GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genome-wide analysis of immunophilin *FKBP* genes and expression patterns in *Zea mays*

W.-W. Wang[1,2], Q. Ma[2], Y. Xiang[2], S.-W. Zhu[2] and B.-J. Cheng[2]

[1]School of Chemistry and Life Sciences, Suzhou University, Suzhou, Anhui, China
[2]Anhui Province Key Lab of Crop Biology, School of Life Sciences, Anhui Agricultural University, Hefei, Anhui, China

Corresponding author: B.-J. Cheng
E-mail: beijiucheng@ahau.edu.cn

Genet. Mol. Res. 11 (2): 1690-1700 (2012)
Received November 25, 2011
Accepted April 18, 2012
Published June 25, 2012
DOI http://dx.doi.org/10.4238/2012.June.25.2

ABSTRACT. The receptors for the immunosuppression drugs FK506 and rapamycin are called FKBPs (FK506-binding proteins). FKBPs comprise a large family; they are found in many species, including bacteria, fungi, animals, and plants. As a class of peptidyl-prolyl cis-trans isomerase enzymes, the *FKBP* genes have been the focus of recent studies on plant stress tolerance and immunology. We identified and analyzed gene families encoding these proteins in maize using computational and molecular biology approaches. Thirty genes were found to encode putative *FKBPs* according to their FK506-binding domain. The *FKBP* genes can be classified into single domain and multiple domain members based on the number of the domains. By analysis of the physical locations, the 30 *FKBP* genes were found to be widely distributed on 10 chromosomes. After analysis of the FKBP phylogenetic tree in the maize genome, we found that the 30 genes revealed two major clades. Gene duplication played a major role in the evolution of *FKBP* genes, which suggests that the *FKBP* genes in maize have a pattern significantly different from that of these genes in rice. Based on semi-quantitative RT-PCR, we found that the 30 *FKBPs* were expressed differently in various tissues in maize, which suggests

that *FKBP* genes play different roles in each tissue. Several *FKBPs* were expressed at higher levels in roots, indicating that these genes in maize may have similar or overlapping functions.

Key words: Maize; FKBPs; Gene duplication; Expression

INTRODUCTION

FK506 is a macrolide immunosuppressant composed of 23 rings. Proteins that can bind it are called FK506-binding proteins (FKBPs). This is a class of highly homologous receptor-binding proteins that are widely present in all organisms, including bacteria, fungi, animals, and plants. FKBPs are mainly divided according to molecular weight difference, such as FKBP12, FKBP15, FKBP22, FKBP55, and so on. As cellular receptors for the immunosuppressant drug ligands FK506 and rapamycin (Siekierka et al., 1989; Harding et al., 1989), FKBPs can catalyze the cis to trans configuration of the N-terminal peptide bond of proline residues in peptide or protein substrates, and consequently the FKBPs have peptidyl-prolyl cis-trans isomerase (PPIase) activity (Fischer et al., 1989; Harding et al., 1989), which may be involved in protein folding processes (Brillantes et al., 1994). In many plants, *FKBP* genes have been found to be vital for regulating normal growth and development, for coping with stress conditions and fundamental considerations for modern cereal farming. Other findings suggest that *FKBP* genes can operate as molecular chaperones (Hartl et al., 2002). All of these points suggest that these proteins participate in important cellular processes. Consequently, *FKBP* genes have been the focus in plant-related subject areas.

FKBPs have been characterized by the FKBP signature residues that form the binding pocket for FK506 or rapamycin (Schreiber, 1991), and the FKBP signature is derived from the available structures of the FKBP12 protein (Michnick et al., 1991; Van Duyne et al., 1991). It was found that the residues required for binding the drug are highly conserved, and the conserved binding site is therefore used as the signature for the FKBP protein identification, which is called the FK506-binding domain (FKBd). Structure analysis of FKBPs has shown that they are composed of at least one FKBd, which forms a highly conserved structure (Galat, 2000). The size of the FKBPs vary significantly, ranging from single-domain (SD) isoforms comprising a single FKBd, to large (>100 kDa) complex multi-domain (MD) proteins (Galat, 2003; Rulten et al., 2006). The most typical SD isoform is FKBP12, which only has 110 amino acids. In maize, FKBP12 binds rapamycin (Agredano-Moreno et al., 2007), forming a complex that inhibits the "target of rapamycin" (TOR) kinase, which can powerfully regulate the germination and the development of the plant (Marivet et al., 1995). Every FKBP has a high sensitivity for different substrates with differences up to a thousand times, showing substrate selectivity. These features imply that PPIase activity of FKBPs plays an important role in the cell. Recently, many studies have begun to examine the function of FKBPs in plants. For example, the disruption of AtFKBP42 gene function causes developmental defect (Kamphausen et al., 2002). Another study has shown that a chloroplast FKBP can interact with a photosynthetic electron carrier, which affects the accumulation of the protein (Gupta et al., 2002). As in animals, different members of FKBPs appear to play different roles in plants.

In recent years, studies have carried out several valuable functional characterizations of individual plant FKBPs, but the identification and analyses of all FKBPs have only been in

Arabidopsis (He et al., 2004; Romano et al., 2005) and *Oryza sativa* (rice) (Gollan and Bhave, 2010) genomes, which have revealed the true size and character of the FKBP family in higher plants. Bioinformatics have opened up new and highly efficient analysis and research approaches (Staskawicz et al., 1995), and can be used to conduct a comprehensive and complete analysis of the basic data of the whole genome with regard to *FKBP* genes, which play an important role in large-scale gene isolation, gene functional annotation and functional genomics studies. *Zea mays* L. (maize) is the most widely cultivated plant in the world, which also provides a model genome for molecular study. Therefore, we took advantage of the sequenced genome of the *Zea mays* L. (maize) and selected bioinformatics applications to perform a genome analysis of the entire FKBP family of maize.

MATERIAL AND METHODS

The genome of maize

In this study, the complete genome sequence of maize was searched in the maize genome website (http://www.maizesequence.org/index.html).

Identification of *FKBP* genes and sequence analysis

The key word "fkbp" was used to search for the *FKBP* genes in NCBI, and through the Pfam database, we then obtained the conserved sequence of the FKBd-c domain. The Hidden Markov model profile of the FKBd-c domain was used as a query in BLASTP searches for possible homologs in maize genome databases. The threshold expectation value was set to 10^{-3}, a value determined empirically to filter out most of the spurious hits. This step was crucial to find the maximum number of candidate genes. By using the Pfam database (Protein family) (http://pfam.janelia.org), we analyzed all the candidate gene sequences to remove genes that did not contain a FKBd-c domain. We then aligned the sequences using the ClustalW software (Thompson et al., 1994).

The molecular weights and isoelectric points (pI) of putative FKBPs were predicted using the Compute pI/MW Tool at the Expert Protein Analysis System (ExPAsy) site (http://au.expasy.org/tools/pi_tool.html).

Physical locations of *FKBP* genes

The physical locations of all regular *FKBP* genes were confirmed by tBLASTn (P value = 0.001) search using a local database of complete *Zea mays* L. genome sequences of each chromosome. Afterwards, the Genome Pixelizer software (http://www.niblrrs.ucdavis.edu/GenomePixelizer/GenomePixelizer_Welcome.html) was used for a graphic portrayal of *FKBP* genes of maize.

Phylogenetic tree construction

Multiple amino acid sequence alignments of the maize *FKBP* genes were performed using the ClustalW software. In order to analyze the evolutionary relationship of the *FKBP*

genes in maize, we constructed the phylogenetic tree using the bootstrap neighbor-joining (NJ) method (1000 bootstrap replicates) with a Kimura 2-parameter model by MEGA 3.1 (http://www.megasoftware.net/).

In order to analyze the evolutionary relationships of the *FKBP* genes between different plants, we compared the *FKBPs* in maize and rice. We obtained the 29 *FKBP* sequences of rice according to Gollan and Bhave (2010). Along with the 30 *FKBPs* in maize, there were 59 *FKBPs* in all for construction of the phylogenetic tree. Multiple alignments of 59 amino acid sequences were performed using ClustalX with default options. Phylogenetic trees were constructed based on the alignment by Clustalxl.81.

Semi-quantitative RT-PCR analysis

The maize seeds of B73 were germinated on plastic plates, and the plants were then transferred to a nutrient solution at 28°C in a culture room under a 16-h light and 8-h dark photoperiod.

Total RNAs were extracted from various tissues (leaves, stems and roots) using the RNAiso reagent (Tiandz Biotechnology) according to manufacturer instructions. First-strand cDNAs were generated from the RNA with the Access RT-PCR system (Promega, USA). The gene-specific primers used for ZmFKBPs were designed to examine the expression patterns of *FKBP* genes. The expression of the actin gene was used as an internal control to normalize the variation in the quality of RNA between different reactions. PCR was performed using Taq polymerase (Tiandz Biotechnology) on a thermal cycler (Tpersonal 48; Biometra, Germany). The RT-PCRs were repeated three times and consistent results were obtained.

RESULTS

Identification and nomenclature of *FKBP* genes in maize

In order to identify the *FKBP* genes, we checked the sequenced genome of maize for these genes using the multiple methods described above; 44 *FKBP* genes were identified in the maize genome. After removal of overlapping sequences, we finally obtained 30 distinct loci encoding putative *FKBP*s containing the characteristic FKBd (Table 1). These genes were then analyzed using the Pfam database; the results showed that the 30 genes all contained highly conserved FKBds. Finally, we checked the FKBds in each of the gene sequences manually. FKBd analysis categorized these *FKBPs* into single and multiple domain genes.

In this study, the nomenclature also adopted the rules that are rather consistent in previously published *FKBPs* in animals and plants. The proteins are designated *FKBP* with the prefix letters “Zm” and a suffix number. The suffix numbers are labeled according to their orthology with reported isoforms in *Oryza sativa* (rice), rather than solely based on their estimated molecular weights. In some cases, the similarities in domain structures and pI were also considered, for example, the putative *FKBP* denoted as ‘ZmFKBP13’ from maize showing the highest amino acid identity with OsFKBP13, despite its predicted molecular mass being 22 kDa (Table 1).

Table 1. Properties of putative FKBPs identified from maize genome.

Maize FKBP name	Maize FKBP locus ID	Rice homologue	Putative length	Molecular mass (kDa)	pI
ZmFKBP12a	GRMZM2G015784_P01	OsFKBP12	112	12.18	8.6
ZmFKBP12b	GRMZM2G015784_P03	OsFKBP12	88	9.6	9.0
ZmFKBP13	GRMZM2G095333_P01	OsFKBP13	213	21.9	9.5
ZmFKBP15-1a	GRMZM2G153991_P01	OsFKBP15-1	155	16.4	8.9
ZmFKBP15-1b	GRMZM2G031204_P01	OsFKBP15-1	155	16.3	8.9
ZmFKBP15-2a	GRMZM2G115757_P01	OsFKBP15-2	148	15.8	6.7
ZmFKBP15-2b	GRMZM2G115757_P02	OsFKBP15-2	142	15	5.4
ZmFKBP16-1	GRMZM2G096585_P06	OsFKBP16-1	239	27.2	8.9
ZmFKBP16-2a	GRMZM2G035708_P02	OsFKBP16-2	181	18.6	8.9
ZmFKBP16-2b	GRMZM2G035708_P03	OsFKBP16-2	226	22.9	9.2
ZmFKBP16-3	GRMZM2G030494_P01	OsFKBP16-3	201	20.4	8.7
ZmFKBP16-4	GRMZM2G001956_P01	OsFKBP16-4	220	22.9	9.9
ZmFKBP17-1	GRMZM2G041060_P03	OsFKBP17-1	474	51	9.0
ZmFKBP17-2a	GRMZM2G057374_P01	OsFKBP17-2	253	26.7	7.8
ZmFKBP17-2b	GRMZM2G068963_P01	OsFKBP17-2	253	26.7	6.8
ZmFKBP18	GRMZM2G150337_P04	OsFKBP18	207	22.1	9.9
ZmFKBP19	GRMZM2G155753_P03	OsFKBP19	240	26.7	9.0
ZmFKBP20	GRMZM2G035922_P01	OsFKBP20-1a	186	20	6.2
ZmFKBP21	GRMZM2G035708_P06	OsFKBP16-2	211	27.2	9.6
ZmFKBP22	GRMZM2G012340_P01	OsFKBP17-2	205	26.8	9.7
ZmFKBP42	GRMZM2G133624_P01	OsFKBP42a	374	42	6.0
ZmFKBP53	GRMZM2G024811_P01	OsFKBP53a	495	53.9	5.2
ZmFKBP27	GRMZM2G104529_P01	OsFKBP53b	246	26.8	9.2
ZmFKBP49	GRMZM2G399313_P01	OsFKBP53b	444	49.1	5.8
ZmFKBP64a	GRMZM2G096585_P04	OsFKBP64	492	54.3	5.1
ZmFKBP64b	GRMZM2G096585_P03	OsFKBP64	553	61.7	5.2
ZmFKBP62	GRMZM2G096585_P05	OsFKBP64	559	62.4	5.2
ZmFKBP65	GRMZM2G154685_P01	OsFKBP65	588	66.3	5.2
ZmFKBP72	GRMZM2G117746_P01	OsFKBP72	631	70.4	5.4
ZmFKBP75	GRMZM2G336858_P01	OsFKBP75	523	58.8	6.1

pI = isoelectric point.

Chromosomal locations and genomic duplications of maize *FKBP* genes

In maize, *FKBP* genes were widely distributed on 10 chromosomes from chromosome 1 to chromosome 10 (Figure 1). Chromosomes 1 and 5 had the largest number of *FKBP* genes (7), and chromosome 7 had 4 *FKBP* genes. On chromosomes 2 and 4, there were 3 *FKBP* genes, respectively, chromosome 9 had 2, and the rest only had 1. According to the definition of gene cluster, there are three or more genes in the same chromosome and the neighboring regions are less than 200 kb (Holub, 2001). From the physical location map (Figure 1), there are 14 genes in 3 gene clusters, and other genes were rather equally distributed on each chromosome. According to He et al. (2004), the gene cluster of the *FKBPs* in *Arabidopsis* is not obvious, which suggests that maize showed a significantly different pattern from *Arabidopsis* and other plants. In the evolution of maize, the *FKBP* gene may have arisen from gene duplication and recombination.

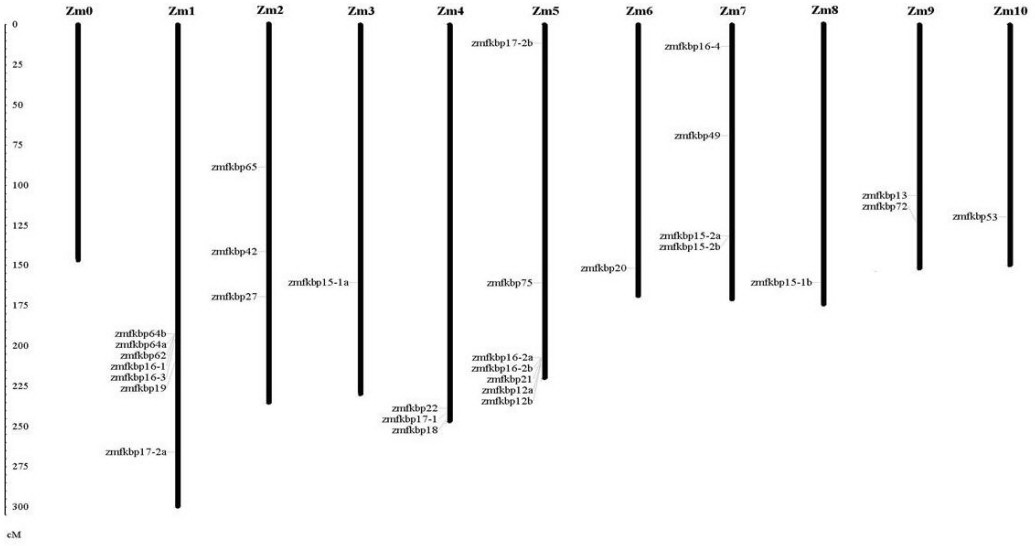

Figure 1. Physical locations of *FKBP* genes in maize.

According to the definition of gene duplication: 1) in a relatively long genome, more than 70% of the nucleotide sequence is arranged, and 2) the similarity of the nucleotide sequence in the arranged area is more than 70% (Zhou et al., 2004). In maize, there were 18 genes produced by gene duplication, which accounted for 60% of the total ZmFKBPs. In addition, one ZmFKBP possibly resembled several other ZmFKBPs, for example, ZmFKBP62 was gene duplicated from ZmFKBP64a, ZmFKBP64b and ZmFKBP16-1. This showed that most of the ZmFKBPs were generated from gene duplication and that large-scale gene duplication occurred during genome evolution. The results showed that gene duplication plays an important role in the quantitative expansion of the *FKBP* genes.

Phylogenetic analysis of the *FKBP* genes in maize

To analyze phylogenetic relationships of the *FKBP* genes among maize, phylogenetic tree were constructed based on the alignments of ZmFKBP protein sequences (Figure 2). Because of the highly conserved amino acid sequences of the *FKBP* genes and the region containing similar motifs, the neighbor-joining method of the MEGA 3.1 software was adopted to construct the phylogenetic trees for the 30 *FKBPs* in maize.

From the phylogenetic tree (Figure 2), the *FKBP* genes in maize showed high sequence similarity between the members of the all family. The tree showed the *FKBP* genes distributed in two distinct clades, and the members in each clade various showed more significant similarity. In addition, the overall sequence similarity between the members showed some differences. Most of the *FKBP* genes had a near relationship, tending to cluster in one branch, but also several genes had a relatively distant relationship and distributed in one branch (Figure 2), which suggests that the evolution of the *FKBP* genes in maize is complex.

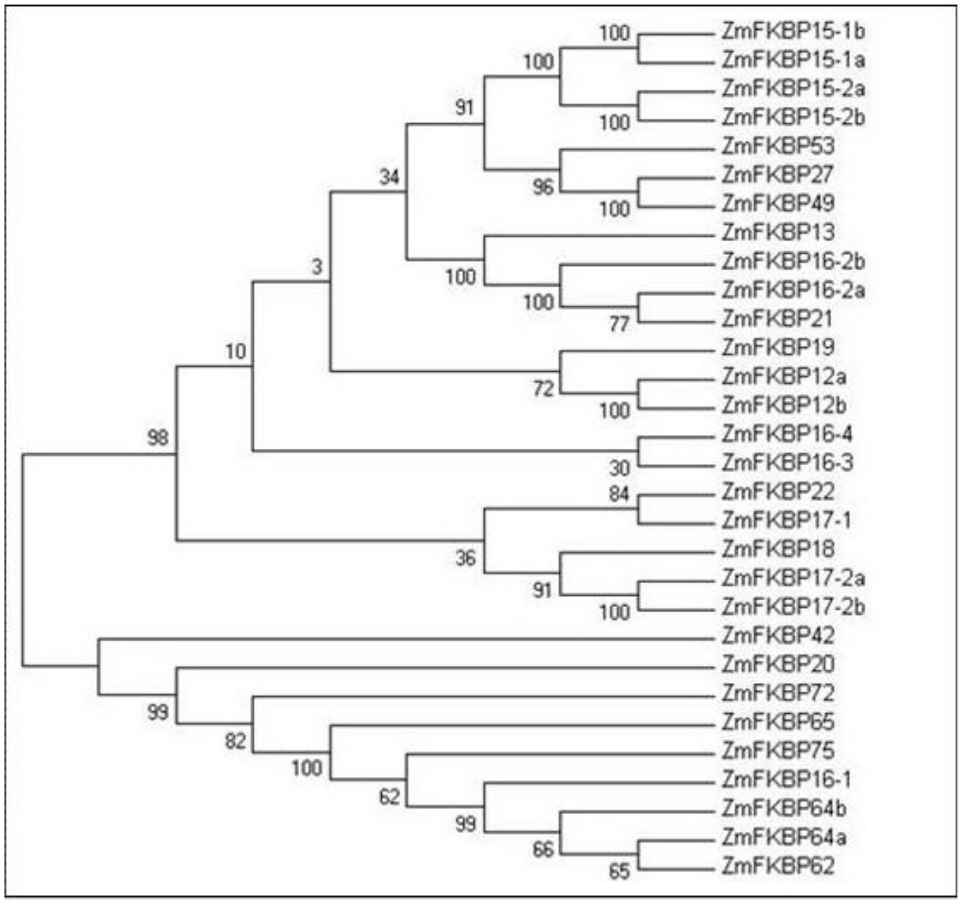

Figure 2. Phylogenetic analyses of the *FKBP* genes in the maize genome.

Evolutionary analysis comparative of the *FKBP* genes between maize and rice

In order to analyze the evolutionary relationships of the *FKBP* genes in different species, the *FKBPs* in maize and rice were compared, and the phylogenetic tree was constructed based on the alignments of ZmFKBP and OsFKBP protein sequences. The tree shows multiple smaller branches but few big branches, which was very different from maize. From the tree (Figure 3), we can see not only the contact between maize and the rice, but also an obvious difference. For almost every *FKBP* gene in rice, there was one or more corresponding ZmFKBPs showing the highest sequence similarity (Figure 3), thereby indicating that the *FKBPs* in maize and rice have high homology. In maize, the number of the *FKBPs* is more than in rice, so it appears that several ZmFKBPs correspond to other ZmFKBPs, but not OsFKBPs, and this may be due to the gene duplication in maize. This also indicates that the evolution of the *FKBPs* in maize is more complex.

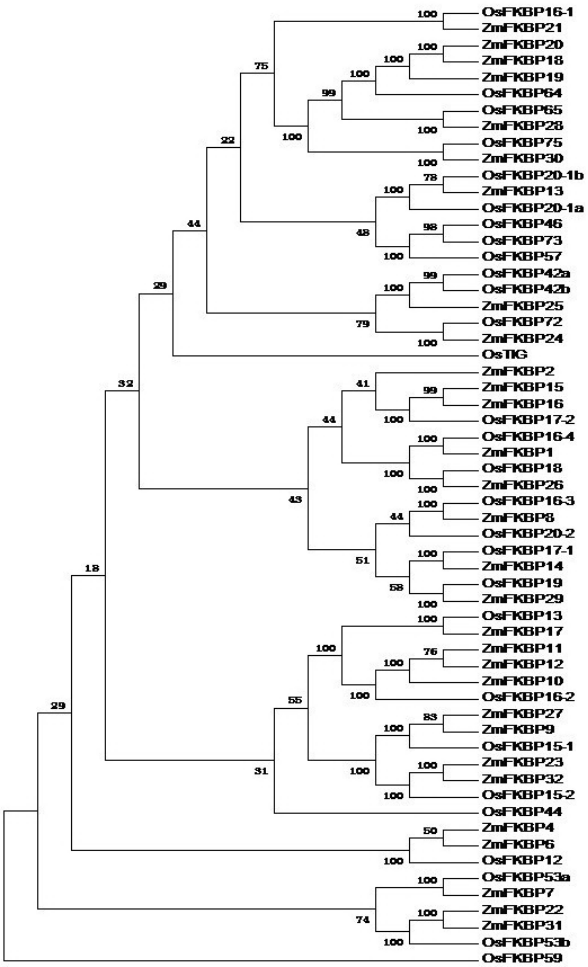

Figure 3. Phylogenetic analyses of the *FKBP* genes in the maize and rice genomes.

Expression of *FKBP* genes

The expression of the *FKBP* genes was examined by RT-PCR. RT-PCR analysis indicated that all 30 *FKBP* genes were expressed in maize plants. The expression patterns of the genes are shown in Figure 4. The genes were expressed in different tissues including roots, stems and leaves, but expression was detected at different levels. From the figure we can clearly see the different expression patterns; the expression of several genes was higher in both leaves and stems compared to roots, such as ZmFKBP18 and ZmFKBP19. There were some special genes that only expressed in one or two tissues and not expressed in the second or third tissue. A special gene, ZmFKBP17-2a, appeared to be only expressed in roots, implicating ZmFKBP17-2a in further specific functions. Moreover, for some close relationship genes, they showed complementary gene expression patterns, such as ZmFKBP64a and ZmFKBP64b. All of the results suggest that the 30 *FKBP* genes are expressed in every tissue of maize but at different levels. This expression pattern provides an important basis for further study of the roles and function of the *FKBP* genes.

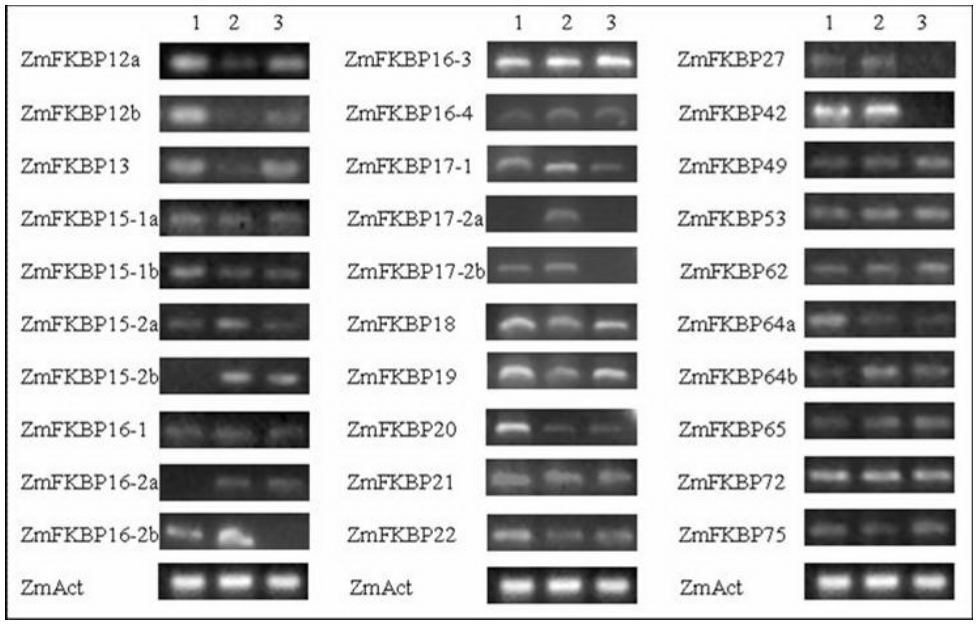

Figure 4. Electrophoresis of *FKBP* RT-PCR products. *Lane 1* = *FKBP* gene in leaves; *lane 2* = *FKBP* gene in roots; *lane 3* = *FKBP* gene in stems, and ZmAct was used as a template control.

DISCUSSION

The FK506- and rapamycin-binding proteins are abbreviated as FKBPs. FKBPs have been identified in all organisms examined, including bacteria, fungi, animals, and plants (Schreiber, 1991; Fruman et al., 1994; Luan, 1998), and they have been extensively studied and described in other species. In this study, we analyzed the sequence of the *Zea mays* L. (maize)

genome and revealed 30 *FKBP* genes, which comprise the largest FKBP family so far reported. For example, the yeast genome contains 4 genes for *FKBPs* (Heitman et al., 1991), and the human genome contains 18 *FKBPs* (Uchida et al., 1999). The large number of genes for immunophilins in plants indicates a diverse array of functions served by these proteins, while this may also reflect a significant degree of functional redundancy among the genes. Among plants, maize also contains a larger number than others, where this may be due to the frequency of the *FKBP* gene duplications in maize. The origin of *FKBP* genes, formation of gene families and the multiplicity of gene expression can be understood by analyzing gene duplication mechanisms. In maize, gene duplication analysis shows that at least 18 *FKBPs* occurring as duplicates in 30 maize FKBP families, the ratio of gene duplications was very high. However, in *Arabidopsis*, the studies reported there were only 4 duplicated genes of the 23 AtFKBPs (He et al., 2004), which were less than the maize. Not only the numbers but also the gene duplications in maize were all significantly increased compared to other plants. Therefore, the evolutionary patterns in *Zea mays* L. were special, which was likely the result of gene duplication events.

Immunophilins consist of a very diverse group of proteins and may have very different origins during genome evolution. Studies in maize report an uneven chromosomal distribution of *FKBP* genes, and the majority of the genes have been found in clusters, which may be due to the high frequency of gene duplication. The *FKBP* genes of maize were widely distributed on 10 chromosomes, and the distribution showed great differences, where chromosome 1 and 5 contained the maximum number, which revealed the important role of the two chromosomes in the evolution of *FKBP* genes. In the maize 30 *FKBP* genes, 60% fell in clusters; however, there were no obvious *c*lusters in the *Arabidopsis* genome. This result indicated that gene duplication played an important role in gene expansion.

Based on the analysis of the phylogenetic trees of the *FKBP* genes, the maize sequences showed two major clades, which was different from rice. Rice sequences exhibited no notable clustering, rather giving rise to several small branches, which may due to its different structure. Furthermore, the gene had high homology, often distributed in one branch, which was very similar to maize. Therefore, we infer that the two monocotyledons have similarities in evolution, and that they may have an analogous mode of selection and evolution. Despite strong homology, the number of the genes and the chromosomal locations in maize are different compared to rice. These differences may reflect different kinds of ancient duplication, which also demonstrate that FKBPs in maize comprise an extraordinary family.

The transcript levels were much lower in roots as compared to those in the green tissues (leaves and stems) in *Arabidopsis*, according to Luan et al. (2004). In our study, FKBPs expressed in every tissue of maize, but their expression does not follow obvious rules, which means every tissue has its strongest expressed gene in maize. This result may be due to different environmental factors and different tissues used for the expression of these genes in this study. We found that ZmFKBP16-2b, ZmFKBP16-3 and ZmFKBP42 were all expressed higher in roots, indicating that these three genes in maize may have similar or overlapping functions. The data presented here indicate that the expression intensity of the 30 *FKBP* genes was different in every tissue, so every *FKBP* plays different roles in each tissue.

ACKNOWLEDGMENTS

Research supported by the Key Projects in the National Science & Technology Pillar

Program (#2009BADA6B00), the National Natural Science Foundation (#11075001) and the Master Scientific Research Foundation of Suzhou University (#2011yss02).

REFERENCES

Agredano-Moreno LT, Reyes dlC, Martinez-Castilla LP and Sanchez de JE (2007). Distinctive expression and functional regulation of the maize (*Zea mays* L.) TOR kinase ortholog. *Mol. Biosyst.* 3: 794-802.

Brillantes AB, Ondrias K, Scott A, Kobrinsky E, et al. (1994). Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein. *Cell* 77: 513-523.

Fischer G, Wittmann-Liebold B, Lang K, Kiefhaber T, et al. (1989). Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. *Nature* 337: 476-478.

Fruman DA, Burakoff SJ and Bierer BE (1994). Immunophilins in protein folding and immunosuppression. *FASEB J.* 8: 391-400.

Galat A (2000). Sequence diversification of the FK506-binding proteins in several different genomes. *Eur. J. Biochem.* 267: 4945-4959.

Galat A (2003). Peptidylprolyl cis/trans isomerases (immunophilins): biological diversity-targets-functions. *Curr. Top. Med. Chem.* 3: 1315-1347.

Gollan PJ and Bhave M (2010). Genome-wide analysis of genes encoding FK506-binding proteins in rice. *Plant Mol. Biol.* 72: 1-16.

Gupta R, Mould RM, He Z and Luan S (2002). A chloroplast FKBP interacts with and affects the accumulation of Rieske subunit of cytochrome bf complex. *Proc. Natl. Acad. Sci. U. S. A.* 99: 15806-15811.

Harding MW, Galat A, Uehling DE and Schreiber SL (1989). A receptor for the immunosuppressant FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* 341: 758-760.

Hartl FU and Hayer-Hartl M (2002). Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295: 1852-1858.

He Z, Li L and Luan S (2004). Immunophilins and parvulins. Superfamily of peptidyl prolyl isomerases in *Arabidopsis*. *Plant Physiol.* 134: 1248-1267.

Heitman J, Movva NR and Hall MN (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* 253: 905-909.

Holub EB (2001). The arms race is ancient history in *Arabidopsis*, the wildflower. *Nat. Rev. Genet.* 2: 516-527.

Kamphausen T, Fanghanel J, Neumann D, Schulz B, et al. (2002). Characterization of *Arabidopsis thaliana* AtFKBP42 that is membrane-bound and interacts with Hsp90. *Plant J.* 32: 263-276.

Luan S (1998). Immunophilins in animals and higher plants. *Bot. Bull. Acad. Sin.* 39: 217-223.

Marivet J, Frendo P and Burkard G (1995). DNA sequence analysis of a cyclophilin gene from maize: developmental expression and regulation by salicylic acid. *Mol. Gen. Genet.* 247: 222-228.

Michnick SW, Rosen MK, Wandless TJ, Karplus M, et al. (1991). Solution structure of FKBP, a rotamase enzyme and receptor for FK506 and rapamycin. *Science* 252: 836-839.

Romano P, Gray J, Horton P and Luan S (2005). Plant immunophilins: functional versatility beyond protein maturation. *New Phytol.* 166: 753-769.

Rulten SL, Kinloch RA, Tateossian H, Robinson C, et al. (2006). The human FK506-binding proteins: characterization of human FKBP19. *Mamm. Genome* 17: 322-331.

Schreiber SL (1991). Chemistry and biology of the immunophilins and their immunosuppressive ligands. *Science* 251: 283-287.

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, et al. (1995). Molecular genetics of plant disease resistance. *Science* 268: 661-667.

Thompson JD, Higgins DG and Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.

Uchida T, Fujimori F, Tradler T, Fischer G, et al. (1999). Identification and characterization of a 14 kDa human protein as a novel parvulin-like peptidyl prolyl cis/trans isomerase. *FEBS Lett.* 446: 278-282.

Van Duyne GD, Standaert RF, Karplus PA, Schreiber SL, et al. (1991). Atomic structure of FKBP-FK506, an immunophilin-immunosuppressant complex. *Science* 252: 839-842.

Zhou T, Wang Y, Chen JQ, Araki H, et al. (2004). Genome-wide identification of NBS genes in japonica rice reveals significant expansion of divergent non-TIR NBS-LRR genes. *Mol. Genet. Genomics* 271: 402-415.