RESEARCH ARTICLE

Genome-wide characterization of the abscisic acid-, stress- and ripening-induced (ASR) gene family in wheat (Triticum aestivum L.)

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Abstract

Background: Abscisic acid-, stress-, and ripening-induced (ASR) genes are a class of plant specific transcription factors (TFs), which play important roles in plant development, growth and abiotic stress responses. The wheat ASRs have not been described in genome-wide yet.

Methods: We predicted the transmembrane regions and subcellular localization using the TMHMM server, and Plant-mPLoc server and CELLO v2.5, respectively. Then the phylogeny tree was built by MEGA7. The exon–intron structures, conserved motifs and TFs binding sites were analyzed by GSDS, MEME program and PlantRegMap, respectively.

Results: In wheat, 33 ASR genes were identified through a genome-wide survey and classified into six groups. Phylogenetic analyses revealed that the TaASR proteins in the same group tightly clustered together, compared with those from other species. Duplication analysis indicated that the TaASR gene family has expanded mainly through tandem and segmental duplication events. Similar gene structures and conserved protein motifs of TaASRs in wheat were identified in the same groups. ASR genes contained various TF binding cites associated with the stress responses in the promoter region. Gene expression was generally associated with the expected group-specific expression pattern in five tissues, including grain, leaf, root, spike and stem, indicating the broad conservation of ASR genes function during wheat evolution. The qRT-PCR analysis revealed that several ASRs were up-regulated in response to NaCl and PEG stress.

Conclusion: We identified ASR genes in wheat and found that gene duplication events are the main driving force for ASR gene evolution in wheat. The expression of wheat ASR genes was modulated in responses to multiple abiotic stresses, including drought/osmotic and salt stress. The results provided important information for further identifications of the functions of wheat ASR genes and candidate genes for high abiotic stress tolerant wheat breeding.

Keywords: Abscisic acid-, stress-, and ripening-induced (ASR), Genome-wide, Tandem and segmental duplication, Phylogenetic analyses, Gene structure, Salt

Background

ASR is a kind of plant specific, small and hydrophilic protein. As the first member of ASR gene family, ASR1 was identified by differential screening a tomato (Solanum lycopersicum L.) fruit cDNA library with cDNA from stressed leaves [1]. Then, a large number of ASR homologs were detected from a wide range of other plant species, including gymnosperms, (e.g., loblolly pine...
In the previous study, one member (i.e. *TaASR4D* here) of the ASR family was characterized in wheat [21]. Nevertheless, a comprehensive characterization of the ASR family in wheat has not been developed. The draft genome of “Chinese Spring” bread wheat has been completed by various sequencing technologies [45–47]. In addition, the physical map (IWGSC, 2018) and a high-quality genome have been published [48], allowing the isolation and analysis of gene families in the genome-wide in wheat. In this study, a total of 33 ASR members were isolated in wheat, and the sequence characteristics, chromosomal distribution and duplication, phylogenetic relationship, gene structure and conserved motif and TF binding sites were analyzed. The tissue specific expression and expression profiles under various abiotic stresses were also examined using the public RNA-seq data and quantitative real-time-PCR (qRT-PCR). These results will provide a better understanding of the wheat ASR family members and important information for subsequent studies and utilization of *TaASRs* in wheat.

**Methods**

**Genome-wide identification of ASR gene family in wheat**

ASR genes reported in other species such as apple and rice were retrieved and downloaded [5, 49]. Their amino acid sequences were used to construct a hidden Markov model (HMM) profile of ASR using the hmmbuild procedure (HMMER3.0) (http://hmmner.org) [50]. The data set of wheat proteins (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0) were searched using BLASTP with the HMM profile of ASR as a query and all possible ASR protein sequences were extracted (e-value \( \leq 1 \times 10^{-10} \)). The self-BLASTP search was first used to remove the redundant sequences among them (e-value \( \leq 1 \times 10^{-10} \)). Subsequently, the PFAM (http://pfam.xfam.org/) and SMART (http://smart.emblheidelberg.de/) website were used to confirm all the *TaASRs* containing the abscisic acid (ABA)/water deficit stress (WDS) domain (PF02496.15). The features of each protein, such as the numbers of amino acids, molecular weight (Da), isoelectric point (pl) and gravity, were calculated using ExPASy (https://web.expasy.org/protparam/) [51]. The trans-membrane structure was obtained using TMHMM Server 2.0 online tool (http://www.cbs.dtu.dk/services/TMHMM/). The subcellular localization of each *TaASR* was predicted using the online tools Plant-mPLoc server (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi) and CELLO v2.5 (http://cello.life.nctu.edu.tw).

**Phylogenetic analysis**

The amino acid sequences of the ASR proteins from wheat and other 7 species including *Brachypodium distachyon*, common bean, foxtail millet, maize, rice,
sorghum and soybean were downloaded from the URGI database (https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Assemblies/v1.0/) and JGI Phytozone (https://phytozone.jgi.doe.gov/pz/portal.html), respectively. These protein sequences and ID loci are listed in Additional file 1: Table S1. To compare the evolutionary relationships among these ASR proteins, the amino acid sequences were aligned using the ClustalW program implemented in MEGA7.0 (http://www.megasoftware.net/). The phylogenetic tree was constructed by using the neighbor-joining (NJ) method based on the JTT matrix-based model with 1000 bootstrap replications [52]. Another phylogenetic tree was constructed using the protein sequences from wheat ASR gene family to understand the evolution of its own members.

Chromosomal locations and gene duplication
The chromosomal localization of each TaASR gene was analyzed by mapping its sequence back to the corresponding chromosome of wheat (IWGSC RefSeq v1.0) using BLAST program with the E-value < 10^-5. To detect the gene homology, the protein sequences of ASR genes in wheat were blasted against each other by BLASTP (E value < 10^-20, identity > 75%) [53, 54]. Tandem duplicated TaASR genes were defined as two or more adjacent homologous genes located on a single chromosome within 150 kb without any intervening gene, while homologous genes among different chromosomes were defined as segmental duplicated genes [18, 55]. The chromosomal distribution and synteny of these ASR genes was visualized by the CIRCOS program [56].

Gene structure and conserved motif analysis
To predict the exon–intron structures of the wheat ASR genes, GSDS (http://gsds.cbi.pku.edu.cn/) was used by comparing the coding/cDNA sequence with its genomic sequence of each gene. To identify the conserved motifs, the MEME program (http://meme-suite.org/) was used with the following parameters: the optimum motif widths of 6–50 amino acid residues and the maximum number of 20 motifs.

Transcription factor binding sites predication
To identify the binding sites of transcription factors in the promoter region of each TaASR gene, 2000-bp genomic DNA sequence upstream of the transcriptional start site used as the promoter sequence was searched via the database PlantRegMap (http://plantregmap.cbi.pku.edu.cn/binding_site_prediction.php) with the following parameters: e-value ≤ 1e^-15 and the top number of 12.

Expression profile analysis of TaASR genes by RNA-seq data
RNA-seq data of five tissues each at three different developmental stages (grain at Z71, Z75, Z85; leaf at Z10, Z23, Z71; root at Z10, Z13, Z39; spike at Z32, Z39, Z65; stem at Z30, Z32, Z65) in bread wheat c.v. Chinese spring with study title “choulet_URGI” was retrieved from expVIP (http://www.wheat-expression.com/), and then the log2 (FPKM + 1) (FPKM, fragments per kilobase transcript per million reads mapped) value of each TaASR was used for visualizing the heat map as a green-yellow–red gradient. The heat map was generated by using the heatmap package in Rversion 3.5.2 (https://www.r-project.org/).

Plant growth, stress treatment and qRT-PCR
Wheat ‘c.v. JM262’ seeds were grown in a growth chamber under controlled conditions as Hu et al. [57] described. For abiotic stress experiments, 15-day-old wheat seedlings were exposed to salt stress (200 mM NaCl solution for 6 and 24 h) and drought stress (23% (w/v) PEG-6000 solution for 6 and 48 h) as described previously [57, 58]. All the treatments were performed with three biological replications. Seedlings grown under the non-stress condition were used as the control. Leaves and roots were collected from ten plants at the above-mentioned time points under both stress and non-stress conditions. Samples were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

Total RNA from all samples was isolated using TRIzol Reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer’s instructions. DNase I was used to eliminate the genomic DNA contamination. Then, the first strand cDNA was synthesized with oligo(dT) primer using the Prime Script II kit (TaKaRa, Dalian). Finally, qRT-PCR was performed in a 20 μl reaction volume using SYBR Green PCR master mix (TaKaRa, Dalian) on ABI 7500 Real-time PCR system (Applied Biosystems, USA), and three technical replicates were conducted for each reaction. The PCR processes were as follows: 95 °C for 30 s, 40 cycles of 95 °C for 3 s and 60 °C for 30 s, followed by a melting curve analysis of 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. For relative quantification, the 2^-ΔΔCT method was used [59], with wheat actin gene used as an internal reference. The quantitative primers were designed using Primer 5.0 and listed in Additional file 2: Table S2.

Results
Identification and characterization of the ASR gene family in wheat
The TaASR4D was previously cloned and characterized [21]; however, the information of other ASR family members is rarely gained in wheat. The recently
Table 1  Characteristics of the ASR gene family members in wheat

| Gene name | ID | Chromosome location | Exon number | ORF (bp) | AA (aa) | Mw (kDa) | PI | Gravity | Subcellular location |
|-----------|----|---------------------|-------------|---------|--------|----------|----|---------|---------------------|
| TaASR1D   | TraesCS3D01G517400.1 | chr3D: 600607844-600608597 | 2          | 663     | 220    | 23.26    | 6.19 | 0.986   | Nuclear             |
| TaASR1B   | TraesCSB01G578500.1  | chr3B: 807930370-807931125  | 2          | 660     | 219    | 23.14    | 6.03 | 0.955   | Nuclear             |
| TaASR1A   | TraesCSA01G509800.1  | chr3A: 730177607-730178363  | 2          | 660     | 219    | 23.18    | 6.25 | 0.977   | Nuclear             |
| TaASR2A   | TraesCSA01G509900.1  | chr3A: 730207889-730208652  | 2          | 657     | 218    | 23.21    | 6.24 | 0.929   | Nuclear             |
| TaASR2D   | TraesCSB01G517300.1  | chr3B: 600594427-600595179  | 2          | 657     | 218    | 23.20    | 6.24 | 0.917   | Nuclear             |
| TaASR2B   | TraesCSB01G578400.1  | chr3B: 807881862-807882916  | 2          | 666     | 221    | 23.45    | 6.10 | 0.950   | Nuclear             |
| TaASR3A1  | TraesCSA01G510100.1  | chr3A: 730279210-730279978  | 2          | 660     | 219    | 23.68    | 6.16 | 1.074   | Nuclear             |
| TaASR3D   | TraesCSB01G517100.1  | chr3B: 600653643-600564143  | 3          | 528     | 175    | 18.86    | 6.51 | 1.106   | Nuclear             |
| TaASR3B   | TraesCSB01G578200.1  | chr3B: 807812908-807813694  | 2          | 684     | 227    | 24.39    | 6.27 | 1.087   | Nuclear             |
| TaASR3A2  | TraesCSA01G510200.1  | chr3A: 730327845-730328798  | 2          | 693     | 230    | 24.91    | 7.79 | 0.991   | Nuclear             |
| TaASR4D   | TraesCSD01G109500.1  | chr4D: 88700513-88701275     | 2          | 414     | 137    | 15.30    | 6.06 | 1.198   | Nuclear             |
| TaASR4B   | TraesCSB01G112000.1  | chr4B: 125481409-125482171  | 2          | 417     | 138    | 15.46    | 6.14 | 1.199   | Nuclear             |
| TaASR4A   | TraesCSA01G208400.1  | chr4A: 50146856-501469147    | 3          | 405     | 134    | 15.00    | 6.11 | 1.043   | Nuclear             |
| TaASR5A   | TraesCSA01G301500.1  | chr5A: 516531874-516532791   | 2          | 795     | 264    | 28.83    | 5.19 | 1.755   | Nuclear             |
| TaASR5B   | TraesCSB01G317600.1  | chr5B: 453075970-453078937   | 2          | 840     | 279    | 30.34    | 4.97 | 1.760   | Nuclear             |
| TaASR5D   | TraesCSB01G300100.1  | chr5D: 382196503-382196510   | 2          | 789     | 262    | 28.65    | 5.20 | 1.735   | Nuclear             |
| TaASR6D   | TraesCSB01G517700.1  | chr3D: 600624824-600625221   | 2          | 285     | 94     | 10.37    | 9.74 | 1.233   | Nuclear             |
| TaASR6A   | TraesCSA01G509700.1  | chr3A: 730521620-73052564    | 2          | 285     | 94     | 10.40    | 9.82 | 1.234   | Nuclear             |
| TaASR9B   | TraesCSA01G506200.1  | chr3A: 697683251-697683662   | 2          | 303     | 100    | 11.04    | 9.99 | 1.349   | Nuclear             |
| TaASR9D   | TraesCSB01G181800.1  | chr3B: 600655220-600655626   | 2          | 294     | 97     | 10.81    | 10.04| 1.366   | Nuclear             |
| TaASR9A   | TraesCSA01G509400.1  | chr3A: 730805673-730806163   | 2          | 294     | 97     | 10.76    | 9.99 | 1.331   | Nuclear             |
| TaASR9B   | TraesCSB01G456200.1  | chr3A: 697683251-697683662   | 2          | 303     | 100    | 11.04    | 9.99 | 1.349   | Nuclear             |
| TaASR10A1 | TraesCSA01G6927000LC.1| chr3A: 730031423-730032153   | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |
| TaASR10A2 | TraesCSA01G157000.1  | chr3A: 730463302-730463736   | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |
| TaASR10U  | TraesCSU1G240200.1   | chrUn: 358203209-358203643   | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |
| TaASR10A3 | TraesCSA01G6937000LC.1| chr3A: 73042877-730443311    | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |
| TaASR10A4 | TraesCSA01G6938000LC.1| chr3A: 730591083-730591517    | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |
| TaASR10A5 | TraesCSA01G509200.1  | chr3A: 730011752-730012186   | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |
| TaASR10A6 | TraesCSA01G509500.1  | chr3A: 730092027-730092461   | 2          | 333     | 110    | 12.34    | 9.88 | 1.286   | Nuclear             |

ORF indicates open reading frame, AA indicates amino acids, Mw indicates protein molecular weight, and PI indicates protein isoelectric point.
Phylogenetic analysis of ASR genes

To compare the evolutionary relationships among these TaASR genes, the phylogenetic tree of TaASRs was constructed using the full length protein sequences (Additional file 3: Figure S1). The 33 TaASR proteins could be clustered into six groups with uneven numbers of family members, 10 in group I (TaASR1D-3A2) representing the largest group of ASRs, 3 in group II (TaASR4D-4A) and III (TaASR5A-5D), 5 in group IV (TaASR6D-7D2) and V (TaASR8B-9D), and 7 in group VI (TaASR10A1-10A6), respectively. To further compare the evolutionary relationships of ASR proteins, a phylogenetic tree was constructed using the protein sequences of ASR genes from wheat (33), *Brachypodium distachyon* (6), common bean (2), foxtail millet (6), maize (10), rice (6), sorghum (7) and soybean (3) (Fig. 1, Additional file 1: Table S1). The wheat ASR proteins in each group (except TaASR9D in group V) were tightly clustered together rather than with ASR proteins from other species, especially those in group IV, V and VI. In addition, wheat group I was clustered with BdASR4, OsASR6, ZmASR4, SiASR5, ZmASR3, PvASR1 and PvASR2. Wheat group II was related to BdASR2, SiASR2, SbASR2, ZmASR2, OsASR4, SbASR1, ZmASR10, ZmASR1 and SiASR1. However,
Chromosomal distribution and gene duplication patterns of wheat ASR genes

Chromosomal distribution analysis showed that, except for TaASR10U located on the unanchored scaffolds, 32 TaASR genes were unevenly mapped on 9 of 21 wheat chromosomes (Table 1, Fig. 2). A total of 12, 6 and 8 TaASR genes were located on the distal of chromosomes 3A, 3B and 3D, which represented 36.4%, 18.2% and 24.2% of total, respectively. Besides, one gene (3.0%) was located on chromosomes 2A, 2B, 2D, 4A, 4B and 4D, respectively. In contrast, no one was located on the remaining 12 chromosomes. The TaASR genes were unevenly distributed among the sub-genomes A, B, and D, with 14, 8 and 10 members, representing 42.4%, 24.2% and 30.3% of total, respectively. Moreover, wheat ASR genes were unevenly distributed among different chromosomal groups. The chromosomal group III carried 26 TaASR genes (78.8%), representing the largest number, followed by the groups II and IV, which carried 3 genes (9.0%). The rest four chromosomal groups including I, V, VI and VII carried no TaASR gene. Furthermore, most

Fig. 2. Chromosomal distribution of TaASR genes and gene duplication analysis in wheat. Different color lines indicate duplicated ASR gene pairs on different chromosome
of these genes were tightly linked and lay within clusters. For example, 12, 5 (except TaASR9B) and 8 TaASR genes were close on chromosome 3A, 3B and 3D within 579.8, 207.2 and 102.5 kb, respectively. Interestingly, all seven group VI members except TaASR10U were tightly linked and lay on the distal of chromosome 3A. Given that the protein sequence of TaASR10U was same with its closed related six members (TaASR10A1, TaASR10A2, TaASR10A3, TaASR10A4, TaASR10A5 and TaASR10A6), we speculated that TaASR10U might be linked with them and it was located on the distal of chromosome 3A.

Tandem and segmental duplications are essential for gene family evolution to generate new gene members [60, 61]. Thus, we analyzed the duplication events of wheat ASR genes. A total of 14 pairs of genes which corresponded to 23 wheat ASR genes were identified as tandem duplication genes and located on chromosome 3 (A, B, D) (Additional file 4: Table S3, Fig. 2, Table 1). Additionally, one group of 5 tandem duplicated genes were located on chromosome 3A within 140.8 kb, two groups of 3 tandem duplicated genes were located on chromosomes 3A and 3D within 148.6 and 7.9 kb, and two groups of 2 tandem duplicated genes were located on chromosomes 3A, 3B and 3D, within 31.0/49.6 kb, 49.3/60.8 kb, and 14.2/10.7 kb, respectively. Furthermore, eight homoeologous gene groups (24 TaASRs genes) might be related to segmental duplication events, which were distributed on chromosomes 2 (A, B, D), 3 (A, B, D) and 4 (A, B, D) (Additional file 4: Table S3, Figs. 1, 2, Table 1). These results showed that there is a high degree of homology between the homologous chromosomes. Interestingly, not every ASR had three homoeologous genes on the homologous chromosomes 3A, 3B, and 3D. 1 pair of TaASRs only had two homoeologous genes (TaASR8B, TaASR8D) on the homologous chromosomes 3B and 3D. Another 1 pair of TaASRs had two genes (TaASR3A1, TaASR3A2) on the homologous chromosomes 3A. Additionally, TaASR7 repeated once (TaASR7D1/D2) only on the chromosome 3D, TaASR10 repeated 6 times (TaASR10A1-A6) only on the chromosome 3A, and one gene (TaASR10U1) distributed on the unanchored scaffolds. These results indicate that there might be independent evolution and repetitive events between the homologous chromosomes. There was still 1 TaASRs (TaASR10U) which was neither tandem nor segmental duplication gene. This result indicated that the tandem and segmental duplication events were essential for the expansion of the wheat ASR gene family.

Gene structure and conserved motifs of ASR genes in wheat

All the examined 33 TaASR genes contained two exons, except TaASR3D and TaASR4A with three exons (Fig. 3, Additional file 5: Table S4). These results are similar to the ASR gene structures of rice and Brachypodium distachyon [5, 8]. Genes in the same group are generally more similar in gene structure and lengths of the full gene, intron and exon. Strikingly, all the members in group VI shared the same lengths of the intron and exon, indicating that gene length varied among diverse groups. Group III shared the longest average lengths of the full gene, exon 1, intron 1 and exon 2, while group IV shared the shortest average lengths except intron 1. Among those 33 TaASRs, TaASR2B and TaASR6B were the longest and shortest for the longest UTR and shorter exon 1.

Conserved motifs were further predicted using the MEME program. A total of 20 conserved motifs were found in 33 wheat ASR family members (Fig. 4, Additional file 6: Table S5, Additional file 7: Table S6). The identified TaASR motifs varied in length from 6 to 50 aa. Wheat ASRs in the same group shared similar conserved motif composition. For examples, group III and VI shared the same 8 and 4 conserved motifs. Additionally, group I shared the same conserved 10 motifs (except that TaASR3D added motifs 15 and 18 while lacked motifs 3, 4 and 19; TaASR3A2 added motif 17 while lacked motifs 2 and 7). Motifs 1, 2 and 5 existed in all the six groups, except for that one or two motifs were absent from some genes. However, the rest motifs were unevenly distributed among different groups. Motif 4 distributed within group I, II and VI and motif 14 distributed within group IV and V. Motifs 3, 6, 7, 10, 12 and 19 only existed in group I. Motifs 8, 9, 11, 13 and 16 uniquely distributed within group III, while motif 20 was only present at group II. In addition, the motifs were unevenly distributed among the proteins, with the number of motifs ranging from 1 (TaASR6B) to 10 (all group I members except TaASR3D). Motifs 1 and 2 were found in 32 of these ASRs, absent from TaASR6B and TaASR3A2, respectively. Motif 17 was only shared by TaASR3A2 and TaASR4A. It should be noted that motifs 15 and 18 each were uniquely identified in TaASR3D, which might be consistent with its special gene structure.

Transcription factor binding sites analysis in the TaASR promoters

Transcription factors are one of the most regulators that regulate gene expressions at transcriptional level by binding to specific DNA sequences. To explore the possible binding sites of TFs, the 2.0 kb upstream promoter regions of the TaASRs were examined via the online database, PlantRegMap. The results showed that a total of 4511 binding sites for 12 TFs including TEOSI 

ETING CELL FACTOR1 (TCP) [34, 62], NAM/ATAF/CUC (NAC [63, 64]), B3 [65], WRKY [66], Ethylene
Response Factor (ERF) [67], Cys2His2 (C2H2) [68, 69], DNA binding with one finger (Dof) [70], basic leucine zipper (bZIP) [71], basic Helix-Loop-Helix (bHLH) [72, 73], Lateral Organ Boundaries Domain (LBD) [74], myeloblastosis (MYB) [75] and GATA [76–78] were discovered (Fig. 5, Additional file 8: Table S7). ERF, C2H2, bHLH, MYB, NAC, bZIP, LBD, TCP and GATA binding sites occurred 780, 561, 474, 464, 440, 321, 313, 292 and 259 times, respectively, and they were present in the promoter of all 33 *TaASR* genes. 285, 207 and 115 binding sites of B3, Dof and WRKY were identified spanning 32, 32 and 29 *TaASR* promoters, which were absent from 1 (*TaASR5A*), 1 (*TaASR2D*) and 4 (*TaASR1B, TaASR5A, TaASR5B and TaASR7D2*) *TaASRs*, respectively. Most of these TFs, such as NAC [63, 64], WRKY [79, 66], C2H2 [68], bZIP [71], bHLH [72, 73], MYB [75] and GATA [76, 77], were involved in regulating plant growth and development, and the responses to multiple abiotic stress, such as drought and salt. All of the *ASR* genes contained GATA binding sites which were involved in light responsive development [78].

**Expression analysis of TaASR genes in various wheat tissues**

Publicly available RNA-seq databases were used to examine the expression profiles of *TaASR* genes in wheat grain, leaf, root, spike and stem. Results showed that all the examined 33 genes expressed in at least one organ and 24 genes were expressed in all the tested tissues (at least one developmental stage), suggesting *ASR* genes significantly contributed to wheat tissue growth and development (Fig. 6, Additional file 9: Table S8). The expression of group II (*TaASR4D-4A*) and group III (*TaASR5A-5D*) genes were higher than that of other group genes in multiple tissues overall. They may be involved in the regulation of wheat growth and development. In contrast, most genes in group IV (*TaASR6D-7D2*) and V (*TaASR8B-9D*) had low or no expression level in almost all tissues, except for *TaASR8B, TaASR8D* and *TaASR9B* with
relatively high expression level in root_Z10, leaf_Z71 and stem_Z32, respectively. Group I (TaASR1D-3A2) genes relatively highly expressed in leaf, root and stem, and lowly expressed in grain and spike. Strikingly, three genes (TaASR1B-2A) did not express in grain. Besides, group VI genes (except TaASR10A2-10U, TaASR10A6) specifically expressed in stem_Z30. TaASR10A6 lowly expressed in grain, leaf and stem, and did not express in root and spike. Additionally, TaASR6B specifically expressed in grain_Z71.

Expression analysis of TaASRs under abiotic stresses by qRT-PCR

The expression patterns of 10 selected TaASRs in root and leaf under PEG and NaCl stress were analyzed by qRT-PCR (Fig. 7). The expressions of TaASR1B, TaASR2B and TaASR2D were significantly down-regulated under both PEG and NaCl stresses in root and leaf. In salt stress (NaCl), TaASR3A1 was down-regulated and up-regulated expressed in leaf at 6 and 24 h, respectively; while remained unchanged in root. In PEG simulated drought stress, the expression of TaASR3A1 was with no change and significantly increased in leaf at 6 and 48 h, and significantly decreased and with no change in root at 6 and 48 h, respectively. The expression levels of TaASR4A, TaASR4B and TaASR4D either remained no significant change or decreased significantly under both NaCl and PEG stresses, except TaASR4A significantly increased under NaCl treatment at 24 h in leaf. Under NaCl treatment, the transcript levels of TaASR5A, TaASR5B and TaASR5D were significantly down-regulated and up-regulated at 6 and 24 h in leaf, respectively; whereas, they were significantly down-regulated in root. Under PEG treatment, they were either down-regulated or not significantly changed in leaf, except for that TaASR5B was up-regulated at 48 h. In root, they were significantly down-regulated. Taken together, five genes (TaASR3A1, TaASR4A, TaASR5A, TaASR5B and TaASR5D) were induced in leaf under NaCl treatment at 24 h, while two genes (TaASR3A1 and TaASR5B) were induced in leaf under PEG treatment at 48 h, suggesting these genes might play a vital role in responses to NaCl and PEG stresses in wheat.

Discussion

Since ASR genes were identified in tomato 20 years ago [1], they have been found in various cereal crops. For example, 5, 6, 6 and 10 ASR genes were identified and characterized at genome-wide level in Brachypodium distachyon [8], foxtail millet [16], rice [5] and maize [7, 9], respectively. In wheat, the information of the ASR gene
family in genomic scale is still lack. Here, a comprehensive analysis on the ASR gene family in the genome-wide level in wheat was conducted.

ASR family members have been identified in various plants, including four members in pine [2], five in tomato and Brachypodium distachyon [8, 15], six in rice and foxtail millet [5, 16], and ten in maize [9]. In the present study, we identified 33 wheat ASR genes, containing a conserved ABA/WDS domain. This suggested that the ASR gene family is small, with no more than 33 members and the number of ASR proteins in wheat was much higher than that in other species. It might be attributed to the allohexaploid genome and complex evolution in wheat [80, 81]. Furthermore, the wheat experienced 2 whole genome duplication events from donors of the A, B, and D genomes [45, 82]. Thus, each wheat gene generally has three homologous loci on sub-chromosomes A, B and D [83]. In this study, each of three TaASR genes from 6 pairs of homoeologous genes, was found to be on each of the A, B and D homoeologous chromosomes 2, 3 and 4, respectively. Interestingly, there was 1 pair of TaASRs with two homoeologous genes (TaASR8B, TaASR8D) on the homologous chromosomes 3B and 3D. Another 1 pair of TaASRs had repeated one time (TaASR3A1, TaASR3A2) on the homologous chromosomes 3A. This might be caused by the independent evolution and repetitive events between the homologous chromosomes. Gene duplication is generally the main factor causing the expansion of the given gene family [84]. Duplication also allows essential genes to undergo mutations in the duplicated copy, suggesting that similar genes would diverge over the long evolution time period, and improve the expansion and evolution of the gene family [85, 86]. The wheat ASR genes in the same group are phylogenetically close to each other rather than with other ASR genes from other species including those from monocots, suggesting that they were the product of recent duplication events rather than orthologs of ASR genes found in other species. Similar
phenomenon was observed in loblolly pine and banana ASR genes [3, 87, 88]. In tomato, all four ASR genes are located next to each other on chromosome IV and in a tandem array [87]. In the present study, all the ASR genes in group VI (except TaASR10U) were linked on chromosome 3A within less than 579.8 kb, while TaASR10U was located on the unanchored scaffolds. Nevertheless, these ASRs from wheat might come from a single gene copy resulted from recent duplication events for the high degree of similarity shared by them in multiple characteristics such as sequence (the same protein sequences), gene structure, chromosomal distribution and phylogeny relationships.

**Fig. 6** Expression profiles of TaASR genes at different developmental stages of five tissues (grain, leaf, root, spike and stem) in wheat
Tandem and segmental duplications of genes have been widely reported for the expansion of different gene families in wheat [89–91]. For examples, 85 tandem or segmental duplications, 22 tandem and 5 segmental duplication events, and 6 tandem and 32 segmental duplication events were identified in WRKY [91], SWEET [89] and OPR [90] gene families in wheat, respectively. Earlier studies have described more frequent tandem duplication events and genes related to stress response have been found to be in distal telomeric segments [92, 93] (IWGSC, 2018). In this study, chromosomal localization revealed that 12, 6 and 8 TaASR genes were located on the distal telomeric ends of chromosomes 3A, 3B and 3D, respectively. As respected, 23 of the 26 (except TaASR3B and TaASR9B on chromosome 3B and TaASR3D on chromosome 3D) TaASR genes were identified as tandem duplication genes. Furthermore, 24 segmental duplication TaASR genes located on chromosomes 2 (A, B, D), 3 (A, B, D) and 4 (A, B, D) were observed in wheat. Tandem and segmental duplication events have also been reported to contribute to the expansion of ASR gene family in other species, such as banana [5, 88], tomato [15] and Brachypodium distachyon [8]. Additionally, tandem and whole genome duplications also contributed to ASR members in rice [5]. Thus, it could be proposed that the tandem and segmental duplication events also contributed to the expansion of the ASR gene family in wheat.

In this study, the expression of these segmental duplication TaASR genes varied under NaCl and PEG stresses. Group II (TaASR4D–4A) had different expression patterns...
in leaf under both NaCl and PEG treatments, and group III only differed in leaf under PEG stress, suggesting the activities of each group genes differentiated after duplication events and they might be functionally important and not redundant. Additionally, TaASR1B and TaASR2B resulted from a gene duplication event, and had uniform expression patterns under NaCl and PEG treatments; however, these genes had similar expression patterns in five tissues (i.e. grain, leaf, root, spike and stems), suggesting that these genes have similar functions.

Most of the ASRs in foxtail millet, maize and rice were ubiquitously expressed in all tested tissues, suggesting the wide functioning of ASRs in many development processes in cereal crops [16, 37]. The TF-binding sites analysis suggested that most TaASR genes were involved in various processes during growth and development. As expected, most wheat ASR genes expressed in multiple tissues and developmental stages, indicating they might play important roles in wheat growth and development. In this study, the expression of group II and III TaASRs was generally high in all five tested tissues. The expression of most group IV, V and VI TaASRs was low or even no expression existed in almost all tissues. Group II proteins contained six motifs of 1, 2, 4, 5, 17 and 20, of which motif 20 was specific for this group proteins. Group III proteins contained 8 motifs of 1, 2, 5, 8, 9, 11, 13 and 16, of which five motifs (motifs 8, 9, 11, 13 and 16) were specific for this group proteins. Group IV and V proteins contained motifs 1, 2, 4 and 14, while motif 14 was uniquely present in these two group proteins. Group VI proteins contained motif 1, 2, 4 and 5. Therefore, the diverse of expression patterns might be resulted from the diversity of motifs that they contained. Most TaASRs in group I had low or no expression in grain and spike. All the group VI TaASRs (except TaASRs, 10A2, 10U and 10A6) rarely expressed in stem_Z30, while TaASR6B only expressed in grain_Z71. Thus, it could be documented that the expression of wheat ASR genes exhibit tissue or development stage-specific pattern. These results were similar to BdASR5, which expressed at relatively high levels in stem and leaf, while it was not the case in the root in Brachypodium distachyon [8]. However, the group I specific motifs of 3, 6, 7, 10, 12, 15, 18 and 19, and motif 2, which was the only motif present in TaASR6B, might contribute to their specific expression patterns. Interestingly, tandem duplication gene pairs TaASR1A/2A, TaASR10A1/10A5, TaASR1B/2B, TaASR1D/2D, TaASR7D1/6D and TaASR8D/9D respectively shared similar motifs and showed similar expression patterns, which might be regulated by a coordinated regulatory mechanism.

ASR genes have been reported to be widely involved in plant responses to various abiotic stresses at the transcriptional level and normally be positively regulated. Overexpression of OsASR5, SiASR1 and wheat ASR1 enhanced osmotic stress and drought tolerance in transgenic plants [16, 21, 34]. SiASR4 and HvASR5-overexpressing transgenic plants exhibited enhanced tolerance to drought and salt stress [33, 94]. Various binding sites of TFs involved in various stresses regulation, like drought, salt, heat and cold, were found in the promoter regions of TaASR genes. Thus, it could be speculated that the wheat ASR genes participated in stress responses. The expressions of TaASR3A1, TaASR4A, TaASR5A, TaASR5B and TaASR5D were up-regulated in leaf under NaCl stress. After exposure to PEG, TaASR3A1 and TaASR5B expression were up-regulated in leaf. Further molecular study of these genes should reveal more functional mechanisms for these genes and contribute to the screening of more candidate genes for contributing to genetic engineering for wheat yield improvement and stress tolerance. Virlouvet et al. [7] reported that PEG decreased ZmASR5 transcript levels in leaf and ZmASR2 and ZmASR7 transcript levels in root. Wang et al. [8] reported that BdASR4 and BdASR1 expression levels remained unchanged under PEG and NaCl stresses, respectively; while BdASR2-3 expression levels decreased in exposure to NaCl. In this study, the rest five tested TaASRs (TaASR1B, TaASR2B, TaASR2D, TaASR4B and TaASR4D) expression levels decreased or remained unchanged in leaf and root under NaCl and PEG treatments. Thus, the functions of these TaASRs might be regulated by multiple elements, and the present of drought/salt associated TF binding sites might not be directly related to the functioning. Hu et al. [21] proved that the TaASR1 expression was up-regulated in leaf when exposed to drought/osmotic stress by PEG-6000 treatment; however, in our study, the TaASR4D (the same gene as TaASR1) transcript levels remained no significantly change in leaf. Wheat varieties, nutrient composition and contents, seedling stage, as well as concentration of PEG-6000 were varied among these studies, and their effects remained inconclusive.

Conclusion

In summary, our study is the first genome-wide analysis of ASR genes in wheat. The chromosomal distribution, phylogenetic relationship, gene structure, composition of conserved motif and TFs binding sites were systematically analyzed. The expansion of the ASR gene family in wheat was mainly due to gene duplication including segmental duplication and tandem duplication. The TFs binding sites analysis suggested that most TaASR genes were involved in various processes during growth and development as well as stress responses in wheat, which will provide abundant resources for functional
characterization of TaASR genes. Taken together, our results will provide a more extensive insight on TaASR gene family, and also contribute to screen more appropriate candidate genes for further investigation on function characterization of ASRs under various stresses.

**Supplementary information**

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s40659-020-02916.

Additional file 1: Table S1. Sequences and ID loci information of ASRs in wheat and other species.

Additional file 2: Table S2. Primers used for qRT-PCR.

Additional file 3: Figure S1. Phylogenetic analysis of 33 ASR proteins from wheat.

Additional file 4: Table S3. Tandem and segmental duplication gene pairs identified in TaASRs.

Additional file 5: Table S4. Gene structure of TaASR genes.

Additional file 6: Table S5. Information of motifs identified from wheat ASR proteins using MEME motif search tool. Note: aa, amino acids.

Additional file 7: Table S6. Conserved motifs identified from the TaASR genes in wheat.

Additional file 8: Table S7. Analysis of TF binding sites in the asr promoters.

Additional file 9: Table S8. FPKM values of wheat ASRs in various developmental tissues.

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Authors’ contributions

HYG and HWL, performed experimental works and data analysis, prepared the original draft: SDL, JSS and QCZ, participated in data analysis; ZSW, BZ, ZW and BF participated in experimental works; LSZ, FHW and LAK helped review and editing the draft. All authors read and approved the final manuscript.

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Availability of data and materials

Please contact author for data requests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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