A genome-wide analysis of the auxin/indole-3-acetic acid gene family in hexaploid bread wheat (*Triticum aestivum* L.)

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The Auxin/indole-3-acetic acid (Aux/IAA) gene family plays key roles in the primary auxin-response process and controls a number of important traits in plants. However, the characteristics of the Aux/IAA gene family in hexaploid bread wheat (*Triticum aestivum* L.) have long been unknown. In this study, a comprehensive identification of the Aux/IAA gene family was performed using the latest draft genome sequence of the bread wheat “Chinese Spring.” Thirty-four Aux/IAA genes were identified, 30 of which have duplicated genes on the A, B or D sub-genome, with a total of 84 Aux/IAA sequences. These predicted Aux/IAA genes were non-randomly distributed in all the wheat chromosomes except for chromosome 2D. The information of wheat Aux/IAA proteins is also described. Based on an analysis of phylogeny, expression and adaptive evolution, we prove that the Aux/IAA family in wheat has been replicated twice in the two allopolyploidization events of bread wheat when the tandem duplication also occurred. The duplicated genes have undergone an evolutionary process of purifying selection, resulting in the high conservation of copy genes among sub-genomes and functional redundancy among several members of the *TaIAA* family. However, functional divergence probably existed in most *TaIAA* members due to the diversity of the functional domain and expression pattern. Our research provides useful information for further research into the function of Aux/IAA genes in wheat.

Keywords: bread wheat genome, Aux/IAA family, chromosome location, expansion pattern, function prediction

Introduction

Auxin, the first phytohormone discovered, controls many aspects of plant physiology and morphology including embryogenesis, lateral root initiation, leaf expansion, inflorescence and fruit set (Vanneste and Friml, 2009), and is involved in gene stimulation and regulating the transcription of multiple genes on the molecular level. Several primer auxin-responsive genes have
been identified containing the Aux/IAA, GRETCHEN HAGEN 3 (GH3), and SMALL AUXIN-UP RNA (SAUR) gene families (Abel and Theologis, 1996). With a key role in the auxin signaling pathway, the Aux/IAA gene is well known as the transcriptional repressor of the Auxin Response Factor (ARF) gene family to regulate downstream auxin-regulated genes (Rogg et al., 2001). Moreover, Aux/IAAs can mediate the pathway interaction between auxin and light signaling (Halliday et al., 2009) or other hormone signaling such as brassinosteroids (Song et al., 2009), jasmonic acid (Kazan and Manners, 2009), and ethylene (Strader et al., 2010).

The Aux/IAA family members encode short-lived nuclear proteins ranging from 18 to 36 kD (Paul et al., 2005).Canonical proteins of the Aux/IAA family share four conserved motifs known as domains I–IV. Domain I at the N-terminus contains a leucine repeat (LxLxLx) motif (Tiwari et al., 2001). Domain II is involved in the instability of proteins (Kepinski and Leyser, 2004). Domains III and IV mediate homo-dimerization and hetero-dimerization between the Aux/IAA and ARF proteins via C-terminal dimerization binding sites (Hagen and Guilfoyle, 2002; Tiwari et al., 2004).

Since the initial isolation of Aux/IAA family genes in soybean (Glycine max) (Walker and Key, 1982), 29 members in Arabidopsis thaliana (Paul et al., 2005), 35 members in Populus trichocarpa (Kalluri et al., 2007), 26 members in tomato (Wu et al., 2012), sorghum (Wang et al., 2010a), and grape (Birsen et al., 2013), and 31 members in rice (Jain et al., 2006), and maize (Wang et al., 2010b) have been identified. Several Aux/IAA family genes control a number of important plant traits. OsIAA2 enhances the resistance of rice to pathogens (Chen et al., 2009); OsIAA5 (Peleg et al., 2011), and OsIAA6 (Jung et al., 2015) are involved in drought tolerance; SbIAA1 relates to stress response (Wang et al., 2010a); and VvIAA4 (Birsen et al., 2013), VvIAA9 (Jung et al., 2014), SlIAA9 (Wang et al., 2005a; Mazzucato et al., 2015), SlIAA15 (Deng et al., 2012), and SlIAA17 (Su et al., 2014) are key regulators of the fruit set process.

Despite extensive studies of Aux/IAA in many other plants, little is known about this gene family in bread wheat (Triticum aestivum L.), one of the most widely grown crops in the world. Until now, only one Aux/IAA gene, TaIAA1, was reported to be regulated by epibrassinolide and light (Singla et al., 2006). Bread wheat (AABBDD; 2n = 6x = 42) is a result of hybridization between T. turgidum (AABB; 2n = 4x = 28), an allotetraploid originating from a cross of T. urartu (AA; 2n = 14) and Aegilops speltoides (SS; 2n = 14), and A. tauschi (DD; 2n = 14) which occurred approximately 0.43 MYA (million years ago). It is problematic to isolate Aux/IAA family genes in bread wheat due to its huge genome that comprises a high scale (>80%) of repetitive sequences (Wicker et al., 2011).

In 2012, the draft genome of bread wheat “Chinese Spring” (“CS”) reading by whole-genome shotgun sequencing was published (Brenchley et al., 2012). Soon afterwards, the sequencing data of T. urartu (Ling et al., 2013) and A. tauschi (Jia et al., 2013), two progenitors of bread wheat, was released in 2013. These resources have provided a wealth of information about the coding genes of wheat (Saintenac et al., 2013). However, little is known about the distribution and position of these genes on each wheat chromosome and their evolution during the two polyploidization events. Recently, a 17-gigabase draft genome of the bread wheat “CS” produced through sequencing isolated chromosome arms was published (Mayer et al., 2014), making possible the isolation and analysis of gene families on a genomic scale.

In this study, a genome-wide isolation of Aux/IAA domains in bread wheat was performed using sequence resources. Eighty-four sequences were isolated and divided into 34 groups based on homology among the A, B and D sub-genomes. Detailed information about wheat Aux/IAA genes (TaIAAs) was acquired. In addition, the expansion pattern and selection pressure analysis of the Aux/IAA family in wheat was deduced.

Materials and Methods

Searching for the Aux/IAA Family in Wheat, T. urartu and A. tauschi

The whole-genome sequences of T. aestivum was downloaded from the wheat genome URGI database (http://wheat-urgi.versailles.inra.fr/) and Ensembl database (http://plants.ensembl.org). Based on these sequences, a local nucleotide and protein database was established by Basic Local Alignment Search Tool (BLAST, ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/). The hidden Markov model (HMM) profile of the Aux/IAA family (PF02309) was extracted from the Pfam database (http://pfam.sanger.ac.uk) and the Aux/IAA HMM profile was used to search the local protein database for target hits with the Aux/IAA-domain by HMMER3.0 (http://hmmer.janelia.org/). All non-redundant sequences with expected values lower than 1E-5 were selected and received a conserved domain check using the Pfam tool (http://pfam.xfam.org/) and SMART (http://smart.embl-heidelberg.de/) web server. According to the sequence ID of the Aux/IAA protein in wheat, the coding sequences and genome sequences were isolated from the local nucleotide database. Using the same method, the Aux/IAA genes of T. urartu and A. tauschi were defined from the T. urartu genomic database (http://gigadb.org/dataset/100050) and A. tauschi genomic database (http://gigadb.org/dataset/100054), respectively.

Phylogenetic Relationships, Gene Structure, and Chromosome Location of Wheat Aux/IAA Genes

The exon/intron organization of each Aux/IAA gene was illustrated in the Gene Structure Display Server program (Hu et al., 2015) by comparing their coding sequences with genomic sequences. The position of each Aux/IAA gene in the wheat chromosomes and the genome sequences of the corresponding wheat chromosomes were determined by BLAST, and the results were displayed using the MapInspect tool (http://mapinspect.software.informer.com/). An unrooted phylogenetic tree for wheat Aux/IAA genes was constructed using MEGA 6.0 (Tamura et al., 2013) via the Neighbor-Joining (NJ) method. Duplicated genes in the branch ends of each group belonging to the A, B or D sub-genomes of wheat were regarded as the homologous copies of one Aux/IAA gene. All of the TaIAAs were named according to their chromosome position and homology among the three wheat genomes.
Physical and Chemical Properties, Secondary Structure Prediction, Motif Display, and Phylogenetic Analysis for the Wheat Aux/IAA Proteins

The basic physical and chemical parameters of TaIAA proteins were predicted by the ProtParam tool (http://www.expasy.org/tools/protparam.html). The secondary structures of TaIAA were analyzed by the net service NPS (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=NPSA/npsa_seccons.html). The conserved domains were investigated using Clustal W (Larkin et al., 2007) by multiple alignment analyses and visualized in Jalview (Waterhouse et al., 2009). Motifs of the TaIAA proteins were displayed with the MEME (Bailey et al., 2009) online tool. MEME found four motifs and the other parameters were defaulted. The phylogenetic analysis of Aux/IAA proteins was performed using the NJ method of MEGA 6.0. The protein sequences of other species such as OsIAAs, AtIAAs, SlIAAs, and VvIAAs were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov/) according to the Genbank number.

Expression Analysis for TaIAAs

The coding sequences of the TaIAAs were submitted to the Plex database (http://www.plexdb.org/) to search for corresponding probes. These probes were then used to query the GeneVestigator database (https://genevestigator.com/gv/) to obtain the expression data of TaIAA genes in 19 organs of wheat. All transcript data was transformed by log2 and the heat map was viewed in the MeV tool (http://www.tm4.org/mev.html).

Expansion Pattern Analysis for TaIAAs

Segmental replication and tandem duplication are the main repeat methods of the gene family among the lineage-specific expansion. In this study, 84 genomic sequences of TaIAAs were investigated for segmental replication and tandem duplication events according to the method established in previous studies (Guo and Qiu, 2013). Paralogs from different sub-genomes of wheat were regarded as segmental replication, while two or more TaIAAs situated in the same location of the chromosome were defined as tandem duplication, based on the wheat genome annotation results in the URGi database.

Collinearity Analysis for the Aux/IAA Family

Phylogenetic trees for the wheat- *T. urartu* and wheat- *A. tauschii* Aux/IAA genes were constructed in MEGA 6.0. Two genes from different species situated in the same branch of the phylogenetic tree were designated orthologs (Koonin, 2005). Based on these orthologous Aux/IAA genes, collinearity maps of the *Tu*-wheat A genome and the *Aet*-wheat D genome were output by the genome visualization tool CIRCOS (Krzywinski et al., 2009).

Ka and Ks Calculations

The rate of Ka (non-synonymous substitution rate)/Ks (synonymous substitution rate) was applied to compare the rates of codon evolution in the sub-genome of wheat using barley as an outgroup (Akhunov et al., 2013). The orthologous gene pairs between barley and wheat were used to calculate Ka and Ks in the PAL2NAL server (Suyama et al., 2006) using the codeml program of phylogenetic analysis by maximum likelihood (PAML; Yang, 1997). The barley Aux/IAA sequences were downloaded from the PlantTF database (http://planttfdb_v1.cbi.pku.edu.cn:9010/).

Results

Searching for Aux/IAA Genes in Wheat

A 22.8 GB local database of wheat nucleotide and protein sequences was built. By retrieving wheat protein sequence databases using the Aux/IAA HMM file, 127 non-redundant sequences were obtained. Next, 84 full-length protein sequences were detected that contained conserved Aux/IAA domains. In addition, corresponding coding sequences and genome sequences were isolated from the local nucleotide database and each Aux/IAA gene was located by searching the wheat chromosome genomic sequences using BLASTn (Table 1). An un-rooted tree of the 84 Aux/IAA full-length coding sequences was constructed (Figure 1A) to reveal the phylogenetic relationship and all the sequences were divided into 34 groups. Among them, 30 groups have two or three genes from different wheat sub-genomes, and these were regarded as different copies of one member of the Aux/IAA gene family.

Genome Distribution of Wheat Aux/IAA Genes

The genome distribution of 84 wheat Aux/IAA genes is shown in Figure 2. Respectively, 31, 27, and 26 Aux/IAA genes are non-randomly distributed in the three wheat sub-genomes. According to chromosome position and genomic homology, all these wheat Aux/IAA genes (TaIAAs) were named TaIAA-A~TaIAA34-D and distributed on every wheat chromosome except for chromosome 2D (Figure 2). Each of the 20 TaIAA genes (TaIAA1, 2, 3, 4, 5, 11, 13, 15, 17, 18, 19, 20, 22, 23, 24, 26, 28, 32, 33, and 34) contains three copies in chromosome A, B and D; 10 TaIAAs have two copies each including TaIAA-A/-B (TaIAA6, 9, 10, and 12), TaIAA-B/-D (TaIAA21), and TaIAA-A/-D (TaIAA14, 25, 27, 29 and 30); and TaIAA7, 8, 16, and 31 have just one copy in wheat chromosomes.

There is a high homology among the scaffolds of one TaIAA member which belongs to the A, B or D sub-genome (Figure 3), proving that the TaIAA genes experienced two segmental replication events in wheat except for the four single-copy TaIAA genes. However, it could also be the case that the segmental replication genes of the four TaIAAs were lost after the expansion event. Furthermore, three genomic loci containing two TaIAA genes each were defined in the A sub-genome, and the B or D sub-genomes have four such loci (Supplementary Table 1), implying that tandem duplication is also an expansion pattern of the TaIAA gene family.

Gene Structure of Wheat Aux/IAA Genes

Schematics of gene structures generated by the GSDD utility are shown in Figure 1C. In the TaIAA gene family, the number of introns ranges from 1 to 5. Among the 30 TaIAAs which contains two or three copies, 17 TaIAA genes (TaIAA1, 2, 3, 6, 10, 12, 13, 17, 19, 20, 22, 23, 24, 27, 29, 30, and 32) have the same gene structures, and the remaining 13 have only one or two differences
| Gene     | Sequence ID | Scaffold Location | ORF bp    | Length AA |
|----------|-------------|-------------------|-----------|-----------|
| TaIAA1-A | TaLoc010775.4 | 1AS:31279430-31280500 | 1071      | 208       |
| TaIAA1-B | TraeS_1EE57162.1 | 1BS:2816364-2815360 | 1042      | 209       |
| TaIAA1-D | Traes_CA1185D111.1 | 1DS:70882162-70883423 | 1079      | 207       |
| TaIAA2-A | Traes_52F80B9.1 | 1AS:163196691-163198564 | 1349      | 233       |
| TaIAA2-B | Traes_E5A12CB09.1 | 1BS:86215073-86216744 | 1366      | 235       |
| TaIAA2-D | Traes_56SB8DDA.1 | 1DS:52657909-52659817 | 1367      | 236       |
| TaIAA3-A | Traes_EADB6A2A.1 | 1BS:113674501-113677207 | 1964      | 199       |
| TaIAA3-B | Traes_8A19C460B.1 | 1AS:16660451-113677207 | 1932      | 199       |
| TaIAA3-D | Traes_0898FA765.1 | 1AS:64904063-64906913 | 1932      | 199       |

(Continued)
TABLE 1 | Continued

| Gene    | Sequence IDa | Scaffold | Location            | ORF bp  | Length AA |
|---------|--------------|----------|---------------------|---------|-----------|
| TaIAA21-D | Traes_C2F7D9273.1 | 4537915  | 5DL:125470184-125470754 | 769     | 160       |
| TaIAA22-A | Traes_F845556C3.1 | 2770845  | 5AL:105778316-105779305 | 1157    | 215       |
| TaIAA22-B | Traes_EC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA22-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA23-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA23-B | Traes_C68AC392A.1 | 5138938  | 5BL:216239503-216242516 | 2165    | 240       |
| TaIAA23-D | Traes_1CFC72F63.1 | 4490955  | 5DL:129481978-129485104 | 2146    | 233       |
| TaIAA24-A | Traes_B7F55FB76.1 | 2770845  | 5AL:124461002-124461327 | 692     | 161       |
| TaIAA24-B | Traes_67E73FC97.1 | 10921140 | 5BL:241395575-241397123 | 743     | 178       |
| TaIAA24-D | Traes_5889C544A.1 | 11024170 | 5BL:241397124-241397125 | 743     | 178       |
| TaIAA25-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA25-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA25-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA26-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA26-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA26-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA27-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA27-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA27-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA28-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA28-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA28-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA29-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA29-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA29-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA30-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA30-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA30-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA31-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA31-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA31-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |
| TaIAA32-A | Traes_AB29EB95D.1 | 2681049  | 5AL:107424300-107426935 | 2136    | 231       |
| TaIAA32-B | Traes_CC006AD0C.1 | 1084556  | 5BL:218543827-218546250 | 1571    | 254       |
| TaIAA32-D | Traes_A9B98305.1 | 4496111  | 5DL:127979413-127980640 | 1759    | 253       |

The sequence name beginning with TaLoc and Traes means downloaded from the wheat genome URGI database and Ensembl database, respectively.

in the number of introns in each group. Overall, a highly similar gene structure is exhibited in the same phylogenetic cluster of the TaIAA genes, suggesting that duplicated genes may have the same function.

Characteristics of TaIAA Protein Sequences

Some physical and chemical properties of wheat Aux/IAA proteins are shown in Supplementary Table 2. The predicted molecular mass varies from 21.9 kD for TaIAA1-A to 37.9 kD for TaIAA15-A amongst the Aux/IAA proteins. The negative grand average of the hydropathicity (GRAVY) index showed that all wheat Aux/IAA polypeptides are hydrophilic except TaIAA26-A, suggesting that they are more likely nucleoproteins than membrane proteins. In addition, 69 out of 84 (82.1%) wheat Aux/IAA proteins possess a stability index of more than 40 and might be unstable in vitro.

The results of multiple alignment and motif distribution analyses of TaIAA proteins showed that 48 out of 84 (57.1%) wheat Aux/IAA proteins contain the same domains, known as domains I, II, III and IV (Supplementary Figure 1). Motifs 1, 2, 3, and 4 are located in these four domains, respectively (Figures 1B, 4). Eighteen (21.4%) wheat Aux/IAA proteins missed one domain (I, II, or IV), while 17 (20.2%) proteins missed domains I & II; just one (1.2%) protein, TaIAA16-B, lacked three domains (I & II & III). Generally, TaIAA proteins within the same phylogenetic group have similar domains and motifs. Domain I (Motif 1) contains the LxLxLx motif, a typical leucine-rich region that was shown in most TaIAA proteins, which has been shown to act as a strong transcriptional repressor (Tiwari et al., 2004). Domain II contains VGWPP, the core sequence of the target site for wheat Aux/IAA protein degradation. Dominant mutation in this region causes Aux/IAA proteins to fail to...
FIGURE 1 | Phylogenetic relationship, motif and gene structure of wheat Aux/IAA genes. (A) The phylogenetic tree of TaIAAs constructed from a complete alignment of 84 wheat Aux/IAA genes using MEGA 6.0 by the neighbor-joining method with 1000 bootstrap replicates. Bootstrap scores are indicated on the nodes and the 34 members of TaIAA, most of which contain duplicated genes, are indicated by blue or pink block. (B) The conserved motifs of TaIAAs. Motifs were identified by MEME software using the deduced amino-acid sequences of the TaIAAs. The relative position of each identified motif in all TaAA proteins is shown as 1, 2, 3, and 4, which represented the four conserved domains of IAA. (C) Exon/intron structures of TaIAA genes. Exons are represented by black boxes and introns by black lines. The sizes of exons and introns can be estimated using the scale below.
FIGURE 2 | Chromosome distribution of TaIAA family in wheat. White ovals on the chromosomes (vertical bar) indicate the position of centromeres. The arrows next to gene names show the direction of transcription. The position of each gene can be estimated using the left scale. The chromosome numbers are indicated at the top of each bar.
resolve via the ubiquitin pathway (Kepinski and Leyser, 2005). Domains III and IV are comparatively more conserved. A βαα structure existing in domain III (Motif 3) appeared among all the TaIAA proteins except TaIAA16-B (Figure 1B). It was found that this fold plays an important role in the dimerization of Aux/IAA proteins. Most of the wheat Aux/IAA proteins include two hypothetical nuclear localization signals (NLS). The first bipartite NLS is comprised of two elements: one between domains I and II, and the other in domain II (Supplementary Figure 1). The second element, the SV40-type NLS, is located in domain IV (Supplementary Figure 1). The possible function of these NLSs may be to transfer TaIAA proteins into the nucleus. Interestingly, the conserved residue in the bipartite NLS is KP in TaIAA proteins, while it is KR in rice Aux/IAA proteins (Jain et al., 2006). Therefore, further study into the bipartite NLS in wheat is required. In addition, the existence of phosphorylation sites in several TaIAA proteins partly indicates that these proteins can be extrapolated as short-lived proteins (Supplementary Figure 1).

Phylogenetic Analysis of TaIAA Proteins

The phylogenetic tree was built with the sequences of 159 Aux/IAA proteins, including 84 TaIAAs, 31 OsIAAs, 29 AtIAAs, and 15 Aux/IAA proteins with known functions from other species. The information of all the above sequences is listed in Supplementary Table 3.

The phylogenetic tree shows that TaIAA proteins can be classified into two major groups: A and B (Figure 5). Based on the
clustering tree of OsIAAs (Jain et al., 2006), groups A and B can be further separated into several subgroups: A1, A3, and A4 contain monocotyledon IAA proteins; A5 contains dicotyledon proteins; and A2, B1, B2, B3, and B4 are all types of IAA protein. Moreover, monocot and dicot IAA proteins are not gathered for one class in every subgroup, while the paralogous proteins of each TaIAA are clustered to the same branch, similar to the clustering result in Figure 1A. Orthologous genes usually have similar biological functions (Li et al., 2005). In group A1, OsIAA5 was induced by drought (Peleg et al., 2011), suggesting that TaIAA12 may be related to drought resistance. In addition, OsIAA2 and OsIAA6 in group B1 were induced by pathogens (Chen et al., 2009), OsIAA1 in group A2 regulates plant type (Song et al., 2009), SbIAA1 in group A3 may be related to stress response (Wang et al., 2010a), and OsIAA4 in group B2 regulates plant tiller (Song and Xu, 2013), implying that their orthologs, such as TaIAA1, 9, 10, 13, and 24, may have similar functions in wheat.

Expression Pattern of TaIAAs in Wheat
The coding sequences of 34 TaIAAs were used to search the Plex database for corresponding probes. All gene copies of each TaIAA gene share one probe. The probes of TaIAA20 and TaIAA34 were not found. Several genes have the same probe, including TaIAA2, 9; TaIAA18, 22, 33; and TaIAA29, 30. Finally, 28 probes for 32 TaIAAs were obtained from the GeneVestigator database (Supplementary Table 4). The expression profile of 32 TaIAAs in 19 wheat organs covering the seedling to adult stage is shown as a heat map (Figure 6). TaIAAs were divided into eight classes according to the subgroups of phylogenetic analysis. In general, the majority of genes in groups A3, B1, B2, and B3 are expressed in vegetative organs of wheat, and genes in group A3 are also expressed in the pistil and embryo. Moreover, all genes in A1, A2 and TaIAA14 of B2 have high expression levels in the vast majority of wheat organs throughout the entire growing stage. In addition, TaIAA6 and TaIAA27 are specifically expressed in the roots, while TaIAA16, TaIAA28, and TaIAA31 are specifically expressed in inflorescence, flag leaf and seed, respectively. TaIAA1, 7, 10, 17, 29, and 30 showed low expression in wheat.

Synchronic Analyses of Aux/IAA Families among T. urartu, A. tauschii, and Wheat
Twenty-seven TuIAA, 31 TaIAA-A, 28 ActIAA, and 26 TaIAA-D sequences were used to construct the phylogenetic tree, and pairs of 22 and 21 Ta-A/Tu and Ta-D/Act orthologs were identified (Supplementary Figure 2). The synchronic analysis results of 16 pairs (just 16 of the 27 TuIAA sequences have chromosome location information (Supplementary Table 5) of TuIAA and TaIAA-A showed that there are 14 (87.5%) homologous genes located on the same chromosome, including 1A, 3A, 4A, 5A, and 6A, while 20 of the 21 pairs (95.2%) between ActIAA and TaIAA-D are located on 1D, 3D, 4D, 5D, 6D, and 7D (Figure 3). In general, there is a good collinearity in Aux/IAA families among T. urartu, A. tauschii, and wheat, which suggests that the evolution of the Aux/IAA family has been conservative following the formation of hexaploid wheat. However, TaIAA27-A and
**Discussion**

**Genome-wide Isolation of Gene Families in Hexaploid Bread Wheat**

Sequencing projects provide an opportunity for the isolation of gene families in a genome-wide scan. However, it is a great challenge for analysis of polyploid genomes because the relatedness of homoeologous sub-genome sequences makes it difficult to assign isolated sequences to the specific chromosome from which they are derived. Until now, some gene families, such as WRKY genes (Okay et al., 2014) and nucleotide-binding site (NBS) domain-containing genes (Bouktila et al., 2015), were isolated in wheat. Yet the position of these family genes on wheat genomes and their homologous relationship was still unknown, which leads to the studies on functional divergence and redundancy of duplicated genes could not be proceeded. So compared with rice or maize, the genomic research into hexaploid wheat has been slow for a long time in crops. With the benefit of the new bread wheat draft genome recently through sequencing isolated chromosome arms, we isolated the Aux/IAA gene family in wheat. Unlike previous studies, all the TaIAA...
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FIGURE 6 | Heatmap of expression profiles for TaIAA genes across different organs of seedling and adult stages. The expression data were generated from GeneVestigator database and viewed in MeV software. The relative expression level of a particular gene in each row was normalized against the mean value by log₂ transformation. The color scale below represents expression values, green indicating low levels and red indicating high levels of transcript abundance.

TABLE 2 | Ka/Ks ratio of the duplicated Aux/IAA genes in wheat using barley as an outgroup.

| Gene   | A-genome | B-genome | D-genome |
|--------|----------|----------|----------|
| TaIAA3 | 0.1181   | 0.1084   | 0.1642   |
| TaIAA5 | 0.0805   | 0.3479   | 0.3058   |
| TaIAA9 | 0.4163   | 0.3521   | –        |
| TaIAA11| 0.4350   | 0.5444   | 0.3777   |
| TaIAA12| 0.2286   | 0.2751   | –        |
| TaIAA13| 0.4020   | 0.4177   | 0.4284   |
| TaIAA18| 0.0887   | 0.1156   | 0.1076   |
| TaIAA19| 0.2721   | 0.2659   | 0.2772   |
| TaIAA22| 0.0023   | 0.1478   | 0.1437   |
| TaIAA23| 0.0620   | 0.0456   | 0.0374   |
| TaIAA28| 0.1320   | 0.0878   | 0.1331   |
| TaIAA32| 0.2021   | 0.2560   | 0.2511   |

Expansion and the Fate of Duplicated Aux/IAA Family Genes in Wheat

In this study, we isolated 27 and 28 Aux/IAA genes from the genomes of T. urartu and A. tauschii, respectively, which are similar in their number of Aux/IAA family genes with tomato (26), sorghum (26), A. thaliana (29), rice (31), and maize (31). We deduce that the allopolyploidization event that crossed T. urartu and A. speltoides resulted in the Aux/IAA family being replicated in T. turgidum. This tetraploid emmer wheat subsequently hybridized with A. tauschii, resulting in the second replication of the Aux/IAA family in hexaploid bread wheat. The tandem duplication also occurred via this process, eventually forming the 84 wheat Aux/IAA sequences including 31, 27, and 26 genes from the A, B, or D sub- genomes of wheat, respectively. A good collinearity of Aux/IAAs was shown between wheat and the A and D genome donors T. urartu and A. tauschii (Figure 3).

After the expansion of Aux/IAAs in wheat, the duplicated genes underwent an evolutionary process of purifying selection that can be inferred by their Ka/Ks ratios (Table 2). Therefore, the duplicated genes from the A, B, or D sub-genomes of wheat have a high conservation and share one probe in the expression database (Figure 6). However, the inversion and crossover also happened in wheat chromosomes during the evolutionary process, causing the different fates of several duplicated Aux/IAA genes. One such fate was the loss of duplicated genes such as
TaIAA14-B or TaIAA17-B (Figure 1). Moreover, the positions of some duplicated genes changed, involving not only the shift of linear array location and transcription direction (Figure 2), but also the exchange of position between two chromosomes. Based on the collinearity of Aux/IAA genes between T. urartu and wheat, we infer that TaIAA27 and TaIAA32 were originally on chromosome 3A and then transferred to chromosome 6A and 7A, respectively by interchromosomal translocation (Figure 3). The translocation phenomenon has appeared in previous research (Salse et al., 2008; Ma et al., 2013). In addition, the other important peculiar feature of duplicated Aux/IAA genes is the altering of the conserved motif, including degeneration and neofunctionalization according to Yang’s view (Yang et al., 2006). For example, TaIAA11-A, TaIAA11-B, the orthologs of the D subgenome and AEGTA27928 all contain the four motifs of the Aux/IAA family, while TaIAA11-D lacks motif 4. Other duplicated genes obtained a new motif. Compared with TaIAA5-A, the motifs of TaIAA5-B and TaIAA5-D transform Motif 1 + 2 + 3 to Motif 2 + 3 + 4, which may bring out a novel function.

Functional Divergence of TaIAs

Based on the phylogenetic tree of Aux/IAA proteins (Figure 5), we infer that the Aux/IAA genes divided before the split of monocots and dicots (90 MYA). Later, the second differentiation happened in a lineage-specific manner in the rice lineage and Triticeae before the divergence of Triticum and Aegilops (3~40 MYA). Therefore, the wheat Aux/IAA family has experienced at least two changes.

Despite several genes, including TaIAA29 and TaIAA30, that may have a functional redundancy because of their identical expression patterns (Figure 6), the functions of most TaIAs are probably diverse due to the diversity of the functional domain and expression pattern.

In this study, TaIAA27 did not contain domain I and the protein may function differently to the classical IAA repressors. Reference on the research in Arabidopsis (Dreher et al., 2006), absent of Domain II in TaIAA proteins may lead to enhanced auxin responses by blocking the degradation of IAA proteins, such as TaIAA14. However, TaIAA1 and TaIAA7 present a very low expression level in various organs throughout the growing period of wheat, suggesting that the degradation of these proteins were independent on domain II- mediate auxin responses. Moreover, TaIAA20, which also lacks domain II, has not had any expression data detected at all, implying that TaIAA20 might be a pseudogene like some AtIAs (Reed, 2001). In addition, TaIAA34 similarly has no expression data but it does have all four domains, which implies that TaIAA34 is expressed through a particular pattern according to similar research into OsIAs (Jain et al., 2006) and ZmAAs (Wang et al., 2010b).

The genes in groups A1 and A2 of the phylogenetic tree exercise an important function in terms of root development and responding to the environment. Some TaIAs in these two groups are highly expressed in various organs and involved in almost the entire growth process of wheat. Among them, TaIAA11 from group A1 is highly expressed in leaves, as well as its orthologous OsIAA3 (Nakamura et al., 2006), which shows that TaIAA11 play a key role in leaf development. TaIAA23 from group A2 shows higher expression levels in leaves and roots than in other wheat organs, and TaIAA23-B shows a sequence similarity as high as 95.3% with TaAux/IAA1 (AJ575098) which is sensitive to light and induced by auxin and brassinosteroids (Singla et al., 2006). In addition, TaIAA27 and TaIAA31 exhibit tissue-specific expression in roots and seeds, respectively.

Conclusion

Bread wheat is an important worldwide crop with a huge genome and highly repetitive transposable elements. In summary, 34 Aux/IAA genes including 84 duplicated genes in total were isolated from the wheat genome and located in 41 wheat chromosomes (except chromosome 2D). The TaIAA family has been replicated twice in the two allopolyploidization events of bread wheat, when the tandem duplication also occurred. The duplicated genes have undergone an evolutionary process of purifying selection, resulting in the high conservation of copy genes among the sub-genomes and functional redundancy among several members of the TaIAA family. However, functional divergence probably existed in most TaIAA members due to the diversity of the functional domain and expression pattern.

Author Contributions

Conceived and designed the experiments: Xyl, ZC, LQ. Performed the experiments: LQ, XZ, HZ, XL, WZ, LC, PL. Analyzed the data: LQ. Contributed reagents/materials/analysis tools: Xyl, LQ, JM, XH. Wrote the manuscript: LQ, LZ, ZC.

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Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2015.00770
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