Plant Growth Regul (2011) 63:225–234
DOI 10.1007/s10725-010-9519-0

ORIGINAL RESEARCH

Genome-wide analysis of the auxin response factor (ARF) gene family in maize (*Zea mays*)

Yan Liu · HaiYang Jiang · Wenjuan Chen · Yexiong Qian · Qing Ma · Beijiu Cheng · Suwen Zhu

Received: 7 May 2010 / Accepted: 26 August 2010 / Published online: 8 September 2010

Abstract Auxin response factors (ARFs) are an important family involved in auxin-mediated response through specific binding to auxin response elements (AuxREs). A few members of the *ARF* family have been functionally characterized in *Arabidopsis*, rice (*Oryza sativa*), Poplar (*Populous trichocarpa*). However, little is known about *ARF* genes in maize (*Zea mays*). We performed a comprehensive bioinformatics analysis of the maize *ARF* gene family including analysis of the genome sequence, conserved domains, chromosomal locations, phylogenetic relationships, gene duplication, and expression profiles. 35 *ZmARF* genes were identified and categorized into four groups (Class I, II, III, and IV). In addition, a segmental *ZmARF* duplication event was shown to play an important role in maize *ARF* gene expansion. 7 *ZmARF* genes had no expression in specific tissues we obtained, but presented in mixed tissues according to the NCBI EST database, respectively. These studies have laid the theoretical foundation for further functional verification of these *ZmARF* genes.

Keywords ZmARF · Phylogenetic analysis · Duplication · EST · Expression

Y. Liu · H. Jiang · W. Chen · Y. Qian · Q. Ma · B. Cheng · S. Zhu (✉)
Anhui Province Key Laboratory of Crop Biology, Anhui Agricultural University, Hefei 230036, China
e-mail: zhusuwen@ahau.edu.cn

Y. Liu
e-mail: lyahau@163.com

H. Jiang
e-mail: jd1998@gmail.com

W. Chen
e-mail: chenchenky@163.com

Y. Qian
e-mail: qianyexiong@yahoo.com.cn

Q. Ma
e-mail: mqqmmq@126.com

B. Cheng
e-mail: beijiucheng@ahau.edu.cn

Introduction

The plant hormone auxin, represented by indole-3-acetic acid (IAA), influences many complex plant processes including apical dominance, vascular elongation, embryogenesis, lateral root initiation, and flower and fruit development (Woodward and Bartel 2005; De Smet and Jürgens 2007). Auxin signaling involves early response genes such as *Aux/IAA, GH3* (*Gretchen Hagen3*), and *SAUR* (small auxin up RNA) family members (Abel and Theologis 1996) and modulation of the interactions of the transcription factors with auxin response elements (AuxREs) of affected genes. AuxREs are found in promoter regions of primary/early auxin responsive genes (Ulmasov et al. 1999; Tiwari et al. 2003).

Auxin response factors (ARFs) are transcription factors in plants that play a vital role in auxin-mediated responses. ARFs can specifically bind to TGTCTC-containing AuxREs and mediate responses to the plant hormone auxin (Li et al. 1994; Hagen and Guilfoyle 2002). *ARF1* was first isolated in *Arabidopsis*, using a yeast one-hybrid screen with the TGTCTC element as bait sequence (Ulmasov and Hagen 1997). ARF1 protein contains three components, upstream of the DNA-binding domain (DBD) in the N-terminal portion, 665 amino acids in the middle and downstream of a protein–protein domain in the C-terminal portion (Ulmasov et al. 1997; Ouellet et al. 2001). In species with sequenced genomes, the structure and function

of *ARF* genes have been identified. Most ARF proteins contain a DBD in the N-terminal portion (classified as a plant-specific B3-type), a characteristic glutamine (Q)—rich middle region that functions as an activation or repression domain, and protein–protein interaction domains III and IV in the C-terminal portion that allow the homo- and hetero-dimerization of ARFs and the hetero-dimerization of ARF and Aux/IAA proteins. Domains III and IV are similar to those found in the C-terminus of Aux/IAAs and can increase in vitro binding (Ulmasov et al. 1999). Much remains to be determined regarding the mechanisms of ARF and Aux/IAA interaction and their regulation at the cellular and whole organism levels.

Sequences derived from large-scale sequencing projects are informative in functional genomics research, providing the opportunity to scan for gene families. Genomic analyses indicated that *Arabidopsis*, rice (*Oryza sativa*), poplar (*Populus trichocarpa*) and *Grapevine (Vitis vinifera)* had 23, 25, 39 and 20 ARF protein family members, respectively (Hagen and Guilfoyle 2002; Udaya et al. 2007; Wang et al. 2007). Structural and phylogenetic comparative analyses of auxin responsive gene families were done between *Arabidopsis* (AtARFs) and rice (OsARFs) (Terol et al. 2006; Wang et al. 2007). The B73 maize genome sequencing project was initiated in 2005 (Bennetzen et al. 2001) and the results were later published (Schnable et al. 2009), enabling us to analyze the maize *ARF* gene family. The complete maize genome sequence also provides a valuable resource for comparative analyses of ARF evolution in different species. A recent genome-wide analysis describes the primary auxin-responsive *Aux/IAA* gene family in maize (Wang Yijun et al. 2010).

In this study, we identified at least 35 putative members of maize *ARF* genes (*ZmARFs*) using a special ARF domain Hidden Markov Model (HMM) of the whole genome. We investigated the maize *ARF* gene number, genomic organization, expansion pattern, motif analysis, phylogenesis, and expression profiles. Our results may be helpful for functional studies of the *ARF* gene family and the relationship between the *ARF* and *Aux/IAA* gene families.

Materials and methods

Identification of maize *ARF* gene family

To identify ZmARFs, maize genome sequences were downloaded from http://www.maizegenome.org/data_portal.html. The Hidden Markov Model (HMM) profile of ARF domains from the Pfam database (http://pfam.janelia.org/search/sequence) was then used to search for maize *ARF* genes using BlastP program (*P*-value = 0.001). The Pfam database was used to confirm whether each predicted *ZmARF* gene encoded the ZmARF domain and if the ZmARF protein sequence was an ARF protein. All confirmed ZmARFs were aligned using Clustal W (Thompson et al. 1994) in MEGA v4.0 (Tamura et al. 2007) to exclude overlapping *ZmARF* genes. Non-overlapping *ZmARF* genes were classified on the basis of different domains.

Phylogenetic analysis of maize *ARF* genes

Complete protein sequences of maize ARFs were merged and then multiple-sequence alignments done with Clustal X (version 1.83) (Thompson et al. 1997). Phylogenetic trees for all complete ZmARF protein sequences were also constructed using MEGA v4.0 (Tamura et al. 2007) by the neighbor-joining (NJ) method. The same methods can be applied to analyze the evolutionary relationships between maize and other plants such as rice, *Arabidopsis*, poplar and grapevine.

Sequence analysis and duplication patterns of ZmARF genes

Based on the Pfam results, the ZmARF amino acid sequences were obtained and complete open reading frames (ORFs) were identified using protein analysis on line (http://www.expasy.ch/tools). Multiple alignments of the motifs III and IV of ZmARF proteins were obtained with Clustal X. Gene duplication events of *ARF* genes in maize B73 were also investigated. All of the confirmed *ARF* genes from maize genomes were aligned using Clustal W and analyzed using MEGA v4.0 on the basis of the phylogenetic tree.

Mapping *ZmARF*s on maize chromosomes

Each non-overlapping *ZmARF* gene sequence was used as a query against the maize sequence (http://maizesequence.org/blast), analyzed by using TBlastN program and positioned on the 11 maize chromosomes. ZmARF names are given according to their position from the top to the bottom on maize chromosomes 1–11. The chromosome map showing the physical location of all *ZmARF* genes was generated by Genome Pixelizer software (http://www.niblrrs.ucdavis.edu/GenomePixelizer/GenomePixelizer_Welcome.html).

Expression profiles of the *ZmARF* gene family

As transcription factors, all *ZmARF* genes were investigated at the transcriptional level. Maize EST database

(http:/www.maizesequence.org/blast) was acquired and the maize expression data was obtained through blast searches against the maize EST database by conducting DNA-TOOLS blast program. *ZmARF* genes were analyzed by using TBlastN program with the following parameters: maximum identity > 95%, length > 400 bp and E value < 10^{-10}. While *ZmARF* genes had no expression specifically in our loaded EST database, these were identified through NCBI EST database (Wang Yijun et al. 2010).

Results

Identification of ZmARFs

We used the multiple sequence alignment of *Arabidopsis* ARF protein domain sequences to build an ARF domain HMM profile. BLASTP searches based on the conserved ARF domain HMM were used to identify the *ARF* genes in maize genomes. 35 potential ARF protein sequences were

Table 1 : List of *ARF* genes in maize

Gene name	Sequence ID	Chr	ORF length (bp)	Deduced polypeptide		
				Length (aa)	MW (kDa)	PI
ZmARF1	GRMZM2G181254_P01	1	1,257	418	46.83	6.57
ZmARF2	GRMZM2G081158_P01	1	2,694	897	98.37	5.84
ZmARF3	GRMZM2G169820_P01	1	3,261	1,086	120.17	5.89
ZmARF4	GRMZM2G153233_P02	1	2,046	681	74.54	8.08
ZmARF5	GRMZM2G078274_P01	2	2,454	817	90.99	5.95
ZmARF6	GRMZM2G034840_P02	2	2,811	936	102.93	5.78
ZmARF7	GRMZM2G017187_P03	2	1,986	661	73.30	5.85
ZmARF8	GRMZM2G352159_P02	3	2,124	707	78.00	7.92
ZmARF9	GRMZM2G352159_P04	3	2,334	777	85.70	5.97
ZmARF10	GRMZM2G338259_P01	3	2,469	822	91.85	6.45
ZmARF11	GRMZM2G056120_P01	3	2,067	688	74.00	6.89
ZmARF12	GRMZM2G437460_P02	3	2,127	708	77.97	6.67
ZmARF13	GRMZM2G378580_P02	4	2,550	849	93.02	6.59
ZmARF14	GRMZM2G137413_P01	4	1,536	511	56.73	6.36
ZmARF15	GRMZM2G081406_P01	4	2,070	689	73.69	6.91
ZmARF16	GRMZM2G028980_P01	4	2,745	914	100.94	5.70
ZmARF17	GRMZM2G159399_P02	5	1,935	644	70.96	6.52
ZmARF18	GRMZM2G035405_P02	5	2,742	913	100.95	6.04
ZmARF19	AC207656.3_FGP002	5	2,151	716	77.52	6.67
ZmARF20	GRMZM2G102845_P01	5	3,444	1,147	127.24	5.79
ZmARF21	GRMZM2G317900_P01	5	3,459	1,152	127.82	6.27
ZmARF22	GRMZM2G702026_P01	5	2,034	677	75.13	5.93
ZmARF23	GRMZM2G390641_P01	6	2,103	700	75.51	7.16
ZmARF24	GRMZM2G089640_P01	6	1,896	631	69.92	5.87
ZmARF25	GRMZM2G441325_P01	6	2,043	680	73.94	6.40
ZmARF26	GRMZM2G441325_P02	6	1,290	429	46.79	6.73
ZmARF27	GRMZM2G030710_P01	6	2,211	736	80.42	7.87
ZmARF28	GRMZM2G075715_P01	7	1,491	496	56.94	9.27
ZmARF29	GRMZM2G116557_P01	8	2,703	900	100.57	7.58
ZmARF30	GRMZM2G160005_P01	9	3,162	1,053	116.75	6.20
ZmARF31	GRMZM2G006042_P03	10	2,481	826	91.14	7.13
ZmARF32	GRMZM2G005284_P01	10	1767	588	63.91	8.88
ZmARF33	GRMZM2G086949_P01	10	2,865	954	104.83	5.93
ZmARF34	GRMZM2G475882_P03	10	2,436	811	89.96	5.94
ZmARF35	GRMZM2G023813_P01	10	1,389	462	50.56	5.38

predicted with a probability E value threshold of 0.001 and identified as *ZmARF* genes using the BLAST program from the Pfam database. 22 protein sequences contained the B3, ARF, and Aux/IAA domains. 10 protein sequences contained the ARF and B3 domains. ZmARF24 protein sequence contained the ARF and Aux/IAA domain. ZmARF26 protein sequence contained only the ARF domain, and ZmARF28 had two protein–protein domains. The overall analysis revealed 35 *ZmARF* gene family members in the complete maize genome. The deduced polypeptide included three fields: number of amino acids (length), molecular weight, and isoelectric point (*PI*). ZmARF ORF lengths ranged from 1257 bp (ZmARF1) to 3459 bp (ZmARF21) and the molecular weights ranged from 46.79 kDa (ZmARF26) to 127.82 kDa (ZmARF21) (Table 1).

Phylogenetic analysis of *ARF* genes

The unrooted phylogenetic tree of all maize ARF full-length protein sequences was generated using MAGE v4.0 program by the N–J method. All ZmARFs could be divided into 4 major classes—I, II, III, and IV (Fig. 1). Class I and Class IV contained 7 and 6 members, respectively. Class II (9 members) and Class III (13 members) were two subgroups from one branch. All 35 ZmARFs were distributed into 12 sister pairs, while the remaining ZmARFs were not matched.

Arabidopsis and rice were chosen to further investigate the phylogenetic relationship between dicot and monocot

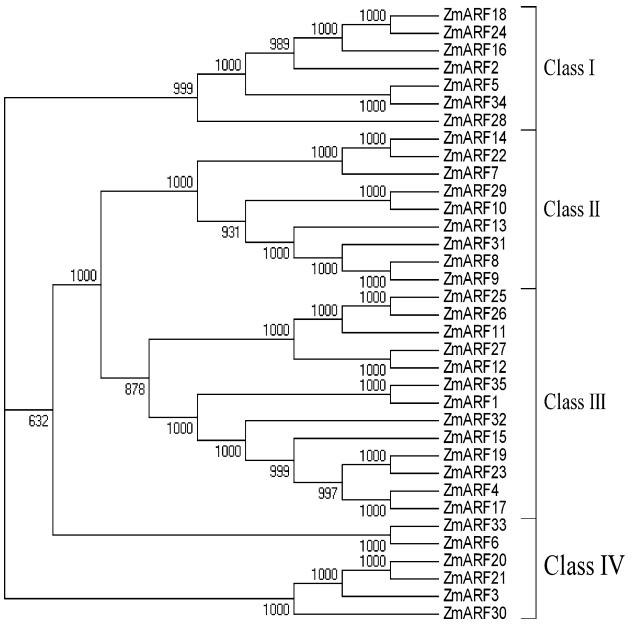


Fig. 1 Phylogenetic tree of maize *ARF* genes. The unrooted tree was generated using the MEGA v4.0 program with the neighbor-joining method

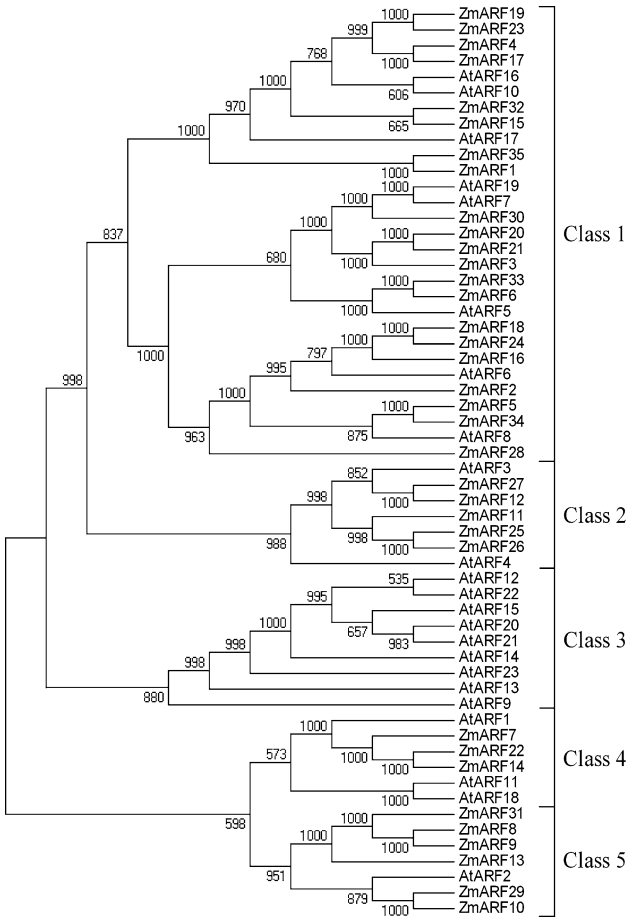


Fig. 2 Phylogenetic tree of maize and *Arabidopsis ARF* genes. The unrooted tree was generated using the MEGA v4.0 program with the neighbor-joining method

ARF genes. The phylogenetic relationship was examined in aligned full-length protein sequences of 35 ZmARFs and 23 AtARFs (Fig. 2). A total of 58 members were divided into 5 groups named Class 1, 2, 3, 4, and 5. Its classification is largely similar to the ZmARFs (Fig. 1). Classes I and II belong to the same branch, but the maize and *Arabidopsis* families do not have a high sequence homology based on the phylogenetic tree. In the joint phylogenetic tree, Classes 1, 2, 3, 4, and 5 contained 29, 7, 9, 6, and 7 members, respectively (Fig. 2). Class 1 is composed of two subgroups. All 18 homologous pairs, including 13 ZmARF-ZmARF and 5 AtARF-AtARF, were confirmed without ZmARF-AtARF pairs. AtARFs and ZmARFs were both found in Classes 1, 2, 4, and 5, but Class 3 only had AtARFs without ZmARFs. Noteworthy is that each family contained a separate branch besides the above paired ZmARFs.

The phylogenetic tree of 35 ZmARFs and 25 OsARFs was aligned and constructed using the same method in order to analyze the relationship among the *ARF* genes of two monocot plants. We also classified all 60 members into

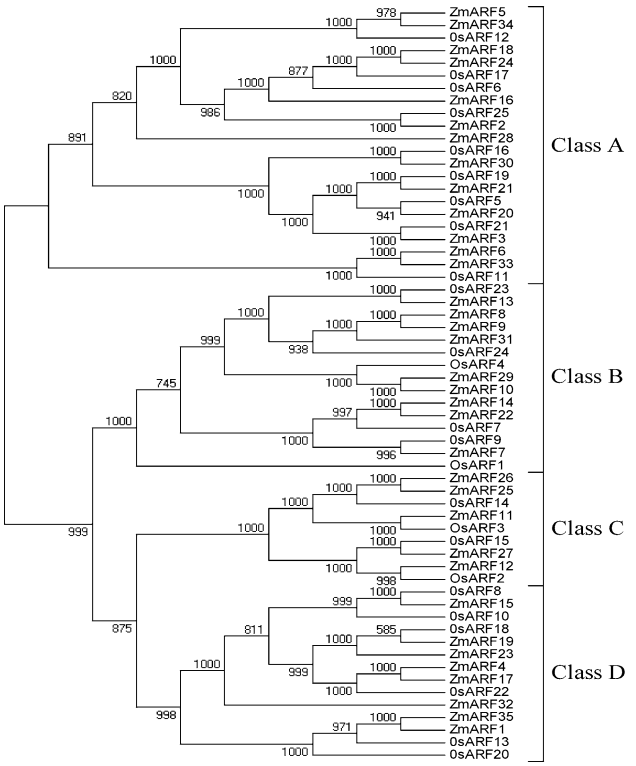


Fig. 3 Phylogenetic tree of maize and rice *ARF* genes. The unrooted tree was generated using the MEGA v4.0 program with the neighbor-joining method

4 classes named Classes A, B, C, and D containing 22, 15, 9, and 14 members, respectively (Fig. 3). Classes C and D were two subfamilies further divided from the same branch. 9 ZmARF-ZmARF and 12 OsARF-ZmARF constituted 21 sister pairs. Every group contained OsARF-ZmARF pairs.

In order to better evaluate the phylogenetic relationship of the same gene family among different species, poplar and grapevine were also selected and, respectively carried out comparative analysis of evolutionary trees with maize. An unrooted phylogenetic tree based on the alignments of 35 ZmARFs and 39 PoptrARFs was constructed by the N–J method (Fig. 4). 74 ARFs fell broadly into three major classes: Classes X, Y and Z containing 27, 30 and 17 members, respectively. They contained only one ZmARF-PoptrARF homologous pair in Class X, 17 PoptrARF-PoptrARF and 11 ZmARF-ZmARF sister pairs.

As the grapevine genome has been announced, but the grapevine *ARF* genes have not been reported. Grape vine assembly and annotation V1.0 were downloaded from http://www.genoscope.cns.fr/externe/English/Projets/Projet_ML/index.html (Yang et al. 2008). Using the same method as above to determine ZmARFs, 20 grapevine *ARF* genes were indentified. Based on the alignment of 35 ZmARFs and 20 GsARFs, a phylogenetic tree was constructed (Fig. 5).

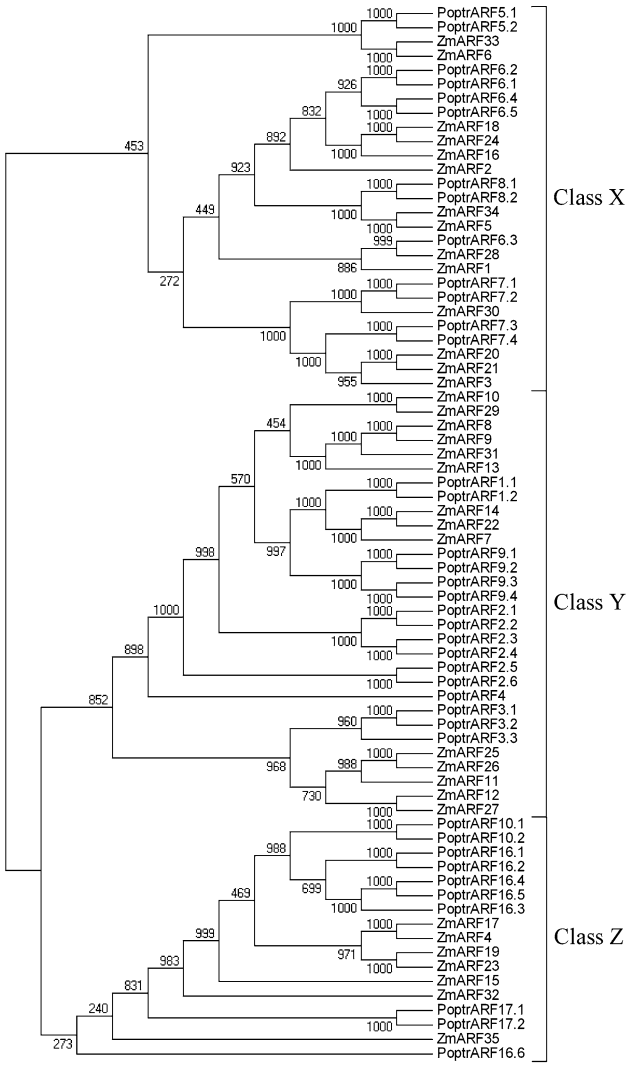


Fig. 4 Phylogenetic tree of maize and poplar (*Populus trichocarpa*) *ARF* genes. The unrooted tree was generated using the MEGA v4.0 program with the neighbor-joining method

As shown in Fig. 5, this phylogram distinguished 3 groups, namely, Classes α (23 members), β (18 members) and γ (14 members). All members included 5 GsARF-GsARF, 12 ZmARF -ZmARF and 3 ZmARF-GsARF sister pairs.

Sequence and conserved region analysis of the ZmARF proteins

According to the Pfam outcome, 33 putative ZmARF protein sequences were identified that had a typical DBD domain. ZmARF24 had no DBD in the N-terminal region. ZmARF26 had only an ARF domain without a DBD in the N-terminal region and a protein–protein interaction domain in the C-terminal portion. Using Clustal X to analyze the full protein sequences and conserved regions of all ZmARFs, we found that the DBD domains of 33 ZmARFs were composed of about 460 amino acids in the N-terminal

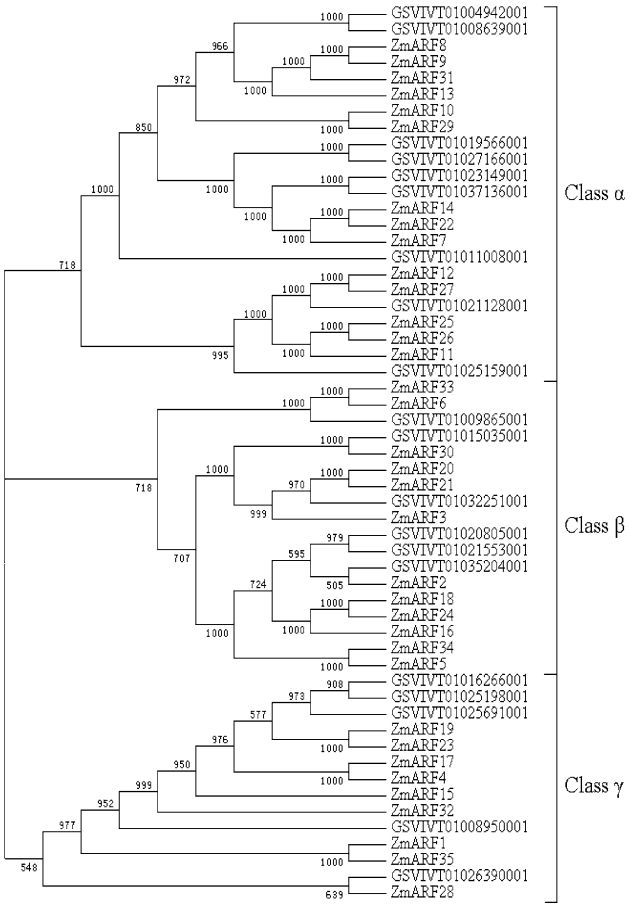


Fig. 5 Phylogenetic tree of maize and grapevine (*Vitis vinifera*) *ARF* genes. The unrooted tree was generated using the MEGA v4.0 program with the neighbor-joining method

portion, and 23 ZmARFs contained motifs III and IV found in the Aux/IAA protein family in the C-terminal portion. These core domains all had high similarity (Fig. 6a, b).

Chromosomal locations of ZmARFs

Based on available information (http://www.maizegenome.org/data_portal.html), standard *ZmARF* genes were positioned on maize chromosomes using Genome Pixelizer software. According to the maize evolutionary tree analysis, each *ARF* gene was divided into one of four categories (Classes I, II, III, and IV) as represented by different colors in Fig. 5. However, the maize gene sequences have not been fully sequenced (about 95% coverage of the whole genome). In addition to the 10 sequenced chromosomes, there is an additional chromosome called unknown or chromosome 0. All 35 *ZmARF* genes can be distributed on chromosomes 1–10. Most *ARF* genes were located on chromosome 5 (6 genes), and five genes were located on chromosome 3 (Fig. 7). The same number of genes was located on chromosomes 6 and 10, with four genes each on chromosomes 1 and 4. These chromosomes all contained

Fig. 6 **a** Alignment profile of maize ARF proteins obtained with the ClustalX program. The height of the *bars* indicates the number of identical residues per position. The *arrows* indicate the core region among DBD regions. Motifs III and IV are found in the Aux/IAA domain. **b** Alignment of Motifs III and IV of ZmARF proteins using Clustal X

the different types of genes. Only one *ZmARF* gene was located on chromosomes 2, 7, 8, and 9, respectively (Fig. 7).

In reference to the nomenclature previously used for AtARFs and OsARFs (Wang et al. 2007), genes were temporarily named from ZmARF1 to ZmARF35 to distinguish every *ARF* gene based on its position from the top to the bottom of maize chromosomes 1–10. This approach has been broadly applied in genome-wide studies for the *ERF*, *GH3*, and *Aux/IAA* gene families in *Arabidopsis* and rice (Jain et al. 2006; Nakano et al. 2006; Terol et al. 2006).

Analyses of *ZmARF* gene duplication

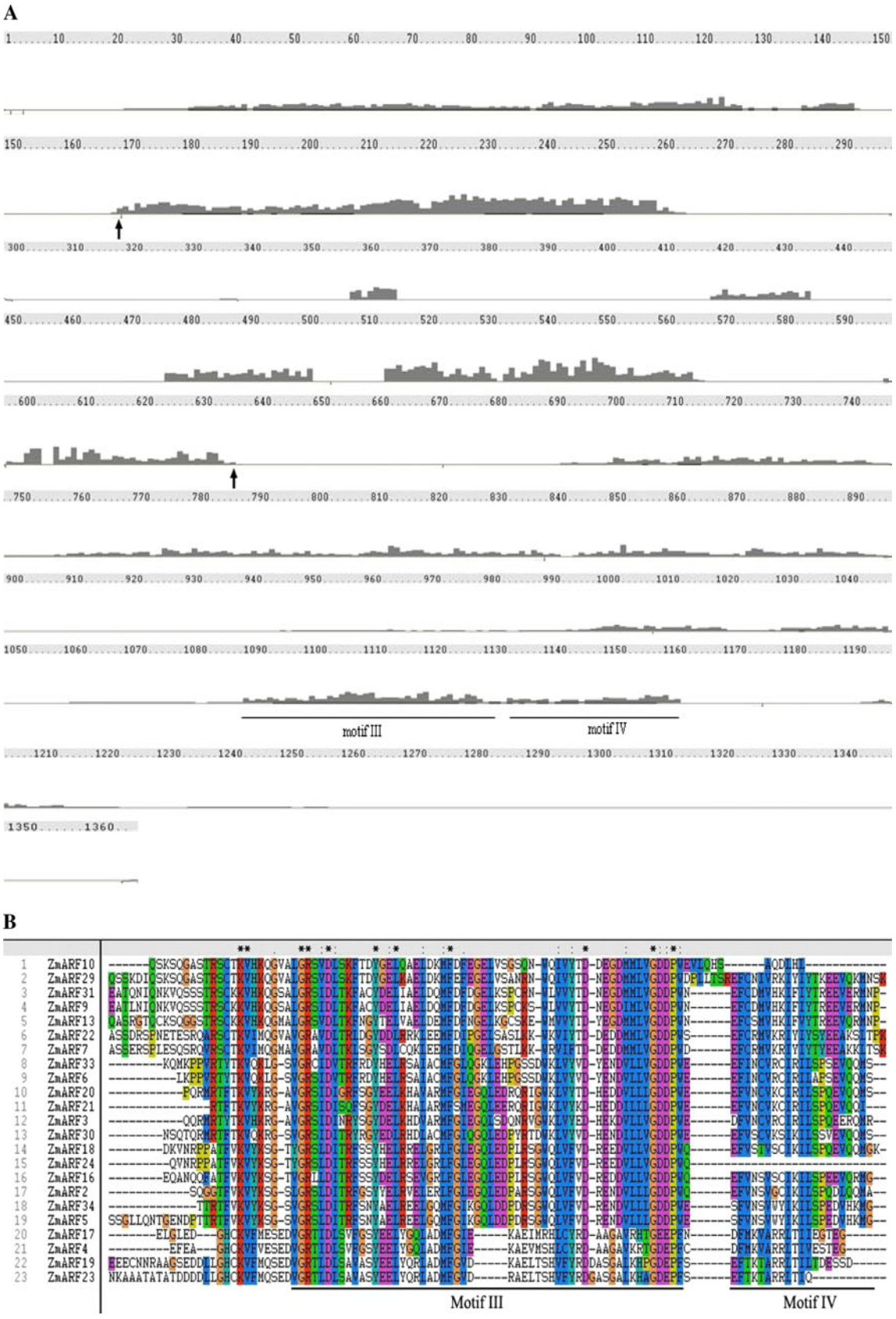
To investigate *ZmARF* gene duplication events, phylogenetic trees of maize *ARF* genes provided some valuable information. A detailed comparison of gene duplications are as follows (Gu et al. 1998; Yang et al. 2008): (1) the length of alignable sequence covering ≥80% of the longer gene, and (2) the similarity of the aligned regions ≥80%. All four *ARF* gene categories had gene expansion. Among the 35 *ZmARF* genes, 24 *ARF* genes (12 pairs) were confirmed and present in the phylogenetic tree (Fig. 1). *ZmARF* gene duplication was prevalent and was the main contributor to the expansion of the *ZmARF* gene family.

Expression patterns of the *ZmARF* family

In order to study the expression of the *ZmARF* gene family, we used the maize EST database to predict ZmARF transcripts. The maize EST were divided into 8 groups (Table 2). According to the NCBI EST expression database, ZmARF1, 18, 19, 23, 24, 32, and 35 have no expression, 9 *ZmARF* genes (ZmARF4, 6, 11, 14, 15, 21, 30, 31, and 33) were found in only one tissue or organ, and the remaining *ZmARF* genes were identified in two or more tissues and organs (Table 2).

Discussion

As key transcription factors, the *ARF* gene family plays an important role in various plant growth and development. The structure of *ARF* gene family have been extensively studied and described in some species. *Arabidopsis*, rice and *P. trichocarpa* are model plants with complete genomic sequences and analyzed *ARF* gene families in the


A
motif III
motif IV
B
Motif III
Motif IV

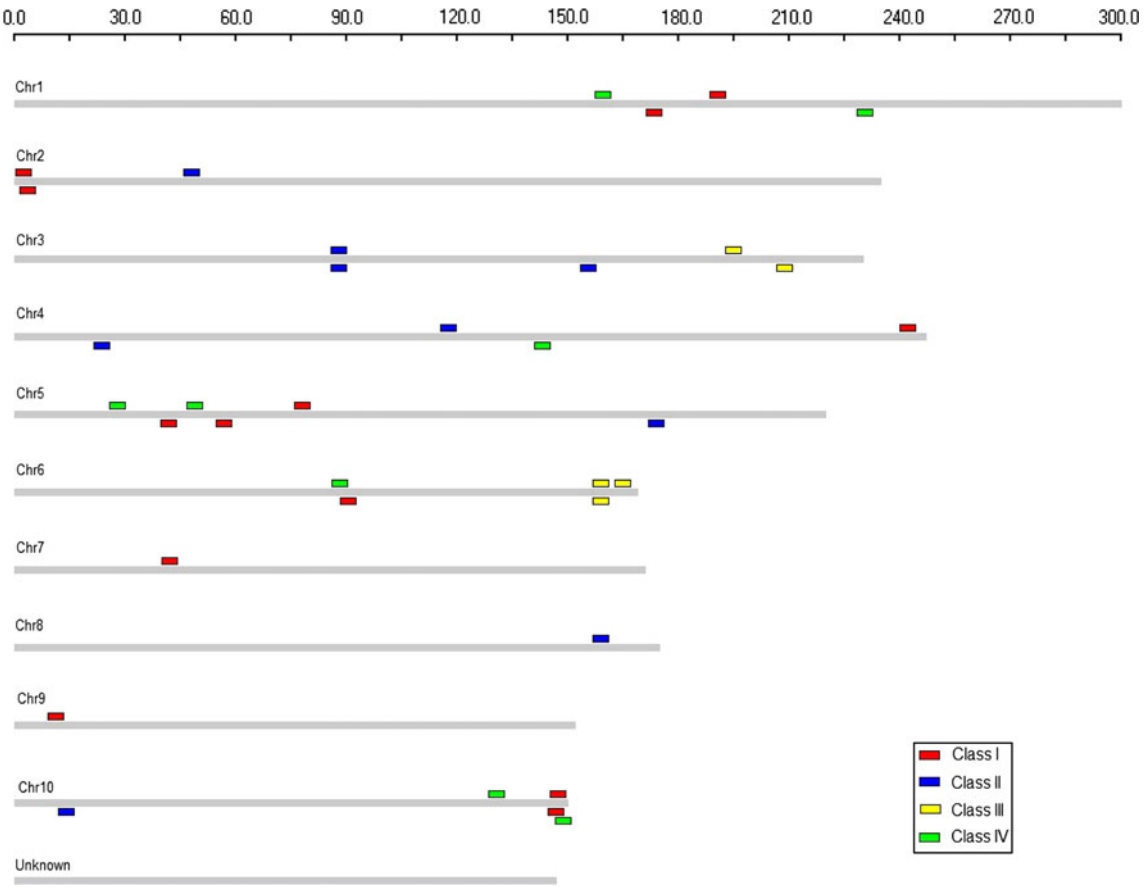


Fig. 7 Genomic distribution of *ARF* genes on maize chromosomes. The four categories of genes corresponding to Fig. 1. The boxes above and below the chromosomes (chr; represented as *gray bars*) designate the approximate locations of the four categories of *ARF* genes

genome-wide (Tiwari et al. 2003; Udaya et al. 2007; Wang et al. 2007). However, there are few studies of *ARF* genes in maize. In this study, we used the *ARF* genes of *Arabidopsis* as queries to determine the largest possible number of maize *ARF* gene sequences and applied various bioinformatics software to find *ZmARF* genes. Through a genome-wide comparative analysis of the evolutionary relationships between maize and other plants such as rice, *Arabidopsis*, poplar and grapevine, the result showed that maize shared more homology with the monocot plant (rice).

In this study, 35 *ARF* genes have been identified in the maize genome, which is higher than in *Arabidopsis* (23), rice (25) and grapevine (20). Therefore, gene duplication can play an important role in a succession of genomic rearrangements and expansions (Vision et al. 2000). Gene duplication included tandem and segmental duplication events, but tandem duplication is an on-going process in poplar genome evolution, whereby two or more genes are located in the same chromosome. Gene duplication between different chromosomes and the same clades are designated as segmental duplication events. This has been found in rice and tandem duplication has been reported in *Arabidopsis* (Lynch and Conery 2000; Simillion et al. 2002; Raes et al. 2003; Wang et al. 2005). 60% of these duplications may help maize to evolve distinct properties from other angiosperms. However, the 35 *ZmARF* genes were distributed on the 10 maize chromosomes without obvious hot spots in chromosomes.

In maize, 35 *ZmARF* genes were divided into four categories and 12 sister pairs were formed. Only 3 of these pairs diverged from their corresponding chromosomal locations on chromosomes 3 (ZmARF8 and 9), 5 (ZmARF20 and 21), and 6 (ZmARF25 and 26). The remaining nine sister pairs were located chromosomes 1 and 5 (ZmARF4 and 17), 1 and 10 (ZmARF1 and 35), 3 and 6 (ZmARF12 and 27), 3 and 8 (ZmARF10 and 29), 4 and 5 (ZmARF14 and 22), 2 and 10 (ZmARF5 and 34, ZmARF6 and 33), and 5 and 6 (ZmARF 18 and 24, ZmARF19 and 23). We found that segmental duplication was common in *ZmARF* genes. The 12 ZmARF sister pairs were the result of the interaction between two kinds of replication events to expand ZmARF number, which were significantly different than for *Arabidopsis* and rice.

Table 2 Expression analysis of *ZmARF* genes in silico

Gene name	Tissue and organ type							
	Endosperm	Tassel	Ear	Embryo	Root	Leaf	Shoot	Mixed
ZmARF1								+
ZmARF2	+	+	+					
ZmARF3	+	+					+	
ZmARF4		+						
ZmARF5		+	+					
ZmARF6			+					
ZmARF7		+	+	+				
ZmARF8	+		+					
ZmARF9	+		+					
ZmARF10		+	+	+				
ZmARF11		+						
ZmARF12	+	+	+		+			
ZmARF13	+		+	+			+	
ZmARF14			+					
ZmARF15			+					
ZmARF16		+	+					
ZmARF17	+			+		+		
ZmARF18								+
ZmARF19								+
ZmARF20		+	+					
ZmARF21				+				
ZmARF22	+	+	+					
ZmARF23								+
ZmARF24								+
ZmARF25		+	+			+	+	
ZmARF26		+	+				+	
ZmARF27	+	+	+					
ZmARF28		+	+					
ZmARF29	+	+	+	+				
ZmARF30	+							
ZmARF31	+						+	
ZmARF32								+
ZmARF33	+							
ZmARF34	+	+	+					
ZmARF35								+

+, Expressed; blank, Not expressed

Analysis of the conserved motifs indicated that 23 of 35 ZmARF proteins contain domains III and IV, which were also found in the C-terminus of Aux/IAAs. These domains have been shown to mediate both homo- and hetro-dimerization between members of the *Aux/IAA* and *ARF* families (Kim et al. 1997). Several interesting points arise from these phylogenies. Seven out of nine ZmARF sister pairs and 9 single ZmARFs related to domains III, and IV in the *Aux/IAA* family (Fig. 6). ARF1 carboxyl terminus has also been shown to interact with Aux/IAA proteins in a yeast one-hybrid screen, and these interactions probably occur through conserved domains III and IV in the carboxyl termini of these proteins. Both *ARF* and *Aux/IAA* families were transcriptional regulators, specially affected auxin signaling transduction (Reed 2001). It has therefore been hypothesized that the Aux/IAA proteins regulate transcription by modifying ARF activity (Guilfoyle et al. 1998a, b).

Potential gene expression patterns were determined using our and NCBI EST databases. Most *ZmARF* genes were expressed in specifical tissues and organs, while 7 *ZmARF* genes had no expression specifically in our EST database but were identified in mixed tissues through NCBI EST database. Whether these gene expressions were

induced by the outside, for example, light-induced and external growth hormone treatment has become a research focus. It has been reported that RT–PCR method was used in Semi-quantitative analysis of *ARF* genes in *Arabidopsis* and rice. (Okushima et al. 2005; Overvoorde et al. 2005; Wang et al. 2007). Additionally, different growth environments may also affect the functional prediction in theory. The comparative and phylogenetic analyses of the *ZmARF* gene family and the expression and structure analysis of ZmARF proteins will lay the foundation for further functional studies.

Acknowledgments This work was supported by grants from the National Transgenic Plants Program of China (No. 2008ZX003-002) and the National Natural Science Foundation of China (No. 30771309). We thank members of the Key Laboratory of Crop biology of Anhui province for their assistance in this study.

References

Abel S, Theologis A (1996) Early genes and auxin action. Plant Physiol 111:9–17

Bennetzen JL, Chandler VL, Schnable PS (2001) National science foundation–sponsored workshop report. Maize genome sequencing project. Plant Physiol 127:1572–1578

De Smet I, Jürgens G (2007) Patterning the axis in plants-auxin in control. Curr Opin Genet Dev 17:337–343

Gu Q, Ferrándiz C, Yanofsky MF, Martienssen R (1998) The *FRUITFULL* MADS-box gene mediates cell differentiation during Arabidopsis fruit development. Development 125: 1509–1517

Guilfoyle T, Hagen G, Ulmasov T, Murfett J (1998a) How does auxin turn on genes? Plant Physiol 118:341–347

Guilfoyle T, Ulmasov T, Hagen G (1998b) The ARF family of transcription factors and their role in plant hormone-responsive transcription. Cell Mol Life Sci 54:619–627

Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385

Jain M, Kaur N, Tyagi AK, Khurana JP (2006) The auxin-responsive *GH3* gene family in rice (*Oryza sativa*). Funct Integr Genomics 6:36–46

Kim J, Harter K, Theologis A (1997) Protein–protein interactions among the Aux/IAA proteins. Proc Natl Acad Sci USA 94:11786–11791

Li Y, Liu ZB, Shi X, Hagen G, Guilfoyle TJ (1994) An auxin-inducible element in soybean SAUR promoters. Plant Physiol 106:37–43

Lynch M, Conery JS (2000) The evolutionary fate and consequences of duplicate genes. Science 290:1151–1155

Nakano T, Suzuki K, Fujimura T, Shinshi H (2006) Genome-wide analysis of the *ERF* gene family in *Arabidopsis* and rice. Plant Physiol 140:411–432

Okushima Y, Overvoorde PJ, Arima K et al (2005) Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: unique and overlapping functions of ARF7 and ARF19. Plant Cell 17:444–463

Ouellet F, Overvoorde PJ, Theologis A (2001) IAA17/AXR3: biochemical insight into an auxin mutant phenotype. Plant Cell 13:829–841

Overvoorde PJ, Okushima Y, Alonso JM et al (2005) Functional genomic analysis of the *AUXIN/INDOLE-3-ACETIC ACID* gene family members in *Arabidopsis thaliana*. Plant Cell 17:2300–3282

Raes J, Vandepoele K, Simillion C, Saeys Y, Van de Peer Y (2003) Investigating ancient duplication events in the *Arabidopsis* genome. J Struct Funct 3:117–129

Reed JW (2001) Roles and activities of Aux/IAA proteins in *Arabidopsis*. Trend Plant Sci 6:420–425

Schnable PS, Ware D, Fulton RS et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115

Simillion C, Vandepoele K, Van Montagu MC, Zabeau M, Van de Peer Y (2002) The hidden duplication past of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 99:13627–13632

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Terol J, Domingo C, Talon M (2006) The *GH3* family in plants: genome wide analysis in rice and evolutionary history based on EST analysis. Gene 371:279–290

Thompson JD, Higgins DG, 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

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Tiwari SB, Hagen G, Guilfoyle TJ (2003) The roles of auxin response factor domains in auxin-responsive transcription. Plant Cell 15:533–543

Udaya C, Stephen P, Amy M (2007) Genome-wide analysis of *Aux/IAA* and *ARF* gene families in *Populus trichocarpa*. BMC Plant Biol 7:59

Ulmasov T, Hagen G, Guilfoyle TJ (1997a) ARF1, a transcription factor that binds to auxin response elements. Science 276:1865–1868

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997b) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971

Ulmasov T, Hagen G, Guilfoyle TJ (1999) Dimerization and DNA binding of auxin response factors. Plant J 19:309–319

Vision TJ, Brown DG, Tanksley SD (2000) The origins of genomic duplications in *Arabidopsis*. Science 290:2114–2117

Wang Y, Deng D, Bian Y, Lv Y, Xie Q (2010) Genome-wide analysis of primary auxin-responsive *Aux/IAA* gene family in maize (*Zea mays L.*). Mol Biol Rep Epub. A head of print Accessed 16 Mar 2010

Wang X, Shi X, Hao B, Ge S, Luo J (2005) Duplication and DNA segmental loss in the rice genome: implications for diploidization. New Phytol 165:937–946

Wang D, Pei K, Fu Y, Sun Z, Li S, Liu H, Tang K, Han B, Tao Y (2007) Genome-wide analysis of the *auxin response factors (ARF)* gene family in rice (*Oryza sativa*). Gene 394:13–24

Woodward AW, Bartel B (2005) Auxin: regulation, action, and interaction. Ann Bot (Lond) 95:707–735

Yang SH, Zhang XH, Yue JX, Tian DC, Chen JQ (2008) Recent duplications dominate NBS-encoding gene expansion in two woody species. Mol Genet Genomics 280:187–198