

## Genome-wide analysis of cyclins in maize (*Zea mays*)

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**ABSTRACT.** Cyclins are primary regulators of the activity of cyclin-dependent kinases and play crucial roles in cell cycle progression in eukaryotes. Although extensive studies have revealed the roles of some cyclins and underlying mechanisms in plants, relatively few cyclins have been functionally analyzed in maize. We identified 59 cyclins in the maize genome, distributed on 10 chromosomes; these were grouped into six types by phylogenetic analysis. The cyclin genes in the maize genome went through numerous tandem gene duplications on five chromosomes. However, no segmental duplications, which occur in rice, were found on maize chromosomes. This information allows us to assess the position of plant cyclin genes in terms of evolution and classification, which will be useful for functional studies of maize cyclins.

**Key words:** Cyclin; Gene duplication; Phylogenetic analysis; *Zea mays*

## INTRODUCTION

Similar to animals and yeasts, progression through the cell cycle is primarily driven by a family of cyclin-dependent kinases (CDKs) in plants. The catalytic activity of CDKs is directly regulated by binding and activation of cyclins, and can be further controlled by several additional mechanisms, including protein phosphorylation/dephosphorylation, proteolysis, and CDK inhibitor protein binding (Stals and Inze, 2001; Rossi and Varotto, 2002; Wang et al., 2004; La et al., 2006). Numerous studies have indicated that cyclins, as essential regulators of CDKs, not only activate CDKs by changing the conformation at their catalytic sites, but also contribute to the subcellular localization and substrate specificity of the complex as well as regulation of protein stability (Booher et al., 1989; Potuschak and Doerner, 2001; Wang et al., 2004).

Since the first discovery of cyclins in sea urchin eggs, at least 14 classes of cyclins have been discovered in animals, such as sea urchin, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens* (Nakamura et al., 1995; Pines, 2002; Wang et al., 2004). Plant cyclins were initially discovered in 1991 (Hata et al., 1991). Since then, more than 70 plant cyclins have been isolated from various plant species. In animals, multiple cyclins have been identified in several species, of which several have been described in the human genome. Mainly based on functions and sequence identity, the human cyclins involved in cell cycle control were classified into A- to I-type, K-, L-, T-type, and UNG2 (uracil DNA glycosylase 2) proteins (Nakamura et al., 1995; Pines, 2002). As in plants, these cyclins are classified as A-, B-, C-, D-, H-, and L-type cyclins (Renaudin et al., 1996; Yamaguchi et al., 2000; Barroco et al., 2003). There are 49 cyclins found in *Arabidopsis*, which are divided into 10 types on the basis of function and sequence analysis, including A- to D-type, H-, L-, T-, U-, SDS-, and J18-types (Wang et al., 2004). Similar to *Arabidopsis*, there are several common types between the two species, except C- and CycJ18-type, which are present in *Arabidopsis* but are absent in rice. However, F-type cyclins are only found in rice, and it has been suggested that this type is specific to monocotyledons (La et al., 2006). In maize, four D-type genes, *Zeama;CycD2;1*, *Zeama;CycD4;1*, *Zeama;CycD4;2*, and *Zeama;CycD5;1* (Quiroz-Figueroa and Vázquez-Ramos, 2006), and five mitotic cyclins, *Zeama;CycB1;1* (formerly cycIaZm), *Zeama;CycB1;2* (formerly cycIbZm), *Zeama;CycA1;1* (formerly cycIIZm), *Zeama;CycB2;1* (formerly cycIIIZm), and *Zeama;CycB1;3* (formerly CycZme1) (Renaudin et al., 1994; Sun et al., 1999), have been reported. Recently, whole genome sequences of maize became available (Schnable et al., 2009), providing an excellent opportunity to study plant cyclins extensively.

Previous studies indicated that a typical animal or plant cyclin contains a conserved 250-amino acid region called the cyclin core region, which consists of two domains: cyclin\_N and cyclin\_C (Nugent et al., 1991). The cyclin\_N domain is also known as the cyclin box with a highly conserved sequence and is found in most of the known cyclins. It is about 100 amino acids long and contains the CDK-binding site. The cyclin\_C domain is less conserved. Some known cyclins, such as the human cyclins G1 and G2, *Arabidopsis* C-, H-, T-, L-, J18-, P-, partial B- and D-type cyclins, have the cyclin\_N domain but not the cyclin\_C domain (Horne et al., 1996), suggesting that the cyclin\_C domain may not be critical for its function. In general, cyclins are well conserved in both plants and animals. Some cyclins (A-, B- and D-type) have been studied in detail not only in yeast and animals, but also in plants. However, several types of animal cyclins, including the E-, G- and K-types, are not found in plants. D-type cyclins, also regarded as G1-specific cyclins because they are the first cyclins expressed in G1 phase, are conserved in animals and plants. They were proposed to be sensors of growth conditions and trigger the G1/S transition by activating the RBR/E2F-DP pathway (Gutierrez et al., 2002; Shen, 2002; Trimarchi and Lees, 2002). Further study revealed that plant D-type cyclins may

function as mediators of internal and environmental stimuli to drive cell division. E-type cyclins are the next cyclins to be expressed in mid to late G1 phase and serve as important regulators in S-phase entry in animals; however, they lack homologues in plants (Obaya and Sedivy, 2002). Both B- and A-type cyclins in plants, known as mitotic cyclins, are expressed during mitosis. A-type cyclins are expressed soon after E-type cyclins at the G1/S boundary and form complexes with Cdk2. The activity of cyclin A/Cdk2 is required for S-phase transition and control of DNA replication. B-type cyclins are expressed within a narrow time window from late G2 to mid M phase, and they contribute greatly to the G2/M transition (Lehner and O'Farrell, 1990; Pagano et al., 1992; Obaya and Sedivy, 2002). In previous studies, only a single A-type cyclin gene was found in invertebrates, and two were found in vertebrates (Nieduszynski et al., 2002). In contrast to animals, plants possess a higher complexity of A- and B-type cyclins, and much research has shown that they have extensive and complex functions. For example, *Nicta*;CYCA3;2 has important functions, analogous to those of cyclin E in animals; the alfalfa A2-type cyclin *Medsa*;CYCA2;2 is expressed in all phases of the cell cycle (Roudier et al., 2000; Yu et al., 2003). In addition, some cyclins such as H-, L- and T-type, have been discovered in both animals and plants, and their functions have been studied in depth (Umeda et al., 1999; La et al., 2006).

In this study, 59 cyclin genes were identified in maize by database searches and were grouped into 6 types according to protein domains. Phylogenetic analyses were performed followed by the complete survey of genomic organization, as well as exon/intron and protein motif structural analyses of the cyclin family genes. On the basis of maize chromosome sequence clone information, these cyclin genes were mapped to 10 different maize chromosomes. Furthermore, tandem gene and segmental duplication events that occurred on maize chromosomes were investigated. The results of these group classifications and investigation of gene duplication will be useful for future studies of cyclin families.

## MATERIAL AND METHODS

### Identification of putative maize cyclins

To retrieve data on maize cyclins, a series of searches in NCBI were performed. Maize protein sequences were downloaded from the website <http://www.maizesequence.org/index.html>, and then Hidden Markov Model (HMM) profiles of cyclin\_N domains and cyclin\_C domains from rice were obtained using the Pfam database (<http://pfam.janelia.org/search/sequence>). As cyclin\_N domains are found in all known cyclins and are highly conserved, we used the BLASTP program to search the maize protein database with the HMM profile of cyclin\_N domain as a query. The e-value chosen in BLASTP was set at  $1e^{-3}$ . Finally, overlapping genes were removed using a complete sequence alignment method in MEGA 3.1 to obtain the final set of non-overlapping set of cyclin genes in maize.

### Nomenclature of the predicted maize cyclin genes

We consulted the principles adopted in other articles (Vandepoele et al., 2002; Wang et al., 2004; Jiang and Ramachandran, 2004) and the nomenclature put forward by Renaudin et al. (1996). The newly identified genes in this study were named in line with the nomenclature used for the previously identified genes and on the basis of their phylogenetic relatedness to other members of the same family. Because both maize and rice are monocotyledons, the nomenclature of the predicted maize cyclin genes was mainly based on the phylogenetic relationship of the protein sequences between rice and maize.

## Phylogenetic analysis of cyclin genes in maize

The phylogenetic tree including maize and rice cyclin genes was generated by MEGA 3.1 as well as the phylogenetic tree including *Arabidopsis* and maize with the following parameters: Poisson correction, pairwise deletion, bootstrap (1000 replicates) (Kumar et al., 2004).

## Conserved motif analysis

To identify the conserved motifs in cyclin proteins, the protein sequences of maize cyclins were merged using the GENESTUDIO software, and then the protein sequences of maize cyclin genes were analyzed using MEME (Multiple EM for Motif Elicitation).

## Mapping cyclins on maize chromosomes

In this study, we used the DNA TOOLS software to set up local databases of the maize complete nucleotide sequences, and the starting position of all cyclin genes on each chromosome was obtained using tBLASTn ( $P = 0.001$ ). Using this method, the physical locations of all cyclin genes were confirmed. Finally, the chromosome map showing the physical location of all cyclin genes was generated using the Genome Pixelizer software ([http://www.niblr.rrs.ucdavis.edu/GenomePixelizer/GenomePixelizer\\_Welcome.html](http://www.niblr.rrs.ucdavis.edu/GenomePixelizer/GenomePixelizer_Welcome.html)).

## Discovery of duplications of maize cyclin genes

In an attempt to detect tandem and segmental duplications of cyclin genes throughout the maize genome, we utilized a modified method described previously to find duplicated blocks (Vision et al., 2000; Vandepoele et al., 2002). First, the local protein databases that consisted of 59 putative maize cyclin protein sequences were constructed with DNA TOOLS, and homologues with high sequence similarities were found by BLASTP search. We then used the highly similar sequences as query to search the maize bacterial artificial chromosome (BAC) database to determine the number and length of the exons. Finally, tandem and segmental duplications were confirmed by sequence alignment, exon comparisons and chromosome location analysis.

## RESULTS

### Identification of cyclins

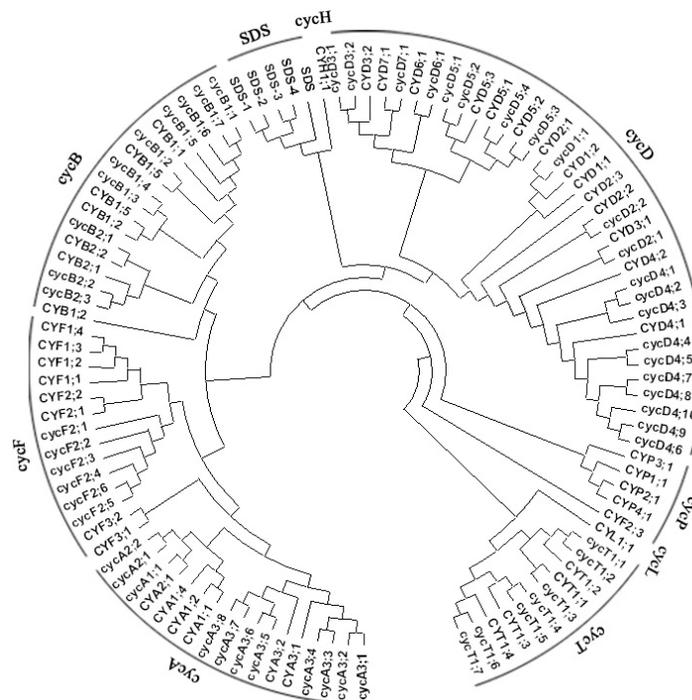
Using the BLAST program available from the Pfam database, the total predicted 79 gene models were confirmed as cyclin genes in maize. After removing the overlapping genes, a total of 59 predicted protein sequences were eventually confirmed as cyclin genes in maize. The overall analysis of the complete genome of maize revealed that the maize cyclin gene family comprises 59 genes (see Material and Methods). All predicted genes and related information are listed in Table 1 including their chromosome locations, sequenced ID, genetic map positions, and number of encoded amino acids. The molecular weights of these deduced cyclin protein sequences generally range from 17.2 (CycD4;9) to 111.7 kDa (CycB1;7).

**Table 1.** Predicted genes and related information including chromosome locations, sequenced ID, genetic map positions, and number of encoded amino acids.

Cyclin name	Sequenced ID	Chromosome	Position (Mb)	Number of AA	MW (kDa)	PI
<i>Zeama;CycA1;1</i>	GRMZM2G017081_P01	5	59.1	489	55.2	9.11
<i>Zeama;CycA2;1</i>	GRMZM2G059922_P01	10	17.6	485	53.7	8.36
<i>Zeama;CycA2;2</i>	GRMZM2G059922_P02	10	17.6	404	44.3	8.88
<i>Zeama;CycA3;1</i>	GRMZM2G026346_P01	1	254.9	305	33.8	8.33
<i>Zeama;CycA3;2</i>	GRMZM2G026346_P02	1	254.9	372	41.4	9.09
<i>Zeama;CycA3;3</i>	GRMZM2G363437_P01	5	18.5	357	40.3	8.51
<i>Zeama;CycA3;4</i>	GRMZM2G363437_P02	5	18.5	351	39.7	8.73
<i>Zeama;CycA3;5</i>	GRMZM2G060690_P01	1	161.5	382	41.6	8.92
<i>Zeama;CycA3;6</i>	GRMZM2G060690_P03	1	161.5	423	46.1	8.85
<i>Zeama;CycA3;7</i>	GRMZM2G180728_P01	3	108.4	433	46.9	8.30
<i>Zeama;CycA3;8</i>	GRMZM2G180728_P02	3	108.4	474	51.6	8.67
<i>Zeama;CycB1;1</i>	GRMZM2G310115_P04	8	170.1	449	50.0	9.19
<i>Zeama;CycB1;2</i>	GRMZM2G034647_P01	8	118.7	446	48.3	9.49
<i>Zeama;CycB1;3</i>	GRMZM2G005619_P01	8	1.5	479	53.3	9.22
<i>Zeama;CycB1;4</i>	GRMZM2G025200_P01	4	15.0	601	67.7	8.63
<i>Zeama;CycB1;5</i>	GRMZM2G073003_P03	3	185.8	228	26.2	6.00
<i>Zeama;CycB1;6</i>	GRMZM2G073003_P01	3	185.8	442	49.5	9.14
<i>Zeama;CycB1;7</i>	GRMZM2G310115_P02	8	170.1	1003	111.7	8.47
<i>Zeama;CycB2;1</i>	GRMZM2G073671_P01	6	81.5	424	47.7	5.75
<i>Zeama;CycB2;2</i>	GRMZM2G138886_P01	2	17.4	424	47.5	5.50
<i>Zeama;CycB2;3</i>	GRMZM2G073671_P01	6	81.5	424	47.7	5.75
<i>Zeama;CycD1;1</i>	GRMZM2G476685_P01	7	99.9	325	34.9	4.72
<i>Zeama;CycD2;1</i>	GRMZM2G075117_P02	4	178.7	360	38.9	5.46
<i>Zeama;CycD2;2</i>	GRMZM2G079629_P01	7	160.5	345	37.8	5.65
<i>Zeama;CycD3;1</i>	GRMZM2G161382_P01	2	160.5	270	28.8	4.67
<i>Zeama;CycD3;2</i>	GRMZM2G161382_P02	2	160.5	391	40.6	4.82
<i>Zeama;CycD4;1</i>	GRMZM2G133413_P01	1	200.6	388	41.9	5.44
<i>Zeama;CycD4;2</i>	GRMZM2G133413_P02	1	200.6	253	26.6	7.50
<i>Zeama;CycD4;3</i>	GRMZM2G133413_P03	1	200.6	187	20.8	9.78
<i>Zeama;CycD4;4</i>	GRMZM2G178229_P01	2	184.5	361	39.1	5.64
<i>Zeama;CycD4;5</i>	GRMZM2G178229_P02	2	184.5	291	32.2	5.60
<i>Zeama;CycD4;6</i>	GRMZM2G178229_P03	2	184.5	290	31.7	7.09
<i>Zeama;CycD4;7</i>	GRMZM2G140633_P01	7	122.1	356	38.8	5.63
<i>Zeama;CycD4;8</i>	GRMZM2G140633_P02	7	122.1	274	30.3	5.37
<i>Zeama;CycD4;9</i>	GRMZM2G140633_P03	7	122.1	158	17.2	4.27
<i>Zeama;CycD4;10</i>	GRMZM2G140633_P05	7	122.1	348	38.0	5.91
<i>Zeama;CycD5;1</i>	GRMZM2G006721_P02	9	143.4	354	37.7	5.41
<i>Zeama;CycD5;2</i>	GRMZM2G007130_P01	1	22.4	345	37.3	5.23
<i>Zeama;CycD5;3</i>	GRMZM2G047637_P01	3	109.6	349	37.9	4.75
<i>Zeama;CycD5;4</i>	GRMZM2G056303_P01	1	253.8	349	36.9	4.83
<i>Zeama;CycD6;1</i>	GRMZM2G050933_P01	7	151.0	307	33.0	5.70
<i>Zeama;CycD7;1</i>	GRMZM2G058410_P01	4	0.8	431	47.4	5.66
<i>Zeama;CycF2;1</i>	GRMZM2G001164_P01	5	181.8	382	43.3	7.67
<i>Zeama;CycF2;2</i>	GRMZM2G401680_P01	5	173.9	244	27.0	5.13
<i>Zeama;CycF2;3</i>	AC212449.4_FGP004	5	174.1	284	31.3	5.61
<i>Zeama;CycF2;4</i>	AC212449.4_FGP001	5	174.1	400	43.0	5.69
<i>Zeama;CycF2;5</i>	AC212449.4_FGP002	5	174.1	343	38.0	5.66
<i>Zeama;CycF2;6</i>	AC212449.4_FGP006	5	174.0	343	38.0	5.46
<i>Zeama;SDS-1</i>	GRMZM2G093157_P01	9	141.1	455	51.0	5.09
<i>Zeama;SDS-2</i>	GRMZM2G093157_P02	9	141.1	459	51.4	5.09
<i>Zeama;SDS-3</i>	GRMZM2G344416_P01	1	28.8	651	72.6	4.99
<i>Zeama;SDS-4</i>	GRMZM2G378422_P01	2	102.7	327	36.3	4.74
<i>Zeama;CycT1;1</i>	GRMZM2G068340_P01	5	188.7	605	67.8	5.18
<i>Zeama;CycT1;2</i>	GRMZM2G101635_P01	4	147.8	608	68.2	5.19
<i>Zeama;CycT1;3</i>	GRMZM2G462118_P01	5	75.2	389	43.8	9.32
<i>Zeama;CycT1;4</i>	AC233979.1_FGP009	10	134.8	487	56.1	7.05
<i>Zeama;CycT1;5</i>	GRMZM2G308570_P02	1	3.9	493	56.7	6.69
<i>Zeama;CycT1;6</i>	GRMZM2G115670_P01	7	82.5	631	71.1	6.18
<i>Zeama;CycT1;7</i>	GRMZM2G377311_P01	1	13.3	592	66.2	6.87

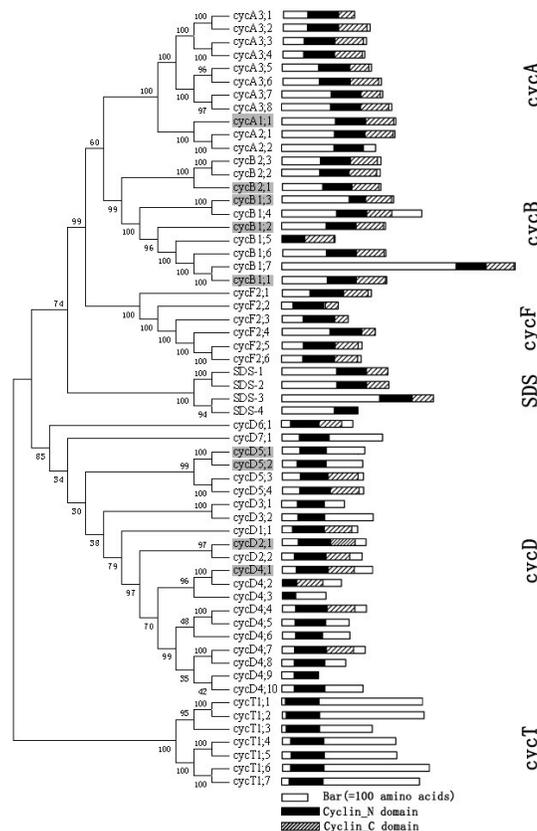
Generic name: designated in previous publications (La et al., 2006). AA = amino acids; MW = molecular weight; PI = isoelectric point of the deduced polypeptide.

To study the evolutionary relationships of cyclin proteins and to classify the newly uncovered members in maize, a phylogenetic tree was generated using 106 putative cyclin sequences, including 59 genes from maize and 47 genes from rice (La et al., 2006) (Figure 1). The maize and rice cyclin genes clustered into nine groups (A, B, D, F, H, L, P, T, and SDS) similar to the phylogenetic tree including rice and *Arabidopsis* (La et al., 2006). There are several common types between the two species, not including the H-, L- and P-types, which are present in rice but not in maize. In the cyclin A group, the types of eight maize genes are closely related to two rice genes, CYA3;1, CYA3;2, while the remaining four maize genes are closely related to four rice genes including CYA2;1, CYA1;1, CYA1;2, and CYA1;4. These genes were designated *cycA1;1-cycA1;8*, *cycA2;1*, *cycA2;2*, and *cycA1;1* on the basis of high sequence similarity to A-type cyclins in rice. Interestingly, two F-type cyclins, CYF3;1 and CYF3;2, in maize are closely related to A-type cyclins belonging to maize and rice. The F-group contains six maize genes and six rice genes, including the CYF2 and CYF1 subgroups, and the members from maize were designated *cycF2;1-cycF2;6* on the basis of sequence similarity with the corresponding F-type genes in rice. Similarly, the nomenclature of other maize cyclin genes, including the B-, D-, T-, and SDS-types, were also confirmed on the basis of the phylogenetic relationship of the rice genes with the corresponding maize cyclin genes. As a result, all maize cyclins were grouped into A-, B-, D-, F-, SDS-, and T-types, and the numbers of genes in these types were 11, 10, 21, 6, 4, and 7, respectively.



## Phylogenetic analysis of predicted maize cyclins

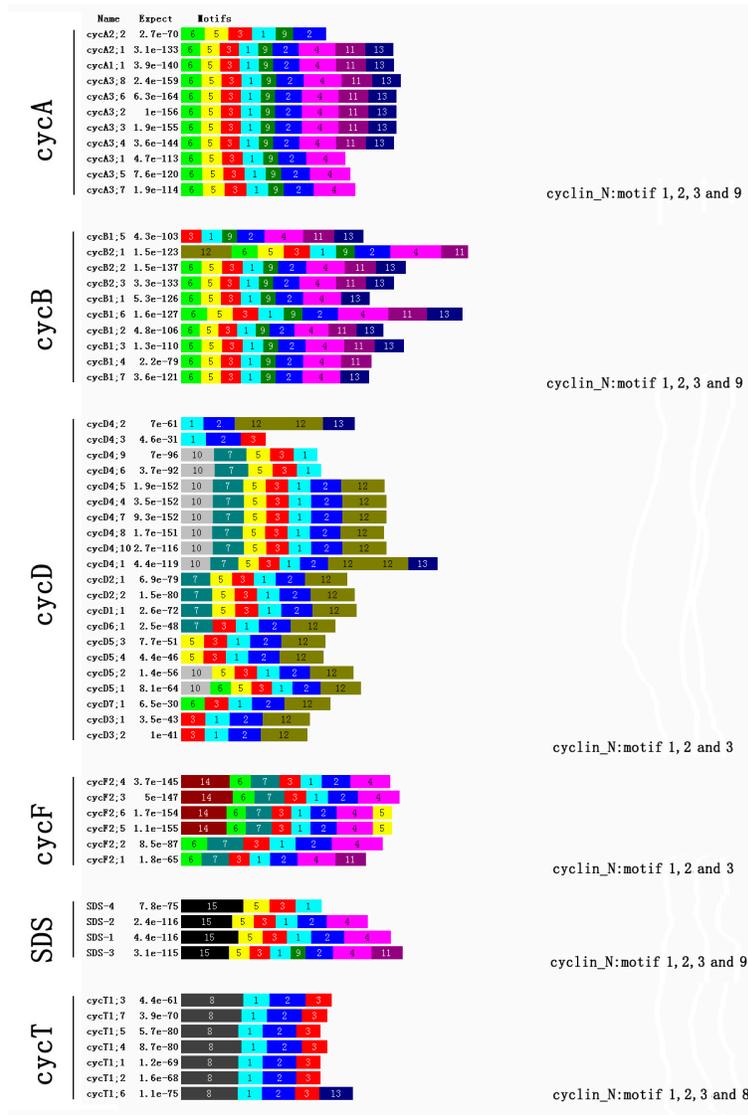
To further study the evolutionary relationships between the genes in the maize cyclin family, a phylogenetic tree was constructed based on maize cyclin\_N domain sequences. As shown in Figure 2, the 59 maize cyclin proteins can be divided into 6 types. Each type of cyclin formed a relatively separate clade, and the relationships among these clades are also generally consistent with that in Figure 1. A- and B-type maize cyclins are more closely related compared with other types. D-type members form the largest cluster in the maize cyclin family, which is closer to the A-, B-, F-, and SDS-type clades. T-type cyclins form a separate clade, making them more independent in the tree. In addition, Figure 2 presents the domain structure with the corresponding cyclin genes in maize. All B- and F-type cyclins contain cyclin\_N and cyclin\_C domains; 91% A-type, 75% SDS-type and 48% D-type members contain both domains. However, all T-type cyclins have only cyclin\_N domains in maize.



**Figure 2.** Phylogenetic tree and domain structure of the 59 predicted maize cyclins. The numbers at the branches represent bootstrap values. All the B- and F-type cyclins possess both cyclin\_N and cyclin\_C domains; most of the A- and SDS-type cyclins also have both domains. However, each of the T-type cyclins has only the cyclin\_N domain without a cyclin\_C domain. Those cyclins highlighted in gray boxes have been described in previous studies.

**Analysis of conserved motifs in maize**

Conserved motifs were investigated by MEME, and 15 conserved motifs were detected (Figure 3). Among these 15 conserved motifs, 5 motifs (1, 2, 3, 8, and 9) are located within the cyclin-box domain. In fact, the conserved motifs encoding the cyclin\_N domain can be found in all 59 cyclin genes of maize, while the cyclin\_C domain is only found in some cyclins. This result confirmed that the C domain is a less conserved region than the N domain in plants.

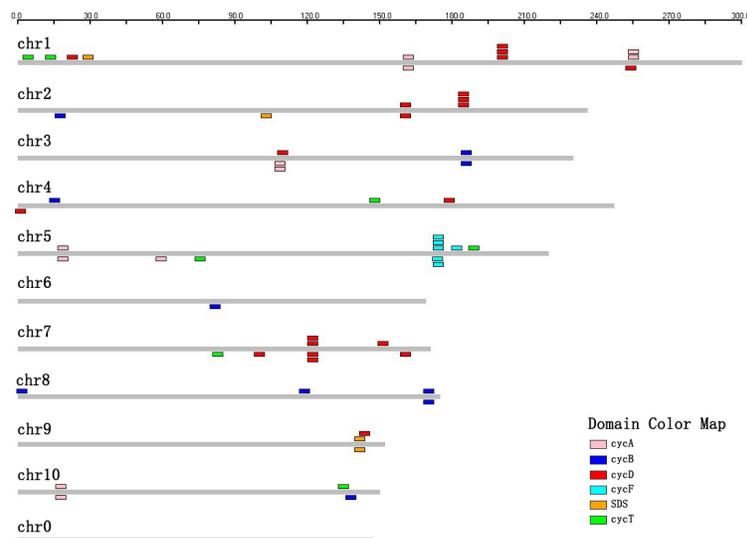


**Figure 3.** Fifteen putative conserved motifs were identified in the cyclin family in maize using the MEME search tool. Different motifs are indicated by different colors, but motif sizes are not related to the length of box.

In A- and B-type cyclin genes, four conserved motifs (1, 2, 3, and 9) are found in all cyclins, suggesting that the phylogenetic relationship of these two types of cyclin genes is close. Most of the D-type genes (except *cycD4;2*, *cycD4;6* and *cycD4;9*) contain three motifs including 1, 2 and 3, and were similar to the F-type genes. Among the T-type genes, all members contain motifs 1, 2, 3, and 8.

### Distribution of predicted cyclin genes on maize chromosomes

For the distribution of predicted cyclin genes on maize chromosomes, searches were performed for map positions and genetic distances of corresponding cyclin genes against maize protein databases. The 59 cyclin family genes are distributed on 10 maize chromosomes; however, no cyclin gene is located on chromosome 0 (Figure 4). In maize, the highest number of cyclin genes is located on chromosome 1 (12 genes), the longest chromosome, whereas only one cyclin gene, *cycB2;1*, is found on chromosome 6. The distribution of cyclin genes on other chromosomes is also uneven: eleven genes on chromosome 5, eight genes on chromosome 7, seven genes on chromosome 2, five genes on chromosome 3, four genes on chromosome 4, 8, 10, and three genes on chromosome 9.



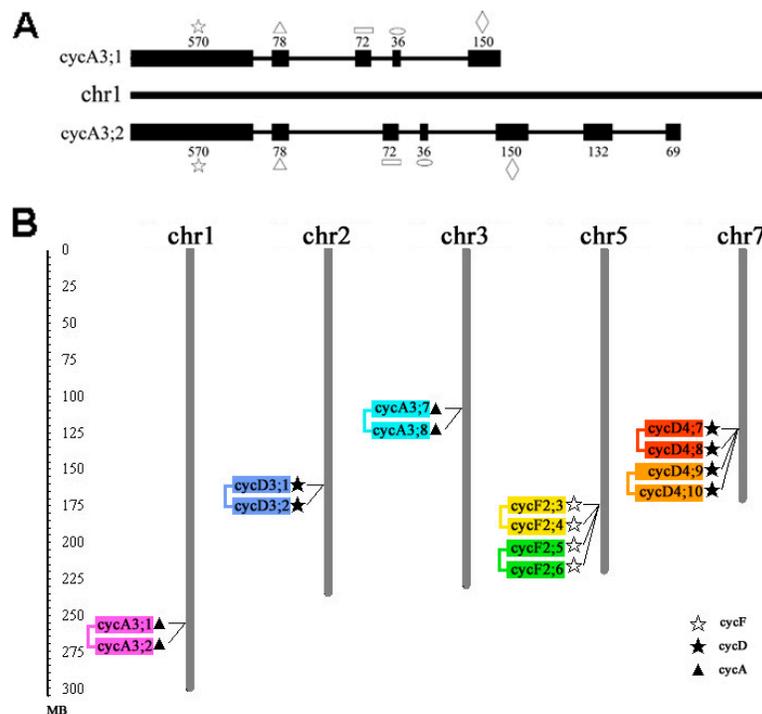
**Figure 4.** Physical locations of maize cyclin genes. The boxes above and below the chromosomes (chr; represented as gray bars) designate the approximate locations of the 11 maize cyclin genes.

Each type of cyclin gene except the F-type is found on at least three chromosomes, and B- and D-type cyclins are dispersed on up to six chromosomes. Interestingly, all F-type cyclin genes are exclusively located on chromosome 5, and 4 of them constitute a gene cluster with high sequence similarity. We also observed that gene clusters are distributed among all chromosomes except chromosomes 0, 4, and 6. Among the 13 clusters found on maize chromosomes, 3 are located on chromosome 1, 2 each on chromosomes 2, 3 and 5, and only 1 cluster on each of chromosomes 7, 8, 9, and 10.

## Genome organization of predicted cyclin genes in maize

In an attempt to study whether gene tandem or segmental duplications led to the acquisition of new cyclin genes in maize, we performed a series of BLASTN searches, sequence alignment and location comparisons on chromosomes as well as comparisons of exon numbers and lengths. We discovered seven tandem duplication events, which occurred on chromosomes 1, 2, 3, 5, and 7. However, no segmental duplications were found on maize chromosomes.

As shown in Figure 5A, *CycA3;1* and *CycA3;2* genes are located on the same BAC clone on chromosome 1 and constitute a gene cluster; the identity of their amino acids is 81.9%. Even in *CycA3;1* and *CycA3;2*, the exon numbers are 5 and 7, respectively, and the lengths of five exons in *CycA3;1* are identical to that of the corresponding exons in *CycA3;2*. Moreover, the exons marked with the same signs in Figure 5 share the same nucleotide sequences. According to these observations, we presumed that one of these genes, *CycA3;1* or *CycA3;2*, was derived from the other gene and formed a tandem gene duplication. Six groups altogether, *CycA3;7* and *CycA3;8*, *CycD3;1* and *CycD3;2*, *CycD4;7* and *CycD4;8*, *CycD4;9* and *CycD4;10*, *CycF2;3* and *CycF2;4*, *CycF2;5* and *CycF2;6*, formed tandem gene duplications (Figure 5B).



**Figure 5. A.** Gene tandem duplication of two A-type cyclins. The black bars represent exons, and spaces between two bars represent introns. The numbers above the exons refer to the number of nucleotides. Those exons marked with the same signs share the same nucleotide sequences. **B.** Distribution of cyclin gene duplications on 5 maize chromosomes. Gene tandem duplications are highlighted with different colors. The colored bands connect corresponding tandem duplicated genes.

## DISCUSSION

Cyclin proteins are components of the regulatory system that controls the orderly progression of the events of cell division in eukaryotes. Pines et al. (2002) found and analyzed at least 22 cyclins in the human genome. In the *C. elegans* genome, 34 cyclins have been found (Plowman et al., 1999). In higher plants, the number of cyclins vary from 49 in *Arabidopsis* to 48 in rice, suggesting that higher plant genomes contain greater numbers of the cyclin genes than those of animals. In our study, 59 predicted cyclins were identified in maize, which contains more members compared with *Arabidopsis* and rice. A reasonable explanation for these facts is that the plant cell cycle has unique features compared to the animal cell cycle, including the need to form unique cytoskeletal structures called preprophase band and phragmoplast, the formation of diffuse mitotic spindles, and distinctive cytokinesis (Cyr and Palevitz, 1995; Smith, 1999; Breyne et al., 2002). We also presumed that the cell cycle genes in higher plants, especially in maize, may be integrated into a more complex system, and plant-specific regulatory pathways of cell division may exist.

In this study, we identified and analyzed a large number of maize sequences by using the conserved cyclin\_N domain from rice (La et al., 2006) as query sequences. A total of 59 cyclin genes in the maize genome were identified, and they belong to the A, B, D, F, T, and SDS families. D-type cyclins perceive the cell's environment, acting as growth sensors to promote initiation and/or establishment of the G1 phase (Sherr and Roberts, 1999). As previously described (Quiroz-Figueroa and Vázquez-Ramos, 2006), CycD4;1 is poorly represented in germinating seeds but highly represented in other proliferating or non-proliferating tissues. CycD5;1 is the least expressed, but has the highest expression in leaf tissue. D-type cyclins contain the highest number (21) of cyclin genes, approximately half of the cyclins in maize, suggesting that there may be many D-type cyclins involved in the G1 phase, and some of these cyclins may be tissue-specific in maize.

Phylogenetic analysis indicated that there are 9 and 6 classes of cyclins in rice and maize genomes, respectively. Six types (A-, B-, D-, F-, T-, and SDS-types) are shared by the two species, whereas rice has the H-, L-, and P-type cyclins that are not found in maize. As shown in Figure 1, the same types of cyclins in rice and maize are located in the same clades, and they share high sequence similarities. By contrast, the H-, L-, and P-types in rice form a separate clade. It is interesting to note, however, that most of the F-type cyclins are grouped together, and one (CYF2;3) that is apart from the other F-type cyclins happens to be closely related to P-type cyclins in rice.

We also studied the evolutionary relationships between maize and *Arabidopsis*, and the results show that *Arabidopsis* and maize share five types of cyclins, A-, B-, D-, T-, and SDS-types, whereas C-, H-, L-, U-, and J18-type cyclins are specific to *Arabidopsis*. However, F-type cyclins are not found in *Arabidopsis*. Compared with humans and animals, plants contain fewer cyclin types, especially maize, which only possesses six types of cyclin genes. Nevertheless, the number of genes in each cyclin type in plants is much more than that of the corresponding type in humans and animals. In humans, none of the cyclin types contains more than three members; however, half of cyclin types in maize possess at least ten cyclins, including 11 A-type, 10 B-type and 21 D-type cyclins. All of these facts suggest that some of the A- B- and D-types may assume the functions that are carried out by the animal-specific cyclins. For example, plants lack E-type cyclins, which are known as very important regulators involved in G1/S checkpoint control in animals. It was

recently reported that *Nicta;CycA3;2* acts as an early G1/S-activated gene with functions that are analogous to those of cyclin E in animals (Yu et al., 2003). Even among the plant species analyzed so far, the classes of cyclins vary significantly. Maize lacks H-, L- and P-type cyclins, but possesses additional F-type cyclins compared with *Arabidopsis*, which may function as H-, L-, and P-type cyclins. In addition, rice and maize share F-type cyclins, while *Arabidopsis* lacks the corresponding type. Previous analysis of the cyclin gene family in rice identified the F-type as a new type of cyclin with no corresponding homologues found in other plant species. Our study reveals that maize is the second plant in which F-type cyclins have been identified. Thus, it is likely that this type of cyclin may be unique to monocotyledons. All the results above confirm that cyclins play complicated and diverse roles in cell cycle progression in plants. Therefore, it is important to analyze the full collection of functional cyclin genes in order to fully understand plant cyclin diversity (John et al., 2001).

We further analyzed the conserved motifs in maize cyclin family by MEME. The majority of cyclin genes in the same type have similar motifs, suggesting that these conserved motifs play crucial roles in subfamily-specific functions. However, SDS-type proteins are very divergent in their structures. For example, *cycSDS-4* contains only motifs 1 and 3 in its cyclin\_N region, whereas *cycSDS-3* has motifs 1, 2, 3, and 9, suggesting the complex nature of the function of SDS-type cyclins in maize. It was interesting that the cyclin\_N domain in T-type cyclins possesses a specific motif (motif 8), which was not found in other type of maize cyclins. A possible explanation is that motif 8 may have a specific function, and that T-type cyclins play specific roles in cell cycle progression.

The analysis of the chromosomal distribution of cyclin genes in maize shows that great differences exist in their distribution. There are 12 cyclin genes located on chromosome 1, which possesses the highest number of cyclin genes, but no cyclin gene is found on chromosome 0. Unlike other cyclin types dispersed throughout the whole genome, all members in F-type cyclins are exclusively located on chromosome 5, which is consistent with the distribution of corresponding homologues in rice (La et al., 2006). According to Vandepoele et al. (2002), studies in rice show that cyclins in *Arabidopsis* experienced large-scale duplications, including segmental and tandem duplications. La et al. (2006) found that there are four D-type, two each of A-type and D-type, and one each of B-type and T-type cyclins organized in segmental or tandem duplications. Similarly, we also found several tandem duplication events located in the maize chromosome. All the results above suggest that the increased number of cyclin genes correlates closely with the frequency of duplication events. It is worth noting that the tandem duplicated genes in maize also formed gene clusters. For example, *CycA3;1* and *CycA3;2* not only formed tandem duplications, but also constituted a gene cluster on chromosome 1. Therefore, we speculate that in maize, tandem duplications may be the basis for the formation of gene clusters. In addition, no segmental duplications were found in the maize chromosomes, unlike in rice and *Arabidopsis*. This analysis reveals that there are potentially more duplication events in rice and *Arabidopsis* genome compared with maize.

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