Identification of the Wheat VQ Protein Family and Expression Analysis of Candidate Genes Associated with Seed Dormancy and Germination

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Abstract

Background: Seed dormancy and germination determine wheat pre-harvest sprouting resistance and thereby affect grain yield and quality. Arabidopsis *VQ* genes have been shown to influence seed germination; however, the functions of wheat *VQ* genes have not been characterized.

Results: In this study, we identified 65 *TaVQ* genes in common wheat and named them *TaVQ1–65*. We identified 48 paralogous pairs, 37 of which had Ka/Ks values larger than 1, suggesting that most *TaVQ* genes have suffered positive selection. Chromosome location, gene structure, promoter element and gene ontology annotation showed that the structure of the genes determined their function and that structural change reflected functional diversity. The transcriptome expression analysis of 62 *TaVQ* genes and microarray analysis of 11 *TaVQ* genes indicated that they played important roles in diverse biological processes. We compared *TaVQ* gene expression and corresponding seed germination index values among wheat varieties with contrasting seed dormancy and germination phenotypes and found that 21 *TaVQ* genes may be related to seed dormancy and germination.

Conclusions: Sixty-five *TaVQ* proteins were identified for the first time in common wheat, and bioinformatics analysis was performed to investigate their phylogenetic relationships and evolutionary divergence. The qRT-PCR data showed that 21 *TaVQ* candidate genes were potentially involved in seed dormancy and germination. These findings provide effective information for further cloning and functional analysis of *TaVQ* genes, as well as useful candidate genes for improvement of PHS resistance in wheat.

Background

Wheat is a widely cultivated gramineous plant and one of the three most important cereals in the world [1]. It is well known that wheat is a heterologous hexaploid derived from three closely related ancestors that have undergone two rounds of natural hybridization. Therefore, the large and complex genome of wheat (17 Gb) poses a significant challenge for wheat genome research [2, 3]. The completion of a whole genome sequence for wheat based on single chromosome sequencing has laid the foundation for wheat genomics research and wheat gene family identification.

VQ proteins are a class of plant-specific proteins with five highly conserved amino acids in the core FxxxVQxLTG sequence of the VQ motif [4], in which x represents any amino acid and VQ is a highly conserved pair of amino acid residues. Research on the VQ proteins has shown that the last three amino acids in almost all species are LTG, although some species have other variants, including FTG, ITG, LTA, and VTG [5]. In some VQ proteins of Gramineae species such as rice, maize, and Moso bamboo, VQ has been mutated to VH in the conserved domain [5, 6]. VQ proteins are generally less than 300 aa in length and contain no or few introns [7]. To date, 34, 40, 61, 18, and 74 VQ proteins have been identified in Arabidopsis, rice, maize, grape, and soybean, respectively [6, 9–11]. According to bioinformatics prediction and experimental verification, some Arabidopsis VQ proteins are located in the nucleus, some in the plastid, and a few partly in the mitochondria [12].

VQ proteins play important roles in the regulation of plant growth and development and the response to abiotic and biotic stress [5–7, 13–17]. For instance, *AtCaMBP25* (*AtVQ15*) negatively regulates osmotic stress response during the early stages of seed germination and growth in Arabidopsis [13]. Likewise, *AtVQ9* expression responds strongly to NaCl treatment, and its mutation enhances salt stress tolerance in Arabidopsis [16]. *VQ54* and *VQ19* in maize, as well as *VQ2*, *VQ16*, and *VQ20* in rice, are highly expressed under drought induction [6, 11]. Soybean *VQ6*

and *VQ53* are highly expressed in roots and stems under low nitrogen conditions [10]. *SIB1* (Sigma factor binding protein 1, also known as *AtVQ23*) was the first VQ motif protein discovered in Arabidopsis and participates in plant disease resistance signaling pathways [15]. *AtVQ21* (*MSK1*) transgenic plants show enhanced resistance to the pathogen *Pseudomonas syringae* but reduced resistance to *Botrytis cinerea* [13, 18]. *AtVQ22* negatively regulates JA-mediated disease resistance signaling pathways [19], and rice *VQ22* shows high expression levels after rice blast infection [5]. *AtVQ14* (*IKU1*) participates in the regulation of endosperm development, thereby affecting the size of Arabidopsis seeds [20]. *AtVQ29* is involved in the photomorphogenesis of Arabidopsis seedlings and flowering time regulation [9]. In addition, the growth of *VQ17*, *VQ18*, *VQ8*, and *VQ22* transgenic Arabidopsis plants is inhibited, indicating that these genes play crucial roles in plant growth and development [8].

VQ proteins came to the attention of researchers because of their interactions with WRKY transcription factors, which are involved in regulating the plant's defense response system [13, 15]. WRKY transcription factors belong to a large gene family and are ubiquitous in plants. Studies have shown that WRKY transcription factors are widely involved in plant growth and development and in resistance to adverse conditions [21–38]. For example, *AtVQ14* and *AtWRKY10* interact to form a protein complex that affects seed size in Arabidopsis [20]. *AtVQ15* and *AtWRKY25* interact and participate in high salt and osmotic stress response [13]. The interaction between *AtVQ22* and *AtWRKY28* negatively regulates JA-mediated disease resistance signaling pathways [19]. *AtVQ23* (*SIB1*) and *AtVQ16* (*SIB2*) interact with *WRKY33* to enhance the binding capacity of *WRKY33* to the W-box, thereby regulating plant disease resistance [6, 15]. *AtVQ21* can form ternary complexes with *AtWRKY33* and *AtMPK4* to regulate plant growth and disease resistance [4, 39]. In brief, VQ proteins are transcriptional regulatory cofactors that participate in growth and developmental processes and stress resistance through their interactions with transcription factors. However, until now, the VQ gene family has not been characterized in common wheat.

Pre-harvest sprouting (PHS) refers to the germination of wheat seeds within the spike of the mother plant that occurs in rainy or high moisture conditions before harvest. A series of physiological and biochemical reactions take place in wheat grains when PHS occurs. The activity of hydrolases such as amylase and proteolytic enzymes is enhanced, leading to starch and protein degradation and seriously affecting wheat processing quality and utilization value. In the international wheat market, when the germination rate of commercial wheat reaches 5%, it is regarded as feed wheat and its price is reduced, causing serious economic losses to producers [40–41]. Seed dormancy and germination traits determine wheat PHS resistance: wheat varieties with higher levels of dormancy or lower germination percentages show higher resistance to PHS. Therefore, the identification of candidate genes that control seed dormancy and germination may help to reduce yield and quality losses caused by PHS. Previously, in Arabidopsis, *VQ18* and *VQ26* were found to be involved in seed germination via the ABA signaling pathway [42]. However, the functions of VQ genes in common wheat are largely unknown.

The objectives of this study were to identify *TaVQ* genes and to perform bioinformatics analysis, including phylogenetic tree construction and characterization of gene structures, conserved domains, chromosome positions, expression patterns, and promoter elements. In addition, we measured the expression levels of *TaVQ* genes in wheat varieties with contrasting seed dormancy and germination phenotypes by qRT-PCR to identify *TaVQ* gene family members that were potentially involved in seed dormancy and germination.

Results

Identification and attribute analysis of VQ candidate genes in wheat

A total of 65 *TaVQ* genes were identified, mapped to wheat chromosomes, and named *TaVQ1–TaVQ65*. The length of the encoded proteins ranged from 127 to 723 aa, with an average length of 220 aa. Their MWs ranged from 13,377.16 Da (*TaVQ52*) to 61,926.76 Da (*TaVQ1*). Information on chromosome positions, ORF lengths, and exon numbers is provided in Table 1. The majority of genes included one exon, and only four genes (*TaVQ13/-17/-18/-53*) contained two exons.

Table 1
Detailed information about the predicted *TaVQ* genes.

Name	Gene ID	Location	ORF length(bp)	Size (aa)	MW(Da)	pI	Exons
TaVQ1	TraesCS1A02G338700	Chr1A:528102140–528103155	2172	723	61926.76	4.99	1
TaVQ2	TraesCS2A01G487800	Chr2A:722518069–722518557	489	162	16310.14	8.11	1
TaVQ3	TraesCS2A01G517000	Chr2A:740346569–740347388	636	211	22232.87	9.77	1
TaVQ4	TraesCS2B01G314600	Chr2B:449669843–449671002	990	329	34759.36	9.97	1
TaVQ5	TraesCS2B01G515400	Chr2B:710280174–710280671	498	165	16828.73	6.09	1
TaVQ6	TraesCS2B01G545500	Chr2B:742454459–742455094	636	211	22348.05	9.87	1
TaVQ7	TraesCS2D01G296200	Chr2D:378525321–378526310	990	329	34841.5	9.97	1
TaVQ8	TraesCS2D01G488100	Chr2D:587696143–587696631	489	162	16649.49	6.92	1
TaVQ9	TraesCS2D01G518600	Chr2D:608745741–608746370	630	209	22044.73	9.93	1
TaVQ10	TraesCS3A01G044200	Chr3A:238666681–23867710	771	256	26516.25	6.49	1
TaVQ11	TraesCS3A01G190200	Chr3A:235380871–235382316	702	233	24220.28	6.11	1
TaVQ12	TraesCS3A01G334400	Chr3A:580608595–580609062	468	155	15944.31	5.77	1
TaVQ13	TraesCS3B01G197900	Chr3B:225257528–225258102	486	161	17544.85	5.7	2
TaVQ14	TraesCS3B01G219100	Chr3B:262901818–262902606	789	262	27481.07	9.16	1
TaVQ15	TraesCS3B01G365300	Chr3B:577490273–577490731	459	152	15724.13	6.07	1
TaVQ16	TraesCS3B01G478300	Chr3B:726621717–726622121	405	134	14557.72	10.15	1
TaVQ17	TraesCS3D01G036600	Chr3D:13254563–13257748	774	257	26587.4	6.7	2
TaVQ18	TraesCS3D01G174600	Chr3D:156240964–156246686	489	162	17816.12	5.45	2
TaVQ19	TraesCS3D01G193700	Chr3D:184379368–184380834	717	238	24770.88	6.12	1

Name	Gene ID	Location	ORF length(bp)	Size (aa)	MW(Da)	pI	Exons
TaVQ20	TraesCS3D02G272900	Chr3D:378529151–378530444	2154	717	61510.13	5	1
TaVQ21	TraesCS4A01G369400	Chr4A:641502472–641502912	441	146	14995.88	11.33	1
TaVQ22	TraesCS4A01G411100	Chr4A:683787345–683787800	456	151	15683.79	10.54	1
TaVQ23	TraesCS4A01G411200	Chr4A:683800842–683801246	405	134	14461.68	10.61	1
TaVQ24	TraesCS4A01G411300	Chr4A:683805840–683806277	438	145	15124.32	10.9	1
TaVQ25	TraesCS4B01G178500	Chr4B:391157880–391158956	438	145	14849.46	7.1	1
TaVQ26	TraesCS4B01G311200	Chr4B:601276598–601277606	435	144	14860.96	11.14	1
TaVQ27	TraesCS4B01G311300	Chr4B:601284457–601284921	465	154	16000.23	10.55	1
TaVQ28	TraesCS4B01G311400	Chr4B:601306252–601306716	465	154	16004.18	10.55	1
TaVQ29	TraesCS4D01G180100	Chr4D:313885547–313886431	450	149	15190.79	8.01	1
TaVQ30	TraesCS4D02G021000	Chr4D:8997650–8998244	1002	333	26481.38	5.19	1
TaVQ31	TraesCS5A01G010900	Chr5A:7167768–7168166	399	132	14359.52	10.15	1
TaVQ32	TraesCS5A01G027100	Chr5A:22485164–22486133	759	252	26758.28	9.44	1
TaVQ33	TraesCS5A01G189700	Chr5A:393252902–393254173	1272	423	42945.76	6.55	1
TaVQ34	TraesCS5A01G427000	Chr5A:611817416–611818023	444	147	15224.72	9.56	1
TaVQ35	TraesCS5B01G008700	Chr5B:9243595–9244008	414	137	14803.08	9.88	1
TaVQ36	TraesCS5B01G026300	Chr5B:25061684–25062761	732	243	25550.96	9.51	1
TaVQ37	TraesCS5B01G193500	Chr5B:349712051–349713313	1263	420	42784.59	6.37	1
TaVQ38	TraesCS5B01G428900	Chr5B:604226071–604227208	444	147	15152.49	9.56	1
TaVQ39	TraesCS5B01G503300	Chr5B:669909164–669909637	474	157	16299.42	11.29	1

Name	Gene ID	Location	ORF length(bp)	Size (aa)	MW(Da)	pI	Exons
TaVQ40	TraesCS5D01G015700	Chr5D:9068493–9069444	399	132	14358.51	10.15	1
TaVQ41	TraesCS5D01G034800	Chr5D:34043996–34045414	735	244	25783.27	9.09	1
TaVQ42	TraesCS5D01G201200	Chr5D:304876446–304878167	1722	573	59359.24	10.4	1
TaVQ43	TraesCS5D01G434900	Chr5D:490166006–490166449	444	147	15110.47	9.56	1
TaVQ44	TraesCS6A01G156200	Chr6A:145133789–145134454	666	221	22431.63	9.85	1
TaVQ45	TraesCS6A01G300700	Chr6A:533466786–533467235	450	149	15970.96	10.16	1
TaVQ46	TraesCS6A01G301100	Chr6A:534747170–534747685	516	171	17845.92	9.49	1
TaVQ47	TraesCS6B01G184400	Chr6B:207154528–207155778	681	226	23005.3	10.08	1
TaVQ48	TraesCS6B01G330400	Chr6B:579549916–579550626	492	163	17065	9.2	1
TaVQ49	TraesCS6D01G146200	Chr6D:116952516–116953190	675	224	22649.97	9.85	1
TaVQ50	TraesCS6D01G280600	Chr6D:388136875–388137396	522	173	18231.26	9.2	1
TaVQ51	TraesCS7A01G326900	Chr7A:475165941–475166708	768	255	26443.91	10.07	1
TaVQ52	TraesCS7A01G349400	Chr7A:512169425–512169814	390	129	13377.16	9.45	1
TaVQ53	TraesCS7A01G375900	Chr7A:549001358–549001981	603	200	21229.86	10.61	1
TaVQ54	TraesCS7A01G401900	Chr7A:581344730–581345350	621	206	22373.4	6.07	1
TaVQ55	TraesCS7A01G478700	Chr7A:671483204–671483848	645	214	21698.87	9.51	1
TaVQ56	TraesCS7B01G227600	Chr7B:427821922–427822689	768	255	26484.95	9.77	1
TaVQ57	TraesCS7B01G233400	Chr7B:436838688–436839071	384	127	13522.39	8.91	1
TaVQ58	TraesCS7B01G277500	Chr7B:507836057–507836671	615	204	21621.29	10.39	1
TaVQ59	TraesCS7B01G302000	Chr7B:538864067–538864690	624	207	22455.42	5.97	1

Name	Gene ID	Location	ORF length(bp)	Size (aa)	MW(Da)	pI	Exons
TaVQ60	TraesCS7B01G381400	Chr7B:646209297–646210514	627	208	21214.45	9.51	1
TaVQ61	TraesCS7D01G323700	Chr7D:413441710–413442474	765	254	26249.67	9.63	1
TaVQ62	TraesCS7D01G329300	Chr7D:420782426–420782812	387	128	13671.46	8.93	1
TaVQ63	TraesCS7D01G372400	Chr7D:481836636–481837615	477	158	16567.59	10.19	1
TaVQ64	TraesCS7D01G395700	Chr7D:510450077–510450709	636	211	22939.95	5.67	1
TaVQ65	TraesCS7D01G466100	Chr7D:579961244–579961867	624	207	21039.19	9.3	1

Phylogenetic trees of VQ proteins from wheat, maize, rice, and Arabidopsis

To explore the evolutionary relationships among *VQ* genes from wheat, Arabidopsis, rice, and maize, we downloaded published *VQ* protein sequences from these species (Table S1) [8–10] and constructed a phylogenetic tree (Fig. 1). Based on the original division and naming of *VQ* subfamilies in Arabidopsis and rice, we divided the 200 *VQ* genes (34 *AtVQ* genes, 40 *OsVQ* genes, 61 *ZmVQ* genes, and 65 *TaVQ* genes) into seven subfamilies (VQI, VQII, VQIII, VQIV, VQV, VQVI, and VQVII). The VQII subfamily contained the largest number of genes (28) (Fig. 1). Members of the *VQ* family from rice, maize, and wheat, which belong to the Gramineae, were interspersed, while those of Arabidopsis, a model plant from the Cruciferae, formed separate clades, probably due to the relatively distant relationship between monocots and dicots.

Structural analysis of the VQ gene family

We constructed a wheat *VQ* phylogenetic tree and a gene structure diagram (Fig. 2a). The structure of each *VQ* gene contained one to three parts: the untranslated region (yellow rectangle), the exon region (green rectangle), and the intron region (solid gray line). Among the 48 *TaVQ* paralogous pairs (Table 2), only two pairs (*TaVQ10/-17* and *TaVQ53/-58*) differed in intron number, having lost or gained one intron (Fig. 2A and Table S2). Further analysis revealed that 94% of the *TaVQ* genes had no introns, and only four genes (*TaVQ13/-17/-18/-53*) contained one intron. This result is consistent with previous studies in other species: 78%, 88%, 89% and 93% of the *VQ* genes in poplar, Arabidopsis, maize, and rice have no introns, respectively, whereas only 28% of moss *VQ* genes have no introns (Fig. 2b). Based on comparisons of many species, including angiosperms (rice, poplar, soybean, Chinese cabbage, etc.) and bryophytes (moss), we speculate that most *VQ* genes tend to lose introns during long-term evolution [6, 8, 9, 43, 44].

Table 2
Paralogous (Ta/Ta) and orthologous (Ta/Os and Ta/Zm)
genes pairs.

Paralogous	Orthologous	
TaVQ1/TaVQ4	TaVQ1/OsVQ8	TaVQ1/ZmVQ14
TaVQ1/TaVQ7	TaVQ1/OsVQ15	TaVQ1/ZmVQ59
TaVQ3/TaVQ6	TaVQ3/OsVQ16	TaVQ4/ZmVQ14
TaVQ3/TaVQ9	TaVQ4/OsVQ15	TaVQ4/ZmVQ59
TaVQ4/TaVQ7	TaVQ6/OsVQ16	TaVQ6/ZmVQ13
TaVQ6/TaVQ9	TaVQ7/OsVQ8	TaVQ7/ZmVQ14
TaVQ10/TaVQ17	TaVQ7/OsVQ15	TaVQ7/ZmVQ59
TaVQ11/TaVQ14	TaVQ9/OsVQ16	TaVQ32/ZmVQ30
TaVQ11/TaVQ19	TaVQ10/OsVQ12	TaVQ41/ZmVQ30
TaVQ12/TaVQ15	TaVQ17/OsVQ12	TaVQ53/ZmVQ35
TaVQ13/TaVQ18	TaVQ26/OsVQ26	TaVQ53/ZmVQ51
TaVQ14/TaVQ19	TaVQ32/OsVQ8	TaVQ58/ZmVQ35
TaVQ21/TaVQ39	TaVQ33/OsVQ35	TaVQ58/ZmVQ51
TaVQ22/TaVQ23	TaVQ36/OsVQ8	TaVQ63/ZmVQ35
TaVQ22/TaVQ26	TaVQ37/OsVQ35	
TaVQ22/TaVQ27	TaVQ41/OsVQ8	
TaVQ22/TaVQ28	TaVQ44/OsVQ5	
TaVQ22/TaVQ30	TaVQ47/OsVQ5	
TaVQ24/TaVQ26	TaVQ49/OsVQ5	
TaVQ24/TaVQ27	TaVQ53/OsVQ23	
TaVQ24/TaVQ28	TaVQ58/OsVQ23	
TaVQ24/TaVQ30	TaVQ63/OsVQ23	
TaVQ26/TaVQ27		
TaVQ26/TaVQ28		
TaVQ26/TaVQ30		
TaVQ27/TaVQ28		
TaVQ27/TaVQ30		
TaVQ27/TaVQ39		
TaVQ28/TaVQ30		

Paralogous	Orthologous
TaVQ28/TaVQ39	
TaVQ32/TaVQ36	
TaVQ32/TaVQ41	
TaVQ33/TaVQ37	
TaVQ33/TaVQ42	
TaVQ36/TaVQ41	
TaVQ37/TaVQ42	
TaVQ44/TaVQ47	
TaVQ44/TaVQ49	
TaVQ47/TaVQ49	
TaVQ51/TaVQ56	
TaVQ51/TaVQ61	
TaVQ53/TaVQ58	
TaVQ54/TaVQ59	
TaVQ55/TaVQ60	
TaVQ55/TaVQ65	
TaVQ56/TaVQ61	
TaVQ59/TaVQ64	
TaVQ60/TaVQ65	

Using published information on characteristics of the VQ domain as a reference, we aligned the protein sequences of wheat and analyzed their VQ domains. The 65 TaVQ proteins all contained conserved VQ domains, but they differed slightly and could be grouped into three types: FxxxVQxLTG (52/65), FxxxVQxFTG (10/65), and FxxxVQxITG (3/65) (Fig. 3a). We further analyzed the VQ domains from multiple species (Fig. 3b) and found that the FxxxVQxLTG sequence was most prevalent and that two additional VQ domain types (LTG/FTG) were also common. There were differences in VQ domain sequence between monocots and dicots. In addition to the more common domain sequences, monocot VQ domains also included ITG, ATG, and LTA, and dicot VQ domains included LTS, LTD, YTG, LTR, and LTV (Table S3).

A total of 20 conserved motifs were identified in the TaVQ gene family (Table S4). All 65 TaVQ proteins shared one conserved motif (core motif 1, Motif 1) (Fig. 3c). The MEME diagram showed that wheat VQ genes from the same subfamily tended to share the same conserved motifs. Only 7 of the 48 *TaVQ* paralogous pairs (*TaVQ12/-15*, *TaVQ32/-36*, *TaVQ32/-41*, *TaVQ44/-49*, *TaVQ47/-49*, *TaVQ55/-60*, and *TaVQ60/-65*) differed in their motifs.

Evolution and divergence of the VQ gene family in wheat, rice, and maize

In total, 48 homologous pairs were identified in wheat, 22 in wheat and rice, and 14 in wheat and maize (Table 2). The Ks values of the wheat paralogous pairs ranged from 0.0163 to 1.5197, indicating that the occurrence of the duplication event in this species was approximately 1.2538 to 116.8985 MYA. The Ks values of orthologous pairs from wheat and rice ranged from 0.5821 to 1.6479, indicating that duplication events occurred approximately 44.78 to 126.7631 MYA. The Ks values of orthologous pairs from wheat and maize ranged from 0.6453 to 1.0257, indicating that the duplication events occurred approximately 49.6415 to 78.8962 MYA (Table 3).

Table 3
Ka, Ks and Ka/Ks ratios of Paralogous and orthologous pairs

Pairs	Ka	Ks	Ka/Ks	Date(MYA)
TaVQ1/TaVQ4	0.0127	0.1185	0.1070	9.1162
TaVQ1/TaVQ7	0.0070	0.0894	0.0790	6.8792
TaVQ3/TaVQ6	0.0240	0.0751	0.3200	5.7800
TaVQ3/TaVQ9	0.3823	0.2480	1.5420	19.0754
TaVQ4/TaVQ7	0.0085	0.0935	0.0900	7.1931
TaVQ6/TaVQ9	0.3781	0.2406	1.5720	18.5046
TaVQ10/TaVQ17	0.0448	0.0747	0.5990	5.7446
TaVQ11/TaVQ14	1.8069	0.9075	1.9910	69.8100
TaVQ11/TaVQ19	1.0034	0.6031	1.6640	46.3915
TaVQ12/TaVQ15	0.2352	0.1872	1.2560	14.4008
TaVQ13/TaVQ18	0.4955	0.3656	1.3550	28.1223
TaVQ14/TaVQ19	2.2662	0.9324	2.4310	71.7200
TaVQ21/TaVQ39	1.1545	0.9827	1.1750	75.5954
TaVQ22/TaVQ23	1.1107	0.9339	1.1890	71.8415
TaVQ22/TaVQ26	0.9021	0.8832	1.0210	67.9385
TaVQ22/TaVQ27	0.9769	0.8069	1.2110	62.0700
TaVQ22/TaVQ28	0.9796	0.8010	1.2230	61.6123
TaVQ22/TaVQ30	0.9757	0.8110	1.2030	62.3854
TaVQ24/TaVQ26	0.8192	0.8369	0.9790	64.3792
TaVQ24/TaVQ27	0.9598	0.8863	1.0830	68.1777
TaVQ24/TaVQ28	0.9475	0.8613	1.1000	66.2500
TaVQ24/TaVQ30	0.4472	0.4703	0.9510	36.1738
TaVQ26/TaVQ27	0.5848	0.5587	1.0470	42.9754
TaVQ26/TaVQ28	0.5786	0.5392	1.0730	41.4792
TaVQ26/TaVQ30	0.9816	0.9084	1.0810	69.8746
TaVQ27/TaVQ28	0.0059	0.0163	0.3640	1.2538
TaVQ27/TaVQ30	0.9130	0.8141	1.1210	62.6192
TaVQ27/TaVQ39	0.6100	0.4961	1.2300	38.1592
TaVQ28/TaVQ30	0.9005	0.7965	1.1310	61.2700

Pairs	Ka	Ks	Ka/Ks	Date(MYA)
TaVQ28/TaVQ39	0.6039	0.4944	1.2210	38.0331
TaVQ32/TaVQ36	0.9418	0.5073	1.8560	39.0254
TaVQ32/TaVQ41	0.8366	0.6503	1.2860	50.0215
TaVQ33/TaVQ37	1.3991	1.0467	1.3370	80.5123
TaVQ33/TaVQ42	2.5055	1.2491	2.0060	96.0877
TaVQ36/TaVQ41	0.8192	0.6094	1.3440	46.8800
TaVQ37/TaVQ42	2.1547	1.5197	1.4180	116.8985
TaVQ44/TaVQ47	0.2084	0.1818	1.1470	13.9815
TaVQ44/TaVQ49	0.2361	0.1606	1.4700	12.3569
TaVQ47/TaVQ49	0.2438	0.1783	1.3670	13.7138
TaVQ51/TaVQ56	0.2983	0.3403	0.8770	26.1769
TaVQ51/TaVQ61	0.2983	0.3403	0.8770	26.1769
TaVQ53/TaVQ58	1.4092	0.5952	2.3680	45.7823
TaVQ54/TaVQ59	0.9133	0.4927	1.8540	37.8962
TaVQ55/TaVQ60	1.2444	0.5470	2.2750	42.0762
TaVQ55/TaVQ65	1.2015	0.4813	2.4960	37.0231
TaVQ56/TaVQ61	0.1112	0.1833	0.6060	14.1023
TaVQ59/TaVQ64	1.0923	0.6707	1.6290	51.5885
TaVQ60/TaVQ65	0.1719	0.1390	1.2370	10.6892
TaVQ1/OsVQ8	2.7423	0.8121	3.3770	62.4654
TaVQ1/OsVQ15	1.7626	1.1060	1.5940	85.0769
TaVQ3/OsVQ16	2.7350	0.8081	3.3840	62.1623
TaVQ4/OsVQ15	1.7960	1.0882	1.6500	83.7077
TaVQ6/OsVQ16	2.5321	0.8356	3.0300	64.2731
TaVQ7/OsVQ8	2.6174	0.8540	3.0650	65.6892
TaVQ7/OsVQ15	1.7711	1.1700	1.5140	89.9977
TaVQ9/OsVQ16	2.1032	0.7993	2.6310	61.4815
TaVQ10/OsVQ12	1.5611	0.9874	1.5810	75.9523
TaVQ17/OsVQ12	1.5725	1.0693	1.4710	82.2562
TaVQ26/OsVQ26	1.8812	1.6479	1.1420	126.7631

Pairs	Ka	Ks	Ka/Ks	Date(MYA)
TaVQ32/OsVQ8	2.3258	0.8401	2.7680	64.6254
TaVQ33/OsVQ35	1.6720	0.9193	1.8190	70.7123
TaVQ36/OsVQ8	2.7247	1.0176	2.6780	78.2731
TaVQ37/OsVQ35	1.6260	0.8935	1.8200	68.7277
TaVQ41/OsVQ8	2.0860	0.9970	2.0920	76.6931
TaVQ44/OsVQ5	1.3857	0.6703	2.0670	51.5615
TaVQ47/OsVQ5	1.1333	0.6778	1.6720	52.1400
TaVQ49/OsVQ5	1.0462	0.5916	1.7680	45.5054
TaVQ53/OsVQ23	1.8754	0.6018	3.1160	46.2923
TaVQ58/OsVQ23	1.6660	0.5821	2.8620	44.7800
TaVQ63/OsVQ23	1.8612	0.7183	2.5910	55.2562
TaVQ1/ZmVQ14	1.7229	0.7294	2.3620	56.1085
TaVQ1/ZmVQ59	2.1932	0.9246	2.3720	71.1208
TaVQ4/ZmVQ14	1.8287	0.7525	2.4300	57.8877
TaVQ4/ZmVQ59	2.3077	0.9715	2.3750	74.7292
TaVQ6/ZmVQ13	1.9158	1.0257	1.8680	78.8962
TaVQ7/ZmVQ14	1.7985	0.7603	2.3660	58.4823
TaVQ7/ZmVQ59	2.3096	0.8740	2.6430	67.2292
TaVQ32/ZmVQ30	1.1674	0.8904	1.3110	68.4954
TaVQ41/ZmVQ30	2.1318	0.8361	2.5500	64.3131
TaVQ53/ZmVQ35	1.0682	0.6453	1.6550	49.6415
TaVQ53/ZmVQ51	2.1978	0.7752	2.8350	59.6277
TaVQ58/ZmVQ35	2.1378	0.8635	2.4760	66.4215
TaVQ58/ZmVQ51	1.3189	0.7664	1.7210	58.9500
TaVQ63/ZmVQ35	3.0729	0.7873	3.9030	60.5592

To investigate the role of natural selection in the evolution of the *VQ* gene family in Gramineae, we analyzed the Ka/Ks ratios of all homologous pairs and generated sliding window graphs (Figure S1 and Table 3). Among the 48 paralogous pairs, 11 had Ka/Ks ratios less than one, and 37 pairs had Ka/Ks ratios greater than one, indicating that wheat *VQ* genes were mainly subjected to positive selection during the evolutionary process. The Ka/Ks ratio of all orthologous pairs was greater than one, indicating that the *VQ* gene family in wheat, rice, and maize had primarily undergone positive selection.

Expression pattern analysis of the TaVQ gene family

Transcriptome data (FPKM-values) were obtained for all *TaVQ* genes, with the exception of *TaVQ13/-18/-45* (Fig. 4a and Table S5). The expression patterns of VQ genes differed among varieties and within time periods in the same variety. Most *TaVQ* genes were highly expressed in J411, especially at 4 hours after seed imbibition, and only four genes (*TaVQ4/-7/-8/-20*) were expressed at a low level. It is worth noting that *TaVQ8* and *TaVQ20* were both highly expressed in HMC21 and expressed at a low level in J411. We further analyzed the expression of 48 paralogous gene pairs. Only one pair showed similar expression patterns in both genes, whereas the rest were differentially expressed among varieties and within different time periods of the same variety.

Microarray data were obtained for 11 *TaVQ* genes to further investigate wheat VQ gene expression (Fig. 4b and Table S6). *TaVQ16*, *TaVQ31*, and *TaVQ35* were highly expressed in Aba and 22 DAP EM, but they showed little expression elsewhere. Further analysis of paralogous pairs showed that three pairs (*TaVQ55/-60*, *TaVQ55/-65*, and *TaVQ60/-65*) had similar expression patterns in different tissues.

Promoter analysis and gene ontology annotation of the TaVQ gene family

Two categories of response element were analyzed in the promoter regions of the *TaVQ* genes (Fig. 5a and Table S7). The first category included elements associated with biotic stress, including ABRE, CGTCA-motif, TGACG-motif, TGA-element, AuxRR-core, TCA-element, GARE-motif, and P-box. The second category included elements associated with abiotic stress, including MBS, LTR, and TC-rich repeats. The most common biotic stress response elements in the *TaVQ* promoters were associated with methyl jasmonate (CGTCA-motif and TGACG-motif) (42.77%) and ABA (ABRE) (41.85%) (Fig. 5b). The drought-associated MBS element (4.31%) was the most common abiotic stress response element (Fig. 5c).

The 65 *TaVQ* genes were annotated with 15 GO terms (Fig. 6 and Table S8): three, three, and nine terms in the molecular function, cellular component, and biological process categories, respectively. Among these terms, GO:0005634 (cellular component), GO:0003674 (molecular function), and GO:0008150 (biological process) were most common and were assigned to 28, 21, and 13 genes, respectively.

Chromosome locations and subcellular localization predictions for the TaVQ gene family

The *TaVQ* genes were unevenly distributed on wheat chromosomes 1–7, and no *TaVQ* genes were present on chromosomes 1B and 1D (Figure S2). One gene was located on chromosome 1A, five were located on chromosomes 5B, 7A, 7B, and 7D, and two to four were located on each of the other chromosomes. Single gene cluster was a chromosomal region of less than 200 Kb containing two or more genes [45]. Two gene clusters containing six genes were identified on chromosomes 4A and 4B (Figure S2).

The TaVQ proteins were assigned to three locations by subcellular localization prediction analysis. Most of them were predicted to be located in the periplasmic region (47, 72.3%), some in the extracellular region (15, 23.1%), and the rest in the cytoplasmic region (3, 4.6%) (Table S9).

Responses of TaVQ genes to water imbibition

We investigated the responses of 65 TaVQ genes in six wheat varieties after water treatment (with different seed dormancy and germination phenotypes at 0 and 10 h). Seeds from three highly dormant varieties (HMC21, YXM, and SNTT) showed no seed germination, whereas most seeds from three varieties with low dormancy (J411,

ZY9507, and ZM895) germinated (average GI of 0.95, 0.92, and 0.94, respectively; Table S10) after 10 h of imbibition. We found that the *TaVQ* genes were differentially expressed in the six wheat varieties. The expression levels of 13 genes (*TaVQ8/-9/-13/-17/-25/-32/-34/-43/-48/-49/-53/-59/-62*) were higher in the low-dormancy varieties than in the high-dormancy varieties. Eight genes showed the opposite expression trend (*TaVQ4/-16/-20/-35/-38/-42/-51/-56*) (Fig. 7).

Discussion

The plant-specific VQ protein initially attracted attention due to its interaction with WRKY transcription factors [15]. Additional in-depth studies showed that the VQ gene family not only participated in responses to biotic and abiotic stress, but also was involved in the regulation of plant growth and development [5–7, 13–17]. Genome-wide surveys of VQ proteins have now been performed in a number of species, although functional research has remained focused on Arabidopsis. VQ genes have not previously been characterized in wheat, and we therefore performed basic bioinformatics analyses to better understand the VQ gene family in wheat.

We identified 65 VQ genes from wheat and classified them into seven subfamilies. The VQ genes of four species (wheat, rice, maize, and Arabidopsis) were distributed in each subfamily, but the number of members in each subfamily differed among species, indicating that the VQ genes had developed in multiple directions over the course of evolution. The VQ genes of monocots (rice, corn and wheat) were interspersed and clustered together, whereas the VQ genes of Arabidopsis were clustered into separate clades, indicating that proteins encoded by wheat VQ genes were highly similar to those of rice and maize [8–9]. These results highlight the evolutionary conservation of the VQ gene family.

Phylogenetic trees represent the genetic relationships among gene families from different species and reflect the similarity of protein-coding genes. Combined with the structural analysis of the VQ gene family, we found that most VQ genes were intron-free [6, 8–11, 43, 44]. Based on comparisons of several species, we speculate that this gene family tends to lose introns during evolution. Amino acid sequence alignment and motif analysis indicated that the sequences of most VQ domains from different species were similar, although a small number of variants existed. In general, members of the same subfamily had similar types and numbers of conserved motifs, but there were also cases in which members of the same family had different types and numbers of conserved motifs. In addition, VQ had mutated to VH in the VQ domain of several Gramineae species [5, 6]. Taken together, these results indicated that the VQ gene family was highly conserved and diverse, reflecting the functional diversity of the gene family members.

With the development of next generation sequencing technology, the genomes of Arabidopsis, rice, maize, and wheat have recently been sequenced [2, 3, 8–10]. Their genome sizes are 164 Mb, 389 Mb, 2500 Mb, and 17 Gb, respectively. Based on genome size and chromosome number, the number of VQ genes among the four species is expected to be the highest in wheat, followed by maize, rice, and Arabidopsis. The numbers of maize, rice, and Arabidopsis VQ genes are 61, 40, and 34, and the number of wheat VQ genes in this study was 65, consistent with predictions based on genome size. By calculating the Ks value of homologous pairs to estimate the time of duplication events, the time range for whole genome duplication events in wheat was approximately 1.2538 to 116.8985 MYA. The Ka/Ks ratio of most paralogous pairs (37, 77%) was greater than one, indicating that the *TaVQ* gene family had undergone positive selection. The sliding window graph demonstrated that the Ka/Ks ratio of homologous pairs was different in different coding segments. There were segments greater than one and segments less than one, indicating that the homologous pairs had undergone different evolutionary selection

pressures. These results show that natural selection plays an important role in the evolution and differentiation of the *VQ* gene family.

TaVQ55/-60/-65 were highly expressed in GSC, GSR, GSE, SR, SL, Fba, and Pba, *TaVQ2/-5/-8* were highly expressed in SL, Aba, 3–5 DAP C, and 22 DAP EM, and *TaVQ16/-31/-35* were highly expressed in SL, Aba, and 22 DAP EM, indicating that the *VQ* gene family was active throughout multiple plant growth and development stages. Previous studies on *Arabidopsis* have shown that *AtVQ14* regulates endosperm development and seed size (Wang et al., 2010) [20] ring time [9]. In this study, *TaVQ48*, which belongs to the same subfamily as *AtVQ14/-29*, was also expressed in floral bracts, before anthesis, in 22 DAP endosperm, and in 22 DAP embryos. Additionally, *TaVQ48* was strongly expressed in germinating seeds, roots, seedling roots, and seedling leaves. These results point the way for further exploring the functions of the *TaVQ* gene family.

GO annotations of 62 *TaVQ* genes were extracted from transcriptome data. The most common GO terms were from the biological process category (43, 38.1%), especially GO:0006952 (13 genes) and GO:0008150 (15 genes). GO:0006952 is related to defense response, and combined with promoter analysis, we found that 5 *TaVQ* genes (*TaVQ1/-2/-32/-41/-51*) had this function in both analyses. GO:0010337 is related to the regulation of salicylic acid (SA) metabolism, but only *TaVQ14* was assigned this annotation. *TaVQ14* also had an SA *cis*-acting element in the promoter analysis. These results indicate that gene structure determines function, and the diversity of structure reflects the diversity of function.

Seed germination and dormancy are important traits in wheat production. They determine PHS resistance, thus affecting the yield and quality of wheat [46]. Identification of candidate genes that control seed dormancy and germination may help to minimize yield and quality losses caused by PHS. Absciscic acid (ABA) synthesis and sensitivity to ABA are clearly involved in the onset of dormancy during seed maturation [47]. ABSCISIC ACID-INSENSITIVE5 (*ABI5*) is a central transcription factor in ABA signaling. Pan et al. (2018) [42] found that *Arabidopsis* *VQ18* and *VQ26* proteins interact with the *ABI5* transcription factor to negatively modulate ABA response during seed germination. Decreasing the expression levels of *VQ18* and *VQ26* at the same time enhanced ABA signal transduction and thus inhibited seed germination, whereas overexpression of these two *VQ* genes led to decreased ABA sensitivity of germinated seeds, confirming the regulatory role of *VQ* proteins, which act antagonistically with *ABI5* to maintain appropriate ABA signaling levels and fine-tune seed germination. Nevertheless, the roles of the *TaVQ* gene remain largely unclear.

In the present study, we tested the 65 *TaVQ* genes expression during seed imbibition of six wheat varieties (HMC21, YXM, SNTT, J411, ZY9507 and ZM895). 13 *TaVQ* genes expression (*TaVQ8/-9/-13/-17/-25/-32/-34/-43/-48/-49/-53/-59/-62*) were consistently higher in low-dormancy varieties than in high-dormancy varieties. By contrast, the expression levels of 8 *TaVQ* genes (*TaVQ4/-16/-20/-35/-38/-42/-51/-56*) were consistently higher in high-dormancy varieties than in low-dormancy varieties. These 21 *TaVQ* genes may be participated in the regulation of seed dormancy and germination. According to the phylogenetic tree analysis, three of these 21 genes (*TaVQ8*, *TaVQ13*, and *TaVQ59*) are members of the *VQV* subfamily. Interestingly, *Arabidopsis* *AtVQ18* and *AtVQ26* involved in seed germination also belong to the *VQV* subfamily. These results suggest that *TaVQ8/-13/-59* may have similar functions in the regulation of seed dormancy and germination, a hypothesis that requires future validation.

Conclusions

Phylogeny and diversification of *VQ* genes in wheat were investigated from different levels, including phylogenetic tree construction and characterization of gene structures, conserved domains, chromosome positions, expression patterns, and promoter elements. In addition, we measured the expression levels of *TaVQ* genes in wheat varieties with contrasting seed dormancy and germination phenotypes by qRT-PCR to identify *TaVQ* gene family members that were potentially involved in seed dormancy and germination. Here, sixty-five *TaVQ* proteins were identified for the first time in common wheat, and bioinformatics analysis was performed to investigate their phylogenetic relationships and evolutionary divergence. The qRT-PCR data showed that 21 *TaVQ* candidate genes were potentially involved in seed dormancy and germination. These findings provide valuable information for further cloning and functional analysis of *TaVQ* genes, as well as useful candidate genes for improvement of PHS resistance in wheat.

Methods

Plant Materials

J411 (Jing 411), HMC21 (Hongmangchun 21), SNTT (Suiningtuotuo), ZM895 (Zhongmai 895), ZY9507 (Zhongyou 9507) and YXM (Yangxiaomai) were selected to test the *TaVQ* gene expression pattern. Their average GI values were 0.96, 0.09, 0.15, 0.92, 0.97 and 0.07 (J411 and HMC21 were provided by Shihe Xiao, Chinese Academy of Agricultural Sciences. ZM895, ZY9507, YXM and SNTT are provided by Xianchun Xia from Chinese Academy of Agricultural Sciences.). The germination index was determined according to previous studies [46].

Identification of wheat *VQ* genes

To determine the number of *VQ* genes in common wheat, we used sequences obtained from the Ensembl database (<http://plants.ensembl.org/index.html>) to build a local wheat database [46]. The *VQ* domain hidden Markov model (PF05678) was used to identify candidate genes by BLAST in the established local wheat database. To ensure the accuracy of the results, all candidate genes were inspected, repetitive sequences were removed, and Pfam, SMART, and NCBI online tools were used to verify the existence of the *VQ* conserved domain in all candidate genes [46, 48]. The ExPASy online tool was used to predict the isoelectric point (PI), protein molecular weight (MW), open reading frame (ORF), and other attributes of the *VQ* proteins.

Phylogenetic tree and multiple sequence alignment

FASTA sequence files were opened in ClustalX2.11 software [49-52] and used to generate a multiple sequence alignment from which a phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replicates in MEGA7.0 [43, 53-55]. The same method was used to build a composite phylogenetic tree of *VQ* protein sequences from corn, rice, Arabidopsis, and wheat.

Intron/exon structure and conserved motif analysis

The distribution and structure of exons and introns were determined by uploading CDS and genomic sequences to the Gene Structure Display Server (<http://gsds.cbi.pku.edu.cn/>) for plotting and analysis [7, 55, 56].

To predict structural differences among the *TaVQ* proteins, all candidate protein sequences were uploaded to the MEME online tool (<http://memesuite.org/tools/meme>) for conserved motif analysis using standard operating parameters [55, 57].

Identification of homologous pairs and calculation of Ka/Ks values

Using previously reported methods for the identification of homologous gene pairs (paralogs and orthologs), the nucleotide sequences of *VQ* genes from wheat and other species were compared using BLASTN [58, 59].

Wheat homologous gene pairs were compared and aligned in ClustalX 2.11, and the aligned sequences were analyzed in MEGA7.0 [60]. The results were uploaded to DnaSP v5.10.1 [61] to calculate the values of Ka (non-synonymous nucleotide mutation rate) and Ks (synonymous nucleotide mutation rate) for all homologous pairs. The formula $T = (Ks/2\lambda) \times 10^{-6}$ was used to estimate the approximate dates of divergence events. To further analyze Ka/Ks values, we used GraphPad Prism 5 software to generate a sliding window graph [7, 62]. A Ka/Ks ratio less than 1 indicates that a DNA mutation is harmful and under purifying selection, whereas a Ka/Ks ratio greater than 1 indicates that a DNA mutation is beneficial and under positive selection. A Ka/Ks ratio of 1 indicates neutral selection.

Chromosome location and gene ontology annotation

The chromosome location of *TaVQ* genes were downloaded from the Ensembl database, and chromosome maps were built using MapGene2Chromosome v2.0 [63].

Gene ontology (GO) annotations were assigned based on our transcriptome data (<http://amigo.geneontology.org/amigo>) in three types: biological_process, molecular_function and cellular_component [55].

Promoter analysis and subcellular location prediction

The 1500 bp sequence upstream of the transcription starting site of each *VQ* gene was downloaded from the Ensembl website, and the *cis*-acting elements in the promoter region were identified using the PlantCARE online tool [64]. WOLF was used to predict the subcellular localization of the *TaVQ* proteins [65].

Tissue expression pattern analysis

We collected three replicate seed tissue samples from HMC21 and J411 at 4, 6, and 10 h after seed imbibition for transcriptome sequencing. In addition, we obtained microarray data of 13 different tissues (three biological replicates) from the Gene Expression Omnibus database (<https://www.ncbi.nlm.nih.gov/geo/>) (accession number GSE12508) [66, 67]. Thermapper Plus was used to generate an expression heat map [45, 68].

RNA extraction and RT-qPCR analysis

Total RNA was extracted from seeds using the TaKaRa MiniBEST Universal RNA Extraction Kit (Takara, Tokyo, Japan). Primer Premier 5.0 was used to design 65 *TaVQ* gene-specific primers, and TaActin was used as the reference gene [69]. The total PCR volume was 10 µl. The following is the reaction process: the first was denaturation at 94 °C for 30 s, then 40–45 cycles for 5 s at 94 °C, 15 cycles at 50–60 °C, and finally an extension step at 72 °C for 10 s. We performed three biological replicates for each sample. Finally, we process the data and create the figure by GraphPad version 5 [70].

Abbreviations

qRT-PCR, quantitative real-time PCR; Ks, number of synonymous substitutions per synonymous site; Ka, number of non-synonymous substitutions per non-synonymous site; CDS, coding sequence; bp, base pair; aa, amino acids; MW, molecular weight; Da, Dalton; PHS, Pre-harvest sprouting; GI, Germination index; pI, isoelectric point; GO, gene ontology; . GSC, germinating seed coleoptile; GSR, germinating seed root; GSE, germinating seed, embryo; SR, seedling root; SC, seedling crown; SL, seedling leaf; II, immature inflorescence; Fba, floral bracts before anthesis; Pba, pistil before anthesis; Aba, anthers before anthesis; 3–5 DAP C, 3–5 DAP caryopsis; 22 DAP EM, 22 DAP embryo; 22 DAP EN, 22 DAP endosperm.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Availability of data and materials

The genome sequences of wheat, maize, rice and Arabidopsis were downloaded from the Ensembl database (<http://plants.ensembl.org/index.html>), PlantTFDB (<http://planttfdb.cbi.pku.edu.cn>), Rice Genome Annotation Project database (http://rice.plantbiology.msu.edu/analyses_search_locus.shtml) and Arabidopsis Information Resource (<http://www.arabidopsis.org>).

Competing Interests

The authors declare that they have no competing interests.

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Authors' contributions

XRC and CG conceived the study, put into effect the main bioinformatics analyses, and drafted the manuscript. DMX and XL took part in the experiments and drafting of the manuscript. XP, WG, SNY, HY, JJC, XYM and JL

processed the experimental data and helped to draft the manuscript. HPZ, CC, and CXM conceived and guided the experiments, and helped in coordinating the project and drafting the manuscript. All authors read and accepted the final manuscript.

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Figures

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