Research Article

Genome-Wide Characterization of OFP Family Genes in Wheat (Triticum aestivum L.) Reveals That TaOPF29a-A Promotes Drought Tolerance

Dezhou Wang,1 Zhichen Cao,1,2 Weiwei Wang,1 Wengen Zhu,1 Xiaocong Hao,1 Zhao Feng Fang,1 Shan Liu,1 Xiaoyan Wang,2 Changping Zhao,1 and Yimiao Tang1,2

1Beijing Engineering and Technique Research Center for Hybrid Wheat, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
2College of Agriculture, Yangtze University, Jingzhou 434023, China

Correspondence should be addressed to Xiaoyan Wang; 215900995@qq.com, Changping Zhao; zhaochangping@baafs.net.cn, and Yimiao Tang; tangyimiao@126.com

Received 30 May 2020; Revised 15 September 2020; Accepted 22 September 2020; Published 7 November 2020

Academic Editor: Sercan Ergn

Copyright © 2020 Dezhou Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

OVATE family proteins (OFPs) are plant-specific transcription factors that play important roles in plant development. Although common wheat (Triticum aestivum L.) is a major staple food worldwide, OFPs have not been systematically analyzed in this important crop. Here, we performed a genome-wide survey of OFP genes in wheat and identified 100 genes belonging to 34 homoeologous groups.

Arabidopsis thaliana, rice (Oryza sativa), and wheat OFP genes were divided into four subgroups based on their phylogenetic relationships. Structural analysis indicated that only four TaOFPs contain introns. We mapped the TaOFP genes onto the wheat chromosomes and determined that TaOFP17 was duplicated in this crop. A survey of cis-acting elements along the promoter regions of TaOFP genes suggested that subfunctionalization of homoeologous genes might have occurred during evolution. The TaOFPs were highly expressed in wheat, with tissue- or organ-specific expression patterns. In addition, these genes were induced by various hormone and stress treatments. For instance, TaOPF29a-A was highly expressed in roots in response to drought stress. Wheat plants overexpressing TaOPF29a-A had longer roots and higher dry weights than nontransgenic plants under drought conditions, suggesting that this gene improves drought tolerance. Our findings provide a starting point for further functional analysis of this important transcription factor family and highlight the potential of using TaOPF29a-A to genetically engineer drought-tolerant crops.

1. Introduction

The shape of produce is an important agronomic trait. Identifying and characterizing the genes that regulate this trait in crops could lead to the improvement of this agriculturally important trait. The first OVATE gene cloned from plants controls fruit shape in tomato (Solanum lycopersicum) [1]. OVATE family proteins (OFPs) have recently been identified in a variety of land plants [2]. OFPs are plant-specific transcriptional regulators containing a common domain designated as the OVATE domain or Domain of Unknown function 623 (DUF623) [3, 4]. These proteins are widely involved in the growth and development of multiple plant tissues and organs, contributing to processes such as secondary cell wall formation, cell elongation, cotyledon development, floral shape formation, and brassinolide (BR) signal transduction [5]. Moreover, a growing body of evidence suggests that OFPs participate in abiotic stress pathways [2, 6, 7].

Many OFP genes have been identified in diverse plant species, including 31 in rice (Oryza sativa) [8], 28 in apple (Malus domestica) [9], 18 in Arabidopsis thaliana [10], 19 CsOFPs in cucumber (Cucumis sativus) [11], 18 CmOFPs in melon [12], 17 ClOFPs in watermelon [11], and 35 RsOFPs in radish [2], laying the foundation for identifying new OFP genes in other plant species. Subsequent studies in Arabidopsis have indicated that OFPs affect various
aspects of plant growth and development, likely by interacting with different types of transcription factors and functional proteins. AtOFP1 functions in the gibberellin (GA) signaling pathway by regulating AtGA20ox1 expression [4]. AtOFP1 and AtOFP4 interact with KNAT7 to regulate secondary cell wall formation in Arabidopsis [13]. AtOFP5 negatively regulates the activity of the BLH1-KNAT3 complex, thereby affecting early embryo sac development [14]. Loss-of-function alleles of AtOFPs did not display any morphological defects, but plants overexpressing AtOFP5 had smaller hypocotyls, siliques, leaves, rosettes, and floral organs than the wild type (WT) [4]. Tomato plants overexpressing SlOFP20 showed several phenotypic defects, including altered fruit shape and floral architecture and reduced male fertility [15]. Among other horticultural crops, RsOFP2.3 is related to tuberous root shape formation in radish, StOFP20 controls tuber shape in potato (Solanum tuberosum), CmOFP1a is a candidate for the fruit size/fruit shape QTL CmFS8.3/CmFS8.3 in melon [12], and CsOFP1a and CIOPFP1a are top candidates for two QTLs related to fruit shape in cucumber and watermelon [11]. OFPs play multiple roles in plants, including development, phytohormone signaling, and responses to stresses such as cold, salinity, and drought stress. In rice, OsOFP1 regulates the levels of proteins involved in BR responses and thereby modulates plant architecture and grain morphology. Overexpressing OsOFP2 in rice resulted in reduced plant height, altered leaf and seed morphology, and changes in the positions of stem vascular bundles. Transcriptome analysis indicated that the expression of genes related to phylogeny, organogenesis, and hormone homeostasis was altered in these plants [16]. OsOFP6 regulates plant development and confers resistance to drought and cold stress in rice. OsOFP6 is involved in lateral root growth and initiation mediated by the auxin IAA. OsOFP6 also functions in the determination of plant height and grain size, lateral root development, and abiotic stress responses [7]. OsOFP8 regulates leaf angle via the BR signaling pathway, and OsOFP30 is also involved in BR responses [17]. Overexpressing OsOFP8 improved disease resistance, drought tolerance, and cold stress tolerance in rice [17]. MdOFP04 and MdOFP20 were significantly upregulated following NaCl treatment in apple (Malus domestica) [9].

Hexaploid bread wheat (Triticum aestivum L.), the most widely cultivated crop worldwide, contains three homologous subgenomes (A, B, and D) [18]. Although the wheat genome was recently sequenced and assembled, the OFP gene family has not been described in wheat. In the current study, we identified 100 OFP genes in wheat and comprehensively investigated these genes. We categorized the Arabidopsis, rice, and wheat OFP genes into four subclasses based on their phylogenetic relationships. We also analyzed the conserved motifs, structures, and duplication patterns of OFP genes in wheat and examined the syntetic relationships between wheat and rice OFP genes. Expression profiling in different tissues and in response to different stress treatments uncovered the possible roles of these genes in regulating plant development and responses to biotic and abiotic stress. We also performed a more detailed examination of the Ovate-like gene TaOFP29a-A (TraesCS1A02G387000). TaOFP29a-A is highly expressed in roots in response to drought stress and is likely involved in drought stress tolerance, as overexpressing this gene in wheat improved tolerance to drought treatment, highlighting its potential as a target for breeding stress-tolerant crops. The results of this study provide a reference for further functional analysis of OFP-related genes in crops.

2. Materials and Methods

2.1. Identification of Wheat OFP Gene Family Members. The wheat genome sequence was downloaded from Ensembl Plants (http://plants.ensembl.org/index.html). The Hidden Markov Model (HMM) profile of the OFP domain (PF04844) was downloaded from the Pfam website (http://pfam.xfam.org) [19]. The OFP proteins were verified individually against the NCBI-CDD database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [20], which was used to verify conserved OFP protein domains, remove OVATE domain-free proteins, and manually delete sequences without complete reading frames. All protein sequences containing the OFP conserved domain were searched. To avoid missing OFP family members, we constructed a new OFP data set for wheat using a high-quality protein set (E value < 1 x 10^-20) for multiple sequence alignment with Clustal (Clustal 2.1; https://www.ebi.ac.uk). Based on the aligned sequences, we constructed a new HMM with HMMER (HMMER 3.1; http://hmm.org/) and used it as a query (E value < 0.01) to search against the Triticum aestivum genome sequencing data. Genes encoding proteins with OFP domains were identified as OFP gene candidates. The ExPaSy online program (http://web.expasy.org/translate/) was used to analyze the physicochemical properties of the TaOFP proteins. The WoLF PSORT online program (https://wolfpsort.hgc.jp/) was used to predict the subcellular localizations of these proteins [21].

2.2. Sequence Analysis and Structural Characterization of TaOFPs. Bioinformatic analysis of TaOFP gene sequences and calculation of the coding sequence (CDS) length, molecular weight (MW), isoelectric point (pI), and open reading frame (ORF) length of each TaOFP were performed using the Compute pl/MW tool at the Expert Protein Analysis System (ExPaSy) site (http://au.expasy.org/tools/pi-tool.html). Gene structures and conserved motifs were analyzed using GSDS (Gene Structure Display Server) and MEME (v.12.0; http://meme-suite.org/tools/meme), respectively. TBools (TBtools-v0.53.jar) was used to analyze and visualize the structures (exons/introns) and conserved motifs of the OFP genes. 2.3. Phylogenetic analysis and classification of the TaOFP gene family. MEGA 7.0 was used to construct an individual phylogenetic tree of the TaOFP gene family [22]. The AtOFP, OsOFP, and TaOFP genes were divided into subfamilies based on their clustering patterns, and a comprehensive phylogenetic tree was constructed based on these genes using MEGA 7.0. All sequences were initially aligned using ClustalW (http://www.ebi.ac.uk/clustalw) with default parameters [22]. Both phylogenetic trees were generated as described by Wang.
et al. [2] with MEGA 7.0 using the Neighbor-Joining method [23] with 1,000 bootstrap replicates [24].

2.3. Chromosomal Location Analysis and Duplication of TaOFP Genes. Each TaOFP gene was mapped onto the 21 wheat chromosomes in the IWGSC RefSeq v1.0 (cv. Chinese Spring) reference genome using Triticaceae Multi-omics Center (http://202.194.139.32/). Ensembl (http://plants.ensembl.org/index.html) was used to extract information about chromosome length, and a physical map was drawn with MG2C (v2.1; http://mg2c.iasm.in/mg2c-v2.1/). Segmental duplications and tandem duplications in the three subgenomes were separately identified using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) [25]. The following criteria were used to identify the tandem duplication events of the TaOFPs: (1) alignment length was >80% of the complete gene sequence, (2) the aligned region had an identity of >80%, and (3) no genes were inserted between these genes. Segmental duplication was defined as follows: (1) alignment length was >1 kb and (2) the aligned region had an identity of >90% [26–29]. Ka/Ks ratios were calculated using Ka/Ks Calculator 2.0 (https://sourceforge.net/projects/kakscalculator2/) [30].

2.4. Analysis of Cis-Acting Elements in the TaOFP Gene Promoters. The cis-acting elements in the TaOFP promoters were analyzed using the TBtools software (v0.6669; http://cj-chen.github.io/tbtools/), which retrieved the upstream region (2 kb) of the CDS of each TaOFP from the wheat genome sequence and converted it into FASTA file format. The sequence was then submitted to PlantCARE (http://bio-informatics.psb.ugent.be/webtools/plantcare) [31] in batches. The results were filtered to retain the response-related cis-acting elements, including drought-responsive, auxin-responsive, jasmonate-responsive, abscisic acid-responsive, and GA-responsive elements. All related cis-elements were visualized using TBtools.

2.5. Expression Analysis of OFP Family Genes in Wheat. To further analyze the expression patterns of the OFP family genes in wheat, the CDS of each gene was submitted to the Wheat Expression Browser website to obtain expression data for different tissues of Chinese Spring wheat, and the expression patterns of OFP family members in wheat were systematically analyzed [32]. The OFP gene expression data were obtained from transcriptome data for Chinese Spring wheat seedlings under drought and heat stress conditions [33]. Chinese Spring wheat transcriptome data under GA, jasmonic acid, abscisic acid, salicylic acid, and cytokinin treatment were used to obtain OFP gene expression data from the Introduction to the Triticaceae Multi-omics Center database [34]. The expression data were used to construct a heat map using TBtool [35].

2.6. Plant Transformation. The wheat (T. aestivum) cultivar Jingdong18 was used as the WT in this study. The full-length cDNA of TaOFP29a-A was cloned into pCUB to generate pCUB-TaOFP29a-A, which was recombined with the Ubi-TaOFP29a-A-3xFLAG-GFP vector to generate the overexpression construct. The pUbi::TaOFP29a-A vector was transformed into Jingdong18 via Agrobacterium-mediated transformation as described previously [36]. Wheat seeds were germinated in plates containing water. Plants were grown in a greenhouse at a constant temperature (20–25°C) under long-day conditions (16 h/8 h light/dark cycle). The sequences of the primer pairs used for vector construction are listed in Supplementary File 1.

2.7. Drought Stress Assay. Transgenic and wild type (WT) plants (N = 30) were grown for 4 weeks and then deprived of water for 2 weeks. To investigate drought tolerance, the plants were rewatered for one week, phenotypic changes were photographed, and the survival rate was calculated. There were three experimental replicates. In addition, three-day-old wheat seedlings were treated with 10% polyethylene glycol (PEG) 8000, and the expression levels of the TaOFPs were detected in whole plants at different time points [37].

2.8. RNA Extraction and qPCR Analysis. Total RNA was isolated from the samples using TRIzol reagent (Invitrogen). For qPCR, first-strand cDNA was synthesized using a Primer Script RT reagent Kit with gDNA Eraser (TaKaRa). The qPCR was conducted in a CFX™ real-time PCR detection system (Bio-Rad). The expression level of the wheat Tubulin gene was used as an endogenous control to normalize gene expression levels. Three biological replicates were performed for each treatment, and three technical replicates were performed for each biological replicate. Relative gene expression levels were calculated using the 2^ΔΔCT method [38]. The sequences of the primer pairs used for qPCR are listed in Supplementary File 1.

3. Results

3.1. Identification of OFP Family Genes in Wheat. We retrieved OFP transcription factor sequences from the wheat genome based on the HMM profile (PF04844) of the OFP family. Initially, 100 nonredundant putative OFP genes were identified. After removing the redundant forms of the genes, 100 genes were identified by HMM analysis (Supplementary File 2). The TaOFP genes were named sequentially from TaOFP1-A to TaOFP31-D based on homologous relationships between the wheat and rice genes. We analyzed the physicochemical properties of the TaOFP genes using the ExPASy online program. Detailed information about the OFP family genes in wheat, including the name and identifier (ID), number of amino acids in the encoded protein, pi, molecular weight (MW), ORF size, chromosome position, rice orthologs, and subcellular localization is provided in Supplementary File 3.

The amino acid composition and physicochemical properties differ among OFP family proteins, and the number of amino acids per protein varies greatly among subfamilies. The predicted sequences of the proteins encoded by the TaOFP genes ranged from 108 (TaOFP24-D.2) to 400 (TaOFP30b-U) amino acids in length, with an average length of 276 amino acids. The predicted MW ranged from 11,662 Da to 41,867.5 Da, with an average MW of 29,558.425 Da. The pI ranged from 4.08 (TaOFP23-D) to 12.12 (TaOFP23-D), with an average of 8.8. The subcellular...
localizations of all the wheat OFPs could be predicted using the online software WoLF PSORT. Among these proteins, 43 were localized to the nucleus, 38 to the chloroplast, 7 to the cytosol, 6 to mitochondria, and 6 to the extracellular space, implying that wheat OFPs are involved in multiple biological processes.

3.2. Phylogenetic Analysis and Classification of the OFP Gene Family. To investigate the phylogenetic relationships among rice, Arabidopsis, and wheat OFP genes, we constructed a phylogenetic tree based on the sequences of their OVATE domains (Figure 1). The 18 OFP family proteins from Arabidopsis, 31 from rice, and 100 from wheat were classified into four subgroups (I–IV) based on their topological structures (Figure 2). However, most TaOFP and OsOFP proteins were clustered into distinct species-specific clades. Only one group of orthologs was identified among wheat, rice, and Arabidopsis: TaOFP2a, TaOFP2b, OsOFP2, and AtOFP14. These results suggest that the main characteristics of OFP proteins in rice, wheat, and Arabidopsis formed prior to the divergence of monocots and dicots and that they subsequently evolved separately in a species-specific manner.

Members within a specific subgroup exhibited a high degree of amino acid sequence identity (Supplementary File 4). In some subfamilies, OFP genes appeared to have expanded in wheat and rice compared to Arabidopsis. For example, subfamily II contains 42 TaOFPs and 14 OsOFPs and only three orthologs (AtOFP7, AtOFP8, AtOFP9) in Arabidopsis. Thus, the OFP genes may have evolved to have different functions in monocotyledons and dicotyledons. The various regions of the OFP proteins may be important for their functions.
Figure 2: Gene structures and conserved protein motifs of TaOFPs. Phylogenetic relationships, gene structures, and the architecture of conserved protein motifs in OFP genes from wheat. (a) Phylogenetic tree constructed based on the full-length sequences of wheat OFP proteins using the MEGA 7.0 software. (b) The motif composition of wheat OFP proteins. The motifs are displayed as different colored boxes. The sequence information for each motif is provided in Supplementary File 4. (c) Exon–intron structures of TaOFP genes. Blue boxes indicate untranslated 5′ and 3′ regions, yellow boxes indicate exons, and black lines indicate introns.
3.3. Genomic Organization and Duplication of Wheat OFP Genes. Physical mapping indicated that the three homologous chromosomes from the wheat A/B/D subgenome contain 100 OFP genes (Figure 3), suggesting that the OFP genes in wheat were retained on homologous chromosomes during the chromosome doubling process (Supplementary File 5). We detected no clear preference for subgenomes or gene loss. Wheat OFP genes are evenly distributed on the three homologous chromosomes (A/B/D) but are unevenly distributed on different chromosomes, with a maximum of seven genes on chromosomes 2, 3, and 4 and only one on chromosome 7A/B/D, regardless of chromosome length (Figure 3). The A homeolog of TaOFP-10 was translocated from chromosome 5AL to 4AL, which is consistent with previous findings [39, 40]. The A homeologs of TaOFP12 and TaOFP30a were translocated from chromosome 4AS to 4AL and 4AL to 4AS, respectively, which was confirmed by examining the 500-Mb up- and downstream regions of these two genes.

On chromosome 2A/B/D, TaOFP-17 originated from tandem duplication events. Tandem and segmental duplication are key factors in the generation of new gene family members during evolution. Compared to the genomes of other grasses, inter- and intrachromosomal duplications in wheat are more commonly detected through interspecific whole-genome analysis [41]. Thus, we investigated the segmental and tandem duplication events in the OFP gene family in wheat.

We identified three pairs of genes among the 100 TaOFPs as tandem duplications and one pair of genes that might have arisen from segmental duplication events, as revealed using Protein Alignment Matrix (Supplementary File 6). Roughly one-to-one correspondences of these tandem duplication and segmental duplication events were observed in the wheat genome.

Figure 3: Physical locations of TaOFP genes on the Triticum aestivum chromosomes. Red dots indicate the positions of TaOFP genes.
A, B, and D subgenomes; thus, tandem duplication or segmental duplication events often occurred in the same locations in the three wheat subgenomes.

The rate of nonsynonymous (Ka) and synonymous (Ks) substitutions provides a basis for evaluating the positive selection pressure of duplication events, where Ka/Ks = 1 indicates neutral selection, Ka/Ks < 1 indicates purifying selection, and Ka/Ks > 1 indicates positive selection. We used Ka/Ks Calculator 2.0 to calculate the Ka/Ks ratios of duplicated TaOFPs. The Ka/Ks ratios of three pairs (TaOFP17-A.1/TaOFP17-A.2, TaOFP17-B.1/TaOFP17-B.2, and TaOFP17-D.1/TaOFP17-D.2) of tandemly duplicated genes were 0.287, 0.306, and 0.379, respectively (Table 1), and the Ka/Ks ratio of TaOFP24-D.2/TaOFP24-D.3, which were derived from segmental duplication, was 90.65. Therefore, duplication events played a pivotal role in the evolution of TaOFPs, and the Ka/Ks average is far less than 1, indicating that OFP gene family members have undergone purifying selection.

### Table 1: Ka/Ks ratios of tandemly duplicated TaOFPs

| Gene name       | Phylogenetic cluster | Chr. NO. | Gene name       | Phylogenetic cluster | Chr | Ka   | Ks   | Ka/Ks | Percent identity (%) |
|-----------------|----------------------|----------|-----------------|----------------------|-----|------|------|-------|-----------------------|
| TaOFP17-A.1     | III                  | 2A       | TaOFP17-A.2     | III                  | 2A  | 0.059244672 | 0.206248703 | 0.287249 | 86.34                   |
| TaOFP17-B.2     | III                  | 2B       | TaOFP17-B.1     | III                  | 2B  | 0.053410651 | 0.174226837 | 0.306558 | 84.96                   |
| TaOFP17-D.2     | III                  | 2D       | TaOFP17-D.1     | III                  | 2D  | 0.069567642 | 0.183393949 | 0.379334 | 84.78                   |

The rate of nonsynonymous (Ka) and synonymous (Ks) substitutions provides a basis for evaluating the positive selection pressure of duplication events, where Ka/Ks = 1 indicates neutral selection, Ka/Ks < 1 indicates purifying selection, and Ka/Ks > 1 indicates positive selection. We used Ka/Ks Calculator 2.0 to calculate the Ka/Ks ratios of duplicated TaOFPs. The Ka/Ks ratios of three pairs (TaOFP17-A.1/TaOFP17-A.2, TaOFP17-B.1/TaOFP17-B.2, and TaOFP17-D.1/TaOFP17-D.2) of tandemly duplicated genes were 0.287, 0.306, and 0.379, respectively (Table 1), and the Ka/Ks ratio of TaOFP24-D.2/TaOFP24-D.3, which were derived from segmental duplication, was 90.65. Therefore, duplication events played a pivotal role in the evolution of TaOFPs, and theKa/Ks average is far less than 1, indicating that OFP gene family members have undergone purifying selection.

#### 3.4. Analysis of Wheat OFP Gene Structures and Conserved Protein Motifs

To study the structures of the TaOFP genes, we analyzed their DNA sequences and determined their intron and exon compositions. We used GSDS 2.0 to map the intron–exon structures of the wheat OFP family. Among the 100 TaOFP genes, 96 genes (96%) contain no introns, and TaOFP14-A, TaOFP14-B, TaOFP14-D, and TaOFP30a-A each contain only one intron (Figure 2(c)). We used MEME to identify the conserved domains of the TaOFP gene family members. Gene structure analysis showed that the lengths of the TaOFP gene family members were quite similar. The structures of OFP family genes were conserved among various subfamilies, and the number and locations of exons were similar among these genes, suggesting that they have similar functions.

All TaOFP genes contain two conserved elements (motif 1 and motif 2) constituting the OVATE domain (Figure 2(b)). Some motifs could be used as markers to identify different subfamilies. For example, motif 8 is only present in subfamily III. These results indicate that during the evolution of the TaOFP gene family, internal differentiation might have led to functional differentiation.

#### 3.5. Identification of Cis-Elements in the Promoters of TaOFP Genes

We identified putative cis-acting regulatory DNA elements in the promoter sequences of TaOFP genes (2,000 bp upstream of the translation start site) based on the Ensembl Plants database (Supplementary File 7). We analyzed the environmental stress- and hormone-responsive elements further (Figure 4). Almost all of these elements are distributed randomly in the promoter sequences of TaOFP genes (Supplementary File 8). Fifty-three genes contain drought-responsive elements (MBS) in their promoters, indicating that the MYB-binding site is involved in drought responsiveness. Forty genes contain temperature-induced response elements known as long terminal repeats (LTRs).

Among the hormone-related cis-acting elements, the MeJA-responsive elements CGTCA and TGACG [42] were the most frequently identified, appearing in 88 TaOFP gene promoters. ABA-responsive elements (ABRE) [43] were present in 94 TaOFP genes. GA-responsive elements, including the GARE-motif and P-box, were present in 24 and 39 TaOFP genes, respectively. These results indicate that the responses of TaOFP genes to environmental factors are quite different, suggesting that a complex mechanism controls the expression of these genes in wheat.

However, some cis-elements are distributed in clusters in certain promoters. For example, the MBS-motif, TGACG-motif, motif I, and G-box were detected only in the promoter of TaOFP4-A, implying that these elements play essential roles in regulating TaOFP4-A expression. The number and distribution patterns of the cis-elements also varied greatly among the promoters of homologous genes assigned the same number, even though their encoded proteins share extremely similar amino acid sequences and domain compositions (Figure 4). For TaOFP-8, 15 and 17 TATA-box cis-elements were identified in the promoters of its B and D homologs, respectively, but none were present in the A homolog. These results suggest that the expression of homologs might be regulated by different mechanisms, pointing to their functional divergence during the polyploidization of the wheat genome.

#### 3.6. Stage- and Tissue-Specific Expression Patterns of OFP Family Genes in Wheat

The TaOFP genes exhibited various tissue-specific expression patterns, as determined using both wheat transcriptome and qPCR data (Figure 5). Little or no expression of these genes was detected in leaves, whereas they were expressed at high levels in spikes and roots and at moderate levels in grains and stems. TaOFP1-B, TaOFP5-A, TaOFP8-B, TaOFP14-A/B/D, TaOFP16-B, TaOFP23-A, TaOFP29a-A, TaOFP29b-U, and TaOFP30b-B were highly expressed in spikes at the second detectable node stage. TaOFP2b-B/D, TaOFP6-B, TaOFP7-B, TaOFP11-A/B, TaOFP13-B/D, TaOFP25-A/D, and TaOFP30a-A/B/D were expressed in spikes at the flag leaf stage.

TaOFP2a-B and TaOFP26-A/D were strongly expressed in spikes at anthesis. TaOFP1-B, TaOFP3-B, TaOFP5-A,
TaOFP9-D, TaOFP13-A/B/D, TaOFP15-B, TaOFP21-A, and TaOFP29b-U were strongly expressed in stems at the heading stage, and TaOFP12-A and TaOFP27-A were significantly expressed in stems at anthesis. Conversely, TaOFP8-B, TaOFP10-D, TaOFP12-D, TaOFP16-B, and TaOFP25-A/D were expressed at the highest levels in grains on the second day after anthesis, whereas TaOFP17-D.1, TaOFP17-D.2, TaOFP24-A, TaOFP24-D.2, and TaOFP24-D.3 were highly

**Figure 4:** Distribution of major stress-related cis-elements in the promoters of TaOFP genes. Putative ABRE, MYB, LTR, MBS, P-box, TATA-box, TCA-element, GARE-motif, TGACG-motif, and CGTCA-motif are represented by different colors as indicated.
expressed in grains at 20 d after anthesis, suggesting that these three genes function in early grain development. TaOFP2b-D, TaOFP4-B, TaOFP10-B/D, TaOFP11-B, TaOFP12-D, TaOFP15-A/B/D, and TaOFP29a-A were expressed at high levels in roots at the three-leaf stage. The expression levels of TaOFP17-A.1/B.1, TaOFP17-A.2/B.2, and TaOFP22-A/D in roots gradually increased over time.

To validate the expression patterns of TaOFP genes in specific tissues based on the wheat transcriptome data, we examined the expression of TaOFPs in five different tissues (root, leaf, stem, spike, and grain) using qPCR. All TaOFP expression profiles are shown in Supplementary File 10, and the tissue-specific expression patterns of ten TaOFPs are shown in Figure 5(b). TaOFP24-D.2 was expressed at
the highest level in grains at 14 d after anthesis; TaOFP8-B, TaOFP9-A, TaOFP14-A, and TaOFP26-D were expressed at the highest levels in spikes at the flag leaf visible stage; TaOFP25-D was expressed at the highest level in grains at 14 d after anthesis; and TaOFP21-A and TaOFP22-D were expressed at the highest levels in leaves at the seedling stage. Furthermore, TaOFP19-A/B/D, TaOFP29a-A, and TaOFP3-B were expressed in roots at the flag leaf visible stage; TaOFP27-A was expressed in stems at the heading stage; and TaOFP13-A/B/D was mainly expressed in stems. The other TaOFP genes were expressed ubiquitously in all tissues or at very low levels. These results indicate that the expression pattern of each TaOFP gene is unique and displays strong spatiotemporal and tissue specificity.

3.7. Expression Analysis of TaOFPs under Stress Conditions. To further explore the expression characteristics of wheat TaOFP family genes under suboptimal conditions, we analyzed both wheat transcriptome and qPCR data (Figure 6). Many TaOFP genes were responsive to a number of environmental stresses (Figure 7). Different TaOFP genes were induced in response to different abiotic stresses such as heat and drought. Here, only genes with more than two-fold differences in transcript levels were
considered to be differentially expressed under various treatments. Whereas TaOFP10-D, TaOFP12-D, TaOFP13-D, TaOFP14-A, TaOFP19-B, and TaOFP29a-A were significantly upregulated in response to drought stress, all of the other OFP genes were either not expressed or downregulated after drought stress. The expression of TaOFP2a-B, TaOFP17-B.1/D.1, and TaOFP17-B.2/D.2 significantly increased in response to heat stress.

Figure 7: Expression of TaOFP gene family members in wheat in response to hormone treatment. DMSO: dimethyl sulfoxide (control); GA: gibberellin; JA: jasmonic acid; 6BA: 6-benzylaminopurine; ABA: abscisic acid; SA: salicylic acid.

BioMed Research International 11
To explore the potential functions of TaOFP genes that are specifically expressed in roots, we analyzed the effects of drought stress on the expression of these genes by qPCR to investigate their roles in environmental stress responses. Under PEG8000-induced osmotic stress conditions, TaOFP3-B and TaOFP12-D were upregulated at 6 h of drought stress, and the expression level of TaOFP3-B increased to sixfold that of untreated seedlings (Figure 6(b)). TaOFP29a-A expression increased during the first three hours of osmotic stress treatment; the greatest increase was detected at 12 h in response to treatment with 10% PEG8000. TaOFP9-D expression significantly increased within the first hour of osmotic stress treatment but then declined to control levels by the 24-h time point. TaOFP3-B, TaOFP9-D, TaOFP12-D, and TaOFP29a-A were upregulated under osmotic stress and clustered together with OsOFP8 in clade II, suggesting they might share the same functions; OsOFP8 was shown to positively regulate drought stress responses [17]. TaOFP19-D was downregulated at the beginning of osmotic stress treatment and returned to normal levels after 3 h to 24 h of treatment. We also examined the expression patterns of TaOFP genes that are expressed in specific tissues under drought stress, such as TaOFP15-D, which is specifically expressed in roots. TaOFP15-D was expressed at similar levels under drought stress vs. control treatment (Figure 6(b)). These results suggest that TaOFP genes may be involved in regulating a variety of stress responses and are preferentially expressed in specific tissues.

The expression levels of most TaOFP genes did not significantly change in response to GA treatment, but TaOFP6-D, 8-B, 10-B, 14-A/B/D, and 17-B.1 were upregulated by this treatment. TaOFP1-A, TaOFP6-D, TaOFP9-A/D, TaOFP10-B, TaOFP22-A/D, TaOFP23-B, TaOFP29b-A/B, TaOFP29a-D, TaOFP30a-D, and TaOFP31-D were significantly upregulated in response to salicylic acid treatment. TaOFP3-A/D, TaOFP4-A, TaOFP6-D, TaOFP10-A, TaOFP11-D, TaOFP12-D, TaOFP13-A, TaOFP14-A/B/D, TaOFP17-D, TaOFP20-A, TaOFP23-B/D, TaOFP24-A/D, TaOFP26-A/D, TaOFP27-A, TaOFP29b-A/U, TaOFP29a-A, TaOFP30b-A/U, and TaOFP31-D were upregulated in response to jasmonic acid treatment.

TaOFP3-A/B/D, TaOFP8-D, TaOFP11-A/B/D, TaOFP13-D, TaOFP14-B, TaOFP15-A/D, TaOFP16-A/B/D, TaOFP17-D, TaOFP19-A/B, TaOFP22-B, TaOFP23-A/D, TaOFP25-B, TaOFP29b-U, and TaOFP31-A were significantly upregulated following treatment with GA, JA, abscisic acid (ABA), 6BA, or SA. TaOFP2a-A, TaOFP12-A/D, and TaOFP17-A/J were upregulated after 3 h of ABA treatment. Although not all OFP family genes were examined, these results suggest that each OFP family member in wheat has a unique inducible expression profile and thus plays specific roles in plant stress responses.

3.8. Overexpressing TaOFP29a-A Improves Drought Tolerance in Wheat. We analyzed the role of TaOFP29a-A in the drought stress response in wheat based on the results of qPCR analysis (Figure 6). To investigate how increased TaOFP29a-A expression enhances drought tolerance in wheat, we generated transgenic lines expressing TaOFP29a-A cDNA from wheat cultivar Jingdong18 under the control of the constitutive ZmUbiquitin1 (Ubi) promoter. We analyzed three independent pUbi:TaOFP29a-A transgenic lines in the T2 generation. The transgenic lines with enhanced TaOFP29a-A gene expression exhibited two- to three-fold higher expression levels of this gene relative to the control (Figure 8(c)). Furthermore, the transgenic plants exhibited higher survival rates than WT plants under drought stress, and their growth recovered to normal levels after rewatering (Figures 8(a) and 8(d)). These results indicate that the transgenic plants had greater drought tolerance than WT plants.

To investigate the potential molecular mechanisms underlying the improved drought tolerance of TaOFP29a-A-overexpression lines, we examined 3-day-old transgenic and WT seedlings after 3 days of PEG8000-induced osmotic stress conditions. Compared to WT plants, the transgenic plants had longer roots (Figure 8(b)). The primary root length and dry root biomass of the transgenic plants were markedly greater than those of the WT under water deficit conditions (Figure 8(e)). We examined the expression levels of three auxin transporter genes, TaARF12, TaRAA1, and TaRMC, which are homologs of OsARF12, OsRAA1, and OsRMC, respectively [44–46]; these genes modulate root growth in rice. TaARF12 was insensitive to drought stress, whereas TaRAA1 and TaRMC were significantly induced by this treatment. Thus, these genes might have contributed to the enhanced development of the root systems of the transgenic plants (Figure 8(f)). These results suggest that the ectopic expression of TaOFP29a-A in plants might affect auxin transport and auxin-related plant development.

Finally, we performed qPCR analysis to examine the expression levels of several drought-responsive marker gene in transgenic and WT plants, including TaP5CSI [44], TaNAC2 [47], TaDREB1A [48], TaMYB2A/B/D [49], TaDOF [50], and TaZAT12 [37, 51]. Under normal conditions, there were no significant differences in the transcript levels of TaDOF, TaMYB2A, or TaZAT12 between TaOFP29a-A-overexpression and WT plants (data not shown). However, the expression levels of TaDREB1A, TaNAC2, and TaP5CSI were significantly higher in TaOFP29a-A-overexpression plants than in the WT (Figure 8(g)).

4. Discussion

4.1. Wheat OFPs Are Diverse, with a Complex Evolutionary History. Common wheat is a hexaploid species with three closely related subgenomes (A, B, and D). In this study, we identified 100 OFP family genes in wheat encoding 34 TaOFPs, which we named based on sequence analysis and domain composition (File 3). Phylogenetic analysis indicated that homologous genes from different subgenomes encoding a single OFP protein always clustered together, as expected (Figure 1). Not every OFP protein is encoded by three homologous genes in subgenomes A, B, and D. For example, TaOFP21 includes two homologous genes (TaOFP21-A and TaOFP21-D), whereas TaOFP10 lacks homologous genes in the B and D subgenomes (Figure 3). This finding suggests that some homologs might have been lost during long-term evolution and natural selection. Indeed, many studies have
Figure 8: Drought stress responses of transgenic wheat plants overexpressing TaOFP29a-A. (a) Phenotypes of transgenic plants before and after osmotic stress treatment. (b) Wheat seeds were germinated in plates containing water for 3 days and transferred to water containing various concentrations of PEG8000 (0 and 10%) for 3 days. (c) The expression levels of TaOFP29a-A in transgenic and wild-type plants under normal conditions. (d) Survival rates of drought-stressed wheat seedlings. Each column represents means (±SD) of three independent experiments. (e) Root biomass in 3-week-old plants. Values are means (±SD) of three biological replicates, with each replicate containing 6 seedlings. (f) The expression levels of root-related genes are altered in transgenic plants under drought stress treatment vs. the control. (g) The expression levels of drought-responsive genes are altered in transgenic plants under drought stress treatment vs. the control. Each data point is the mean (±SE) of three experiments (30 seedlings per experiment). Significant differences from the WT are denoted by two asterisks corresponding to $P < 0.01$ by Student’s t-tests.
shown that in allopolyploid species, genetic variations including gene rearrangements, structural variation, DNA sequence loss or amplification, and transposon activation occurred frequently during genome polyploidization [52]. Alternatively, perhaps there was incomplete coverage in the wheat reference genome.

Gene duplication, fusion, and/or exon shuffling have commonly occurred in eukaryotes, leading to the biological diversity and functional divergence of certain gene families during plant evolution [53–55]. These processes were also involved in the expansion of the OFP gene family in plants. In the current study, we identified one TaOFP gene pair located within segmental duplication blocks and three pairs (Table 1, Table 2) that were tandemly duplicated.

The typical OFPs share high degrees of sequence similarity in the conserved domains but obvious diversity in terms of gene structure and protein size (file 2, Figure 2). TaOFPs also show very high degrees of sequence similarity in the conserved domains compared to other TaOFPs; although, there are still some differences in the same conserved amino acids (Figure 2), implying that they share a conserved evolutionary relationship. Interestingly, both TaOFP24-D.2 and TaOFP24-D.3 harbor partial OVATE domains (Figure 2). Phylogenetic analysis indicated that TaOFP24-D.2 and TaOFP24-D.3 are closely related to TaOFP24-D.1, suggesting they might have been derived from this typical TaOFP and likely experienced DNA sequence changes during gene duplication. Together, these findings suggest that wheat OFPs have a complicated evolutionary history involving gene expansion and functional divergence.

### 4.2. Functional Prediction of Wheat OFP Genes

The OVATE gene was first identified as a major QTL controlling pear-shaped fruit in tomato [1, 33]. Subsequently, studies in Arabidopsis and rice indicated that OFPs control multiple aspects of plant growth and development [3, 5, 13, 14]. The wheat genome contains more OFPs than the Arabidopsis and rice genomes, but the roles of OFPs in growth and development remain in wheat remain unclear. AtOFP1 functions in GA signaling by repressing the expression of GA20ox1, a gene encoding a key enzyme in GA biosynthesis, whereas OsOFP8 is involved in the BR signaling pathway and shows normal responses to GA treatment [17], highlighting the functional diversity of these genes in Arabidopsis and rice. OsOFP19 modulates plant architecture by integrating cell division patterns and BR signaling [37]. Phylogenetic analysis of OFP genes from rice, Arabidopsis, and wheat showed that TaOFP19 is a homolog of AtOFP1. Similar to its ortholog OsOFP19, which is expressed in various tissues and in response to GA treatment, TaOFP19 is specifically expressed in roots and in response to GA treatment. These findings suggest that this gene might share a similar function with OsOFP19 in the BR or GA signaling pathway.

OsOFP8 regulates leaf angle via the BR signaling pathway [8]. Overexpression of OsOFP8 improved drought resistance in rice [17]. The function of OFP8 is conserved between rice and wheat, as TaOFP8 positively regulates drought stress responses in wheat. Interestingly, TaOFP8-B was downregulated under drought stress (Supplementary File 11), whereas TaOFP8-A and TaOFP8-D transcripts were not detected; thus, TaOFP8 plays a different role from its ortholog OsOFP8 during drought stress. We identified several genes (including TaOFP8, TaOFP20, TaOFP31, TaOFP29a, and TaOFP29b) of OFP subgroup II that are closely related to OsOFP8 (Figure 1). Moreover, TaOFP29a-A was highly expressed in roots, and TaOFP29a-A expression significantly increased under drought stress (Figure 5), whereas TaOFP29a-B and TaOFP29a-D transcripts were not detected under normal conditions. TaOFP20, TaOFP31, and TaOFP29b were not induced by drought stress treatment (Supplementary File 11). These results suggest that there are some differences between rice and wheat with respect to which OFP subfamily genes are induced by a particular stress.

### 4.3. Root-Specific Expression of TaOFP29a-A Improves Drought Stress Tolerance in Wheat

We observed that TaOFP29a-A was more highly expressed in roots than in other tissues, suggesting that TaOFP29a-A might function in root development. In this study, we identified TaOFP29a-A and established that it is specifically expressed in roots and upregulated by drought stress. We generated TaOFP29a-A overexpression lines to investigate the role of this gene in drought stress tolerance. The TaOFP29a-A overexpression lines showed greater tolerance to drought stress than the corresponding nontransgenic plants, including longer roots during germination and greater dry root weight in seedlings, suggesting that the role of TaOFP29a-A in regulating root growth might depend on the drought stress pathway.

Optimizing root system architecture (RSA) can overcome yield limitations in crop plants caused by water stress [45]. Many RSA-related genes have recently been identified [56]. In rice, knockout of OsARF12 led to decreased primary root length [45]. In OsRMC RNAi transgenic rice, the primary roots were shorter, and the number of adventitious roots was higher but the number of lateral roots was lower compared to the WT, suggesting that OsRMC might be involved in RSRPR10-mediated JA signaling [57–59]. Overexpression of OsRAA1 resulted in reduced primary root growth and an increased number of adventitious roots [46, 60]. To explore the mechanism underlying the role of TaOFP29a-A in drought stress, we measured TaARF12, TaRAA1, and TaRMC expression levels in transgenic and

---

**Table 2: Ka/Ks ratios of segmentally duplicated TaOFPs**

| Gene name | Phylogenetic cluster | Chr. NO. | Gene name | Phylogenetic cluster | Chr | Ka | Ks | Ka/Ks | Percent identity (%) |
|-----------|---------------------|---------|-----------|---------------------|-----|----|----|-------|----------------------|
| TaOFP24-D.2 | I | 5D | TaOFP24-D.3 | I | 5D | 0.063574285 | 0.139652008 | 0.455234 | 90.65 |
WT seedlings under normal conditions or drought stress. The expression patterns of TaRAAl and TaRMC were consistent with that of TaOFP29a-A, whereas of TaDREB1A, TaNAC2, and TaP5CSI, like TaOFP29a-A, were upregulated in response to drought stress. These results suggest that TaOFP29a-A might function as a core regulator that activates downstream signaling to maintain drought stress. These results suggest that TaOFP29a-A functions via a complex mechanism that remains to be elucidated. These observations suggest that TaOFP29a-A functions via a complex mechanism that could potentially be used for genetic engineering to enhance drought tolerance in crops.

5. Conclusions

In this study, we comprehensively analyzed the OFP gene family in wheat, including their gene structures, evolution, and expression patterns. We also determined that overexpressing TaOFP29a-A improved drought tolerance in wheat. These results provide fundamental resources for both evolutionary and functional studies and suggest that TaOFP29a-A could be useful for engineering drought-tolerant plants.

Data Availability

All datasets presented in this study are included in the article/Supplementary Material.

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

Dezhou Wang and Zhichen Cao contributed equally to this work.

Acknowledgments

This work was financially supported by the Beijing Academy of Agriculture and Forestry Sciences (KJX201907-2), Beijing Postdoctoral Research Foundation (ZZZ2019-41), Beijing Academy of Agriculture and Forestry Sciences Postdoctoral Research Foundation (BAAFS2019-ZZ-005), and Foundation for Youths of BAAFS (QNJJ201805).

Supplementary Materials

Supplementary 1. Supplementary file 1: Primers used in this study.

Supplementary 2. Supplementary file 2: A complete list of wheat OFP gene sequences identified in the present study.

Supplementary 3. Supplementary file 3: Features of OFP genes identified in Triticum aestivum.

Supplementary 4. Supplementary file 4: Sequence logos for the conserved motifs of wheat OFP domain proteins.

Supplementary 5. Supplementary file 5: Characteristic features of 100 TaOFPs on the chromosome.

Supplementary 6. Supplementary file 6: Percent Identity Matrix of 100 TaOFPs Protein sequence.

Supplementary 7. Supplementary file 7: A complete list of TaOFP promoters sequences identified in the present study.

Supplementary 8. Supplementary file 8: Predicted cis-elements in TaOFP promoters.

Supplementary 9. Supplementary file 9: Transcriptome data of specific TaOFPs wheat tissue specific expression.

Supplementary 10. Supplementary file 10: The expression of TaOFPs in normal 1 and 2.

Supplementary 11. Supplementary file 11: The expression of OFP genes under osmotic stress treatment.

References

[1] J. Liu, J. Van Eck, B. Cong, and S. D. Tanksley, “A new class of regulatory genes underlying the cause of pear-shaped tomato fruit,” Proceedings of the National Academy of Sciences of the United States of America, vol. 99, no. 20, pp. 13302–13306, 2002.

[2] Y. Wang, Q. Wang, W. Hao, H. Sun, and L. Zhang, “Characterization of the OFP Gene Family and its Putative Involvement of Tuberous Root Shape in Radish,” International Journal of Molecular Sciences, vol. 21, no. 4, p. 1293, 2020.

[3] J. Hackbusch, K. Richter, J. Muller, F. Salamini, and J. F. Uhrig, “A central role of Arabidopsis thaliana ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins,” Proceedings of the National Academy of Sciences, vol. 102, no. 13, pp. 4908–4912, 2005.

[4] S. Wang, Y. Chang, J. Guo, and J.-G. Chen, “Arabidopsis Ovate Family Protein 1 is a transcriptional repressor that suppresses cell elongation,” The Plant Journal, vol. 50, no. 5, pp. 858–872, 2007.

[5] Y. Xiao, D. Liu, G. Zhang, H. Tong, and C. Chu, “Brassinosteroids Regulate OFP1, a DLT Interacting Protein, to Modulate Plant Architecture and Grain Morphology in Rice,” Frontiers in Plant Science, vol. 8, 2017.

[6] D. Liu, W. Sun, Y. Yuan et al., “Phylogenetic analyses provide the first insights into the evolution of OVATE family proteins in land plants,” Annals of Botany, vol. 113, no. 7, pp. 1219–1233, 2014.

[7] Y. Ma, C. Yang, Y. He, Z. Tian, and J. Li, “Rice OVATE family protein 6 regulates plant development and confers resistance to drought and cold stresses,” Journal of Experimental Botany, R. Sunkar, Ed., vol. 68, no. 17, pp. 4885–4898, 2017.

[8] Y. Hu, “The genes cloning and functional analysis of OsOFP transcription factor family in rice(Oryza sativa L.),” Jilin University, 2015.

[9] R. R. Xu, R. Li, X. F. Wang, and Y. J. Hao, “Identification and expression analysis under abiotic stresses of OFP gene family in apple,” Scientia Agricultura Sinica, vol. 51, no. 10, pp. 1948–1959, 2018.

[10] S. Wang, Y. Chang, J. Guo, Q. Zeng, B. E. Ellis, and J.-G. Chen, “Arabidopsis Ovate Family Proteins, a Novel Transcriptional Repressor Family, Control Multiple Aspects of Plant Growth.
and Development,” *PLoS One*, V. N. Uversky, Ed., vol. 6, no. 8, p. e23896, 2011.

[11] Y. Pan, Y. Wang, C. McGregor et al., "Genetic architecture of fruit size and shape variation in cucurbits: a comparative perspective,” *Theoretical and Applied Genetics*, vol. 133, no. 1, 2020.

[12] S. Wu, B. Zhang, N. Keyhaninejad et al., "A common genetic mechanism underlies morphological diversity in fruits and other plant organs," *Nature Communications*, vol. 9, no. 1, p. 4734, 2018.

[13] E. Li, S. Wang, Y. Liu, J.-G. Chen, and C. J. Douglas, "Ovate family protein4 (OPF4) interaction with KNA17 regulates secondary cell wall formation in *Arabidopsis thaliana*," *The Plant Journal*, vol. 67, no. 2, pp. 328–341, 2011.

[14] G. C. Pagnussat, H.-J. Yu, and V. Sundaresan, "Cell-fate switch of synergid to egg cell in *Arabidopsis costre* mutant embryo sacs arises from misexpression of the BEL1-like homeodomain gene BLH1," *The Plant Cell*, vol. 19, no. 11, pp. 3578–3592, 2007.

[15] S. Zhou, Z. Hu, F. Li et al., "Overexpression of SIOFP2 affects floral organ and pollen development,” *Horticulture Research*, vol. 6, no. 1, p. 125, 2019.

[16] A. J. Schmitz, K. Begcy, G. Sarath, and H. Walia, "Rice Ovate Family Protein 2 (OPF2) alters hormonal homeostasis and vasculature development," *Plant Science*, vol. 241, pp. 177–188, 2015.

[17] C. Yang, W. Shen, Y. He, Z. Tian, and J. Li, "Ovate family protein 8 positively mediates brassinosteroid signaling through interacting with the GSK3-like kinase in rice," *PLoS Genetics*, S. Hake, Ed., vol. 12, no. 6, article e1006118, 2016.

[18] M. Spannagl, M. M. Martin, M. Pfeifer, T. Nussbaumer, and K. F. X. Mayer, "Analysing complex Triticaceae genomes—concepts and strategies,” *Plant Methods*, vol. 9, no. 1, p. 35, 2013.

[19] R. D. Finn, "Pfam: the protein families database,” *Nucleic Acids Research*, vol. 42, no. D1, pp. D222–D230, 2005.

[20] A. Marchler-Bauer, Y. Bo, L. Han et al., "CDD/SPARCLE: functional classification of proteins via subfamily domain architectures,” *Nucleic Acids Research*, vol. 45, no. D1, pp. D200–D203, 2017.

[21] C.-S. Yu, C.-J. Lin, and J.-K. Hwang, "Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions,” *Protein Science*, vol. 13, no. 5, pp. 1402–1406, 2004.

[22] S. Kumar, G. Stecher, and K. Tamura, "MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets,” *Molecular Biology and Evolution*, vol. 33, no. 7, pp. 1870–1874, 2016.

[23] J. Felsenstein, "Confidence limits on phylogenies: an approach using the bootstrap,” *Evolution*, vol. 39, no. 4, pp. 783–791, 1985.

[24] Z. Gu, A. Cavalcanti, F.-C. Chen, P. Bouman, and W.-H. Li, "Extent of gene duplication in the genomes of Drosophila, nematode, and yeast,” *Molecular Biology*, vol. 19, no. 3, pp. 256–262, 2002.

[25] F. Madeira, Y. m. Park, J. Lee et al., "The EMBL-EBI search and sequence analysis tools APIs in 2019,” *Nucleic Acids Research*, vol. 47, no. W1, pp. W636–W641, 2019.

[26] Q. Zhao, Z. Zhu, M. Kasahara, S. Morishita, and Z. Zhang, "Segmental duplications in the silkworm genome,” *BMC Genomics*, vol. 14, no. 1, p. 521, 2013.

[27] X. Kong, L. Lv, S. Jiang et al., "Genome-wide identification and expression analysis of calcium-dependent protein kinase in maize,” *BMC Genomics*, vol. 14, no. 1, p. 433, 2013.

[28] C. Meng, B. Kuster, A. C. Culhane, and A. Gholami, "A multivariate approach to the integration of multi-omics datasets,” *BMC Bioinformatics*, vol. 15, no. 1, p. 162, 2014.

[29] M. Lynch and J. S. Conery, "The Evolutionary Fate and Consequences of Duplicate Genes,” *Science*, vol. 290, no. 5494, pp. 1151–1155, 2000.

[30] D. Wang, Y. Zhang, Z. Zhang, J. Zhu, and J. Yu, "KaKs-Calculator 2.0: A Toolkit Incorporating Gamma-Series Methods and Sliding Window Strategies,” vol. 8, no. 1, pp. 77–80.

[31] M. Lescot, P. Déhais, G. Thijs et al., "PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences,” *Nucleic Acids Research*, vol. 30, no. 1, pp. 325–327, 2002.

[32] F. Choulet, A. Alberti, S. Theil et al., "Structural and functional partitioning of bread wheat chromosome 3B,” *Science*, vol. 345, no. 6194, article 1249721, 2014.

[33] Z. Liu, M. Xiu, J. Qin et al., "Temporal transcriptome profiling reveals expression partitioning of homeologous genes contributing to heat and drought acclimation in wheat (*Triticum aestivum* L.)," *BMC Plant Biology*, vol. 15, no. 1, p. 152, 2015.

[34] J. Liu, L. Huang, C. Wang et al., "Genome-wide association study reveals novel genomic regions associated with high grain protein content in wheat lines derived from wild emmer wheat,” *Frontiers in Plant Science*, vol. 10, p. 464, 2019.

[35] C. Chen, H. Chen, Y. Zhang et al., "TBoots, a toolkit for biologists integrating various HTS-data handling tools with a user-friendly interface,” *BioRxiv*, vol. 289660, 2018.

[36] K. Wang, H. Liu, L. Du, and X. Ye, "Generation of marker-free transgenic hexaploid wheat via an agrobacterium-mediated co-transformation strategy in commercial Chinese wheat varieties,” *Plant Biotechnology Journal*, vol. 15, no. 5, pp. 614–623, 2017.

[37] A. Sallam, A. M. Alqudah, M. F. A. Dawood, P. S. Baenziger, and A. Börner, "Drought Stress Tolerance in Wheat and Barley: Advances in Physiology, Breeding and Genetics Research,” *International Journal of Molecular Sciences*, vol. 20, no. 13, p. 3137, 2019.

[38] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method,” *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[39] K. M. Devos, G. Moore, and M. D. Gale, "Conservation of marker synteny during evolution,” *Euphytica*, vol. 85, no. 1–3, pp. 367–372, 1995.

[40] T. G. Lee, Y. J. Lee, and Y. W. Seo, "Expression analysis of individual homoeologous wheat genome- and rye genome-specific transcripts in a 2B(‘Delta Delta C(T)) Method,” *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[41] International Wheat Genomic Sequencing Consortium, "A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome,” *Science*, vol. 345, no. 6194, article 1251788, 2014.

[42] J. Rouster, R. Leah, J. Mundy, and V. Cameron-Mills, "Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain,” *The Plant Journal*, vol. 11, no. 3, pp. 513–523, 1997.

[43] Q. Shen and T.-H. D. Ho, "Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-
responsive complexes each containing a G-box and a novel cis-acting element,” *Plant Cell*, vol. 7, no. 3, pp. 295–307, 1995.

[44] K. Dudziak, M. Zapalska, A. Börner, H. Szczepan, K. Kowalczuk, and M. Nowak, “Analysis of wheat gene expression related to the oxidative stress response and signal transduction under short-term osmotic stress,” *Scientific Reports*, vol. 9, no. 1, p. 2743, 2019.

[45] Y. H. Qi, S. K. Wang, C. J. Shen et al., “OsARF12, a transcription activator on auxin response gene, regulates root elongation and affects iron accumulation in rice (Oryza sativa),” *New Phytologist*, vol. 193, no. 1, pp. 109–120, 2012.

[46] L. Ge, H. Chen, J.-F. Jiang et al., “Overexpression of OsRAA1 causes pleiotropic phenotypes in transgenic rice plants, including altered leaf, flower, and root development and root response to gravity,” *Plant Physiology*, vol. 135, no. 3, pp. 1502–1513, 2004.

[47] M. Nazari, K. J. Goharrizi, S. S. Moosavi, and M. Maleki, “Expression changes in the TaNAC2 and TaNAC69-1 transcription factors in drought stress tolerant and susceptible accessions of *Triticum aestivum*,” *Plant Genetic Resources*, vol. 17, no. 6, pp. 471–479, 2019.

[48] S. Yousefi, A. J. Márquez, M. Betti, J. L. Araus, and M. D. Serret, “Gene expression and physiological responses to salinity and water stress of contrasting durum wheat genotypes,” *Journal of Integrative Plant Biology*, vol. 58, no. 1, pp. 48–66, 2016.

[49] X. Mao, D. Jia, A. Li et al., “Transgenic expression of TaMYB2A confers enhanced tolerance to multiple abiotic stresses in Arabidopsis,” *Functional Integrative Genomics*, vol. 11, no. 3, pp. 445–465, 2011.

[50] Y. Liu, N. Liu, X. Deng et al., “Genome-wide analysis of wheat DNA-binding with one finger (Dof) transcription factor genes: evolutionary characteristics and diverse abiotic stress responses,” *BMC Genomics*, vol. 21, no. 1, p. 276, 2020.

[51] X.-Y. Cui, Y.-T. Du, J.-d. Fu et al., “Wheat CBL-interacting protein kinase 23 positively regulates drought stress and ABA responses,” *Bmc Plant Biology*, vol. 18, no. 1, p. 93, 2018.

[52] N. Piperidis, J.-w. Chen, H.-h. Deng, L.-P. Wang, P. Jackson, and G. Piperidis, “GISH characterization of *Erianthus arundinaceus* chromosomes in three generations of sugarcane interspecific hybrids,” *Genome*, vol. 53, no. 5, pp. 331–336, 2010.

[53] M. Morgante, S. Brunner, G. Pea, K. Fengler, A. Zuccolo, and A. Rafalski, “Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in sugarcane chromosomes in three generations of *Erianthus arundinaceus*,” *Genome*, vol. 53, no. 5, pp. 331–336, 2010.

[54] V. Croset, R. Rytz, S. F. Cummins et al., “Ancient protostome origin of chemosensory ionotropic glutamate receptors and the evolution of insect taste and olfaction,” *Plos Genetics*, vol. 6, no. 8, article e1001064, 2010.

[55] A. A. Shetty, S. Magadum, and K. Managanvi, “Vegetables as Sources of Antioxidants,” *Journal of Food Nutritional Disorders*, vol. 2, no. 1, 2013.

[56] T. Werner, E. Nehnevajova, I. Kollmer et al., “Root-specific reduction of cytokinin causes enhanced root growth, drought tolerance, and leaf mineral enrichment in *Arabidopsis* and sugar beet,” *Plant Cell*, vol. 22, no. 12, pp. 3905–3920, 2011.

[57] J. Jiang, J. Li, Y. Xu et al., “RNAi knockdown of *Oryza sativa* root meander curling gene led to altered root development and coiling which were mediated by jasmonic acid signalling in rice,” *Plant, Cell Environment*, vol. 30, no. 6, pp. 690–699, 2007.