Genome-wide characterization and expression and co-expression analysis suggested diverse functions of WOX genes in bread wheat

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1. Introduction

Cell division, cell differentiation and morphogenesis are the most important developmental processes in the multi-cellular organisms. Plants growth and developments are regulated by numerous important genes and factors (Li et al., 2019a; Ajdanian et al., 2019). The homeobox superfamily comprises various important groups of genes that play critical roles in growth and development (Gehring et al., 1994). It consisted of 14 gene families including KNOX, BEL, WOX, HD-ZIP I-IV, and SAWADEE etc (Mukherjee et al., 2009). This classification was performed based on conserved regions of the homeodomain (HD) along with other characteristic domains and motifs (Mukherjee et al., 2009). The nomenclature of these families was according to the combination of specific domains and motifs.

The WUSCHEL-related homeobox (WOX) gene family was firstly discovered in Arabidopsis thaliana (Laux et al., 1996). These genes are known to be involved in the plant growth and organ developmental processes (van der Graaff et al., 2009). They also regulate the proliferation of shoots and root apical meristems along with the stem cell homeostasis (Endrizzi et al., 1996; Haecker, 2004). The presence of WOX genes in the quiescent centre and organising centre of root and shoot meristems suggested their role in deciding the fate of cell differentiation in these organs, respectively (Sarkar et al., 2007; Forzani et al., 2014). It was reported that an Arabidopsis WOX mutant, wus was failed to develop shoot meristem (Endrizzi et al., 1996).

The WOX genes have been classified into three clades; ancient, intermediate and modern, during the evolution (van der Graaff et al., 2009). The ancient clade was termed due to their emergence in lower plants. The intermediate clade includes those WOX genes, which emerged in the ferns and are related to the vascular plants. The most recent clade i.e. the modern clade, also known as WUS clade, is responsible for the formation of seeds. This clade is exclusively identified in the seed-bearing gymnosperms and angiosperms (van der Graaff et al., 2009; Lian et al., 2014). However, it has been suggested that all the three clades are equally ancient depending upon their functional and selective adaptation (Wu et al., 2019). The number of WOX genes is independent of the genome size of plants. For instance, Oryza sativa consisted of 13 WOX genes, while A. thaliana has 15 WOX genes (Zhang et al., 2010). Moreover, a variable number of WOX genes in different plants usually depend upon the level of ploidy and duplication events (Wu et al., 2019;
Wang et al., 2019; Li et al., 2019b). It has been observed that higher number of WOX genes are present in more evolved plants as compared to algae, which may be attributed to the neo-functionality of genes (Wu et al., 2019; Li et al., 2019b). The largest WOX gene family has been reported in the tetraploid genome of Brassica napus with 52 members (Li et al., 2019b), while only five WOX genes are present in Phyllostachys edulis (moso bamboo) (Xu et al., 2019). These genes have been found to be involved in various developmental processes including stem cell maintenance, embryo patterning, organ formation, flowering, etc. (Laux et al., 1996; Zuo et al., 2002; Haecker, 2004; Breuninger et al., 2008; Zhang et al., 2011; Nakata et al., 2012). The WOX gene family has been identified in several plant species including tomato, potato, pea, cotton, rice, maize, sorghum, etc (He et al., 2019; Zhang et al., 2010). In a previous study, WOX5 has been isolated and characterized in common wheat (Zhao et al., 2014). Herein, we performed genome-wide identification and comprehensive characterization of the TaWOX gene family in bread wheat (Triticum aestivum L.). They were used for the analysis of gene and protein structure, the occurrence of conserved motifs and domains and their phylogenetic relationship. Further, Kα/Ks ratio, cis-acting regulatory elements, expression and co-expression analyses were carried out during various tissues developmental stages and in the presence of biotic and abiotic stress conditions. While our manuscript writing was in progress, Li et al. (2020) has also reported TaWOX genes. Moreover, they performed expression analysis for only a few genes and in the limited tissues. They lack several analyses including rigorous phylogenetic analysis, expression analyses in embryo developmental stages, stress conditions and co-expression analyses. The co-expression analysis suggested their interaction with a wide range of other genes involved in the diverse molecular processes. In the current study, we explored various characteristic features of WOX genes that will be helpful for their functional characterization in future studies.

2. Materials and methods

2.1. Identification of TaWOX proteins

Identification of TaWOX proteins was carried out by the BLASTp search using the available WOX protein sequences of A. thaliana against the T. aestivum protein model sequences downloaded from the Ensembl Plants (http://plantsensembl.org/index.html), at e-value 10-10. The HMMER search and the Pfam BLAST were used to further confirm the identified sequences (https://pfam.xfam.org/) (Finn et al., 2014). To validate the presence of signature homeodomain, the Simpler Modular Architecture Research Tool (SMART) (Letunic et al., 2015) and the NCBI CDD search were used (Marchlerbauer et al., 2017).

2.2. Chromosomal distribution and nomenclature

The EnsemblePlants (http://plants.ensembl.org/Triticum aestivum/) was used to find out the chromosomal localization of each identified gene. A bidirectional BLAST search was performed to find the homologous TaWOX genes having sequence similarity ≥90%. The diagrammatic representation of the chromosomal distribution of the TaWOX genes was created using the MapInspect software (http://www.plantbreeding.wur.nl/uk/software/mapinspect.html). For the nomenclature of TaWOXs, we followed the standard procedure prescribed as per the international rules for gene symbolization in T. aestivum (http://wheat.pw.usda.gov/ggpages/wgt/98/intro.htm) as performed in earlier studies (Kaur et al., 2020).

2.3. Identification of duplication events

The prediction of duplication events was based on ≥80% sequence similarity, which was conducted through the bidirectional BLAST search at e-value 10-10. Tandem and segmental gene duplication events were classified based on distance between paralogous gene pairs as suggested in the earlier studies (Shumayla et al., 2016, 2019; Sharma et al., 2020).

2.4. Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of the WOX proteins was performed using the MultAlin and the MUSCLE programs at default parameters to get the conserved residues (Edgar, 2004). Phylogenetic relatedness of the TaWOX protein sequences with the other plants such as A. thaliana, O. sativa, Sorghum bicolor and Zea mays was studied through an evolutionary tree. A phylogenetic tree was constructed with the full length protein sequences using the Molecular Evolutionary Genetics Analysis (MEGA) 7 software following the Neighbor-joining method with 1000 bootstrap replicates (Kumar et al., 2016).

2.5. Gene structure analysis

The occurrence of exon and intron in the TaWOX genes was analysed by comparing the CDS and their corresponding genomic sequence as done in earlier studies (Taneja et al., 2016; Taneja and Upadhyay, 2018). The Gene Structure Display Server (GSDS 2.0) was used for the intron phase analysis and pictorial presentation of gene architecture (Fu et al., 2014).

2.6. Physicochemical properties of TaWOX proteins

The physicochemical properties of TaWOX proteins such as peptide length, molecular weight (MW) and isoelectric point (pI) were computed using the ExPasy tool (web.expasy.org/compute pi/) (Gasteiger et al., 2005). Subcellular localization was analysed using the CELLOv.2.5 (http://cello.life.nctu.edu.tw/cello2go/) and the Protcomp9.0 (http://www.softberry.com/berry.phtml?topic=protcompl&group=programs&subgroup=proloc). The transmembrane regions were predicted using the TMHMMv.2.0, Phobius and Topcons tools (Krogh et al., 2001; Kall et al., 2004; Bernal et al., 2009). The conserved motifs were determined using the Multiple EM for Motif Elicitation (MEME) Suite version 4.11.2 (Bailey et al., 2009).

2.7. Ka/Ks analysis

The ClustalOmega server (https://www.ebi.ac.uk/Tools/msa/clustalo) was used to align the nucleotide and protein sequences of the duplicated TaWOX genes. For the calculation of non-synonymous substitution per non-synonymous site (Ka), synonymous substitution per synonymous site (Ks), and the Ka/Ks ratio, we used PAL2NAL server (Suyama et al., 2006). To know the divergence time of each duplicated TaWOX gene pair, we used formula T = Ks/2r where divergence time and rate were denoted by T and r, respectively. The value of divergence rate was kept as 6.5 × 10^-9 as reported for cereals and used in earlier studies (Gaut et al., 1996, Tyagi et al., 2020a, 2021).

2.8. cis-acting regulatory elements

The cis-acting regulatory elements were analysed in 1500 bp upstream sequence from the translation start site of each TaWOX gene. These sequences were retrieved and searched at the PlantCARE database for the analysis of the occurrence of cis-acting regulatory elements following the standard procedure (Lescot et al., 2002).

2.9. Expression analysis in tissue development stages

For the expression analysis, publicly available high throughput RNA sequence (RNA seq) data of T. aestivum was downloaded from the Expression ATLAS for different tissue developmental stages of leaf, stem, root, spike, grain and embryo (Papadomphilou et al., 2018; Choulet et al., 2014; Pingault et al., 2015). The expression value was calculated in term
of the fragment per kilobase per million (FPKM) using the Trinity package (Haas et al., 2013). Expression analysis of each gene was also carried out by using the data available at the WheatExp server (Pearce et al., 2015) (http://wheat.pw.usda.gov/WheatExp/; accession number: ERP004714) from similar stages.

2.10. Expression analysis during biotic and abiotic stress conditions

Expression analysis under biotic stress was carried out using publicly available high throughput RNA-seq data (PRJNA243835) generated in triplicates after 24, 48, and 72 h post-inoculation of Puccinia striformis f. sp. tritici (Pst) and Blumeria graminis f. sp. tritici (Bgt), separately (Zhang et al., 2014). Moreover, RNA-seq data (accession number SRP062745) generated by Zhang et al. (2016) from the salt (150 mM NaCl) treated root tissue were used for the expression profiling of TaWOX genes under salt stress conditions after 6, 12, 24, and 48 h of treatment. Effect of heat (HS), drought (DS) and combined heat drought (HD) stresses were studied by using the publicly available RNA-seq data (accession number SRP062745) generated after one and six hours of treatment (Liu et al., 2015). The differential expression analysis was performed using the Trinity package at FDR <0.05 with 0.001 p-value (Haas et al., 2013). The Hierarchical clustering Explorer tool (HCE.3.5) was used to generate heat maps (Seo et al., 2006).

2.11. Co-expression analysis

We used the CoExpress v.1.5 to identify the TaWOX genes co-expressing with the other genes in the genome of T. aestivum (Nazarov et al., 2010) at the following parameters: correlation power 1, threshold filter >0.9 with Pearson correlation coefficient. The co-expression analysis was performed using the expression data from tissue developmental stages, and various abiotic and biotic stress conditions. The functional annotation and gene ontology (GO) mapping of co-expressed transcripts were performed using the Blast2GO tool (Conesa and Götz, 2008; Nazarov et al., 2010). The interaction network was visualized through Gephi 0.91 (Bastian et al., 2009).

3. Results and discussion

3.1. Identification and chromosomal distribution TaWOX genes

A total of 43 TaWOX genes were identified in the genome of T. aestivum after an extensive BLASTp search (Additional file 1). In the allohexaploid genome of T. aestivum, these genes were found to be distributed on all the chromosomes except chromosome number six and seven (Figure 1; Table 1). Majority of TaWOX genes were localized on the chromosome group three. The chromosome group four carried only one gene in each A, B and D subgenome. Three genes were present on the 2A chromosome, while two genes were found on the 2B and 2D chromosomes. Rest of the chromosomes were found to be carrying an equal number of genes. Furthermore, the location of TaWOX-8Un gene was unknown. This may be due to the unavailability of complete genomic information. A total of 15, 13, and 14 TaWOX genes were distributed on the A, B, and D subgenomes, respectively. The results suggested an even distribution of the TaWOX genes on the A, B, and D subgenomes (Figure 1). A similar result was found by Li et al. (2020), but they found only two genes on the chromosome 2A while we found three genes (TaWOX3, TaWOX4 and TaWOX5). In the case of diploid genome of O. sativa and A. thaliana, 13 and 15 WOX genes have been reported, respectively (Bi et al., 2016). The higher number of TaWOX genes could be due to the allohexaploid nature of the T. aestivum genome. Further, number of genes at each A, B and D subgenome was comparable to the diploid genome. The results suggested a direct correlation of the number of gene to the ploidy level of the genome.

3.2. Homeologous and paralogous TaWOX genes

TaWOX genes were clustered into 14 homeologous groups, which were equally contributed by all the three subgenomes expect TaWOX-4. It was only found on the chromosome 2 of A subgenome. The results indicated that the other homeologs from B and D subgenomes might have lost during evolution.

A total of five pair of paralogous TaWOX genes were predicted. All the duplication events among TaWOX genes were found to be tandemly duplicated (Figure 1; Additional file 2). The paralogous genes TaWOX9-A, TaWOX10-A1 and TaWOX10-A2 were found on the chromosome 3A with high degree of similarity, and in close proximity as tandem duplicated genes. Similarly, tandem duplication events were predicted among TaWOX9-D, TaWOX10-D1 and TaWOX10-D2, as well. Further, TaWOX9-B and TaWOX10-B tandem duplicated genes were present on the chromosome 3B (Figure 1). Interestingly, all the duplication events were confined to the WUS clade and chromosome group 3 during evolution. No duplication events were traced from the ancient and intermediate clades. Survival of duplicated genes suggested their advantageous selection. These might have been kept for acquiring new functions such as for producing higher protein, required more in different tissues with different functions.

3.3. Ka/Ks analysis

We estimated the Ka/Ks ratio for the above five pairs of paralogous genes. The Ka/Ks computation is used for calculating type of selection pressure on the protein. Here, the resulted ratio can be < 1, = 1 or >1 where less than 1 means the functional constraint with negative or purifying selection of the genes. However, more than one indicates accelerated evolution with the positive selection (Nekrutenko et al., 2002).
### Table 1. Various characteristic features of TaWOX genes and proteins of *T. aestivum*.

| GENES ID | Gene name | Chromosome | Exon | Protein length (AAs) | Isoelectric point | Mol.Wt. (kDa) | Subcellular localization |
|----------|------------|------------|------|----------------------|-------------------|---------------|--------------------------|
| Ancient Clade | | | | | | | |
| TraesCSA02G341700.1 | TaWOX8-A | 3A | 3 | 265 | 6.0964 | 29.5 | Nuclear |
| TraesCSB02G373800.1 | TaWOX8-B | 3B | 4 | 261 | 6.0964 | 29.2 | Nuclear |
| TraesCS1D02G335500.1 | TaWOX8-D | 3D | 2 | 263 | 6.8294 | 29.4 | Nuclear |
| TraesCS1D02G204800.1 | TaWOX8-Un | Un | 2 | 156 | 7.4367 | 17.7 | Nuclear |
| Intermediate Clade | | | | | | | |
| TraesCS1A02G427400.1 | TaWOX2-A | 1A | 4 | 486 | 7.7375 | 50.6 | Nuclear |
| TraesCS1B02G427400.1 | TaWOX2-B | 1B | 4 | 485 | 7.7252 | 50.6 | Nuclear |
| TraesCS2B02G177900.1 | TaWOX3-A | 2A | 3 | 265 | 6.7135 | 27.8 | Nuclear |
| TraesCS2B02G177900.1 | TaWOX3-B | 2B | 3 | 261 | 6.7135 | 27.4 | Nuclear |
| TraesCS3B02G272200.1 | TaWOX3-D | 2D | 3 | 264 | 6.8625 | 27.6 | Nuclear |
| TraesCS3D02G244300.1 | TaWOX6-A | 3A | 3 | 260 | 6.8407 | 27.9 | Nuclear |
| TraesCS3A02G2087800.1 | TaWOX6-B | 3B | 3 | 261 | 6.7444 | 28 | Nuclear |
| TraesCS3A02G2073300.1 | TaWOX6-D | 3D | 3 | 261 | 7.0197 | 28.1 | Nuclear |
| TraesCS2B02G514000.1 | TaWOX5-A | 2A | 3 | 234 | 9.5634 | 25.3 | Nuclear |
| TraesCS2B02G514000.1 | TaWOX5-B | 2B | 3 | 237 | 9.529 | 25.8 | Nuclear |
| TraesCS3A02G2087800.1 | TaWOX5-D | 2D | 3 | 237 | 7.8833 | 25.4 | Nuclear |
| TraesCS3B02G174400.1 | TaWOX12-A | 4A | 3 | 307 | 7.8699 | 31.5 | Nuclear |
| TraesCS3B02G174400.1 | TaWOX12-B | 4B | 3 | 309 | 8.2579 | 31.8 | Nuclear |
| TraesCS3D02G176400.1 | TaWOX12-D | 4D | 3 | 306 | 7.7696 | 31.4 | Nuclear |
| WUS Clade | | | | | | | |
| TraesCS1A02G052000.1 | TaWOX1-A | 1A | 2 | 263 | 8.1704 | 28.5 | Nuclear |
| TraesCS1B02G069000.1 | TaWOX1-B | 1B | 2 | 264 | 8.1153 | 28.6 | Nuclear |
| TraesCS1D02G064000.1 | TaWOX1-D | 1D | 2 | 267 | 8.3764 | 29.1 | Nuclear |
| TraesCS2A02G177900.1 | TaWOX4-A | 2A | 3 | 308 | 8.2945 | 32.7 | Nuclear |
| TraesCS2A02G177900.1 | TaWOX5-A | 2A | 3 | 234 | 9.5634 | 25.3 | Nuclear |
| TraesCS2B02G542600.1 | TaWOX5-B | 2B | 3 | 237 | 9.529 | 25.8 | Nuclear |
| TraesCS2D02G515600.1 | TaWOX5-D | 2D | 3 | 237 | 9.6965 | 25.6 | Nuclear |
| TraesCS3A02G358100.1 | TaWOX9-A | 3A | 2 | 301 | 8.8572 | 33.4 | Nuclear |
| TraesCS3B02G391100.1 | TaWOX9-B | 3B | 2 | 299 | 9.409 | 33 | Nuclear |
| TraesCS3D02G352500.1 | TaWOX9-D | 3D | 2 | 298 | 8.4395 | 33.3 | Nuclear |
| TraesCS3A02G358200.1 | TaWOX10-A1 | 3A | 2 | 288 | 10.048 | 31.9 | Nuclear |
| TraesCS3A02G358400.1 | TaWOX10-A2 | 3A | 2 | 290 | 9.5964 | 32.3 | Nuclear |
| TraesCS3B02G391200.1 | TaWOX10-B | 3B | 2 | 290 | 10.0961 | 32.3 | Nuclear |
| TraesCS3D02G352600.1 | TaWOX10-D1 | 3D | 2 | 285 | 9.5715 | 31.6 | Nuclear |
| TraesCS3D02G352700.1 | TaWOX10-D2 | 3D | 2 | 291 | 9.0153 | 31.9 | Nuclear |
| TraesCS3D02G368100.1 | TaWOX11-A | 3A | 2 | 212 | 7.6866 | 24.5 | Nuclear |
| TraesCS3D02G368100.1 | TaWOX11-B | 3B | 2 | 209 | 7.7325 | 24.1 | Nuclear |
| TraesCS3D02G361100.1 | TaWOX11-D | 3D | 2 | 210 | 7.6866 | 24.2 | Nuclear |
| TraesCS3A02G088500.1 | TaWOX13-A | 5A | 2 | 318 | 10.3524 | 34.2 | Nuclear |
| TraesCS3A02G091000.1 | TaWOX13-B | 5B | 2 | 321 | 10.916 | 34.5 | Nuclear |
| TraesCS3A02G097400.1 | TaWOX13-D | 5D | 2 | 322 | 10.6937 | 34.4 | Nuclear |
| TraesCS3A02G157300.1 | TaWOX14-A | 5A | 2 | 241 | 8.8212 | 25.8 | Nuclear |
| TraesCS3A02G156400.1 | TaWOX14-B | 5B | 2 | 241 | 8.8212 | 25.8 | Nuclear |
| TraesCS3D02G162600.1 | TaWOX14-D | 5D | 2 | 242 | 8.3999 | 25.9 | Nuclear |

### Table 2. The Ka/Ks ratio and divergence time of duplicated TaWOX gene pairs.

| Gene A | Gene B | Ka | Ks | Ka/Ks | T – Ks/2r | Selection pressure |
|--------|--------|----|----|-------|-----------|-------------------|
| TaWOX9-A | TaWOX10-A1 | 0.1418 | 0.3223 | 0.4428 | 25.5 | Purifying |
| TaWOX9-A | TaWOX10-A2 | 0.2054 | 0.4300 | 0.4777 | 33.1 | Purifying |
| TaWOX9-B | TaWOX10-B | 0.1860 | 0.3939 | 0.4723 | 30.3 | Purifying |
| TaWOX9-D | TaWOX10-D1 | 0.0950 | 0.2825 | 0.3362 | 21.7 | Purifying |
| TaWOX9-D | TaWOX10-D2 | 0.1954 | 0.3856 | 0.5066 | 29.6 | Purifying |

Ka-non-synonymous substitutions per non-synonymous site, Ks-synonymous substitutions per synonymous site, T-Divergence time and r-Divergence rate.
The occurrence of Ka/Ks ratio less than 1 for each TaWOX paralogous gene pair suggested the negative selection pressure or strong purifying selection. Further, the occurrence of these duplication events was predicted in the range of 21–33 Million years ago (MYA) (Table 2).

3.4. Phylogenetic analysis

We constructed a phylogenetic tree to understand the evolutionary relatedness among the A. thaliana, O. sativa, S. bicolor, T. aestivum and Zea mays WOX proteins. We found three distinct clades (Figure 2), as reported in previous literature (Zhang et al., 2010; Lian et al., 2014; Li et al., 2018; Wang et al., 2018, 2019; Chang et al., 2020). The ancient clade consisted of 13 WOXs which are orthologous to Arabidopsis WOX10, WOX13 and WOX14, out of which four WOXs belong to T. aestivum. The intermediate clade contained 34 WOXs, 15 TaWOXs orthologous to Arabidopsis WOX8, WOX9, WOX11 and WOX12. However, the modern or WUS clade consisted of 55 WOXs including eight from Arabidopsis (WOX1, WOX2, WOX3, WOX4, WOX5, WOX6, WOX7 and WUS) and 24 from T. aestivum. The ancient clade carried the lowest number of genes, while WUS clade comprised of nearly half of all sequences, making it the largest clade, as reported in other plants (Li et al., 2019b). Further, the homeologous and paralogous TaWOXs were tightly clustered, which suggested their structural homology. The paralogous genes might have maintained their conserved structure even after the duplication events. Our analysis supported the notion that the WOX gene family is monophyletic in origin and suggested their conserved role (Lian et al., 2014). Further observation suggested that the gene duplication events had occurred to expand the TaWOX gene family.

3.5. Gene structure analysis

We analysed the structural features of TaWOX genes including the exon-intron organization and the intron phase distribution. In the TaWOX genes, the number of exons ranged from one to four. In the ancient clade, three and four exons were found in TaWOX8-A and TaWOX8-B, respectively. While TaWOX8-D and TaWOX8-Un carried only two exons. All the introns were predicted to present in phase 1 in the ancient clade genes. In the intermediate clade, only TaWOX2 group genes carried four exons, while resting all TaWOX3, TaWOX6, TaWOX7 and TaWOX12 group genes carried three exons (Figure 3; Additional file 3). Moreover, in this clade the majority of introns were found in phase 1 followed by phase 0. In the WUS clade, TaWOX1, TaWOX9, TaWOX10, TaWOX11, TaWOX13 and TaWOX14 consisted of two exons while only two genes had 3 exons (Figure 3, Additional file 3). In addition to it, the maximum number of introns was predicted to be in phase 1. Further, we found conserved exon-intron structure in all three clades supporting our phylogenetic tree. On the other hand, the phenomenon of exon loss in WUS clade could also be seen in TaWOX genes as reported previously in
rice and soybean WOX gene family (Hao et al., 2019). Gene structure variation in the members of ancient clade suggesting that the ancient genes might be associated with the process of rapid evolution (Wang et al., 2019).

3.6. Physicochemical characterization of TaWOX proteins

We analysed various physicochemical properties of WOX proteins including peptide length, MW and pl (Additional file 3, Table 1). The majority of TaWOX proteins consisted of 212–301 amino acid residues (aa), except TaWOX2 and TaWOX7 group proteins from the intermediate clade, which were of ~486 and ~515 aa, respectively. The longest and the heaviest protein was TaWOX7 (MW 53.7 kDa) while TaWOX11 was the smallest and the lightest one (MW 24.1 kDa). The average molecular weight of the ancient, intermediate and modern clades was 29.3, 38.2 and 29.5 kDa, respectively. We found great variation in the isoelectric point of TaWOX proteins (6.09–10.91).

By knowing the subcellular localization of WOX protein, we could correlate between its position and targeted function. All the TaWOX proteins were predicted to be localized in the nuclear region. Hence implying their specific function confined in nucleus and suggesting them as transcription factors. Similar features of WOX proteins have been reported from other plant species (Chang et al., 2020; Li et al., 2018; Wang et al., 2018).

3.7. Motifs and domain analyses

Multiple sequence alignment (MSA) gives intuitive format to concisely constraint evolutionary relationship between residues of protein for their characterization and in predicting function. We constructed MSA using the MultAlin with 43 fulllength TaWOX protein sequences. We found that the signature motif of the homeodomain with the helix-turn-helix-loop-helix was conserved in all the 43 TaWOX protein sequences (Figure 4). It was further confirmed using the SMART server (Additional file 4). We found 15 highly conserved amino acid residues within the homeodomain. In the first helix, five amino acid residues i.e. R, W, P, Q and L at 97, 98, 100, 104 and 107 position were found to be highly conserved. In the second helix, P and L at 120 and 132 positions were most conserved residues. However, in the third helix N, V, W, F, Q and N were the six conserved amino acid residues (Figure 4). Similar conservation of amino acid residues in the homeodomain with slight variation have been reported in various plant species (Haecker, 2004; Li et al., 2018). We found that the G is the most conserved amino acid residue present in both turn and loop.

Earlier studies in Arabidopsis reported the presence of other functional domains known as the WUS box and the Ear-like motif, which are present in WUS or modern clade, and responsible for transcription inhibition of some genes. We could also find conserved WUS box sequences in all the 24 TaWOX proteins of WUS clade (Figure 4). We observed TLELFPL as conserved motif in the majority of sequences, with slight variations. Such as the T was replaced by the L in the TaWOX9, TaWOX10 and TaWOX13 group proteins, while the last L was replaced by the Q or T in certain sequences. Similar variations are reported in other plants also (Zhang et al., 2010; Li et al., 2018).

We found amino acid residues ‘PLELRLC’ in TaWOX11-A, TaWOX11-B, and TaWOX11-D, and ‘GTSPELTLR’ in TaWOX4-A as an Ear-like motif beside the occurrence of WUS box (Figure 4) as reported in the many other plant species as well (Zhang et al., 2010; Li et al., 2018).

To understand the conserved structure of TaWOX proteins, we further analysed the occurrence of 15 conserved motifs. We found that
the motifs one, two and six were the most conserved motifs present in the all 43 TaWOX proteins (Figure 5). The motif two and motif six corresponded to the alpha-helix 1 and the alpha-helix 3, respectively. However, motif 1 starting from the helix 1, ends in the helix 3, by accommodating both turn and loop as well. Uniformity in the motifs alignment of all the clades confirmed their close relatedness in the phylogeny. The ancient clade of wheat comprised of TaWOX8 group proteins clustered together with the presence of motif number 15 at the N-terminus, which was completely absent in other clades. It might have possible role in the early period of evolution. Genes of the intermediate clade had the motifs 10, 11, 12 and 13 in common (Figure 5). Furthermore, motifs 4, 5, 7 and 9 were only present in the WUS clade, suggesting introduction of new motifs during duplication and diversification process for the neo-functionalization particularly in the seed plants (Jain et al., 2008; Li et al., 2019b). Conserved motifs distribution in each clade implies conserved phylogenetic relatedness. The results further suggested that the ancient and intermediate clades have motifs responsible for transcriptional activation activities and thereafter developed WUS-motifs responsible for transcriptional repressive activities in the WUS clade (Zhou et al., 2018).
3.8. Cis-acting regulatory elements

The cis-acting regulatory elements in the 41 TaWOX genes were identified using the PlantCARE database (Lescot et al., 2002). All the identified elements were broadly categorized into four groups such as (1) light response (2) hormone (3) stress and (4) growth and development related (Additional file 5). The cis-regulatory elements ACE, G-Box, Box 4, TCT-motif, MRE, Sp1, AE-box, ATTAAT were identified as the light responsive elements. Moreover, G-box (CAGGTG) element also plays an important role in response to abscisic acid, anaerobiosis and methyl-jasmonate, and it has an active role in ethylene induction along with seed specific expression. ABA response element (ABRE in 34 TaWOX genes) and AGGTG (in 29 TaWOX genes) were the most prevalent hormone responsive cis-acting regulatory element as compared to other elements related to the hormone response such as P-box, TATC-box, gibberelhin-response element (GARE-motif), Auxin response element (AuxRR-core), TGA-box. Under stress response elements, methyl jasmonate-response elements (CGTCA-motif in 30 WOX genes and TGACG-motif in 29 WOXs), anaerobic responsive elements having GC and GT motifs active during plant defence responses (ARE in 29 WOX genes), CAACGT, MBS and LTR were frequently detected. The growth and development related cis-regulatory elements CAT-box, GCCACT involved in meristem-specific activation (present in 20 WOX genes), O2 site confers role in zein metabolism and circadian motif (present in 18 WOX genes), RY-elements specific for seed and shoot development (in 15 WOX genes; CATGCATG, MSA-like were predicted in the majority of WOX genes). The results revealed that the TaWOX genes have diverse cis-regulating elements that could response to a variety of stress, hormone and development-related factors that further suggested their roles in various biological pathways. Similar results are reported in tea and soybean (Wang et al., 2019; Hao et al., 2019).

3.9. Tissue specific expression analysis

TaWOX genes exhibited differential expression in various developmental stages of root, stem, leaf, spike, grain and embryo tissues. In-total 35 TaWOX genes showed expression in different tissues of wheat (Figure 6a,b). The majority of genes were highly expressed in spike and grain tissues, which suggested their role in the reproductive tissue and embryo development. For instance, genes belonging to WUS clade, TaWOX1 group genes were highly expressed in spike tissue as compared to other tissues, while TaWOX10 group genes showed significant expression in grain tissue. Similarly, Arabidopsis AtWOX2 is involved in embryo and cotyledon boundary formation (Haecker, 2004). Further, several genes including TaWOX11 and TaWOX5 group genes were highly expressed in the root and stem developmental stages. Phylogenetically these genes were grouped with AtWUS and AtWOX4, where AtWUS plays a key role in the development of shoot and floral meristems, and AtWOX4 is responsible for lateral growth in Arabidopsis. Further, TaWOX3 of the intermediate clade showed expression for root development which is very similar with the result of OsWOX11 of rice (Zhao et al., 2009). These results suggested conventional roles for these similar genes in wheat (Laux et al., 1996).

To get further insight into the role of TaWOX, we analysed their expression in the endosperm, aleurone layer and transfer cell. A total of 12 TaWOX genes showed expression in these tissues. We observed developmental stage specific expression of TaWOX genes in the endosperm, which suggested specific roles of each TaWOX gene. For instance, TaWOX8-A of the ancient clade could express in all the endosperm developmental stages but specifically at the central region. Similar results are reported for the Arabidopsis (AtWOX13) ancient clade genes (Deveaux et al., 2008). TaWOX10-A1, TaWOX10-A2 and TaWOX10-D1 of the WUS clade were highly expressed at the later developmental stages of the endosperm and aleurone layer (Figure 6b) coinciding results with

![Figure 6](image.png)

Figure 6. Relative expression profiles of TaWOX genes in various tissue development stages and in the presence of biotic and abiotic stress conditions. Figures 6a and b show expression profiling in various tissue development stages. Figure 6c shows expression under biotic (Bgt and Pst) stress conditions, figure 6d under heat, drought and their combination stresses, while figure 6e under salt stress.
AtWOX2 of the modern clade (Haecker, 2004). However, leaf developmental stages were deprived of significant expression of the majority of TaWOX genes.

TaWOX8 group genes belonging to the ancient clade also exhibited expression in all tissues, which suggested their role throughout the life cycle of plant. Broad expression pattern of the ancient clade genes reflected their conserved functions during evolution (Wang et al., 2019). Moreover, no such trend was found in the other clades. These assorted expressions of various TaWOX genes suggested their precise roles in various tissue developmental stages. Variable expression of WOX genes in different tissue has also been observed in other plants (Li et al., 2018, 2019; Rahman et al., 2017; Ramkumar et al., 2018).

3.10. Expression analysis under biotic stress

To study the expression pattern of TaWOX genes under biotic stress, we used the publicly available RNA seq data generated in response to fungal infection of triplicates after 24, 48, and 72 h post-inoculation of Puccinia striiformis f. sp. tritici (Pst) and Blumeria graminis f. sp. tritici (Bgt), separately (Zhang et al., 2014). Under biotic stress, only the ancient clade genes (TaWOX8) exhibited differential expression. The results indicated that the other genes might not have any relevant function in biotic stress response. All the TaWOX8 group genes were upregulated after both Bgt and Pst infection, except TaWOX8-B. Moreover, the level of upregulation was higher in case of Bgt as compared to Pst infection (Figure 6c). Such differential expression of ancient clade genes suggested their function in biotic stress response which needs to be further established in future studies.

3.11. Expression analysis under abiotic stress

In case of abiotic stress, the expression of TaWOX genes was analysed under heat (HS), drought (DS) and combined heat drought (HD) stress after one and six hours of treatments (Liu et al., 2015), and under salt stress conditions after 6, 12, 24, and 48 h of treatment (Zhang et al., 2016). Out of 43 genes, only five TaWOX genes showed differential expression in heat and drought stresses; four genes of them belong to the ancient clade (Figure 6d). All the ancient clade genes were significantly upregulated after 6 h of DS, while all the genes were downregulated at other treatments. Similar differential expression pattern of

Figure 7. Interaction networks of the TaWOX genes in (a) various tissue development stages, (b) in the presence of biotic stress, (c) in the presence of heat and drought stress and (d) in the presence of salt stress. The interaction network was generated using the Gephi 0.9.1 tool.
$GmWOX$s genes are reported under abiotic stress conditions (Hao et al., 2019).

Salt stress is a major threat to the plants that adversely affects the development and growth of plants. Hence, the expression profiling of $TaWOX$ genes were performed to understand their putative role during salt stress. $TaWOX3$ group genes of the intermediate clade were upregulated with the increase in the hours of salt treatment (Figure 6e). While the ancient clade $TaWOX8$ group genes were highly upregulated at initial hour of treatment while normalized at 48 h that might be due to adaptation of plants at the later hours. Similarly, variable expression of other $TaWOX$ genes such as $TaWOX5$ and $TaWOX12$ of the intermediate clade and $TaWOX11$ of the WUS clade was also observed. These results of differential expression of $WOX$ genes suggested revisiting the function of these genes. They are not only related to the development, but might also be an important player in stress management, which need to be validated in future studies.

3.12. Co-expression and interaction analysis

To understand the interaction network of $TaWOX$ genes, co-expression analysis was carried out in tissue developmental stages, and under abiotic and biotic stress conditions as well. In tissue developmental stages, 18 $TaWOX$ genes exhibited co-expression with a total of 391 transcripts. Among them, $TaWOX12-A$ of the intermediate clade was found to be co-expressed with 77 transcripts (Figure 7a; Additional file 6). These co-expressed transcripts encode the proteins related to the membrane integrity, fruit development, polarity specification, shoot apical meristem, transmembrane transport, DNA binding, Hydrolase activity, etc., which depicted their putative role in tissue development and signalling pathways (Figure 7a; Additional file 6).

In biotic stress, $TaWOX-A$ and $B$ of the intermediate clade and and $TaWOX8-Un$ of the ancient clade exhibited co-expression with a total of 169 other transcripts. These transcripts encode for various receptor-like kinases, transcription factors, chalcone synthase, flavonoid pathway related and chloroplast genes, which are related to transference activity, regulation of transcription, oxidation and reduction process etc. The results suggested their involvement in various physiological and signal cascade mechanisms (Figure 7b; Additional file 7).

In case of heat and drought stresses, a total of 15 $TaWOX$ genes showed co-expression with 157 different transcripts (Figure 7c; Additional file 8). These transcripts belong to various metabolic processes, transmembrane transport, post embryonic development, regulation of transcription, transmembrane transport activity related proteins, which depicted their role in defence and signalling pathways.

In salt stress, 13 $TaWOX$ genes were found co-expressing with 167 other transcripts, in which $TaWOX8-D$ of the ancient clade exhibited co-expression with 47 transcripts (Figure 7d; Additional file 59). The GO mapping of co-expressed transcripts suggested their role in water deprivation (GO:0009414), oxidation-reduction process (GO:0055114), regulation of transcription (GO:0006355), salt stress response (GO:00065634), peroxidase activity (GO:0004601), metal ion binding (GO:0046872) etc.

These results suggested diverse roles of $TaWOX$ genes in plant development, stress responses, signalling cascade, and various physiological processes. Moreover, the functional validation of each candidate gene needs to be done in future studies.

4. Conclusions

The WUSCHELrelated homebox (WOX) genes are known to play vital function during development in plants. Here, we performed identification of 43 TaWOX genes in an important crop plant $T. aestivum$. Phylogenetically, TaWOXs were clustered into three major groups termed as the intermediate, ancient and WUS or modern clades, confirmed by conserved exon-intron structure and motif composition in each clade. Tandem duplication events suggested that this phenomenon has contributed for the expansion of WOX gene family in wheat. Promoter analysis revealed the occurrence of cis-acting regulatory elements involved in growth and development as well as in stress response. The continuous expression of certain genes in all tissues reflects their conventional role as housekeeping genes in wheat. Moreover, the high expression of some TaWOX genes in various tissues developmental stages including spike, grain, endosperms etc. suggested their specific involvement in the growth and development of those tissues or organs. The differential expression of a few $TaWOX$ genes under biotic and abiotic stress conditions revealed their roles in stress response. Co-expression analyses further suggested the involvement of $TaWOX$ genes in the growth and development, and stress signalling. Moreover, the functional validation of each gene is necessary in the future studies. The current study would help in selecting the candidate $TaWOX$ genes for functional validation in wheat to get improved crop variety.

Declarations

Author contribution statement

M. Rathour and A. Sharma: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
A. Kaur: Performed the experiments; Analyzed and interpreted the data.
S.K. Upadhyay: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

Ajdanian, L., Babaei, M., Aroiee, H., 2019. The growth and development of cress ($Lepidium sativum$) affected by blue and red light. Heliyon 5, 02109.
Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., Noble, W.S., 2009. Meme suite: tools for motif discovery and searching. Nucleic Acids Res. 37 (suppl_2), W202–W208.
Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks. In: Third International AAAI Conference on Weblogs and Social media.
Bernal, A., Viklund, H., Hennerdal, A., Elofsson, A., 2009. TOPCONS: consensus prediction of membrane protein topology. Nucleic Acids Res. 37 (suppl_2), W465-W468.
Breuning, H., Rikirsch, E., Hermann, M., Ueda, M., Laux, T., 2008. Differential expression of WOX genes mediates apical-basal axis formation in the Arabidopsis embryo. Dev. Cell 14 (6), 867–876.
Chang, Yingying, Song, Xiaobo, Zhang, Qixiang, Liu, Hui, Bai, Yongchao, Lei, Xiaoshuo, Pei, Dong. 2020. Genome-wide identification of WOX gene family and expression analysis during rejuvanational rhizogenesis in wheat (Agropyron rigens L.). Forests 11, 16.

Choulet, F., Alberti, A., Theil, S., Glover, N., Barbe, V., Daron, J., Pingault, L., Sourdille, P., Gouloux, A., Paux, E., Leroy, P. 2014. Structural and functional partitions of Bowman-Science 345 (6194).

Conesa, A., Götz, S. 2008. Blast2GO: a comprehensive suite for functional analysis in plant genomics. Int. J. Plant Genom. 2008.

Deveau, Y., Toffano-Nioche, C., Clainse, G., Tharreau, V., 2014. Genes of the most conserved WOX clade in plants affect root and flower development in Arabidopsis. BMC Evol. Biol. 8 (1), 291.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Endrizzi, K., Mounin, B., Haeccker, A., Levin, J., Laux, T. 1996. THE SHOOT MERISTEMLESS gene is required for maintenance of undifferentiated cells in Arabidopsis shoot and floral meristems and acts at a different regulatory level than the meristemo gene WUSCHEL and ZWILLE. Plant J. 10 (6), 967–979.

Finn, R.D., Alex, B., Jody, C., Penelope, C., Eberhardt, R.Y., Eddy, S.R., Andreas, H., Yoon, S., Sun, Q. 2017. The Pfam protein family database. Nucl. Acids Res. 45 (1), D265–D270.

Finn, R., Tate, J., Mistry, J., Coggill, P., L School, E., Lopez, R., Goerzen, J., Wu, C., Mercer, M., Sonnhammer, E.L., Gil, M., Chothia, C., Orengo, C.A. 2006. The SCOP database in 2006: refinements, new developments and 10 years of progress. Nucl. Acids Res. 34, D238–D243.

Finn, R.D., Mistry, J., Bouzid, M., Coggill, P., G. L., Eddy, S.R., Sonnhammer, E.L., Lomsadze, A., Rapp, B., Durbin, R., Bateman, A. 2008. Pfam: clans, web tools and mUltiPalm–an improved version of the multiple sequence alignment program. Nucl. Acids Res. 36, 1–12.

Jeon, S.-W., Lee, C.-Y., Park, J.-H., Kim, M.-J., Jung, K.-T., Kim, J.-H. 2014. Classification and evolutionary divergence analysis of homeobox genes in rice. FEBS J. 281 (10), 1725–1737.

Letunic, I., Doerks, T., Bork, P. 2015. SMART: recent updates, new developments and a portal to tools for in silico analysis of promoter sequences. Nucl. Acids Res. 43 (1), 275–279.

Marchlerbauer, A., Bo, Y., Han, L., Jure, J., Lanzczycki, C.J., Lu, S., Chitaz, F., Feavon, S., Marchler, M.K.R., Greer, R.L., Lu, Z., Frank, S. 2017. PRINTS/PROSITE: an update. J. Mol. Biol. 429 (12), 2110–2137.

Marchlerbauer, A., Polson, J., Dischler, D., Blatov, A., Couteau, P., Hwang, H., Wu, G., Wu, C., Mercier, J.-C. 2015. W2D20: an updated database of conserved protein families to support the study of human and animal genomes. Nucleic Acids Res. 43 (1), D286–D291.

Mazumder, D., Tewari, M., Tiwari, A., Rahman, M., Patra, S., Mandal, S. 2012. Comparative analysis and comprehensive characterization of the WOX family genes in plant kingdom. Sci. World J. 2014.

Marchlerbauer, A., Bo, Y., Han, L., Jure, J., Lanzczycki, C.J., Lu, S., Chitaz, F., Feavon, S., Marchler, M.K.R., Greer, R.L., Lu, Z., Frank, S. 2017. PRINTS/PROSITE: an update. J. Mol. Biol. 429 (12), 2110–2137.

Nekrutenko, A., Makova, K.D., Li, W.H. 2002. The K(A)/K(S) ratio test for assessing the coding potential of genomic regions: an empirical and simulation study. Genome Res. 12, 198–202.

Papadopoulos, I., Fonseca, N.A., Keays, M., Tang, Y.A., Barrera, B., Bazant, W., Burke, M., Furlag, A., Fuentes, A.M.P., George, N., Huerta, L. 2018. Expression Atlas gene and protein expression across multiple studies and organisms. Nucleic Acids Res. 46 (D1), D246–D251.

Pearce, S., Vazquez-Gross, H., Hermit, S.Y., Hane, D., Wang, Y., Gu, Y.Q., Dubovsky, L. 2015. WheatExp: an RNA-seq expression database for polyploid wheat. BMC Plant Physiol. 8 (1), 1–8.

Pingault, L., Choulet, F., Alberti, A., Glover, N., Wincker, P., Freiillet, C., Paux, E. 2015. Deep transcriptome sequencing provides new insights into the structural and functional organization of the wheat genome. Genome Biol. 16 (1), 29.

Rahman, M.M., Asan, S.M., Liu, X., Chen, J., Zhao, J. 2017. Expression profiles of WUSCHEL-related homeobox gene family in pineapple (Ananas comosus L.) Trop. Plant Biol. 4 (10), 204–215.

Ramkumar, T.R., Raman, M., Upadhyay, S.K., Semb, J.K. 2018. Identification and characterization of WOX gene family: a conserved gene family that is important orchid species Phalaenopsis equestris and Dendrobium catenatum. Plant Gene 14, 37–45.

Raskar, A.K., Luitjen, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., Scheres, B., Heidstra, R., Veen, T. 2007. Genes are regulated by spatiotemporal expression in Arabidopsis thaliana shoot and root stem cell steers. Nature 446 (7137), 811–814.

Seo, J., Gordish-Dressman, H., Hoffman, E.P. 2006. An interactive power analysis tool for whole-genome association studies. Bioinformatics 22 (7), 808–814.

Sharma, A., Tha, A., S., Alok, S., Singh, K., Upadhyay, S.K., 2020. Thaumatin-like protein kinases: molecular characterization and transcriptional profiling in five cereal crops. Plant Sci. 290, 110317.

Shumayla, S., Sharma, S., Pandey, A.K., Singh, K., Upadhyay, S.K. 2016. Molecular characterization and global expression analysis of lectin receptor kinases in bread wheat (Triticum aestivum). PLoS One 11 (4), e0153925.

Shumayla, A., Tyagi, S., Sharma, A., Singh, K., Upadhyay, S.K. 2019. Genomic dissection and transcriptional profiling of Cysteine-rich receptor-like kinases in five cereals and functional characterization of TaCRK68-A. Int. J. Biol. Macromol. 134, 316–329.

Suyama, F., Torrons, D., Bork, P. 2006. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. Nucleic Acids Res. 34, W609–W612.

Taneja, M., H. J., Taneja, M., Tyagi, S., Singh, K., Upadhyay, S.K. 2020. Molecular characterization and differential expression suggested diverse functions of P-type II Ca2þ-ATPases in Triticum aestivum L. Front. Plant Sci. 17, 777.

Taneja, M., Tyagi, S., Sharma, S., Upadhyay, S.K. 2016. Ca2þ/cation antiporters (CaCA): identification, characterization and expression profiling in bread wheat (Triticum aestivum L.). Front. Plant Sci. 7, 1775.

Taneja, M., Tyagi, S., Sharma, S., Upadhyay, S.K. 2016. Ca2þ/cation antiporters (CaCA): identification, characterization and expression profiling in bread wheat (Triticum aestivum L.). Front. Plant Sci. 7, 1775.

Taneja, M., Tyagi, S., Upadhyay, S.K. 2018. Molecular characterization and differential expression suggested diverse functions of P-type II Ca2þ–ATPases in Triticum aestivum L. BMC Genom. 19 (1), 389.

Tyagi, S., Shumayla, A., Singh, K., Upadhyay, S.K. 2020. Molecular Characterization of Ascorbate Peroxidase (APX) and APX-Related (APX-R) Genes in Triticum aestivum L. Genomics. Tyagi, Shihv, Shumayla, Madhu, Singh, Kashmir, Upadhyay, Santosh Kumar, 2021. Molecular characterization revealed the role of catalases under abiotic and arsenic stress in bread wheat (Triticum aestivum L.). J. Hazard. Mater. 403, 123589.

Wang, M., Wang, R., Liu, M., Wang, X., Wang, J., 2014. The WUSCHEL-related homeobox (WOX) gene family in plant kingdom. Sci. World J. 2014.

Zhang, H., Yang, Y., Wang, C., Liu, M., Li, H., Fu, Y., Wang, Y., Nie, Y., Liu, J., Ji, W., 2014. Large-scale transcriptional comparisons reveals distinct gene activations in wheat responding to water stress and powdery mildew. BMC Genom. 15, 891.
Zhang, X., Zong, J., Liu, J., Yin, J., Zhang, D., 2010. Genome-wide analysis of WOX gene family in rice, sorghum, maize, Arabidopsis and poplar. J. Integr. Plant Biol. 52 (11), 1016–1026.

Zhang, Y., Wu, R., Qin, G., Chen, Z., Gu, H., Qu, L.J., 2011. Over-expression of WOX1 leads to defects in meristem development and polyamine homeostasis in Arabidopsis F. J. Integr. Plant Biol. 53 (6), 493–506.

Zhang, Y., Liu, Z., Khan, A.A., Lin, Q., Han, Y., Mu, P., Liu, Y., Zhang, H., Li, L., Meng, X., Ni, Z., Xin, M., 2016. Expression partitioning of homeologs and tandem duplications contribute to salt tolerance in wheat (Triticum aestivum L.). Sci. Rep. 6, 21476.

Zhao, Yu, Hu, Yongfeng, Dai, Mingqiu, Huang, Limin, Zhou, D.-X., 2009. The WUSCHEL-Related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. Plant Cell 21 (3), 736–748.

Zhao, S., Jiang, Q.T., Ma, J., Zhang, X.W., Zhao, Q.Z., Wang, X.Y., Wang, C.S., Cao, X., Lu, Z.X., Zheng, Y.L., Wei, Y.M., 2014. Characterization and expression analysis of WOX2 genes from wheat and its relatives. Gene 537 (1), 63–69.

Zhou, X., Guo, Y., Zhao, P., Sun, M., 2018. Comparative analysis of WUSCHEL-related homeobox genes revealed their parent-of-origin and cell type-specific expression pattern during early embryogenesis in tobacco. Front. Plant Sci. 9, 311.

Zuo, J., Niu, Q., Frugis, G., Chua, N., 2002. The WUSCHEL gene promotes vegetative-to-embryonic transition in Arabidopsis. Plant J. 30 (3), 349–359.