Genome-Wide Identification and Expression Profiling Analysis of WOX Family Proteins Encoded Genes in *Triticeae* Plant Species

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Abstract

Background: Genotype dependence of plant regeneration is an important factor restricting the genetic improvement of *Triticeae* plant species. The WUSCHEL-related homeobox (WOX) is a group of plant specific transcription factor, which play an important role in plant growth and development, and cell division and differentiation. Recent studies revealed that the application of regeneration-related genes such as *WOX* and *BABY BOOM* (*BBM*) could improve plant regeneration. The application of *WOX* genes is one of the ways to improve the genetic transformation system of *Triticeae* and other species, but there are rare studies in this area.

Results: From the available genome sequence database, in total 136 WOX transcripts were identified for the *Triticeae* plants, including 43 in *Triticum aestivum*, 30 in *Triticum turgidum*, 25 in *Triticum dicoccoides*, 17 in *Hordeum vulgare*, 13 in *Aegilops tauschii*, and 8 in *Triticum urartu*. All of the WOX family genes were distributed on the chromosomes of homologous groups 1 to 5 in the six *Triticeae* species, part of which were confirmed by their specific PCR markers using a set of *T. durum-T. aestivum* genome D substitution lines. All of the WOX proteins in the six *Triticeae* species could be grouped into three clades, similar to those in rice (*Oryza sativa* L.) and *Arabidopsis*. WOX family members were conserved among these *Triticeae* plants, all of them contained the conserved HOX DNA-binding homeodomain, and WUS clade members contained the characteristic WUS motif, while only TaWUS and TaWOX9 in all the six *Triticeae* plant species contained the ERF-associated amphiphilic repression (EAR) motif. The expression profiles of *TaWOX* genes by quantitative real-time PCR (qPCR) showed obvious difference among WOX family members.

Conclusions: Totally 130 *WOX* genes were identified in the six *Triticeae* plant species. The WOX family genes were located on the chromosomes in the five homologous groups except groups 6 and 7 in the *Triticeae* species, and their expression profiles were different in different tissues, indicating that each of them had diverse function. The findings in this study could provide a basis for evolution and functional investigation and practical application of the WOX family genes in *Triticeae* plant species.

Background

Triticeae tribe belongs to the *Poaceae* family, and is made up of more than 350 plant species in 30 genera around. In *Triticeae* plants, a series of species such as *Triticum aestivum* (bread wheat), *Hordeum vulgare* (barley), *Secale cereale* (rye), *Triticum urartu*, *Triticum dicoccoides*, and *Triticum turgidum* have been cultivated as crops and provides necessary nutrition for more than two billion people in the world [1, 2]. For a long time, people are interested in understanding the origination, genetic basis and evolution of *Triticeae* plants for their better improvement. Clear interpretation on genetic information of *Triticeae* plants may bring us closer to achieve the aforementioned objective. The assembling of wheat genome is a milestone in interpreting the genetic information of *Triticeae* plants. However, due to the large genome size of up to 16 Gb, the genomic study on wheat is legged behind of rice and maize [3]. The application of modern biotechnology tools such as transgene and gene editing in plant breeding can help us to increase yield, improve quality, and enhance biotic or abiotic resistance of major crops, but the realization of these aims depends on genetic transformation. The ability of regenerating new plantlets from *in vitro* tissues is a big limitation that restricts the application of genetic transformation and gene editing systems [4, 5].

Regeneration ability is one of important genetically physiological traits for most plants, which enables plants recover from wound tissues and form new organs. For modifying plants using genetic-engineering strategy, shoot or somatic embryo production from isolated tissues or cells is an indispensable step to achieve transgenic plants. But, it is still difficult to obtain regenerated plants in the process of genetic transformation from most genotypes (especially the extensively commercial varieties) of wheat and other *Triticeae* species [5–7]. During plant regeneration, a series of genes express in an orderly manner under the regulation of auxin and cytokinin. These regeneration-related genes

include *WUSCHEL-RELATED HOMEOBOX* (*WOX*),*AUXIN RESPONSE FACTOR (ARF)*, *BABY BOOM* (*BBM*), *SCARECROW* (*SCR*), *SHORT ROOT* (*SHR*), *PLETHORA* (*PLT*), *CUP-SHAPED COTYLEDON* (*CUC*), and *YUCCA* (*YUC*), which express during the progress of embryonic patterning, somatic embryogenesis, cell differentiation, wound reparation, and epigenetic reprogramming [5, 8–12]. An in-depth understanding of regeneration-related genes in molecular level will make it possible to break through the bottleneck in genetic transformation and build a more efficient transformation system with less genotype-dependent. The application of regeneration-associated genes including *WUS2* and *BBM* in crop transformation has achieved a great success, by which various maize inbred lines and tissues, and recalcitrant genotypes of *Indica* rice, sugarcane, and sorghum can be efficiently transformed for getting transgenic plants [13, 14].

The WOX family is a group of plant specific transcription factors and belongs to the homeobox (HB) transcription factor family [15]. All the identified *WOX* genes contain a conserved sequence of amino acids (60–66 residues), which is called as homeodomain (HD) encoded by the *HB* DNA sequence [16, 17]. The distinctive WUS-box motif forms as T-L-X-L-F-P-X-X(T-L-[DEQP]-L-F-P-[GITVL]-[GSKNTCV]), of which the consensus structure is TLELFPLH [15]. These homolog sequences fold into a DNA-binding domain. Update published data suggests that *WOX* genes act as pivotal regulators during the progress of embryonic development and polarization, plant growth and development, stem cell differentiation, embryo patterning, and flower development [18–22]. There are 15 WOX genes in *Arabidopsis thaliana*, 13 in rice, and 21 in maize [15, 23, 24]. In *Arabidopsis*, as a stem cell regulator, *AtWUS* expresses in the organizing-center (OC) cells in the shoot apical meristem and regulates plant growth and shoot stem cell maintaining [25, 26]. Ectopic overexpression of *WUS* genes promotes cell dedifferentiation in shoot meristem, somatic embryo formation, adventitious shoot and lateral leaf origination [26–28].

It is found that AtWOX1 possibly regulates the activity of S-adenosylmethionine decarboxylase polyamine homeostasis and/or the expression of *CLAVATA3*(*CLV3*), and has an important function in meristem development in *Arabidopsis*. Overexpression of *AtWOX1* leads to abnormal meristem development and polyamine homeostasis [29]. Normally, *AtWOX2* expresses in the zygote and early embryogenesis formation, and performs functions in correcting the apical domain development of the embryos [23]. AtWOX2 triggers the expression of *PINFORMED1* (*PIN1*), which is an auxin transport and localizes auxin to the cotyledonary tips of early embryo and root pole [18]. *AtWOX3* (*PRESSED FLOWER1, PRS1*) expresses in the peripheral layer of shoot meristem and regulates cells to form the lateral domain in vegetative and floral organs [30]. The expression of *AtWOX2* and *AtWOX3* are regulated by Leafy Cotyledon2 (LEC2), and AtWOX2 and AtWOX3 play essential roles in somatic embryogenesis [31]. *AtWOX4* expresses in a narrow domain in cambial cells, and AtWOX4 coordinating with PHLOEM INTERCALATED WITH XYLEM (PXY) acts as a key regulator for cambium activity in the main stem [32]. *AtWOX5* expresses in the QC of meristematic zone in root tips, regulates the columella stem cell (CSC) identity, and helps to maintain the root stem cell niche [33]. *AtWOX6* (*PRETTY FEW SEEDS2*, *PFS2*) expresses in developing ovules and primordials and differentiating organs, regulates ovule development, and affects differentiation and maturation of leaves, outer integuments and floral primordial [34]. *AtWOX7* expresses during all development stages of lateral root, but primarily involves in the initiation of lateral root [35]. *AtWOX8* (*STIMPY-LIKE*) and *AtWOX9* (*STIMPY*) are closely homologs [36, 37] and responsible for maintaining the normal development of both basal and apical embryo lineages at early development stage [18]. The expression of *AtWOX8* is induced by AtWRKY2 in the basal cell lineage at the initiation stage of embryogenesis [38]. *AtWOX11* plays a key role in the course of vascular cambium differentiation to new lateral root founder cells. *AtWOX11* is strongly induced expressed in de novo root organogenesis, which is the same as its homologous *AtWOX12* [39, 40]. *AtWOX13* expresses mainly in meristematic tissues to promote replum development and orchestrate fruit patterning [41]. *AtWOX14* is regulated by the CLAVATA3/ESRLIKE41/PHLOEM INTERCALATED WITH XYLEM (CLE41/PXY) pair, expresses in the procambium during stem maturation, and promotes xylem differentiation, vascular cell differentiation and lignification in inflorescence stems [42, 43].

Based on the phylogenetic analysis in *Arabidopsis*, plant WOX proteins are naturally divided into three clades: WUS and WOX1 to WOX7 in the WUS clade; WOX8, 9, 11, and 12 in the intermediate clade; and WOX10, 13, and 14 in the ancient clade [15]. But, the *WOX* genes in *Triticeae* plant species have not been fully identified and characterized yet. Therefore, the objectives of this study are, (1) identifying *WOX* genes in the six *Triticeae* plant species including *T. aestivum*, *T. turgidum*, *T. dicoccoides*, *H. vulgare*, *A. tauschii*, and *T. urartu*, and aligning them onto chromosomes; (2) dividing all of the WOX proteins in the six *Triticeae* species into groups by phylogenetic analysis using deduced protein sequences from all the *WOX* genes and the sequences of *OsWOX* genes from rice and *AtWOX* genes from *Arabidopsis*; and (3) analyzing the differential expression of *TaWOX* genes in different tissues by RNA sequencing (RNA-seq) and quantitative real-time PCR (qPCR). Our results would provide insights for further understanding the functions and evolution clarification of *WOX* family genes in *Triticeae* plants, and facilitate their application in gene transformation for the improvement of *Triticeae* plants.

Results

Identification of WOX genes in Triticeae plant species

Totally, 43 *TaWOX* transcripts were obtained using the recently released IWGSC wheat genome [3], and there were still 6 pseudo gene copies (Table 1). Specifically, 15 *WOX* transcripts in *H. vulgare* (Table 2), 13 *WOX* transcripts in *A. tauschii* (Table S1), 23 *WOX* transcripts in *T. dicoccoides* (Table S2), 28 *WOX* transcripts in *T. turgidum* (Table S3), and 8 *WOX* transcripts in *T. urartu* (Table S4) were identified from IWGSC genome database, respectively. Some homologous alleles of *WOX* genes were not annotated as transcripts in the database, but were also collected and listed in the tables. For example, *TaWUSb* and *TaWUSd* were located on chromosomes 2B and 2D in *T. aestivum*, respectively (Table 1). The *WUS* genes in other five *Triticeae* plant species were also located on their group 2 chromosomes (Table 2, Table S1-S4). *TdWOX12a*, *TdWOX12b*, *TdWOX7b* and *TdWOX13b* were located on chromosomes 1A, 1B, and 3B in *T. dicoccoides*, respectively (Table S2).

Table 1
Characteristics of *TaWOX* gene family members in *T. aestivum*.

Gene	Gene locus	Chromosome	Gene stretch region	mRNA length (bp)	Protein Sequence Length (aa)	UniProt ID
TaWOX2a	TraesCS1A02G052000	1A	33,397,501 – 33,398,955:-1	1314	263	A0A1D5S1T3
TaWOX12a	TraesCS1A02G399400	1A	563,818,671 – 563,823,103:1	1854	486	A0A1D5RPD4
TaWOX2b	TraesCS1B02G069000	1B	53,364,615 – 53,365,864:-1	1119	264	A0A1B1XWM5 A0A1B1XWM7 W5ABB5
TaWOX12b	TraesCS1B02G427400	1B	652,781,930 – 652,786,496:1	1983	485	A0A1D5SDQ8
TaWOX2d	TraesCS1D02G054000	1D	35,059,826 – 35,061,088:-1	1138	267	W5ANF9
TaWOX12d	TraesCS1D02G406900	1D	470,219,711 – 470,224,514:1	2028	486	A0A1D5SWV6
TaWUS	TraesCS2A02G491900	2A	724,513,458– 724,514,647:1	927	308	A0A1D5TC72
TaWOX4a	TraesCS2A02G514000	2A	738,371,677– 738,372,966:1	1061	234	A0A1D5TF70
TaWOX11a	TraesCS2A02G100700	2A	53,782,606 – 53,785,288:1	1380	265	A0A1D5TJV0
TaWOX11b	TraesCS2B02G117900	2B	81,755,546 – 81,758,516:1	1366	261	A0A1D5U6K9
TaWOX4b	TraesCS2B02G542600	2B	740,320,190– 740,321,561:-1	1002	237	W5BBK8
TaWOX11d	TraesCS2D02G100200	2D	52,227,203 – 52,229,885:1	1379	264	A0A1D5TJV1 A0A1D5V0E6
TaWOX4d	TraesCS2D02G515600	2D	606,709,221– 606,710,431:1	979	237	A0A1D5UH04
		2D	590146287– 590147498:1			
TaWOX10a	TraesCS3A02G073500	3A	45,776,166 – 45,777,448:1	992	260	A0A1D5VKG7
TaWOX7a	TraesCS3A02G247200	3A	465,225,214– 465,228,773:1	1968	515	A0A1D5V4S9
TaWOX8a	TraesCS3A02G341700	3A	588,932,808 – 588,937,056:1	2230	265	A0A077RTA5 A0A1D5VD81 A0A1D5VQY0 A0A1D5WIZ6 A0A1D6RQB3 A0A1D6RQB4 A0A1D6RQB5 W5CGX8

Gene	Gene locus	Chromosome	Gene stretch region	mRNA length (bp)	Protein Sequence Length (aa)	UniProt ID
TaWOX14.1a	TraesCS3A02G358200	3A	606,515,981 – 606,519,197:-1	1162	288	A0A1D5VFV1
TaWOX13a	TraesCS3A02G358100	3A	606,444,775 – 606,446,830:-1	1138	301	A0A1D5VA42
TaWOX14.2a	TraesCS3A02G358400	3A	606,573,438– 606,576,220:-1	1133	290	A0A1D6RQ92
TaWOX9a	TraesCS3A02G368100	3A	617,060,395– 617,061,453:-1	949	212	T1WFN3
TaWOX10b	TraesCS3B02G087800	3B	56,055,903 – 56,057,760:-1	1196	261	A0A1D5VWS6
TaWOX7b	TraesCS3B02G272200	3B	438,378,936 – 438,382,259:-1	1776	515	A0A077RSZ6
TaWOX8b	TraesCS3B02G373800	3B	586,694,870 – 586,698,391:1	1216	261	A0A077S168 A0A1D5WT92
TaWOX13b	TraesCS3B02G391100	3B	616,425,121– 616,426,978:-1	900	299	A0A1D5VST7
TaWOX14b	TraesCS3B02G391200	3B	616,645,332– 616,647,892:-1	1216	290	A0A1D5WB93
TaWOX9b	TraesCS3B02G399800	3B	631,036,656 – 631,037,718:-1	948	209	D8L9N7
TaWOX10d	TraesCS3D02G073300	3D	33,294,918 – 33,295,992:1	786	261	A0A077RHG9 A0A1D5WSB5 A0A341T564
TaWOX7d	TraesCS3D02G244300	3D	339,473,290– 339,476,679:-1	1834	513	A0A1D5WHW6
TaWOX8d	TraesCS3D02G335500	3D	447,560,283– 447,562,999:1	792	263	A0A1D5VD82 A0A341TAX4
TaWOX13d	TraesCS3D02G352500	3D	463,197,196– 463,199,275:-1	1112	298	A0A1D5WMN9
TaWOX14.1d	TraesCS3D02G352600	3D	463,227,796 – 463,230,501:-1	895	285	A0A1D5WPP9
TaWOX14.2d	TraesCS3D02G352700	3D	463,378,560 – 463,381,808:-1	942	291	A0A1D5WNX7
TaWOX9d	TraesCS3D02G361100	3D	474,614,857 – 474,615,873:-1	901	210	T1WGQ3
TaWOX6a	TraesCS4A02G130200	4A	170,708,103– 170,711,065:-1	1350	307	A0A341TSN5
TaWOX6b	TraesCS4B02G174400	4B	382,691,977 – 382,694,806:1	1254	309	A0A1D5XNI6 A0A1D5Y4Y9
TaWOX6d	TraesCS4D02G176400	4D	306,795,298– 306,798,208:1	1262	306	A0A341UK30

Gene	Gene locus	Chromosome	Gene stretch region	mRNA length (bp)	Protein Sequence Length (aa)	UniProt ID
TaWOX5a	TraesCS5A02G085000	5A	111,588,730 – 111,590,895:1	1220	318	A0A341UT17
TaWOX3a	TraesCS5A02G157300	5A	336,949,988 – 336,951,183:1	1060	241	A0A1D5YD57
TaWOX5b	TraesCS5B02G091000	5B	118,451,983 – 118,454,221:1	1302	321	A0A1D5ZG91 A0A1D6A0K9
TaWOX3b	TraesCS5B02G156400	5B	288,891,901 – 288,893,003:-1	968	241	W5F9A2
TaWOX5d	TraesCS5D02G097400	5D	108,103,399 – 108,105,722:1	1381	322	W0Z680
TaWOX3d	TraesCS5D02G162600	5D	254,023,305 – 254,024,410:1	1006	242	W5FQU4
TaWOX8u	TraesCSU02G204800	Un	304,503,012– 304,503,827:1	617	156	A0A077RQB3 A0A096UQ47 A0A1D6RTL8 A0A1D6RTL9
TaWUSb		2B	714,777,526– 714,778,733:1	921	306	
TaWUSd		2D	590,146,287– 590,147,498:1	927	308	
		1D	6,219,571- 6,220,231:1			
		3A	64,319,914 – 64,325,218:-1			
		3B	83,465,544 – 83,470,232:-1			
		3B	83,471,253 – 83,471,941:-1			
		3D	52,801,752 – 52,812,298:-1			
		3D	463,261,309– 463,261,744:-1			

Table 2
Characteristics of *HvWOX* gene family members in *H. vulgare*

Gene	Gene locus	Chromosome	Gene stretch region	mRNA length (bp)	Protein Sequence Length (aa)	Uniprot ID
HvWOX2	HORVU1Hr1G010580	1H	24,444,001 – 24,445,742:1	1742	279	A0A287ELV0
HvWOX12	HORVU1Hr1G087940/50	1H	540,693,806 – 540,698,431:-1	1470	489	A0A287GM87 A0A287GM65
HvWOX11	HORVU2Hr1G017270	2H	40,107,707 – 40,111,565:1	927	308	A0A287H773
HvWOX4	HORVU2Hr1G113820	2H	729,806,496– 729,808,073:1	1151	228	A0A287JHP1
HvWOX10.1	HORVU3Hr1G013290	3H	28,673,837 – 28,674,948:-1	786	261	M0Y8G7
HvWOX10.2	HORVU3Hr1G013330	3H	28,785,048 – 28,786,156:-1	815	261	A0A287K575
HvWOX7	HORVU3Hr1G060950	3H	464,417,446– 464,421,050:1	2027	516	A0A287L9L2
HvWOX8.1	HORVU3Hr1G080660	3H	589,829,423– 589,834,968:-1	3229	267	M0 × 0 × 0
HvWOX8.2	HORVU3Hr1G080690	3H	590,115,430– 590,116,290:1	584	130	A0A287LWD8
HvWOX9	HORVU3Hr1G085050	3H	610,834,437– 610,835,788:-1	1165	209	F2E473
HvWOX14	HORVU3Hr1G086430	3H	616,993,938 – 616,996,482:-1	1216	283	M0XTJ6
HvWOX13	HORVU3Hr1G086450	3H	617,085,484– 617,087,698:1	824	274	A0A287M365
HvWOX6	HORVU4Hr1G051530	4H	423,508,136– 423,511,456:-1	1710	306	M0Y4Z0
HvWOX5	HORVU5Hr1G022120	5H	111,001,136 – 111,003,388:1	1046	276	A0A287QMF0
HvWOX3	HORVU5Hr1G049190	5H	381,765,625 – 381,766,908:1	1126	186	A0A287R4V3
HvWUS		2H	717,822,805 – 717,905,740:-1	942	313	

Identification of WUS homologous genes in Triticeae **plant species**

In the six *Triticeae* plant species, only one transcript of *WUS* gene was annotated as *TaWUSa* on chromosome 2A in wheat in the database (Table 1). We found the homologous fragments of *TaWUSa* on chromosomes 2B and 2D in wheat (Table 1), 2D in *A. tauschii* (Table S1), 2A and 2B in *T. dicoccoides* and *T. turgidum* (Tables S2 and S3), and 2H in barley (Table 2). According to the results of multiple sequence alignment, the full length of the open reading frame (ORF) of these homologous genes can be achieved, and their deduced amino acid sequences were highly consistent

with *TaWUS* (Fig. 1A). To understand if these genes can normally transcribe and express, promoter analysis was performed. It was showed that the promoter region of the *WUS* genes in the six *Triticeae* plant species all contained core promoter elements including transcription start TATA-box and AT ~ TATA-box,indicating they possessed potential transcriptional activity (Fig. 1B). In the promoter region of *TaWUSa*, *TdWUSa*, *TtWUSa*, and *TuWUS*, a fragment of GGTCCAT was existed, which is a cis-acting regulatory element involved in auxin responsiveness. Nevertheless, this element was not detected in the promoter of *AtaWUS*, *TaWUSb*, *TaWUSd*, *TdWUSb*, and *TtWUSb*.

Chromosomal location of WOX **genes in** Triticeae **plant species**

In general, no *WOX* gene was found on homologous groups 6 and 7 for the genomes of the six *Triticeae* plant species, i.e., *T. aestivum*, *T. turgidum*, *T. dicoccoides*, *H. vulgare*, *A. tauschii*, and *T. urartu*, (Tables 1 and 2, and Tables S1-S4). In *T. aestivum*, except *TaWUS*, all the *TaWOX* genes had three copies of transcripts on its genomes A, B, and D. Three homologous alleles of *TaWUS* were located on chromosomes 2A, 2B, and 2D. The homologous genes of *TaWOX2* or *TaWOX12* were located on chromosomes 1A, 1B, and 1D. Three copies of *TaWOX4* or *TaWOX11* were located on chromosomes 2A, 2B, and 2D. The three homologous genes of *TaWOX7* to *TaWOX10*, *TaWOX13* and *TaWOX14* were all located on chromosomes 3A, 3B, and 3D. The three alleles of *TaWOX6* were located on chromosomes 4A, 4B, and 4D. The three alleles of *TaWOX3* or *TaWOX5* were located on chromosomes 5A, 5B, and 5D. Further investigation would be needed for the unknown chromosomal location of an incomplete transcript of *TaWOX8*. No *WOX* gene was found on homologous groups 6 and 7 (Table 1, Fig. 2A). The *HvWOX* genes in *H. vulgare* showed the similar chromosomal localization to the *TaWOX* genes in *T. aestivum* and *AtaWOX* genes in *A. tauschii*. *HvWOX2* and *HvWOX12* were located on chromosome 1H; *HvWOX4* and *HvWOX11* were located on chromosome 2H; *HvWOX7* to *HvWOX10*, *HvWOX13*, and *HvWOX14* were located on chromosome 3H; *HvWOX6* was located on chromosome 4H, and *HvWOX3* and *HvWOX5* were located on chromosome 5H. (Table 2; Fig. 2B). There are additional copies of *HvWOX8* and *HvWOX10* on chromosome 3H. The *HvWOX10.1* and *HvWOX10.2* showed complete sequence consistency, but *HvWOX8.2* was shortened compared with *HvWOX8.1*.

Similar situation was observed in *A. tauschii*. *AtaWOX2* and *AtaWOX12* were located on chromosome 1D. *AtaWOX4* and *AtaWOX11* were located on chromosome 2D. *AtaWOX7* to *AtaWOX10*, *AtaWOX13*, and *AtaWOX14* were all located on chromosome 3D. *AtaWOX6* was located on chromosome 4D, *AtaWOX3* and *AtaWOX5* were located on chromosome 5D (Table S1, Fig. S1A). Similar results were also obtained in *T. dicoccoides* and *T. turgidum*. As expected, all the *TdWOX* and *TtWOX* genes were located on the corresponding chromosomes of their genomes A and B because the two species only have the two genomes (Table S2, Table S3, Fig. S1B, Fig. S1C). Additional copies of *TdWOX8a* and *TtWOX14a* were also existed on the corresponding chromosomes.

To verify the chromosomal locations of those *WOX* genes in the six *Triticeae* species, partial sequences of some of the *WOX* genes were amplified by their specific primers using a set of *T. durum-T. aestivum* genome D substitution lines (Fig. 3). The *TaWUSa* and its two homologs (named as *TaWUSb* and *TaWUSd*) were detected in *T. aestivum* L. *cv* CS (ABD genome), *T. durum cv* Langdon (AB genome), and other substitution lines except 2D(2A), indicating that the two copies *TaWUSa* and *TdWUSa* were located on chromosome 2A. *TaWUSb* was amplified in CS, Langdon, and other substitution lines except 2D(2B), indicating that *TaWUSb* was located on chromosome 2B. *TaWUSd* only appeared in CS, 2D(2A) and 2D(2B), indicating that it was located on chromosome 2D (Fig. 3). Similarly, *WOX2a*, *WOX2b, WOX6a*, and *WOX6b* were absent in 1D(1A), 1D(1B), 6D(6A), and 6D(6B), respectively. *WOX2d* and *WOX6d* were only detected in CS and the substitution lines which contain chromosome 1D or 4D (Fig. 3).

Evolution of WOX family proteins in Triticeae **plant species**

Phylogenetic trees of WOX family proteins in *Triticeae* species were constructed based on the deduced protein sequences. From the phylogenetic trees, it was suggested that WOX proteins in *Triticeae* plants were also divided into three clades, like those in many other plant species [44, 45]. However, the WOX protein classification in wheat was closer to that in rice in comparison with that in *Arabidopsis*. TaWUS, TaWOX2 to TaWOX5, TaWOX9, TaWOX13, and TaWOX14 were assigned to the same clade with the homologous proteins in rice, corresponding to *Arabidopsis* WUS clade (AtWUS and AtWOX1 to AtWOX7). TaWOX6, TaWOX7, and TaWOX10 to TaWOX12, and their homologous proteins from rice were classified into a clade, corresponding to an *Arabidopsis* intermediate clade (AtWOX8, 9, 11, and 12). TaWOX8 and OsWOX8 were clustered in separated branches, showing correspondence to an *Arabidopsis* ancient clade (AtWOX10, 13, and 14) (Fig. 4).

Barley WOX proteins were also divided into three clades: the first clade harbored HvWOX2, 3, 5, 9, 13 and 14; the second clade was for HvWOX8 only; and the third clade included HvWOX6, 7, and 10 to 12 (Fig. S2A). Similar to wheat, one branch in *A. tauschii* contained AtaWOX2 to AtaWOX5, 9, 13 and 14. AtaWOX6, 7, and 10 to 12 were clustered into the same branch, but AtaWOX8 was belonged to another branch alone (Fig. S2B). In *T. turgidum*, TtWOX proteins were also divided into three clades: TtWOX2 to TtWOX5, 9, 13 and 14 were in the first branch; TtWOX6, 7, and 10 to 12 were in the second branch; and the three copies of TtWOX8 were clustered into the same group with OsWOX8 (Fig. S2C). In *T. dicoccoides*, TdWOX2 to TdWOX5, 9, 13 and 14 were clustered in one branch, TdWOX8 was in other branch alone, and TdWOX6, 7, and 10 to 12 were in another branch (Fig. S2D). In *T. urartu*, only eight sequences coding WOX family proteins were retrieved because there was no complete genome information on *T. urartu* yet. The deduced protein sequences from gene sequences of *TuWOX* and *OsWOX* were used to construct a phylogenetic tree, in which TuWOX2, 5, and 9 were grouped together, and TuWOX10 and TuWOX6/11 were in the same branch, and the two homologous sequences of TuWOX8 were clustered together (Fig. S2E).

The phylogenetic tree of the WOX family proteins from the six *Triticeae* species was also constructed via maximum likelihood method (Fig. 5). Based on the tree, it was clearly seen that the WOX proteins with the same names from the six *Triticeae* species were clustered together (Fig. 5), indicating that the WOX proteins were conserved in these plant species.

Analysis for the conserved motifs of WOX proteins in Triticeae species

All the amino acid sequences of WOX proteins in the six *Triticeae* species were deduced from their transcripts mentioned above. Each member contained HOX homeodomain, which were the most noteworthy symbol and defining feature of this protein family (Fig. 6, Fig. S3). Sequences of HOX homeodomain of the three clades of WOX proteins were conserved in the six *Triticeae* species (Fig. 7A). The conserved WUS-box motif TLXLFPXX (TL-[DEQP]-LFP-[GITVL]-[GSKNTCV]) was found in TaWUS, WOX2 to WOX5, and WOX9 in these *Triticeae* species (Fig. 6A, Fig. 7B). While, there was one amino acid residue change in ELXLFPXX of TaWUS and LLXLFPXX of WOX13 and WOX14 in the *Triticeae* species (Fig. 7B). The carboxy-terminal ERF-associated amphiphilic repression (EAR) domain of L-[ED]-L-[RST]-L only exists in WUS and WOX9 (Fig. 6A), and EAR domain of WOX9 in these *Triticeae* species showed highly conserved (Fig. 7C).

Expression patterns of TaWOX genes in different organs of wheat

The *WOX* genes mainly expressed in the meristematic region, and played a regulatory role in the process of plant growth and tissue differentiation. We retrieved the data from expVIP website (http://wheat-expression.com), and sketched the contours of expression pattern of *TaWOX* genes. It is showed that *TaWUS* expressed in root during seedling stage, in spike during vegetative stage, and in spike and leave/shoot during productive stage. Its expression level was higher in spike than other organs (Fig. S4A). All the three homologous of *TaWOX2* to *4*, *7*, *8*, and *12* showed higher expression

level in developing spike than other organs, and even higher at vegetative stage than reproductive stage (Fig. S4B-D, G, H, and L). The expression level of *TaWOX5* was higher in grain than that in other organs at reproductive stage (Fig. S4E). *TaWOX6*, *9* to *11* showed a high transcriptional activity in root (Fig. S4F, I-K). The transcripts of *TaWOX10* and *TaWOX11* mainly accumulated in root at seedling stage while the expression level of *TaWOX9* was high in root at vegetative stage (Fig. S4I-K). The transcript levels of *TaWOX6b* and *TaWOX6d* in root were increased at productive stage compared with vegetative stage (Fig. S4F).

Further, we used wheat root, stem, leave, spike at booting stage, and anther at heading stage as well as immature embryo, callus derived from the immature embryos at proliferative and differential stages as materials to perform expression profiling analysis of *TaWOX* genes by qPCR assay. The results indicated that expression patterns of *TaWOX* genes changed greatly in different organs at different stages (Fig. 8). The expression levels of *TaWUS* and *TaWOX6* to *8* were relative high in spike (Fig. 8A, B), and the expression levels of *TaWOX9* and *TaWOX11* were high in root (Fig. 8B, C). Additionally, *TaWOX2* showed high activity in embryo, and *TaWOX3* and *TaWOX4* showed high expression levels in embryogenic callus and differential callus, respectively (Fig. 8A).

Discussion

In *Triticeae* plant species, wheat and barley are two important crops globally, which account for a large proportion of food production in the world. With the completion of assemble and annotation of the colossal wheat genome, a great progress on functional genomic study in *Triticeae* plants, especially in wheat, has been achieved [46–49]. It is well-known that wheat genome was originated from the natural hybridization of its three ancestor species. Therefore, wheat genome consisting of three genomes of A, B, and D has a large number of repeated gene sequences, and most wheat genes have three or more copies [50]. In present study, we identified 43 *WOX* gene copies in the genome of *T. aestivum*, 42 of which was consistent with the result reported by Li et al. [51], and a new locus of *TaWOX8* was added to the results of TaWOX family. Particularly, we firstly identified 17 *WOX* genes in *H. vulgare*, 13 in *A. tauschii*, 30 in *T. turgidum*, 25 in *T. dicoccoides*, and 8 in *T. urartu*. There were still several duplicated copies of *WOX* gene such as *TaWOX14a, TaWOX14d, HvWOX10*, and *TdWOX14*. A few of *WOX-like* pseudo genes were found to be scattered over *Triticeae* genomes, which might be a duplication of *WOX* genes or the other genes losing transcriptional activity during their evolution progress.

WUS plays an indispensable role on the stem cell niche maintenance in shoot apical meristem (SAM), lateral primordia differentiation and other diverse cellular processes [26]. The deficiency of *WUS* gene will lead to the loss of function of SAM and terminated plant growth [25]. However, only the allele of *TaWUS* located on chromosome 2A was annotated as a transcript. *TdWOX12a*, *TdWOX12b*, *TdWOX7b* and *TdWOX13b*, which have a high sequence identity with their homologous genes from wheat, were also not annotated as transcripts in the database. The DNA sequences and deduced protein sequences of four genes *TdWOX12a*, *TdWOX12b*, *TdWOX7b*, and *TdWOX13b* were added into the WOX members in the six *Triticeae* species (Table S2). In barley, the annotation of *HORVU1Hr1G087940* and *HORVU1Hr1G087950* and their deduced protein sequences A0A287GM87 and A0A287GM65 are actually originated from *HvWOX12* (Table 2).

In previous studies, the classification and naming of *WOX* genes in wheat were confused to some extent. This might be attributed to the different naming scheme of *WOX* genes in *Arabidopsis* and rice [15, 23, 24]. For example, the *TaWOX5* reported by Zhao et al. [52] were regarded as *TaWOX9* due to their highly similarity to *OsWOX9*, even though it showed a close similarity to *AtWOX5* in all the WOX members in *Arabidopsis* (Fig. 4). Several reported *TaWOX* members such as *TraesCS3A02G358100*, *TraesCS3B02G391100*, *TraesCS3D02G352500*, *TraesCS3A02G358200*, *TraesCS3A02G358400*, *TraesCS3B02G391200, TraesCS3D02G352600*, and *TraesCS3D02G352700* on chromosomes 3A, 3B, and 3D, respectively, were named as *TaWOX13* and *TaWOX14* [51] according to new nomination regulation. However, *TaWOX13*

was not similar to *AtWOX13* or *OsWOX13*, and *AtWOX14* was also not similar to *AtWOX14* in transcripts. While, *TaWOX13* and *TaWOX14* were similar to the homologs of *TaWOX5* according to phylogenic analysis (Fig. 4). The WOX13 and WOX14 in other *Triticeae* species showed the similar phylogenetic relationship with WOX5 members (Fig. 5).

All the *TaWOX* genes in wheat have three or more copies. Due to their sequence similarity, it is difficult to distinguish the expression level of each copy of *TaWOX* genes. A feasible approach was applied to estimate the amount of mRNA by calculating transcript amount of each copy. Zhao et al. indicated that the transcriptional level of individual *TaWOX5* allele was varied during the period of callus growth in wheat [52]. Based on the results in the present investigation, the expression profiles of other *WOX* alleles were also changed in different wheat organs, which need to be justified by further research.

Conclusions

To our knowledge, this is the first study on genome-wide and contrastive analysis on *WOX* family genes in *Triticeae* plant species. In total, 130 *WOX* genes were identified, including 43 in *T. aestivum*, 28 in *T. turgidum*, 23 in *T. dicoccoides*, 15 in *H. vulgare*, 13 in *A. tauschii*, and 8 in *T. urartu*. The homologous genes of *TaWUSb*, *TaWUSd*, and *WUS* in other five *Triticeae* species were annotated, which were predicted to express normally according to promoter element analysis. Four novel homologous alleles of *TaWOX* genes including *TdWOX12a*, *TdWOX12b*, *TdWOX7b*, and *TdWOX13b* were also identified in *T. dicoccoides*. All of these WOX members showed evolutionary conservation and same chromosomal location arrangement. Based on the RNA-seq data in wheat-expression database and qPCR array results, *TaWOX* genes were found to have tissue-specific expression feature. The results showed in this study would be helpful to further understand the molecular function and evolutionary relationship of *WOX* family genes in *Triticeae* plants, and potentially apply them in plant genetic transformation in the future.

Methods

Materials and cultivation conditions

Wheat line Chinese Spring (CS) stored in our laboratory was used as the plant material to conduct gene identification and expression analyses. A set of *T. durum-T. aestivum* genome D substitution lines and their genetic background Langdon (LD), which were kindly provided by Dr. Steven Xu at the Northern Plains Crop Science Laboratory of the USDA-ARS, North Dakota, USA, and genetically identified by Prof. Zhishan Lin at the institute of Crop Sciences (ICS), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China, were used to verify the chromosomal localization of the *WOX* genes identified in this study. In each of these disomic substitution lines, a pair of A-genome or B-genome chromosome in the tetraploid wheat *T. durum* was replaced by a corresponding pair of D-genome chromosomes from *T. aestivum.* For example, in substitution line 1D(1A) chromosome 1D from *T. aestivum* D replaces the chromosome 1A in *T. durum*. Thirty seeds of those wheat materials were planted as a trail with 1 m in length and 20 cm in width in the experimental station of ICS, CAAS, Beijing, China, under natural soil conditions without stress.

Rna Isolation And Qpcr Analysis

The wheat samples for roots, stems, and leaves were collected at three-leaf stage, for young spikes at booting stage, and for anthers at heading stage. The immature embryo samples were collected 15 days post anthesis (DPA). Callus samples were induced from the immature embryos on MS medium containing 2,4-D 2.0 mg L^{-1} under dark condition

and collected after cultured for one week and two weeks, respectively. The calli were cultured for differentiation on 1/2 MS medium containing 5.0 mg L^{-1} Zeatin in a photoperiod of 14 h-light and 10 h-darkness and sampled one week later.

Total RNA was extracted using TRIzol™ Reagent Kit (Invitrogen 15596026), and reverse transcription reaction was performed using the PrimeScript™ RT reagent (Takara) according to the manufacturer's protocol. The qPCR was performed on ABI7500 Thermal Cycler using 2 × RealStar Green Fast Mixture (with ROX II, Genestar). *TaActin* (Genbank: AB181991) was used as internal controls, and three biological replicates were adopted. Gene-specific primers were designed with premiere primer 6.0 (Table S5). Each qPCR reaction system (20 μL) contained 10 μL of 2 × RealStar Green Fast Mixture, 0.4 μL of forward primer (10 mM), 0.4 μL of reverse primer (10 mM) and 1 μL of diluted cDNA (200 ng μL^{-1}). The thermal cycling conditions were 95 ℃ for 5 min, 40 cycles of amplification (95 ℃ for 15 s, 60 ℃ for 15 s, and 72 ℃ for 30 s), and 95 ℃ for 10 s at dissociation stage, followed by 65–95 ℃ with increments of 0.5 ℃ for 0.05 s.

Database used for searching WOX **family genes in** Triticeae **plants**

Twenty-six predicted WOX family protein sequences of wheat were obtained from Plant TFDB database (http://planttfdb.cbi.pku.edu.cn), and retrieved Genbank (https://www.ncbi.nlm.nih.gov/genbank) with AtWOX of *Arabidopsis*, OsWOX of rice, and ZmWOX of maize (*Zea mays*). Using all of the protein sequences above as queries to conduct TBLASTN search on Gramene (http://ensembl.gramene.org/Tools/Blast) and URGI (https://urgi.versailles.inra.fr) for the identification of WOX proteins encoded genes in wheat genomes. Then, BLASTN search with sequences of *TaWOX* genes was performed in the genomes of *H. vulgare*, *T. urartu*, *T. dicoccoides*, *T. turgidum*, and *A. tauschii.* All the genetic analysis was carried out using these protein sequences of the six *Triticeae* plants. Based on the BLAST results from Gramene and URGI, the *WOX* genes from the *Triticeae* plants were located on exact chromosomes. The location chart was made by MapGene2Chrom web v2.1 (http://mg2c.iask.in/mg2c_v2.1/).

Dna Isolation And Pcr Analysis

Wheat genomic DNA was isolated by NuClean Plant Genomic DNA kit (Cwbio, CW0531M) from the leaf samples at three-leaf stage. PCR reaction system (20 μL) contained 10 μL of 2 × Taq Master Mix (containing Mg^{2+} and dNTP, Vazyme, China), 0.5 μL of each forward primer and reverse primer (10 mM), and 1 μL of gDNA (1 μg μL^{-1}), adding ddH_2O up to 20 °C. Sequences of all the primers used for the detection were shown in Table S6. The thermal cycling conditions were 94 ℃ for 5 min, 35 cycles of 94 ℃ for 20 s, 60 ℃ for 20 s, 72 ℃ for 30 s, and then 72 ℃ for 10 min.

Phylogenetic Trees Construction

The full-length of the WOX proteins of *Triticeae* species were aligned by ClustalW algorithm. Phylogenetic analysis and phylogenetic tree construction were performed by the MEGA X program (https://www.megasoftware.net/) using maximum approach and 1000 bootstrap replicates. Sequences of TaWOX proteins were aligned with AtWOX and OsWOX proteins, and phylogenetic tree was constructed to confirm classification and phylogenetic relationship of the identified TaWOX members. Then taking OsWOX proteins as model, the phylogenetic trees were constructed between OsWOX and HvWOX, OsWOX and TdWOX, OsWOX and TtWOX, OsWOX and AtaWOX members to name and classify the WOX members in the six *Triticeae* species.

Conserved Protein Motif Analysis

The conserved domain HD, was identified by SMART software (http://smart.embl-heidelberg.de/). The distinctive WUS-box motif as TLXLFPXX(T-L-[DEQP]-L-F-P-[GITVL]-[GSKNTCV]) and the EAR domain as LXLXL(L-[ED]-L-[RST]-L) were both defined in a strict sense. TEXshade software was employed to perform the multiple sequence alignments for HD domains, WUS-box motifs, and EAR motifs. The logo diagrams were drawn by canonical conserved residues including HD domains, WUS-box motifs, and EAR motifs by SeqLOGO in TBTools.

Expression analysis of TaWOX genes using RNA-seq data

RNA-seq data of 43 *TaWOX* genes was downloaded from expVIP (http://wheat-expression.com/). The expression level in root and leaves/shoot at seedling stage, in root and leaves/shoot spike at vegetative stage, and in root, spike, grain at vegetative stage were analyzed and compared.

Statistical analysis

The SPSS 19.0 software package was employed to statistically analyze the expression data of the target genes achieved by qPCR. Statistical comparisons of multiple sets of data was carried out by Duncan's multiple range test. The histogram was made using the Excel software.

Abbreviations

ARF: Auxin response factor; BBM: BABY BOOM; CLE41: CLAVATA3/ESRLIKE41; CLV3: CLAVATA3; CS: Chinese Spring; CSC: columella stem cell; CUC: CUP-SHAPED COTYLEDON; DPA: days post anthesis; EAR: ERF-associated amphiphilic repression; HB: homeobox; HD: homeodomain; LEC2: LEAFY COTYLEDON2; OC: organizing-center; ORF: open reading frame; PFS2: PRETTY FEW SEEDS2; PIN1: PINFORMED1; PLT: PLETHORA; PRS1: PRESSED FLOWER1; PXY: PHLOEM INTERCALATED WITH XYLEM; qPCR: quantitative real-time PCR; RNA-seq: RNA sequencing; SAM: shoot apical meristem; SCR: SCARECROW; SHR: SHORT ROOT; WOX: WUSCHEL-related homeobox; YUC:YUCCA.

Declarations

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Availability of data and material

All data used or analyzed in this study are included in this published article and additional files. Twenty-six predicted WOX family protein sequences of wheat could be downloaded from Plant TFDB database (http://planttfdb.cbi.pku.edu.cn). Genome sequences and annotation of *WOX* genes in the six *Triticeae* species could be downloaded from Gramene (http://ensembl.gramene.org/Tools/Blast) and URGI (https://urgi.versailles.inra.fr). Transcriptome data used for gene expression analysis could be downloaded from expVIP (http://wheat-expression.com/).

Authors' contributions

The experiment was conceived by XY and HL. LS and HL analyzed the data, KW and XS assisted with bioinformatics analysis. LS performed the PCR and qPCR experiments. The manuscript was drafted by LS, XY and HL, and corrected and approved by all authors.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. FAOSTAT (2020) http://www.fao.org/faostat/en/, Accessed date: 10 Augest 2020.
2. Feuillet C, Muehlbauer GJ. Genetics and Genomics of the *Triticeae*. Springer, 2009.
3. IWGSC, et al. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science. 2018; 361.
4. Singh RK, Prasad M. Advances in *Agrobacterium tumefaciens*-mediated genetic transformation of graminaceous crops. Protoplasma. 2016; 253: 691–707.
5. Wang K, Gong Q, Ye X. Recent developments and applications of genetic transformation and genome editing technologies in wheat. Theor Appl Genet. 2020; 133: 1603–1622.
6. Ye X, Shirley S, Xu H, Du L, Clement T. Regular production of transgenic wheat mediated by *Agrobacterium tumefaciens*. Agri Sci China. 2002; 1: 239–244.
7. Wang K, Liu H, Du L, Ye X. Generation of marker-free transgenic hexaploid wheat via an *Agrobacterium*-mediated cotransformation strategy in commercial Chinese wheat varieties. Plant Biotechnol J. 2017; 15: 614–623.
8. Kareem A, Durgaprasad K, Sugimoto K, Du Y, Pulianmackal AJ, Trivedi ZB, et al. PLETHORA Genes Control Regeneration by a Two-Step Mechanism. Curr Biol. 2015; 25: 1017–1030.
9. Kumar V, Jha P, Van Staden J. LEAFY COTYLEDONs (LECs): master regulators in plant embryo development. Plant Cell Tiss Org. 2020; 140: 475–487.
10. Pan J, Zhao F, Zhang G, Pan Y, Sun L, Bao N, et al. Control of de novo root regeneration efficiency by developmental status of *Arabidopsis* leaf explants. J Genet Genomics. 2019; 46: 133–140.
11. Xu J, Hofhuis H, Heidstra R,1 Sauer M, Friml J, Scheres B. A molecular framework for plant regeneration. Science.2006; 311: 386–388.
12. Xu L. De novo root regeneration from leaf explants: wounding, auxin, and cell fate transition. Curr Opin Plant Biol. 2018; 41: 39–45.
13. Lowe K, Wu E, Wang N, Hoerster G, Hastings C, Cho MJ, et al. Morphogenic regulators Baby boom and Wuschel improve monocot transformation. Plant Cell. 2016; 28: 1998–2015.
14. Lowe K , La Rota M , Hoerster G ,Hastings C, Wang N, Chamberlin M, et al. Rapid genotype "independent" *Zea mays* L. (maize) transformation via direct somatic embryogenesis. In Vitro Cell Dev-Pl. 2018; 54: 240–252.

15. Van der Graaff E, Laux T, Rensing SA. The WUS homeobox-containing (WOX) protein family. Genome Biology 2009; 10**:** 248.
16. Gehring WJ, Muller M, Affolter M, Percival-Smith A, Billeter M, Qian YQ, et al. The structure of the homeodomain and its functional implications. Trends Genet, 1990; 6: 323–329.
17. Gehring WJ, Qian YQ, Billeter M, Furukubo-Tokunaga K, Schier AF, Resendez-Perez D, et al. Homeodomain-DNA recognition. Cell. 1994; 78: 211–223.
18. Breuninger H, Rikirsch E, Hermann M, Ueda M, Laux T. Differential expression of *WOX* genes mediates apical-basal axis formation in the *Arabidopsis* embryo. Dev. Cell. 2008; 14: 867–876.
19. Hao Q, Zhang L, Yang Y, Shan Z, Zhou XA. Genome-wide analysis of the *WOX* gene gamily and gunction exploration of *GmWOX18* in soybean. Plants. 2019; 8: 215.
20. Costanzo E, Trehin C, Vandenbussche M. The role of WOX genes in flower development. Ann Bot. 2014;114: 1545–1553.
21. Dolzblasz A, Nardmann J, Clerici E, Causier B, van der Graaff E, Chen J, et al. Stem cell regulation by Arabidopsis WOX genes. Mol Plant. 2016; 9: 1028–1039.
22. Honda E, Yew CL, Yoshikawa T, Sato Y, Hibara K, Itoh JI. *LEAF LATERAL SYMMETRY1*, a member of the *WUSCHEL-RELATED HOMEOBOX3* gene family, regulates lateral organ development differentially from other paralogs, *NARROW LEAF2* and *NARROW LEAF3* in rice. Plant Cell Physiol. 2018; 59: 376–391.
23. Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, et al. Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. Development. 2004; 131: 657–568.
24. Zhang X, Zong J, Liu J, Yin J, Zhang D. Genome-wide analysis of *WOX* gene family in rice, sorghum, maize, *Arabidopsis* and poplar. J. Integr. Plant Biol. 2010; 52(11): 1016–1026.
25. Ma Y, Miotk A, Šutiković Z, Ermakova O, Wenzl C, Medzihradszky A, et al. WUSCHEL acts as an auxin response rheostat to maintain apical stem cells in *Arabidopsis*. Nat Commun, 2019; 10: 5093
26. Jha P, Ochatt SJ, Kumar V. WUSCHEL: a master regulator in plant growth signaling. Plant Cell Reports. 2020; 39: 431–444.
27. Zuo J, Niu QW, Frugis G, Chua NH. The *WUSCHEL* gene promotes vegetative-to-embryonic transition in *Arabidopsis*. Plant J. 2002, 30: 349–359.
28. Gallois JL, Nora FR, Mizukami Y, Sablowski R. WUSCHEL induces shoot stem cell activity and developmental plasticity in the root meristem. Gene dev. 2004; 18(4): 375–380.
29. Zhang Y, Wu R, Qin G, Chen Z, Gu H, Qu L. Over-expression of *WOX1* leads to defects in meristem development and polyamine homeostasis in *Arabidopsis*. J Integr Plant Biol. 2011; 53: 493–506.
30. Shimizu R, Ji J, Kelsey E, Ohtsu K, Schnable PS, Scanlon MJ. Tissue specificity and evolution of meristematic WOX3 function. Plant Physiol. 2009; 149: 841–50.
31. Wang F, Shang G, Wu L, Xu Z, ZhaoX, Wang J. Chromatin accessibility dynamics and a hierarchical transcriptional regulatory network structure for plant somatic embryogenesis. Dev Cell. 2020; 54: 1–16.
32. Suer S, Agusti J, Sanchez P, Schwarz M, Greb T. WOX4 imparts auxin responsiveness to cambium cells in *Arabidopsis*. Plant Cell. 2011; 23: 3247–3259.
33. Kong X, Lu S, Tian H, Ding Z. WOX5 is shining in the root stem cell niche. Trends Plant Sci. 2015; 20(10): 601–603.
34. Park SO, Zheng Z, Oppenheimer DG, Hauser BA. The *PRETTY FEW SEEDS2* gene encodes an *Arabidopsis* homeodomain protein that regulates ovule development. Development. 2005; 132: 841–849.

35. Kong D, Hao Y, Cui H. The WUSCHEL related Homeobox protein WOX7 regulates the sugar response of lateral root development in *Arabidopsis thaliana*. Mol Plant. 2016; 9: 261–270.
36. Wu X, Dabi T, and Weigel D. Requirement of homeobox gene *STIMPY/WOX9* for arabidopsis meristem growth and maintenance. Curr Biol. 2005; 15: 436–440.
37. Wu X, Chory J, and Weigel D. Combinations of WOX activities regulate tissue proliferation during *Arabidopsis* embryonic development. Dev Biol. 2007; 309: 306–316.
38. Ueda M, Zhang Z, Laux T. Transcriptional activation of Arabidopsis axis patterning genes *WOX8/9* links zygote polarity to embryo development. Dev Cell. 2011; 20: 264–270.
39. Baesso B, Chiatante D, Terzaghi M, Zenga D, Nieminen K, Mahonen AP, et al. Transcription factors PRE3 and WOX11 are involved in the formation of new lateral roots from secondary growth taproot in *A. thaliana*. Plant Biol. 2018; 20: 426–432.
40. Liu J, Sheng L, Xu Y, Li J, Yang Z, Huang H, et al. WOX11 and 12 are involved in the first-step cell fate transition during de novo root organogenesis in *Arabidopsis*. Plant Cell. 2014; 26: 1081–1093.
41. Romera-Branchat M, Ripoll JJ, Yanofsky MF, Pelaz S. The *WOX13* homeobox gene promotes replum formation in the *Arabidopsis thaliana* fruit. Plant J. 2013; 73: 37–49.
42. Denis E, Kbiri N, Mary V, Claisse G, Conde ESN, Kreis M, et al. WOX14 promotes bioactive gibberellin synthesis and vascular cell differentiation in *Arabidopsis*. Plant J. 2017; 90: 560–572.
43. Etchells JP, Provost, CM, Mishra L, Turner SR. WOX4 and WOX14 act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. Development. 2013; 140: 2224–2234.
44. Lian G, Ding Z, Wang Q, Zhang D, Xu J. Origins and evolution of WUSCHEL-related homeobox protein family in plant kingdom. Sci World J. 2014; 534140
45. Wu CC, Li FW, Kramer EM. Large-scale phylogenomic analysis suggests three ancient superclades of the WUSCHEL-related homeobox transcription factor family in plants. PLoS ONE. 2019; 14(10): e0223521.
46. Hong, Seo. Genome-wide transcript analysis of inflorescence development in wheat. Genome, 2019; 62(9): 623–633.
47. Jighly A, Joukhadar R, Sehgal D, Singh S, Ogbonnaya FC, Daetwyler HD. Population-dependent reproducible deviation from natural bread wheat genome in synthetic hexaploid wheat. Plant J. 2019; 100: 801–812.
48. Jiang W, Yin J, Zhang H, He Y, Shuai S, Chen S, et al. Genome-wide identification, characterization analysis and expression profiling of auxin-responsive GH3 family genes in wheat (*Triticum aestivum* L.). Mol Biol Rep. 2020; 47: 3885–3907.
49. Zhao Y, Li J, Zhao R, Xu K, Xiao Y, Zhang S, et al. Genome-wide association study reveals the genetic basis of cold tolerance in wheat. Mol Breeding, 2020; 40: 36.
50. Brenchley R, Brenchley R, Spannagl M, Pfeifer M, Barker GLA, D'Amore R, et al. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature. 2012; 491: 705–710.
51. Li Z, Liu D, Xia Y, Li Z, Jing D, Du J, et al. Identification of the *WUSCHEL-Related Homeobox* (*WOX*) gene family, and interaction and functional analysis of *TaWOX9* and *TaWUS* in wheat. Int. J. Mol. Sci. 2020; 21: 1581.
52. Zhao S, Jiang Q, Ma J, Zhang X, Zhao Q, Wang X, et al. Characterization and expression analysis of *WOX5* genes from wheat and its relatives. Gene. 2014; 537: 63–69.

Figures

Figure 1

Multiple sequence alignment and element prediction of the promoters among TaWUS and other predicted WUS genes from the six Triticeae species. A. Multiple sequence alignment among TaWUS and other predicted WUS proteins. Alignment of protein sequences was conducted by ClustalW algorithm using MEGA X. The position of conserved WUS-box motif was shown in red box, and the position of EAR domain was shown in blue box. B. Element prediction of the promoter regions of TaWUS and other predicted WUS genes in the six Triticeae species. TATA-BOX elements and their positions in the promoters were displayed in green oval, AT~TATA-BOX elements and their positions in yellow oval, cis-acting regulatory elements involved in auxin responsiveness AuxRR-core and their positions in red oval, and auxin-responsive TGA-elements and their positions in blue oval.

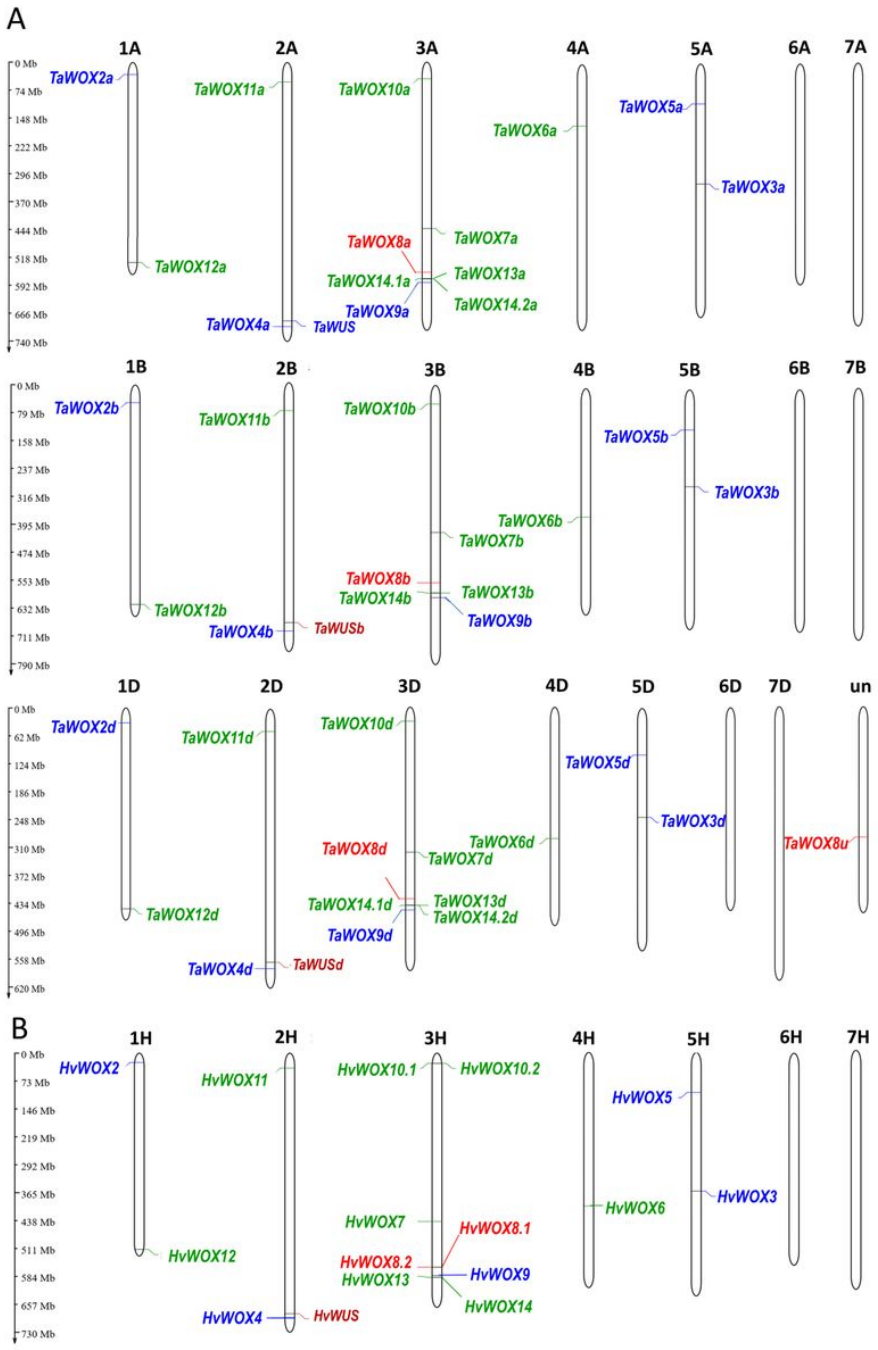

Figure 2

Chromosomal locations of WOX genes in T. aestivum (A) and H. vulgare (B). The number of chromosomes was labeled on the top of each chromosome. The location of each WOX genes was marked on the chromosome. The WOX members in WUS, intermediate and ancient clades were shown as blue, green and red types, respectively. The newly collected WOX members were shown as brown type.

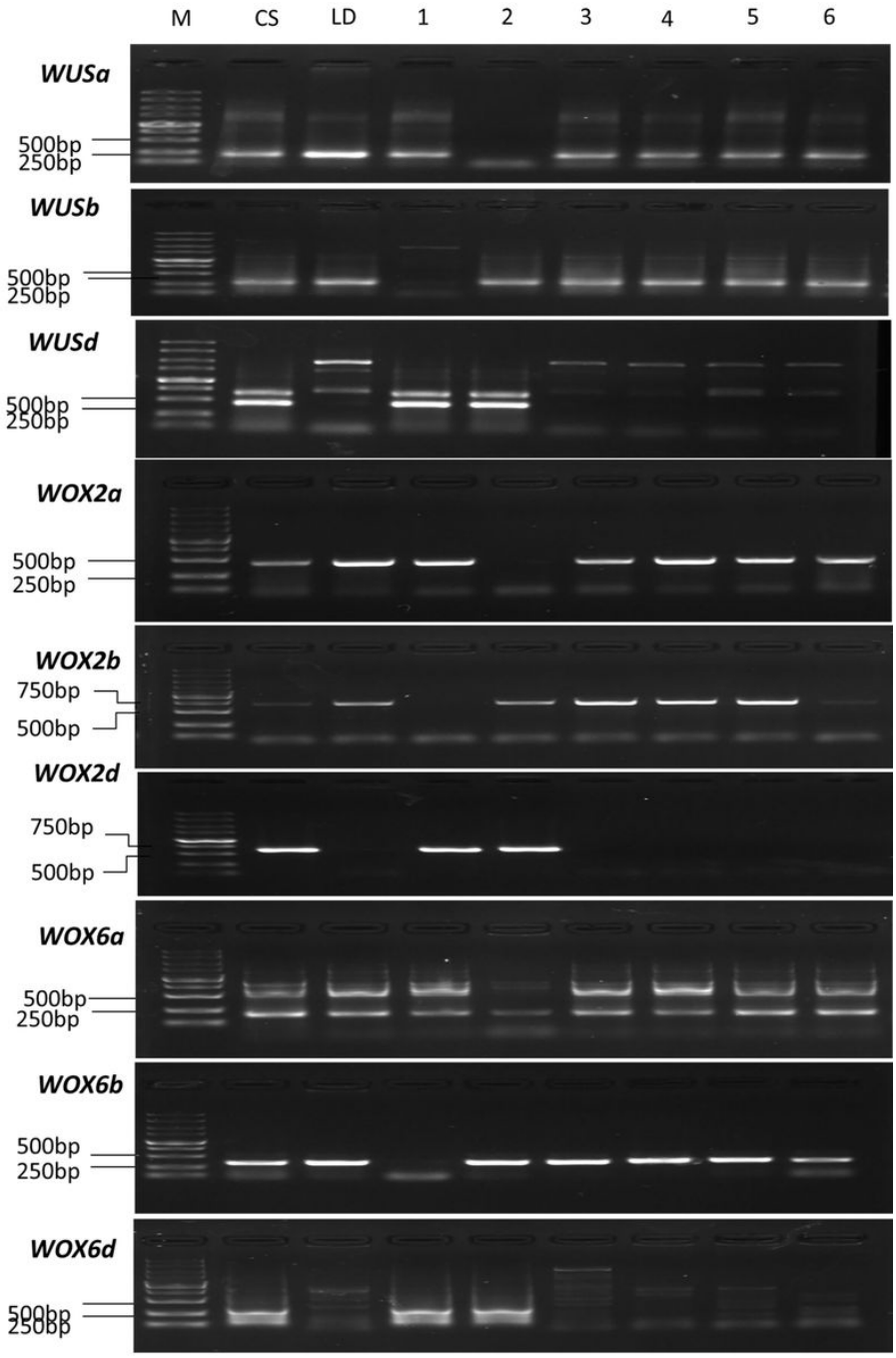

Figure 3

Verification of chromosomal locations of several TaWOX alleles by specific PCR amplification. Gel electrophoresis of specific fragments of TaWUS, TaWOX2 and TaWOX6 alleles. M, DNA molecular marker; CS, PCR product of Chinese Spring (T. aestivum); LD, PCR product of Langdon (T. durum); samples 1-6 in row WUSa were: substitution lines 2D(2B), 2D(2A), 1D(1A), 3D(3A), 4D(4A), and 5D(5A); samples 1-6 in row WUSb were: 2D(2B), 2D(2A), 1D(1B), 3D(3B), 4D(4B), and 5D(5B); samples 1-6 in row WUSd were: 2D(2B), 2D(2A), 1D(1A) and 1D(1B), 3D(3A) and 3D(3B), 4D(4A) and 4D(4B), and 5D(5A) and 5D(5B); samples 1-6 in row WOX2a were substitution lines 1D(1B), 1D(1A), 2D(2A), 3D(3A), 4D(4A), and 5D(5A); samples 1-6 in row WOX2b were 1D(1B), 1D(1A), 2D(2B), 3D(3B), 4D(4B), and 5D(5B); samples 1-6 in row WOX2d were 1D(1B), 1D(1A), 2D(2A) and 2D(2B), 3D(3A) and 3D(3B), 4D(4A) and 4D(4B), and 5D(5A) and 5D(5B); samples 1-6 in row WOX6a were substitution lines 4D(4B), 4D(4A), 1D(1A), 2D(2A), 3D(3A), and 5D(5A);

samples 1-6 in row WOX6b were 4D(4B), 4D(4A), 1D(1B), 2D(2B), 3D(3B), and 5D(5B); samples 1-6 in row WOX6d were: 4D(4B), 4D(4A), 1D(1A) and 1D(1B), 2D(2A) and 2D(2B), 3D(3A) and 3D(3B), and 5D(5A) and 5D(5B).

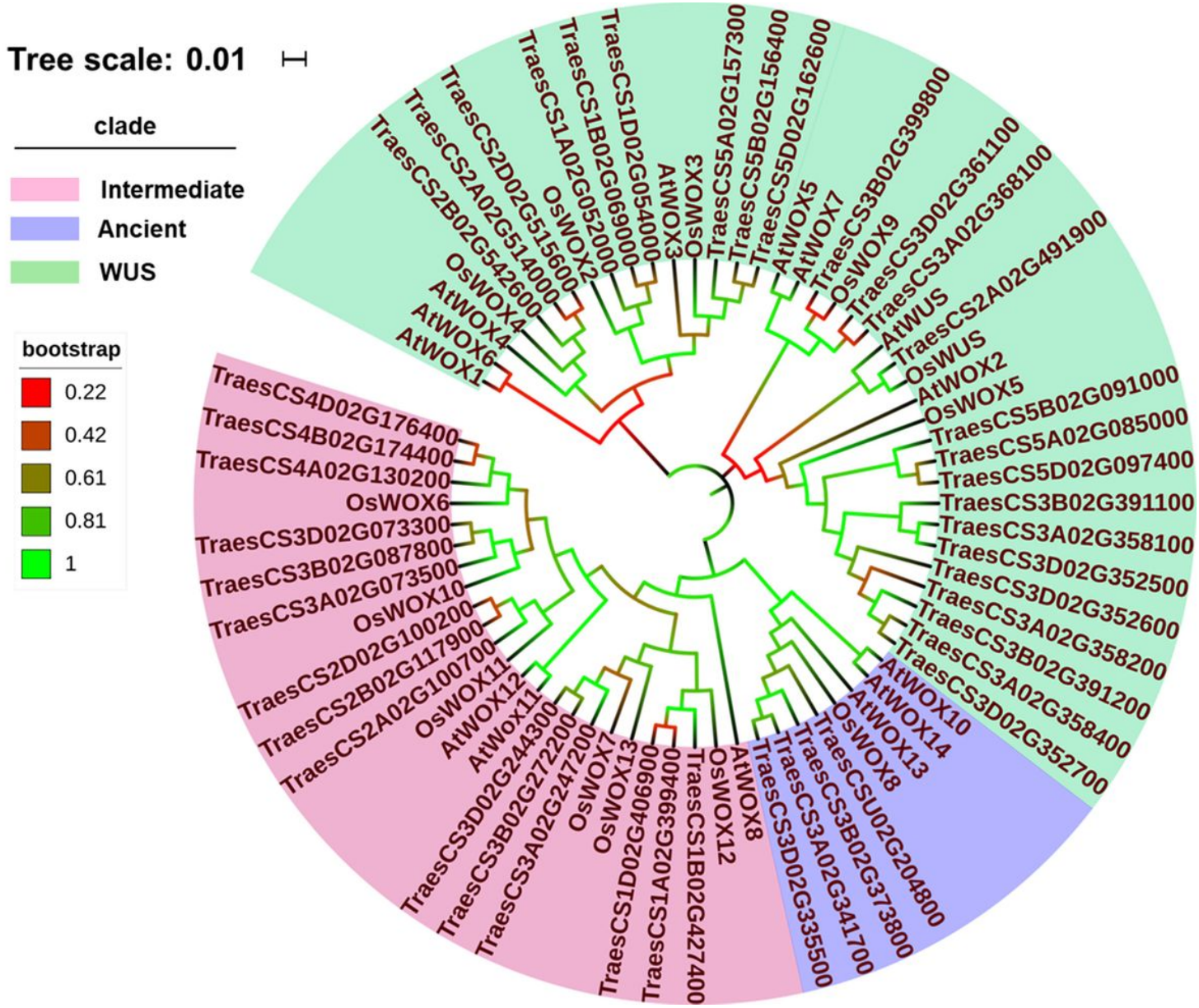

Figure 4

Phylogenetic relationships among WOX proteins in wheat, rice and Arabidopsis. Phylogenetic tree was constructed based on the sequences of WOX proteins in wheat, rice and Arabidopsis, performed by the MEGA X using neighbor-joining approach with 1000 bootstrap replicates. WUS clade harbors WUS, WOX2 to WOX5, WOX9 in wheat and rice, TaWOX13, TaWOX14, AtWUS and AtWOX1 to AtWOX7; intermediate clade contains WOX6, WOX7, and WOX10 to WOX12 in wheat and rice, AtWOX8, 9, 11, and 12; and ancient clade contains WOX8 in wheat and rice, AtWOX10, 13, and 14. Scale plate and legend in upper left displayed tree scale and bootstrap value.

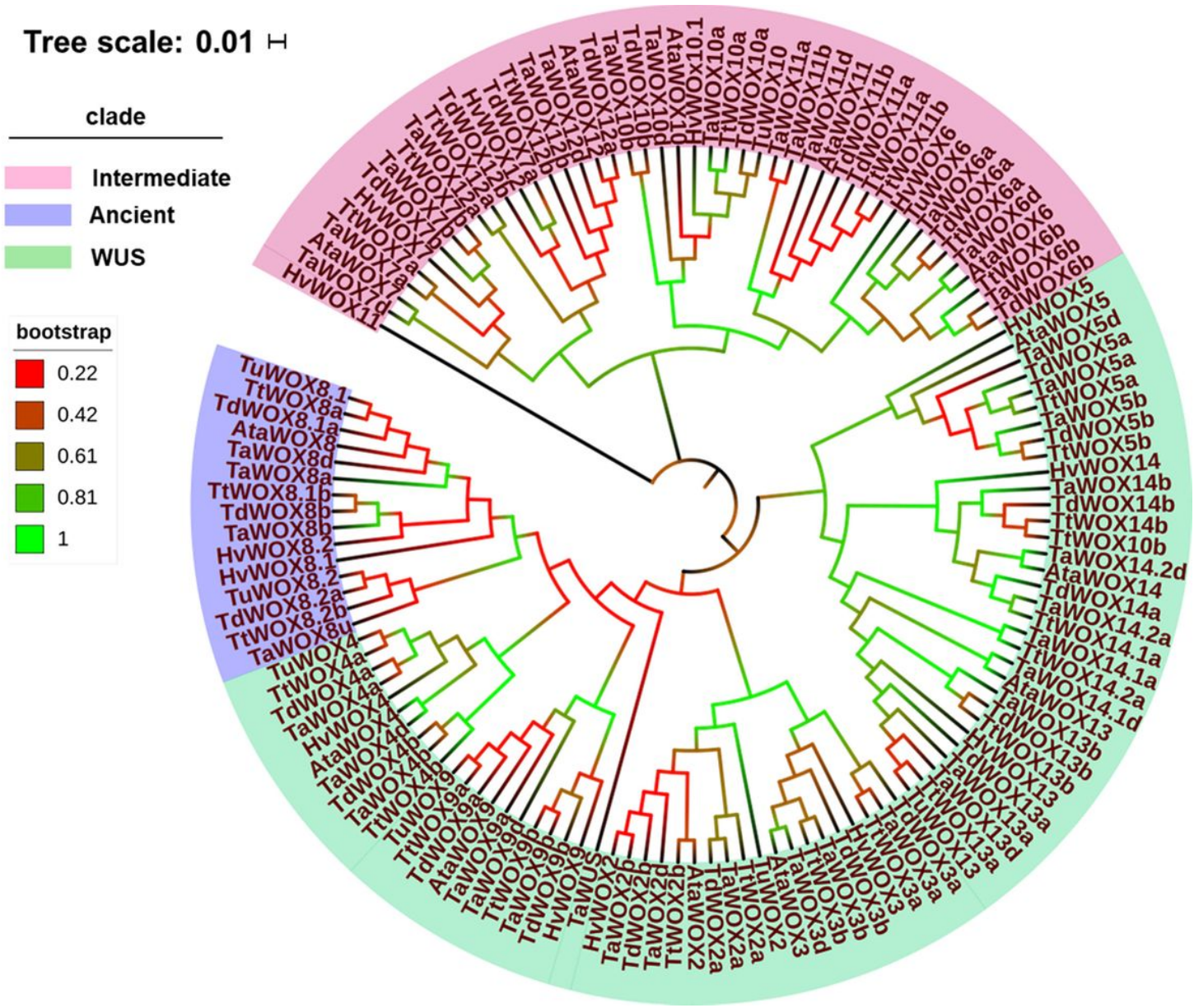

Figure 5

Phylogenetic relationships among WOX proteins from wheat, barley, T. dicoccoides, T. turgidum, A. tauschii, and T. urartu. Phylogenetic tree was constructed based on the sequences of WOX proteins in six Triticeae species implemented by the MEGA X software using maximum likelihood method. Legend in upper left displayed colored ranges of WOX members.

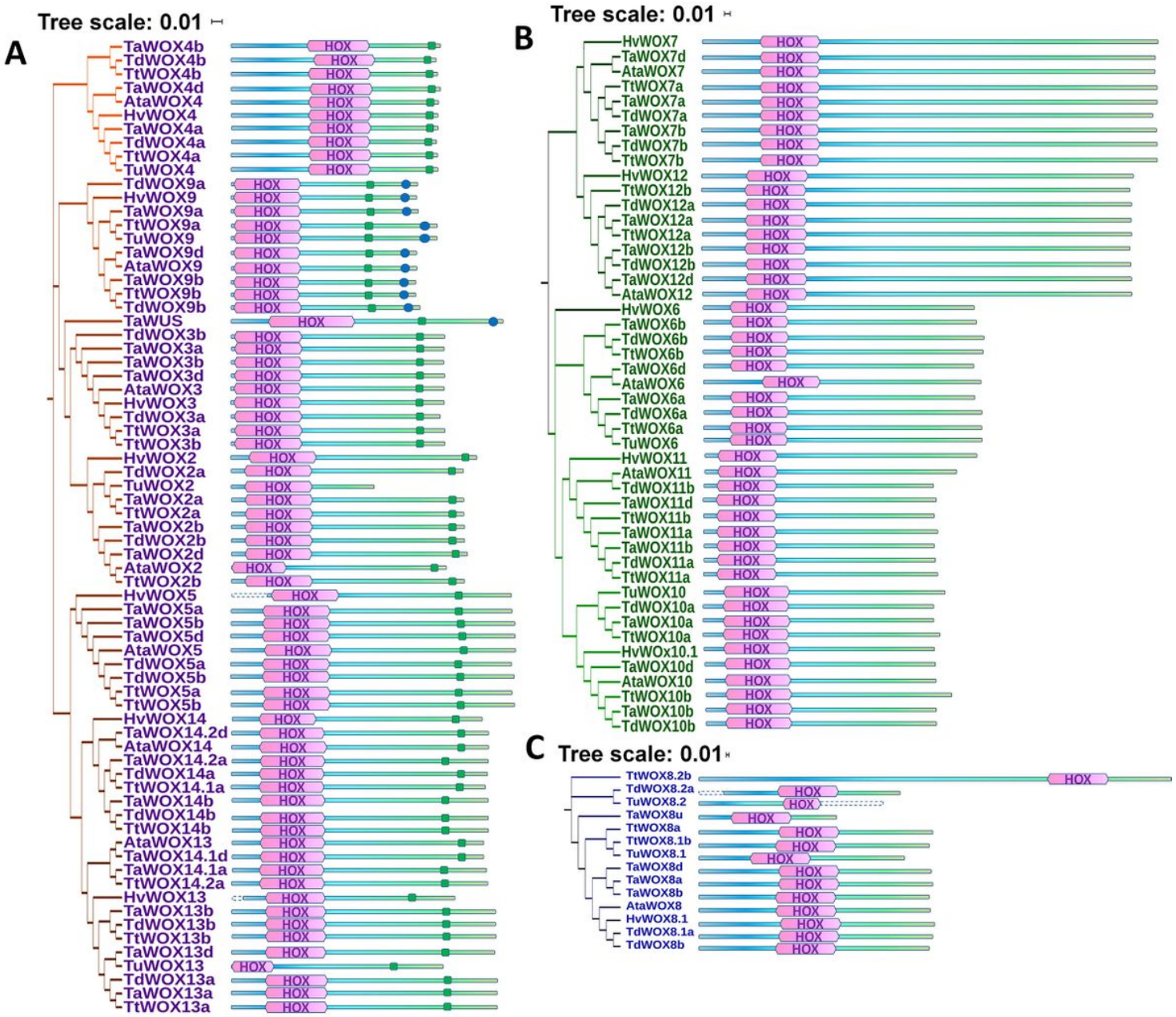

Figure 6

Depiction of the domain structure of WOX proteins in six Triticeae species. Positions of HOX homeodomain, WUS-Box motif and EAR domain in WOX protein of the six Triticeae species including wheat, barley, T. dicoccoides, T. turgidum, A. tauschii, and T. urartu. WOX members were divided by their phylogenetic relationship. There were HOX homeodomain, WUS-Box motif and EAR domain in WUS clade WOX proteins in the six Triticeae species (A). Positions of HOX homeodomain in intermediate clade WOX proteins in the six Triticeae species (B). Positions of HOX homeodomain in ancient clade WOX proteins in the six Triticeae species (C). Length of WOX members were displayed by the bar length, positions of HOX homeodomain were displayed as pink hexagon, WUS-Box motif was displayed as green round dot, and EAR domain was displayed as blue round dot.

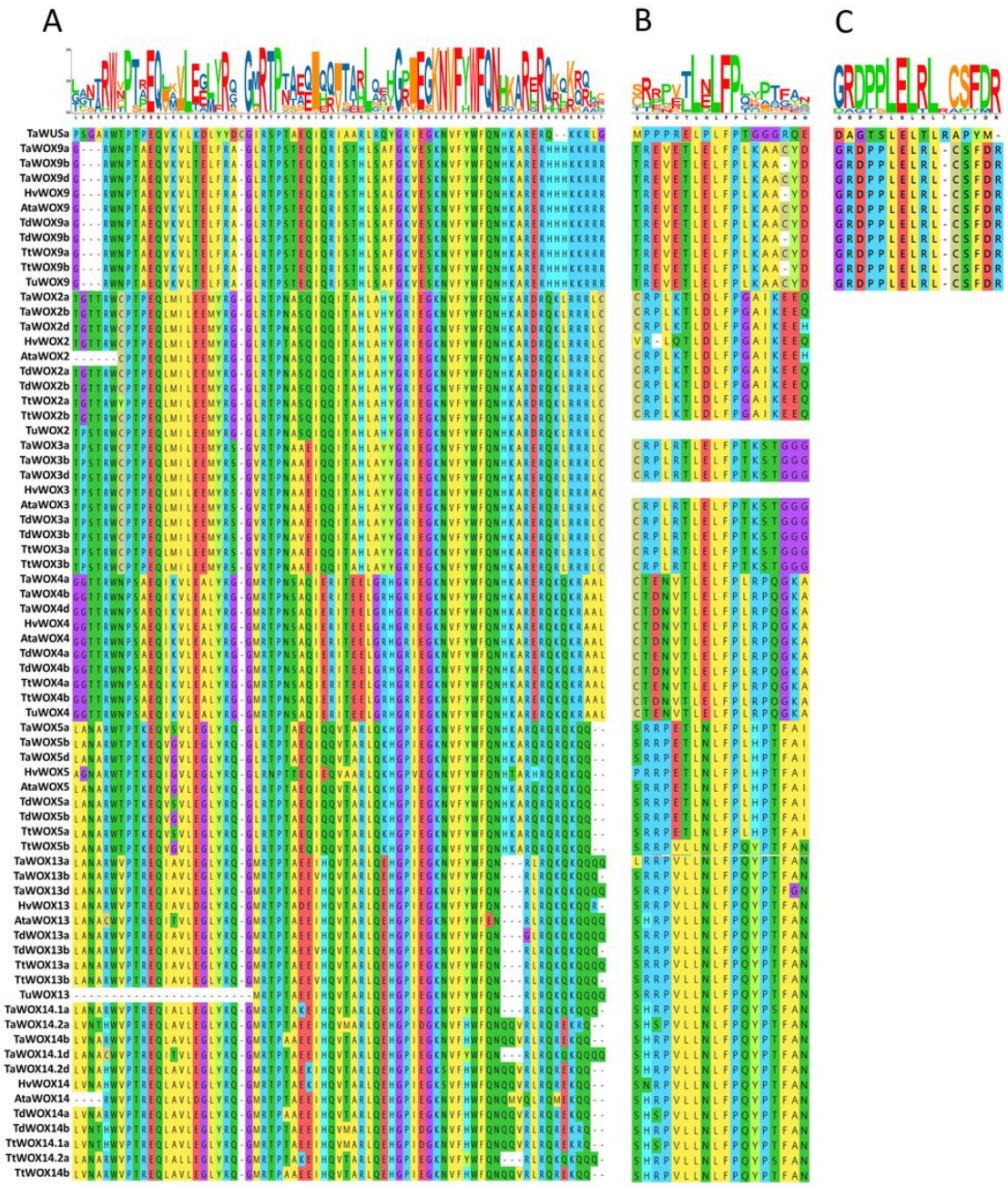

Figure 7

Alignment of WOX homeodomains from WUS clade of 6 Triticeae plant species. Phylogenetic alignment of homeodomain sequences was conducted by ClustalW algorithm using MEGA X software. LOGO of protein sequences represent the relative frequency of an amino acid at the corresponding position, and the content of the aligned sequences at a position in bit (max. 4.322 bit for proteins, i.e. log220). Multiple sequence alignment among the HOX homeodomain (A), WUS-motif (B) and EAR domain (C) of WOX proteins in WUS clade in the six Triticeae species.

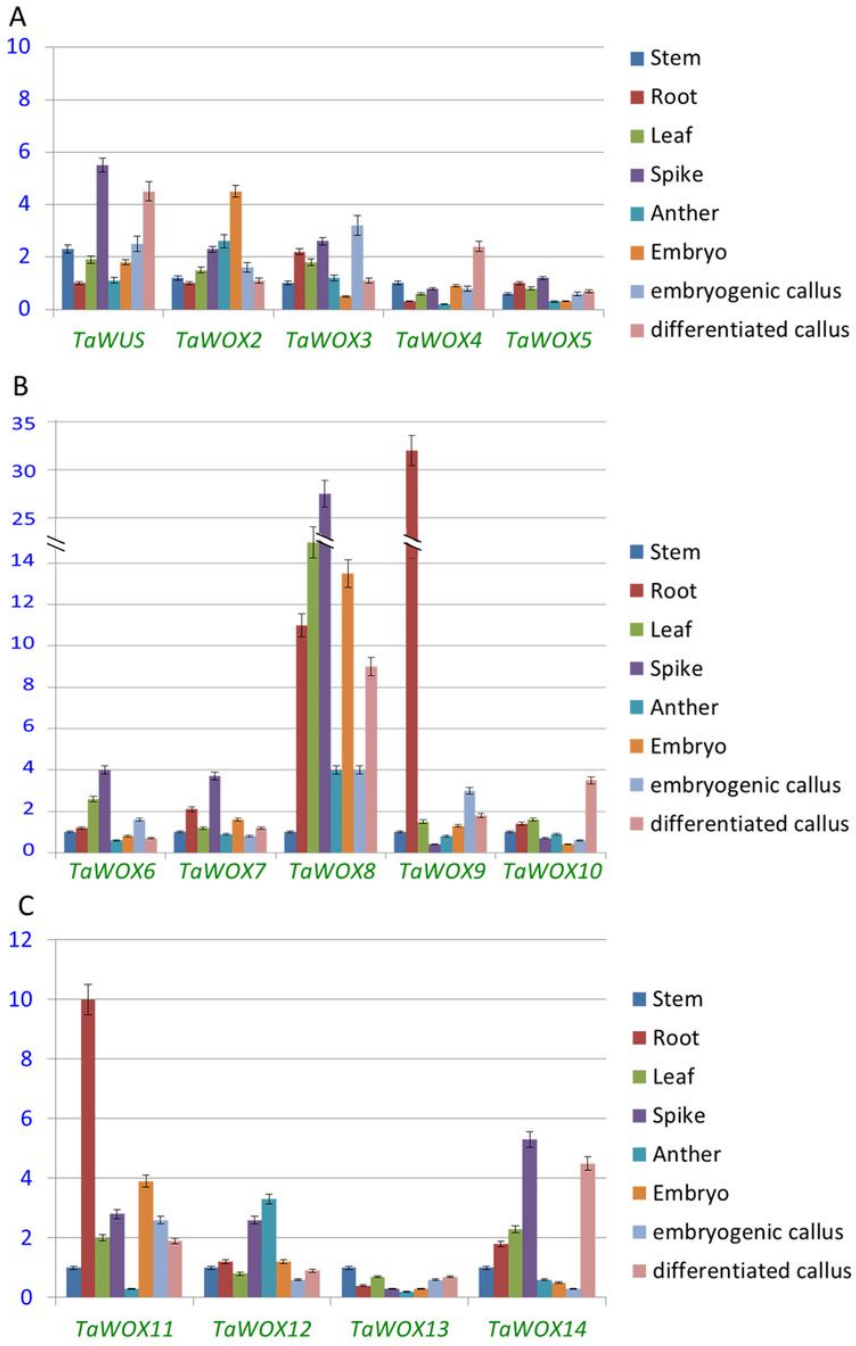

Figure 8

Expression pattern of TaWOX genes in various tissues of wheat. Gene expression level was examined using qPCR. The qPCR data was normalized using wheat TaActin gene. Values were means ± sd of three biological replicates.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile2.docx
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