF5A-2* and *TaeIF5A-3* have 896 and 672 bp, respectively, and they share 59% homology.

Expression of *TaeIF5A-1* in wheat tissues

To determine differential tissue expression of eIF5A gene in wheat tissues, *TaeIF5A-1* gene was screened in six plant organs of *T. aestivum* cv. Chinese Spring including young leaf, root, pollen, young spike, young embryo and flag leaf by Semi-quantitative RT-PCR and *β-tubulin* gene was used as a control. At the same time, semi-quantitative RT-PCR amplification products were cloned and sequenced to confirm that the gene used was not the other isoforms of wheat eIF5A but was only *TaeIF5A-1*. As shown in Figure 8, the level of *TaeIF5A-1* expression differed significantly, with high level of expression found in young leaf, pollen, young spike and young embryo, and the lowest level in root. In addition, *TaeIF5A-1* transcript level was low in flag leaf.

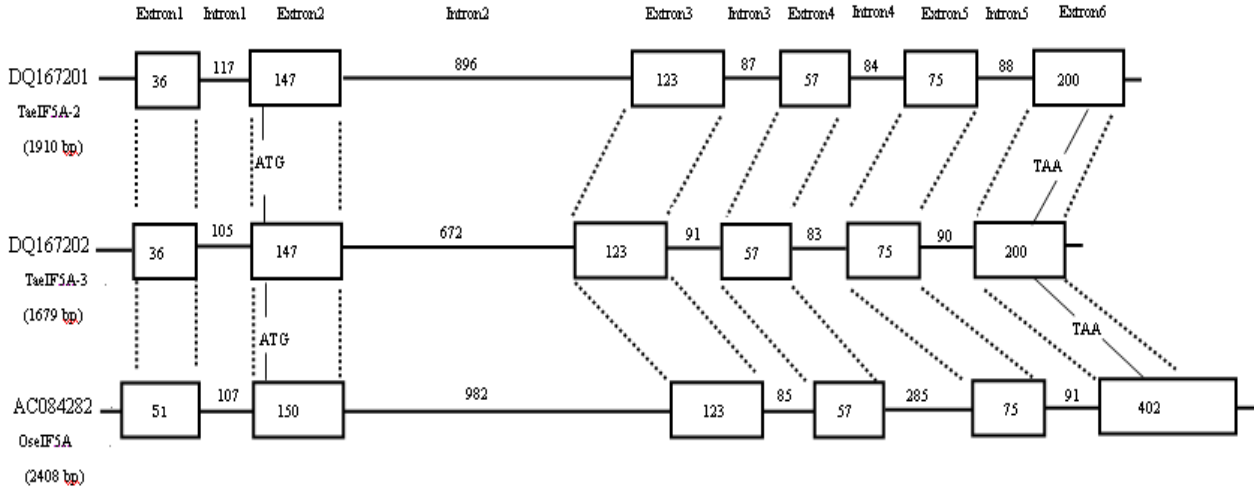

Figure 7. The structure and organization of *TaeIF5A-2* (DQ167201), *TaeIF5A-3* (DQ167202) and OseIF5A (AC084282) at genomic nucleotide level. Boxes represent exons, horizontal lines between the boxes are introns. Lengths are given as numbers of nucleotides.

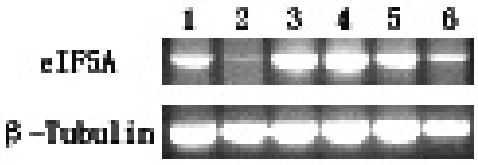

Figure 8. The result of Semi-quantitative PCR from mRNA of young leaf (1), root (2), pollen (3), young spike (4), young embryo (5) and flag (6), using β-tubulin as acontrol. Using 30 cycles of PCR amplification and 2 μL first-strand cDNA as template.

DISCUSSION

Phylogenetic analysis of eIF5A

We reported the isolation and characterization of wheat homologues of eIF5A, a hypusine-containing protein gene. The phylogenetic tree based on the amino sequence in this study is consistent with the report of Dresselhaus et al. (1999) and Chou et al. (2004). Within plants systems, duplication of the eIF5A genes antedated the divergence of dicots and monocots. The basal clade occurring in wheat represents ancestral types of the gene family. Like the existence of two genes for eIF5A in humans (Jenkins et al., 2001), all plants possess two or more genes. Only two clades occur in monocots, whereas the other clades exist in dicots, a pattern associated with the shorter evolutionary history of monocots.

Phylogenetic analysis of ten species of triticeae tribe according to the second intron of eIF5A

Like exons, intron sequences proved to provide important information for the development of DNA-based typing strategies and can be used to assess the evolutionary relationships (Voorter et al., 2005). In this investigation, the phylogeny of ten species of triticeae tribe constructed according to the second intron of eIF5A indicate that the evolution of eIF5A is consistent with species, identifying four major clades of A, B, C and D. In group A, the relationship of *A. tauschii* (DQ279897) and *T. aestivum* (*TaeIF5A-3*) is closer than the others, supporting that the *A. tauschii* (DD, 2n=14) is the donor of D genome to common wheat (AABBDD, 2n=42). Moreover, the relationship of *T. aestivum* (*TaeIF5A-2*) and *T. elongatum* (DQ279894) is also close. Based on the results of the next section- physical locating of wheat eIF5A, we can know that B genome of *T. aestivum* (AABBDD) is relation to genome of *T. elongatum* (EE). In addition, two Dasypyrum species, *D. villosum* (DQ279900) and *D. breviaristatum* (DQ414518) were also clustered together in group B. Our research of construction phylogeny using intron can be used to investigate the evolutionary relationships of the species of the *Triticeae* tribe that contains 350 to 450 species (Dewey, 1984).

Physical locating of wheat eIF5A

Analysis of gene physical mapping and comparative genome is great help to know the function, interaction, expression and regulation of genes living systems. To date, more than 7000 wheat Expressed Sequence Tags (ESTs) has been mapped on chromosomes (Lazo et al., 2004). In the present study, the BLAST result of *TaeIF5A-1* on the wheat EST website (http://wheat.pw.usda.gov/wEST/blast) indicated that *TaeIF5A-1* share 84.1% identity with the EST BE517627 which has four copies located on C-2AL1-0.85, C-2BL2-

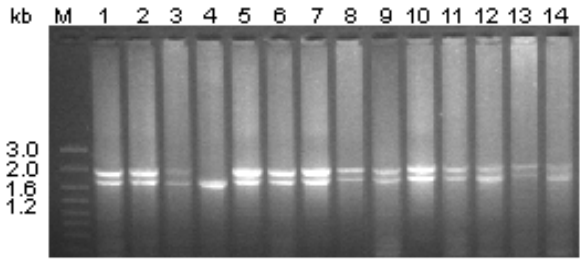

Figure 9. The PCR products of Langdon D-genomic disomic substitution lines genomic DNA with Primers P1 and P2. 1. 1D (1A), 2. 1D (1B), 3. 2D (2A), 4. 2D (2B), 5. 3D (3A), 6. 3D (3B), 7. 4D (4A)+4AL, 8. 4D (4B), 9. 5D (5A), 10. 5D (5B), 11. 6D (6A), 12. 6D (6B).12. 7D (7A), and 14. 7D (7B).

0.36, C-2DL3-0.49 and 7BL10-0.78-1.00 suggesting that the wheat eIF5A genes locate on the chromosome 2AL, 2BL, 2DL and 7BL. In addition, the genomic sequence of *TaeIF5A-2* was located on chromosome 2B (Figure 9) which was confirmed by using Langdon D-genomic disomic substitution lines (generously provided by doctor Joppa, University of North Dakota, USA). Moreover, the result of the Chinese Spring ditelosomic lines analysis shows that the genomic sequence of *TaeIF5A-2* was also located on the long arm of chromosome 2B (figure not shown). Although the genomic sequence of *TaeIF5A-3* has not yet located using above materials, we may infer that *TaeIF5A-3* have three copies which lie on the 2AL, 2DL and 7BL.

Expression pattern of *TaeIF5A-1*

In this investigation, cDNA encoding *TaeIF5A-1* was isolated. Characterized and the expression of *TaeIF5A-1* was researched by semi-quantitative RT-PCR and β-tubulin as internal control. The eIF5A gene enabled the examination of the abundance of mRNA. The *TaeIF5A-1* exhibited down-regulated expression pattern during the flag leaf senescence, suggesting that the *TaeIF5A-1* might play role for leaf development, which was also supported by the investigations in tomato (Wang et al., 2001) and in rice (Chou et al., 2004). It is interesting to note that the expression level of wheat eIF5A gene is lower in root than in other tissues. The observed patterns of the transcripts of the eIF5A in wheat tissues suggested that the expression of *TaeIF5A-1* has shown spatial and temporal difference.

The current model of the involvement of *TaeIF5A-1* in cell growth and protein synthesis suggests that it is required for correct translation of a subset of mRNAs. Identification of eIF5A in wheat potentially involved in developmental regulated mRNA translational control is of great interest. Biochemical approaches and molecular genetics will probably provide insight into the role (s) which eIF5A performs in plants, especially in polyploidy species.

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