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Identification of the Valine-Glutamine gene family in *Chenopodium quinoa* Willd and analysis of its expression pattern and subcellular localization under drought stress

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Abstract

Background *Chenopodium quinoa* Willd (Quinoa) is highly tolerant to drought, cold, and salt stress. Gene editing technology development, and research on quinoa's drought resistance have attracted much attention. The transcriptional cofactor VQ plays an important role in the drought response in plants, but its role in quinoa has not been reported.

Results Bioinformatics identified 23 members of the quinoa VQ gene family, mainly located in the nucleus and unevenly distributed on 10 chromosomes. Gene structure and phylogenetic analysis indicated that the *VQ* genes were closely related and highly conserved, forming three subfamilies. The cis-acting elements of the promoter reveal its involvement in the response to light and hormonal stress. qRT-PCR analysis showed that all *VQ* genes were differentially expressed under drought stress, among which *CqVQ13* was significantly up-regulated, and subcellular localization indicated that it was localized to the nucleus.

Conclusion This study conducted a systematic bioinformatics analysis of the basic physicochemical properties and chromosome localization of 23 members of the CqVQ gene family. Combined with transcriptome gene expression profiling and qRT-PCR, we found that *CqVQ13* was significantly up-regulated under drought stress and localized in the nucleus. This discovery provides an important candidate gene for drought response studies in quinoa and lays the foundation for further exploration of the molecular mechanisms of the VQ gene family in response to drought stress.

Keywords Quinoa, VQ gene family, Bioinformatic analysis, Subcellular localization analysis, Drought stress

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Introduction

Quinoa has attracted much attention due to its unique biological characteristics and rich nutritional value. As a heterotetraploid annual dicot of Chenopodium [1], it originates from the Andes Mountains, and its growth environment endows it with excellent characteristics such as drought tolerance, cold tolerance, and salt tolerance [2]. These characteristics enable quinoa to grow and reproduce under extreme ecological conditions, making it one of the important crops in agricultural production. Quinoa seeds are rich in various amino acids, minerals, and vitamins required by the human body, especially lysine, which has a much higher content of essential amino acids than other grains. Therefore, they are known as the "nutritional gold and king of vegetarianism" [3]. With the rapid development of biotechnology, the high-quality reference genome of quinoa has been released, which not only contributes to the mining of stress-related genes in quinoa but also provides valuable data resources and important theoretical support for crop improvement through modern biotechnology such as gene editing in the future [4]. Drought stress has become one of the main threats to global crop yield in nature [5], which has caused great damage to agricultural production, leading to crop reduction and endangering food security [6]. Therefore, cultivating high-yield drought-resistant crops has become an urgent task. However, due to its excellent stress resistance and high nutritional value, quinoa has shown great potential in cultivating arid and semi-arid areas, which is of non-negligible importance for maintaining ecological balance, promoting economic development, and ensuring nutritional security [7]. Thus exploring the biological mechanisms of stress resistance in quinoa and uncovering stress-related genes are of great significance for improving crop adaptability to drought stress. The transcription factor VQ plays a crucial role in plant stress resistance. Transcription cofactor VQ (VQ-motif-containing protein) is a class of plant-specific proteins with a conserved amino acid sequence of "FxxhVQxhTG". Moreover, it contains two highly conserved dipeptides valine, and glutamine in its sequence, so it is called VQ protein [8]. VQ proteins are not only widely present in monocot and dicot plants but also can be detected in some fungi, lower animals, and bacteria, where they respond to abiotic and biotic stresses and other biological processes through various mechanisms [9–11]. The first VQ protein was reported to have been identified from Arabidopsis using the yeast two-hybrid [12], and since then, using bioinformatics methods, 18, 49, and 39 VQ members were identified in grape [13], apple [14], and rice [15], respectively. Previous studies have shown that *VQ* gene expression is induced by salt, drought, and temperature stress, as well as ABA [11, 16, 17]. Among them, the expression of most genes in cotton, maize, and rice is induced under drought, salt, cold, and heat stress [15, 18, 19]. PEG and NaCl treatments lead to significant expression of *IbVQ4*, indicating that *IbVQ4* positively responds to drought and salt stress [20]. Transgenic *Arabidopsis thaliana* overexpressing *AtVQ15* is highly sensitive to salt stress, and the mutant of this gene shows strong salt tolerance [21]. For example, under salt stress, *AtVQ9* expression is increased and then interacts with *WRKY8* to repress the expression of its target genes, thereby regulating salt stress tolerance [17]. Arabidopsis WRKY2 and WRKY34 transcription factors interact with VQ20 protein to regulate pollen development and function [22]. As transcription cofactors, VQ proteins can not only interact with WRKY transcription factors, but also with ABI5, PIF1, and other transcription factors, and play an important role in plant growth and development and stress response. For example, *AtVQ18* or *AtVQ26* can interact with ABI5 and inhibit its transcriptional activation ability, thereby negatively regulating ABA response during seed germination and seedling establishment [23]. In Arabidopsis, *AtVQ29* interacts with PHY interacter1 (PIF1) to promote transcriptional activity in early seedling development [24]. *TaVQ4-D* interacts with *TaMPK3* and *TaMPK6* and plays a role in plant drought stress as a phosphorylation substrate of *TaMPK3* and *TaMPK6* [25]. Although the exploration of VQ protein has been involved in a variety of model crops, it has not been reported in quinoa species, which provides a new direction for this study.

In a word, this research aims to analyze the biological characteristics and expression patterns of the transcription cofactor VQ gene family in quinoa under drought stress through systematic bioinformatics analysis and qRT-PCR verification, as well as to find out the drought resistance-related genes for subcellular localization analysis to further understand their functions in cells. We expect to be able to screen the *VQ* drought resistance gene to lay the foundation for the functional characterization of this gene in the later stage. This study will not only help us to better understand and utilize the stress resistance of quinoa but also provide scientific support for crop variety improvement and stress resistance research.

Materials and methods

Materials and treatment

In this research, Longquinoa No. 1 (from Gansu Academy of Agricultural Sciences, Gansu, China) was used as cultivation material, the specific disinfection method was as follows: The seeds were first disinfected in 75% ethanol for 30 s, then rinsed twice with sterile water, then disinfected in 5% NaClO for 15 min, then rinsed 5–6 times with sterile water. The seeds were planted in POTS with a diameter of 15 cm. The composition of cultivated

soil was a mixture of nutrient soil and vermiculite (mixture ratio 3:1). Ten seeds were sowed in each pot, covered with vermiculite, irrigated, and cultivated in a solar greenhouse with light intensity of 4000 LX, day and night temperature of (15–27)°C /(8–18)°C, and humidity of (70%±10%). When the seedling age reached 30 days, the seedlings were thinned to 5 plants per pot. In addition, to ensure adequate nutrition, 300 mL of 1/2 Hoagland nutrient solution was watered to replenish nutrients. When seedlings were approximately 40 d old, seedlings were treated with drought (20% PEG6000) for 3, 6, 9, and 12 h, and the control group was well watered. Three replicates were set up for each treatment, and the treated seedlings were randomly divided into three groups with six plants in each group to sample the leaves and roots of quinoa seedlings. After all samples were collected, they were flash-frozen in liquid nitrogen and placed in a -80 °C refrigerator. It was used for subsequent quantitative tests [26, 27].

Screening and identification of the VQ gene family members in quinoa

The genome sequence and structural annotation information of quinoa were obtained from the Phytomoze12 database (https://phytozome-next.jgi.doe.gov/) [28]. *Arabidopsis thaliana* VQ family protein sequences from the TAIR database (https://www.arabidopsis.org/) to download [26], Using the Arabidopsis VQ protein sequence as a reference, the complete genome protein sequence of Quinoa was aligned using BLAST-based on the conserved domain of VQ (PF05678). With the help of Pfam (http://pfam.xfam.org/family), NCBI-CDD (https://www.ncbi.nlm.nih.gov/cdd/), and SMART (web site: http://smart.embl-heidelberg.de/) multiple online tools to further validate and confirm these candidate genes [29], and finally 23 VQ family members were identified in quinoa.

Basic physicochemical properties of proteins and phylogenetic analysis

The number of amino acids, isoelectric point, and molecular weight of quinoa VQ protein were derived by the online software ExPASy (https://web.expasy.org/protparam/) [30], and analysis of the basic physical and chemical properties such as hydrophobicity. Through the Port-Prediction (https://web.expasy.org/protparam/) to predict the subcellular localization [31].

Respectively from the Phytozome and Ensembl (http://plants.ensembl.org/index.html) [32] database download quinoa, Arabidopsis, and cabbage genome-wide data, protein sequences of the VQ gene family in quinoa, Arabidopsis, and Chinese cabbage were obtained. The ClustalW method with default parameters in MEGA7 (https://www.megasoftware.net/MEGA7-CC-Quick-Start-Tutorial) software was used to align the amino acid sequences of the identified VQs family proteins of Chinese cabbage, Arabidopsis, and quinoa. The alignment results were used to construct the phylogenetic tree using the maximum likelihood method (ML), the bootstrap was repeated 1000 times, and other parameters were defaulted. The evolutionary tree was beautified by Itol (http://itol.embl.de/) [33].

Gene structure and construction of conserved motifs

The *VQ* gene structure information was extracted from the quinoa genome and submitted to the GSDS 2.0 (http://gsds.gao-lab.org/index.php) website [34]. The format was selected as GTF/GFF3, draw the gene structure map. The amino acid sequence of quinoa VQ was submitted to MEME (http://meme-suite.org/tools/meme) online software for amino acid conservative Motif analysis. The number of motif searches was set to 10, and other parameters were defaulted [35].

Gene duplication and chromosomal localization

Based on the gene annotation information (Cq_PI614886_gene_V1_pseudomolecule.gff), Tbtools (version 1.09876) was used to map the chromosome location of the *VQ* gene. Based on quinoa Fasta sequence of *VQ* gene in NCBI BLAST (https://blast.ncbi.nlm.nih.gov) [36] gene replication analysis, calculation of duplicate genes on Ka/Ks values, evaluation of evolutionary selection. PAML (pal2nal) CODEML program (http://www.bork.embl.de/PAL2NAL) in the estimation of synonymous (Ks) and non-synonymous (Ka) replacement rate [37], using the following formula to determine each SlCAMTA gene duplication of time (millions of years ago, MYA: $T = Ks/2\lambda(\lambda = 6.56E\text{-}9)$ [38].

Construction of interaction network diagram between upstream cis-acting elements and proteins

The sequence 2000 bp upstream of the transcription start site of the VQ gene family members of quinoa was obtained from the quinoa genome database. Then through PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [39]the retrieval and analysis of the cis of *VQ* gene promoter element composition. Finally, cis-acting elements related to light response, hormone response, tissue-specific expression, and stress response were screened for analysis. The protein sequences of quinoa VQ family members were submitted to the STRING (http://STRINGdb.org/) website [40], and then the STRING software was used to construct the VQ network with Arabidopsis as the template to further predict the protein interaction network map of quinoa.

Expression pattern analysis of transcriptome data as well as RNA extraction and real-time quantitative PCR

Quinoa different tissues and organs (No: PRJNA394651) and different processing (No: PRJNA306026) transcriptome data from Bioproject database (http://www.ncbi.nlm.nih.gov/bioproject). PrimerPremier 5 online software was used to design primers for quinoa VQ family fluorescence quantitative PCR (Table S1). Design parameters included amplicon length, 100–200 bp; Primer length, 15–25 bp; Melting temperature (Tm), 56–70 °C. RNA was extracted using the plant extraction kit RNAplant-RTR2303 (Beijing Zhongke Ruitai Biotechnology Co, LTD.) according to the operating instructions. RNA integrity and purity were determined using an Agilent 2100 Bioanalyzer and NanoDrop, respectively. After passing the test, the RNA was placed at -80 °C for later use. cDNA was obtained by reverse transcription of Primer ScriptTM RT Regent Kit with gDNA Eraser (TaKaRa, Shanghai) kit and quantified by SYBR Primer Ex TaqTM II (TaKaRa) kit. Quantitative PCR was performed on a Light Cycler® 96 Real-Time PCR System (Roche, Switzerland). The reaction system consisted of 20 μL of total amplification reaction, including 1 μL of template cDNA, 1 μL of each upstream and downstream primer (10 μmol/L), 10 μL of EvaGreen 2×qPCR MasterMix, and 7 μL of ddH_2O. The reaction was performed using a two-step procedure as follows: 40 cycles of predenaturation at 95 °C for 30 s, denaturation at 95 °C for 15 s, and annealing/extension at 60 °C for 30 s [41]. *TUB-9* actin was used as a reference gene. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [42]. One-way analysis of variance was used to compare and analyze the process with SPSS 22.0 software, and the significance level was $p < 0.05$ [27]. Each experiment was repeated three times with independent RNA samples.

Subcellular localization analysis

The cloned *CqVQ13* gene was integrated into the expression vector containing the green fluorescent protein (GFP) gene pCAMBIA1302-GFP by homologous recombination to construct the pCAMBIA1302-CqVQ13-GFP fusion expression vector, and the vector plasmid was transferred into *Agrobacterium Tumefacium* GV3101. The transformed *Nicotiana benthamiana* leaves (10 mm×16 mm) were cut with scissors and laid flat on the slide with the back side up. The cover glass was gently covered with forceps to drive away the bubbles on the leaves, and the fixed liquid seeping from the edge of the cover glass was gently wiped with clean filter paper. Then, the distribution of the GFP green fluorescence signal emitted by the transient expression vector was observed under a laser confocal scanning electron microscope (LSM800) at the excitation wavelength of 488 nm and the emission wavelength of 510 nm to determine the expression location of *CqVQ13* in tobacco cells [43].

Results

Analysis of basic physical and chemical properties of the CqVQ gene family

Using the known *VQ* genes of Arabidopsis, the whole quinoa genome was aligned and analyzed, and 23 VQ family members were finally identified, all containing VQ protein domains. The number of amino acid residues in this family is between 90 (CqVQ11/22) and 506 (CqVQ23), the molecular weight is between 10755.04 (CqVQ11/22) and 57263.59 (CqVQ23), and the isoelectric point is between 4.15 (CqVQ20) and 11.43 (CqVQ04). The hydrophobicity index of CqVQ proteins was less than 0, indicating that these proteins were hydrophilic proteins. The prediction of subcellular localization showed that most CqVQ proteins were localized in the nucleus, while CqVQ20 protein was localized in the cell membrane, cytoplasm, and nucleus. Similarly, the CqVQ23 protein was localized in the chloroplast and nucleus. And functions at these sites (Table S2).

Phylogenetic analysis of the CqVQ gene family

To clarify the relationship among the *VQ* genes in quinoa, a phylogenetic tree was constructed based on the protein sequences encoded by quinoa (CqVQ), Arabidopsis (AtVQ), spinach (SoVQ), and Chinese cabbage (BrVQ) (Fig. 1).

Phylogenetic analysis indicates that the VQ gene family can be divided into four distinct subfamilies, with Group IV being the largest branch. The VQ gene family of quinoa mainly exists in Groups I, II, and IV, while the VQ gene family members of *Arabidopsis thaliana* and *Spinacia oleracea* are distributed in all four Groups. And the results showed that quinoa is more closely related to spinach than to Arabidopsis and Chinese cabbage. There are 19 pairs of paralogs on the side branches of the phylogenetic tree, including *CqVQ11/CqVQ12*, *CqVQ16/CqVQ23*, *BrVQ22-1/BrVQ22-2*, and *SoVQ06/SoVQ07*, and 29 pairs of orthologs on the main trunk, including *AtVQ15/BrVQ15*, *CqVQ04/SoVQ08*, *AtVQ02/SoVQ02*, and *SoVQ10/CqVQ06*.

Conserved motifs and gene structure analysis of the CqVQ gene family

The basic motifs of VQ family proteins were predicted by MEME online software, and 10 conserved motifs were retrieved from 23 VQ proteins in quinoa (Fig. 2).

Each quinoa VQ protein has a different motif (1–5 Motifs), and the genes with the same motif are located in the same branch. Motif 1 was present in all protein family members, indicating that Motif 1 was highly conserved in each family member. Motif 10 was only found

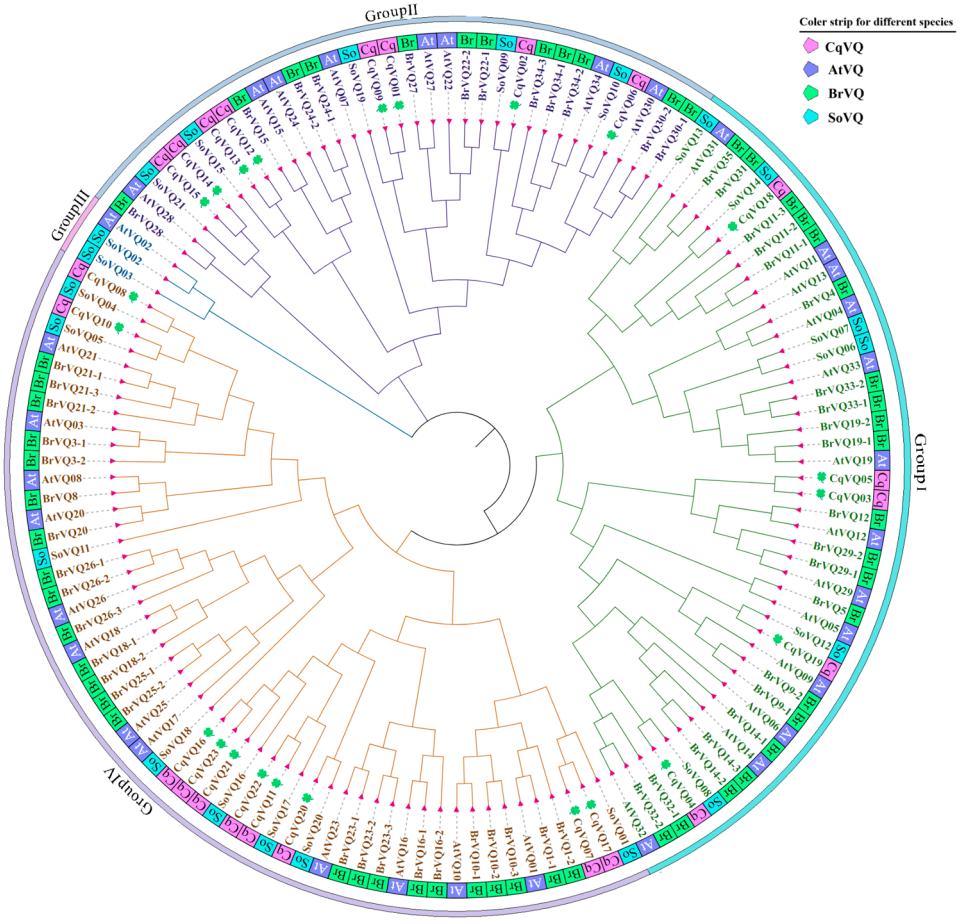

Fig. 1 Phylogenetic tree of VQ gene family in Quinoa, Chinese cabbage, Spinach and Arabidopsis

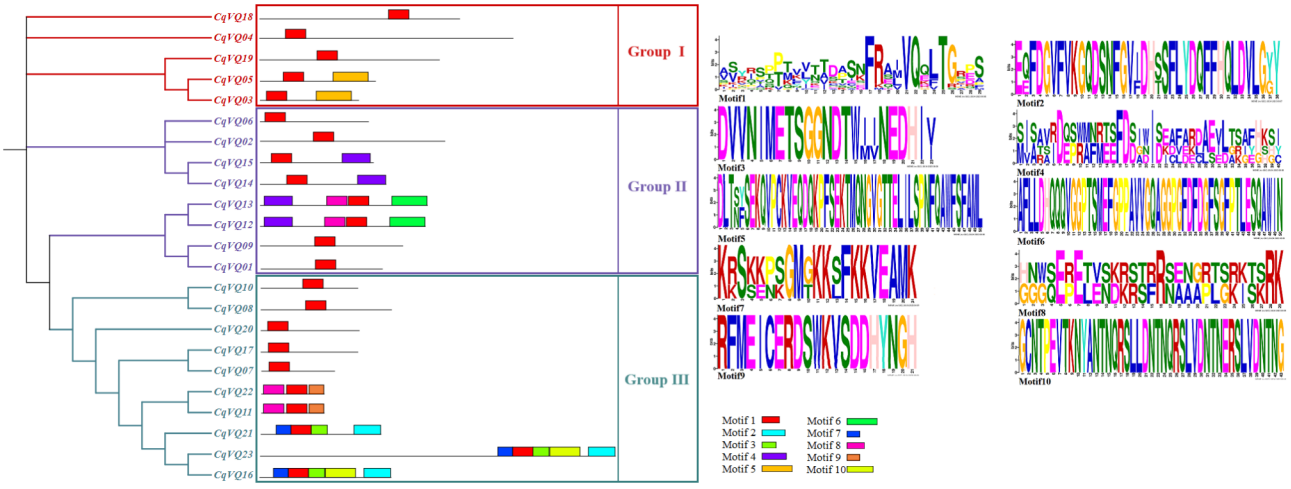

Fig. 2 The conserved motifs of quinoa VQ family proteins

in two protein family members, CqVQ16 and CqVQ23, and CqVQ16 and CqVQ23 were located in the same branch. Similarly, Motif 9 was only found in two protein family members, CqVQ11 and CqVQ22, which were also located in the same branch. Motif 5 is present only in two protein family members, CqVQ03 and CqVQ05, which are also located in the same branch. The presence of motifs 2, 3, 7, and 10 in both CqVQ16 and CqVQ23 proteins indicated that these four motifs shared conserved regions and similar functions in quinoa. Secondly,

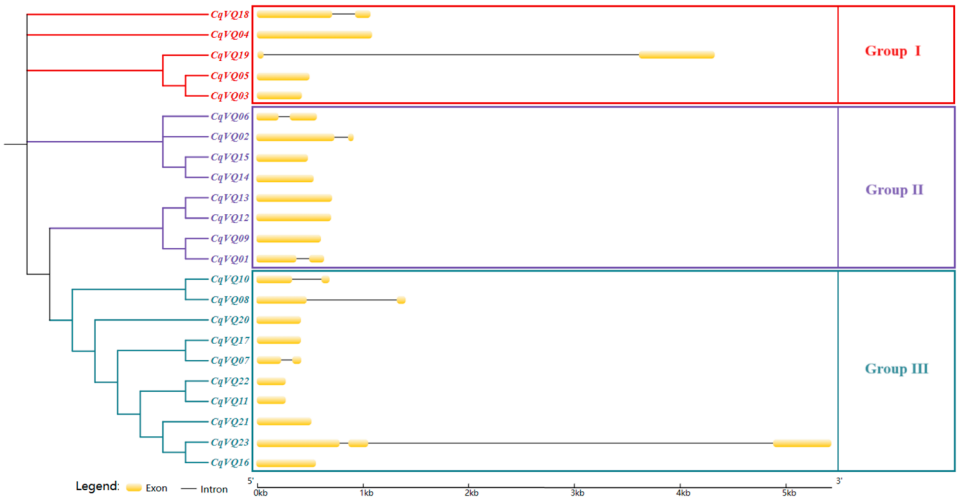

Fig. 3 The intron-exon structure of quinoa *VQ* genes was mapped by phylogenetic tree

Table 1 Gene duplication analysis of the VQ gene family in quinoa

Gene1	Gene2	Ka	Ks	Ka/Ks	MYA	Ks/2x	Purifying selection	Duplicate type
CqVQ03	*CqVQ05*	0.009	0.126	0.075	8.404	8404008.840	Yes	Segmental
CqVQ13	*CqVQ15*	0.019	0.201	0.094	13.413	13413199.330	Yes	Segmental
CqVQ16	*CqVQ17*	0.050	0.111	0.451	7.414	7414110.119	Yes	Segmental

CqVQ16 and CqVQ23 had the most motifs, while 11 VQ family members including CqVQ01 and CqVQ02 had the least motifs, indicating that different *VQ* genes possess different numbers of motifs, which may lead to large differences in traits.

By studying the exon-intron organization, the evolutionary and structural diversity of the *VQ* genes in quinoa was explored. From the genetic structure map, the number and position of the introns and exons of the quinoa *VQ* gene were thoroughly analyzed (Fig. 3). All the VQ gene family members had no UTR region. *CqVQ23* contained three exons, while *CqVQ01*, *CqVQ02*, *CqVQ06-CqVQ08*, *CqVQ10*, *CqVQ18* and *CqVQ19* (34.7%) contained two exons. The other 14 family members (*CqVQ03-CqVQ05*, *CqVQ09*, *CqVQ11-CqVQ17*, and *CqVQ20-CqVQ22*) contained only one exon.

Gene duplication analysis and chromosomal localization of the CqVQ gene family

During evolution, since orthologous genes usually maintain the same function in different species during evolution, orthologous relationships between quinoa VQ gene family members were established, 23 and orthologous sequences with more than 75% sequence identity in cDNA and amino acid composition were screened for further evolutionary analysis (Table 1).

The Ka, Ks, and Ka/Ks ratio of the quinoa *VQ* gene were calculated as the ratio of nonsynonymous substitution rate (Ka) to synonymous substitution rate (Ks). The potential differentiation functions and purifying selection pressures of the VQ gene family members in quinoa were explored. We observed that group 3 (*CqVQ16/CqVQ17*) had higher Ka values between genomes than the other orthologs, indicating that the proteins of these orthologous pairs evolved more rapidly. In addition, group 3 (*CqVQ16/CqVQ17*) had a higher ratio of Ka/Ks (0.451) among all the orthologous genes, indicating that it had undergone higher evolutionary pressure. In the above chart, we found that among the three groups of orthologous genes, the earliest group 2 (*CqVQ13/CqVQ15*) duplication occurred about 13.4 million years ago, and the latest group 3 (*CqVQ16/CqVQ17*) duplication occurred about 7.4 million years ago. The first group of homologous genes (*CqVQ03/CqVQ05*), occurred about 8.4 million years ago.

To verify the relationship between genetic differentiation and gene duplication, we chromosomal mapped and analyzed the *VQ* genes obtained from the quinoa database (Fig. 4).

Twenty-three *VQ* genes were found to be located on 10 chromosomes. There was only one *VQ* gene on each of the four chromosomes Chr01(B), Chr04(A), Chr13(A) and Chr17(B), and *CqVQ21* was located on chromosome Chr01(B). *CqVQ16* was located on chromosome Chr04(A), *CqVQ04* on chromosome Chr13(A), and *CqVQ07* on chromosome Chr17(B). There were two *CqVQ* genes on both chromosomes Chr00 and Chr07(A), of which *CqVQ22* and *CqVQ23* were located on chromosome Chr00, and *CqVQ12* and *CqVQ15* were located on chromosome Chr07(A). There were three *CqVQ*

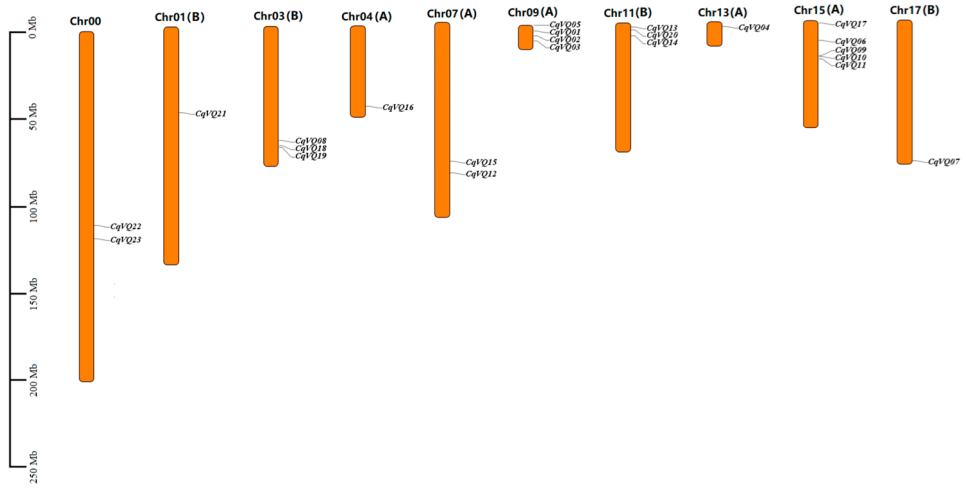

Fig. 4 Chromosomal location of quinoa VQ gene family

genes on Chr03(B) and Chr11(B), of which *CqVQ08*, *CqVQ18*, and *CqVQ19* were located on Chr03(B), and *CqVQ08*, *CqVQ18*, and *CqVQ19* were located on chr03 (b). *CqVQ13*, *CqVQ20* and *CqVQ14* were located on Chr11(B). *CqVQ01*, *CqVQ02*, *CqVQ03* and *CqVQ05* were located on Chr09(A). Five genes, *CqVQ06*, *CqVQ09*, *CqVQ10*, *CqVQ11* and *CqVQ17* were located on chromosome Chr15(A).

Analysis of physicochemical properties of protein secondary structure of CqVQ gene family

By predicting the secondary structure of the quinoa VQ protein family, the study showed that the secondary structure of the quinoa VQ protein family contained α-helix, extended chain structure, and random coil, and most protein families were in the random coil state, followed by α-helix structure and extended chain structure (Table S5).

In the random coil structure, 6 proteins (CqVQ01-CqVQ04, CqVQ18 and CqVQ19) accounted for more than 70%, and CqVQ05, CqVQ06, CqVQ09, CqVQ10, CqVQ14 and CqVQ15 accounted for between 60% and 70%. The proportion of other CqVQ proteins (CqVQ07, CqVQ08, CqVQ11-CqVQ13, CqVQ16, CqVQ17 and CQVQ20-CQ23) ranged from 42 to 60%. In the α-helix structure, the number of proteins accounting for more than 18% was the largest, reaching 13 (CqVQ06, CqVQ07, CQVQQ10-CQVQ17, and CqVQ21-CqVQ23). CqVQ03, CqVQ05, CqVQ08, CqVQ18 and CqVQ20 all accounted for about 15%, while CqVQ01, CqVQ02, CqVQ04, CqVQ09 and CqVQ19 all accounted for less than 10%. In the extended chain structure, CqVQ01, CqVQ07-CqVQ09, CqVQ11, CqVQ13, CqVQ16, CqVQ17 and CqVQ20-CqVQ23 accounted for more than 18%. CqVQ02, CqVQ03, CqVQ05, CqVQ06, CqVQ10, CqVQ12, CqVQ15 and CqVQ18 accounted for 10–18%, and CqVQ04, CqVQ14 and CqVQ19 accounted for less than 10%.

Cis-acting element analysis and protein interaction network analysis of the CqVQ gene family

The study of cis-acting elements in the promoter region of the VQ gene family in quinoa revealed a total of 52 cis-acting elements related to plant hormones, stress, light response, and tissue-specific expression, and different genes had different types and numbers of cis-acting elements (Fig. 5).

The *CqVQ* gene contained the most cis-acting elements, up to 25 kinds, of which G-box, Box4, TCT-motif, GT1-motif, and Sp1 were present in multiple copies in multiple *CqVQ* genes. *CqVQ04*, *CqVQ09*, *CqVQ14*, and *CqVQ19* were the only four genes without the G-box element, and *CqVQ15* and *CqVQ23* were the only two genes without the Box4 element. GTGGC-motif elements only existed in the *CqVQ23* gene, AAAC-motif elements only existed in the *CqVQ18* gene and ACA-motif elements only existed in the *CqVQ14* gene. There were 13 cis-acting elements related to plant tissue-specific expression. Among them, the cis-regulatory element ARE, which was essential for anaerobic induction, was present in multiple genes in multiple copies, while the WUN-motif was only present in the gene *CqVQ05* in a single copy. MBS1, a cis-regulatory element involved in flavonoid biosynthesis, and AT-rich elements, a cis-acting element involved in the binding site of AT-rich DNA-binding protein (ATBP-1), were both present in the quinoa *VQ* gene in single copy form. The activation element AT-rich sequence mediated by the maximum elicitor is present as a single copy in gene *CqVQ12*, whereas it is present as a multiple copy in gene *CqVQ13*. Circadian, RY element, and GCN4_motif were the two cis-acting elements involved in circadian rhythm control, seed-specific regulation, and

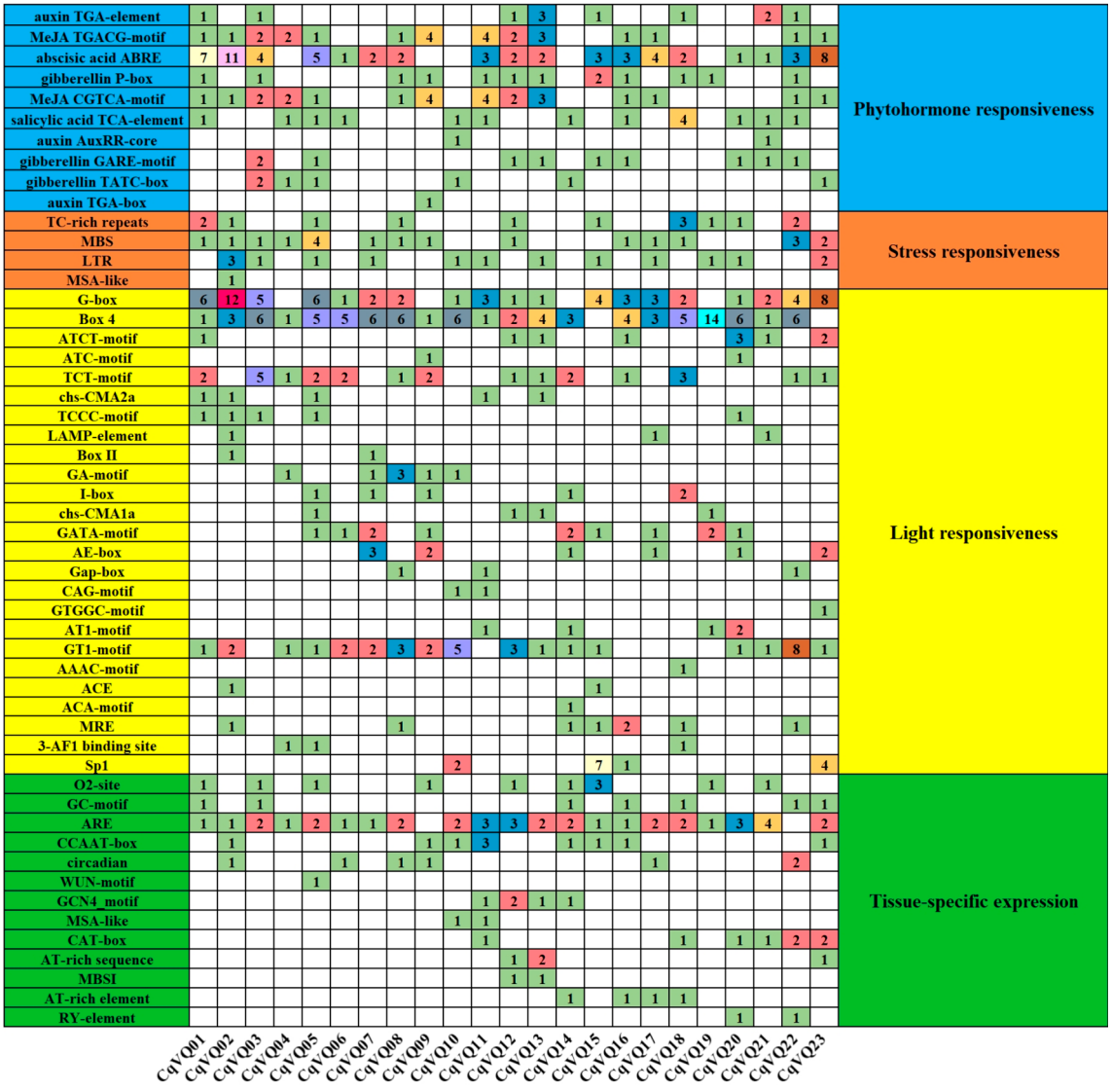

Fig. 5 Identification of the cis-regulatory element in the 2000 bp promoter region of the *CqVQ*

endosperm expression, respectively. There were 10 cis-acting elements related to plant hormones, among which abscisic acid-responsive element (ABRE) existed in multiple genes in the form of multiple copies. The salicylic acid response element is the TCA element, which was present in multiple copies of the *CqVQ18* gene. However, *CqVQ01*, *CqVQ04-CqVQ06*, *CqVQ10*, *CqVQ11*, *CqVQ14*, *CqVQ16* and *CqVQ20-CqVQ22* genes only existed in single copy form. The P-box, GARE-motif, and TATC-box were all GA responsive elements, among which the GARE-motif and TATC-box elements were present in the form of multiple copies in gene *CqVQ03* and the form of a single copy in gene *CqVQ05*. Similarly, P-box and TATC-box elements were present as single copies in genes *CqVQ12*, *CqVQ13*, *CqVQ16*, and *CqVQ22*. The auxin response elements included TGA-box, TGA-element, and AuxRR-core. The TGA-box element was only present in the *CqVQ09* gene and was present as a single copy. The AuxRR-core element was only present in the genes *CqVQ10* and *CqVQ21* in the form of a single copy, and the methyl jasmonate response elements TGACG-motif and CGTCA-motif were present in multiple copies in several genes. There were four cis-acting elements involved in stress-related responses, including drought-inducing element MBS, low-temperature response element LTR, MSA-like cis-acting elements involved in cell cycle regulation and TC-rich repeats involved in defense and stress response.

The study of protein interaction networks not only helps to understand the function of the individual proteins that constitute the network but also helps to explain the biological function of most proteins in the cell and to identify key proteins in the protein interaction network, which allows for the study of proteins associated with

stress resistance. Therefore, to further understand the interaction of *VQ* genes in quinoa, in this study, a protein interaction network map was constructed based on Arabidopsis homologous proteins (Fig. 6A). All quinoa VQ-like proteins were predicted to participate in the known protein-protein interaction network of Arabidopsis VQ, indicating a close relationship between the two proteins.

Among them, we found that AtVQ22 acts as a positive regulator of plant growth in Arabidopsis, so CqVQ01 and CqVQ09 may have a similar function in this study. In addition, studies have shown that AtVQ10 and AtVQ11 might regulate the activity of WRKY transcription factors, and AtVQ10 and AtVQ11 might play a regulatory role in the ABA signaling pathway in plants, and participate in the regulation of plant adaptation to high salt or high osmotic stress, as well as the adaptation of plants to low or high-temperature stress. In this study, AtVQ10 CqVQ07 and CqVQ17 proteins played corresponding functions. However, the AtVQ11 protein and CqVQ06 protein played similar functions [44]. We understood that the protein MKS1 acts as a regulator of plant defense responses and that MKS1 can function as an MPK4 adaptor or coupling protein, affecting the activity of WRKY factors and other proteins. However, the sequence of the MKS1 protein is similar to that of the CqVQ10 protein in the protein interaction network diagram, so the CqVQ10 protein might have similar functions to the MKS1 protein [12]. CaMBP25, a calmodulin-binding protein, acted as a negative regulator of osmotic stress tolerance, and *AtCaMBP25* gene expression was induced by various stress signals, including dehydration, low temperature, and high salt because CaMBP25 protein sequence is highly similar to CqVQ13 protein sequence. Therefore, the CqVQ10 protein may also have similar functions to the CaMBP25 protein [21]. AtVQ9 acts as a negative regulator of the salt stress response and acts as a repressor of WRKY8 transcription factor by reducing WRKY8 DNA binding activity and acting against WRKY8 to regulate sodium and potassium homeostasis under salt stress. The sequence of the AtVQ9 protein was highly similar to that of the CqVQ19 protein. Therefore, we hypothesized that the CqVQ19 protein sequence might also have a similar function [17]. IKU1 regulates seed size by negatively regulating the cellularization of syncytial endosperm and might act by binding and regulating the activity of the WRKY10 transcription factor. However, the IKU1 protein and CqVQ04 protein had similar sequence similarities, so it was possible that the CqVQ04 protein sequence also had a similar function to the IKU1 protein [45]. As shown in the figure, there were interactions between all Arabidopsis family members, so we speculated that there are also interactions between members of its similar quinoa McKein family. In addition, we made more extensive predictions, not only limited to the interaction between VQ proteins but also extended to other genes that interact with VQ proteins, such as transcription factors. In particular, we focused on the WRKY transcription factors that interacted with VQ proteins, and the prediction results showed that CqVQ01/04/07/09/10/13/17 all interacted with WRKY8/10/25/33/51, which was of great significance for understanding the role of VQ proteins in gene regulatory networks.

Expression profiling of the CqVQ gene family

The *CqVQ* genes showed tissue specificity in roots and shoots under different treatments (Fig. 6B). Among them, *CqVQ07*, *CqVQ11*, *CqVQ12*, *CqVQ17*, *CqVQ20*, and *CqVQ21* were expressed at low levels in all treatments. However, eleven genes, *CqVQ02*, *CqVQ03*, *CqVQ05*, *CqVQ06*, *CqVQ08-CqVQ10*, *CqVQ13*, *CqVQ14*, *CqVQ18*, and *CqVQ19*, were found to have low expression levels in various treatments of young shoot tissues. Secondly, the expression levels of *CqVQ01*, *CqVQ09*, *CqVQ13*, *CqVQ16*, and *CqVQ18* genes were higher

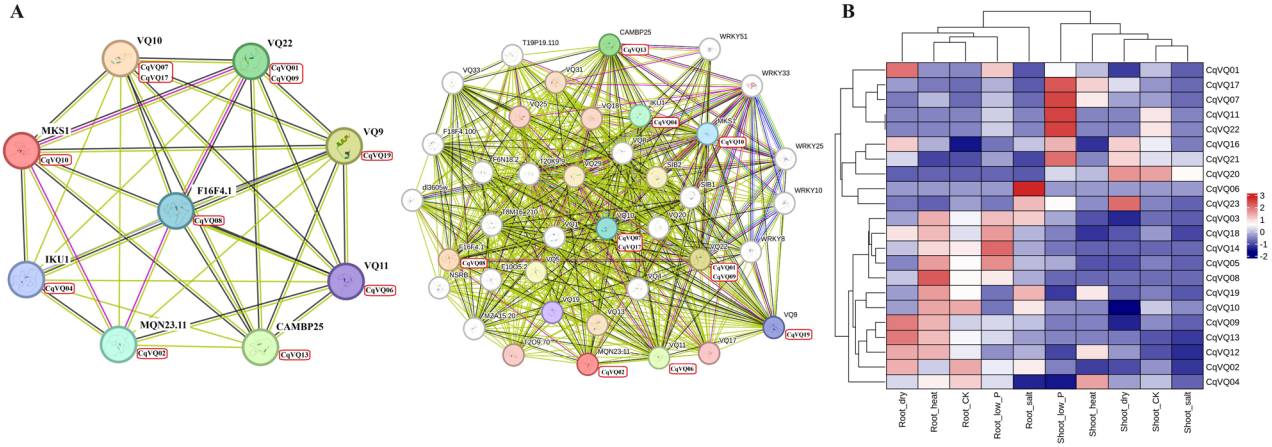

Fig. 6 **A**: Protein-protein interaction network analysis of CqVQ proteins; **B**: Relative expression profiles of *VQ* genes in different tissues of quinoa

under drought stress than those in the control group. Nine genes (*CqVQ03*, *CqVQ05*, *CqVQ08*, *CqVQ09*, *CqVQ12-CqVQ14*, *CqVQ18*, and *CqVQ19*) were highly expressed under high-temperature stress, and four genes (*CqVQ03*, *CqVQ06*, *CqVQ19*, and *CqVQ23*) were highly expressed under salt stress. The expression levels of *CqVQ01*, *CqVQ03*, *CqVQ05*, *CqVQ08*, *CqVQ14*, and *CqVQ18* were higher under low P stress. Compared with the control group, the expression levels of *CqVQ16*, *CqVQ21*, and *CqVQ23* genes were higher under drought stress, while the expression levels of *CqVQ11* and *CqVQ22* genes were lower under drought stress. The expression levels of *CqVQ04*, *CqVQ07*, *CqVQ12*, *CqVQ17*, and *CqVQ19* were higher under high-temperature stress, while the expression levels of *CqVQ11*, *CqVQ16* and *CqVQ20-CqVQ22* were lower under high-temperature stress. The expression levels of *CqVQ01*, *CqVQ07*, *CqVQ11*, *CqVQ16*, *CqVQ17*, *CqVQ21*, and *CqVQ22* genes were higher under low P stress, but the expression level of *CqVQ20* gene was lower under low P stress. Compared with the control group, the expression levels of all genes under salt stress were lower. The results also showed that the expression of *CqVQ06* was the most significant under salt stress in root tissue, and the expression of *CqVQ07*, *CqVQ11*, *CqVQ17*, and *CqVQ22* were the most significant under low phosphorus stress in shoot tissue. The expression levels of the above five genes were the highest under various stresses in both root and shoot tissues. These results suggested a potential role of the quinoa *VQ* gene in improving the tolerance of quinoa to water stress.

Analysis of the expression pattern of the CqVQ gene family under drought stress

After 20%PEG6000 treatment, the expression of *CqVQ02*, *CqVQ04*, *CqVQ12*, *CqVQ15*, *CqVQ18-CqVQ20*, and *CqVQ22* genes were up-regulated with increasing treatment time compared with the control.

The expression levels of *CqVQ02*, *CqVQ04*, *CqVQ12*, and *CqVQ18-CqVQ20* genes were significantly up-regulated at 9 h and 12 h compared with the control, and the expression level of *CqVQ02* at 12 h was significantly higher than that of the control and at 3 h, 6 h, and 9 h. The expression levels of *CqVQ04*, *CqVQ12*, *CqVQ15*, *CqVQ18*, *CqVQ20*, and *CqVQ22* genes at 9 h were significantly higher than that at 3 h, 6 h, and 12 h, except that the expression level of *CqVQ19* at 3 h was higher than that of the control and at 6 h, 9 h, and 12 h. The expression levels of *CqVQ01*, *CqVQ03*, *CqVQ05*, *CqVQ07*, *CqVQ08*, *CqVQ09*, *CqVQ10*, *CqVQ11*, *CqVQ13*, and *CqVQ16* were significantly up-regulated at 9 h and 12 h compared with the control. The expression levels of *CqVQ07*, *CqVQ10*, *CqVQ11*, and *CqVQ16* at 9 h were significantly higher than those at 3 h, 6 h, and 12 h in the control group. The expression levels of *CqVQ01*, *CqVQ03*, *CqVQ05*, *CqVQ08*, *CqVQ09*, and *CqVQ13* at 12 h were significantly higher than those at 3 h, 6 h and 9 h in the control group.

After 20%PEG6000 treatment in the root tissue structure, with the increase of treatment time, The 13 genes *CqVQ01*, *CqVQ04*, *CqVQ07*, *CqVQ08*, *CqVQ09*, *CqVQ10*, *CqVQ11*, *CqVQ14*, *CqVQ16*, *CqVQ17*, *CqVQ18*, *CqVQ20* and *CqVQ22* were detected at 3 h, 6 h, 9 h, and 12 h. The expression of *CqVQ01* at 6 h was higher than that at 3 h, 9 h, and 12 h, and the expression of *CqVQ09*, *CqVQ10*, and *CqVQ20* at 9 h were higher than that at 3 h, 6 h, and 12 h. The expression levels of the other 9 genes (*CqVQ04*, *CqVQ07*, *CqVQ08*, *CqVQ11*, *CqVQ14*, *CqVQ16*, *CqVQ17*, *CqVQ18*, and *CqVQ22*) at 12 h were higher than those at 3 h, 6 h, and 9 h. The expression level of the *CqVQ07* gene at 12 h was the highest among all the quinoa *VQ* genes in root tissue, which was 55.46 times the control. The expression levels of *CqVQ03*, *CqVQ05*, *CqVQ06*, *CqVQ15*, and *CqVQ23* were lower at 3 h than those of the control, but higher at 12 h than those of the control, 3 h, 6 h, and 9 h. The expression levels at 12 h were 16.28, 29.38, 3.43, 11.71, and 4.08 times the control, respectively.

In summary, the results showed that the expression levels of *CqVQ03*, *CqVQ05*, *CqVQ08*, and *CqVQ13* genes at 12 h were higher in the leaf and root tissues than in the control. The expression levels of *CqVQ10*, *CqVQ20*, and *CqVQ21* at 9 h were higher than those of the control, 3 h, 6 h, and 12 h in both leaf and root tissues. The expression of *CqVQ13* at 9 h and 12 h was the highest among all the *VQ* genes in leaf and root tissues, which were 77.17 and 81.76 times the control, respectively (Fig. 7).

Subcellular localization analysis of the *CqVQ13* gene

To further study the function and mechanism of the *CqVQ13* gene, the pCAMBIA1302-CqVQ13-GFP fusion expression vector was constructed and transferred into Agrobacterium Tumefacium GV3101 to infect tobacco leaves. Laser confocal microscopy was used to observe the expression of *CqVQ13*. The results showed that the 35 S::CqVQ13-GFP vector was transiently expressed in tobacco benzenoid. Compared with the GFP empty vector as the control, the leaves transformed with the GFP empty vector showed strong green fluorescence in the nucleus, cells, and cell membrane, while the leaves transformed with *CqVQ13* showed strong green fluorescence mainly in the nucleus (Fig. 8). This suggested that *CqVQ13* may have nuclear localization properties, which was consistent with the general properties of transcription factors.

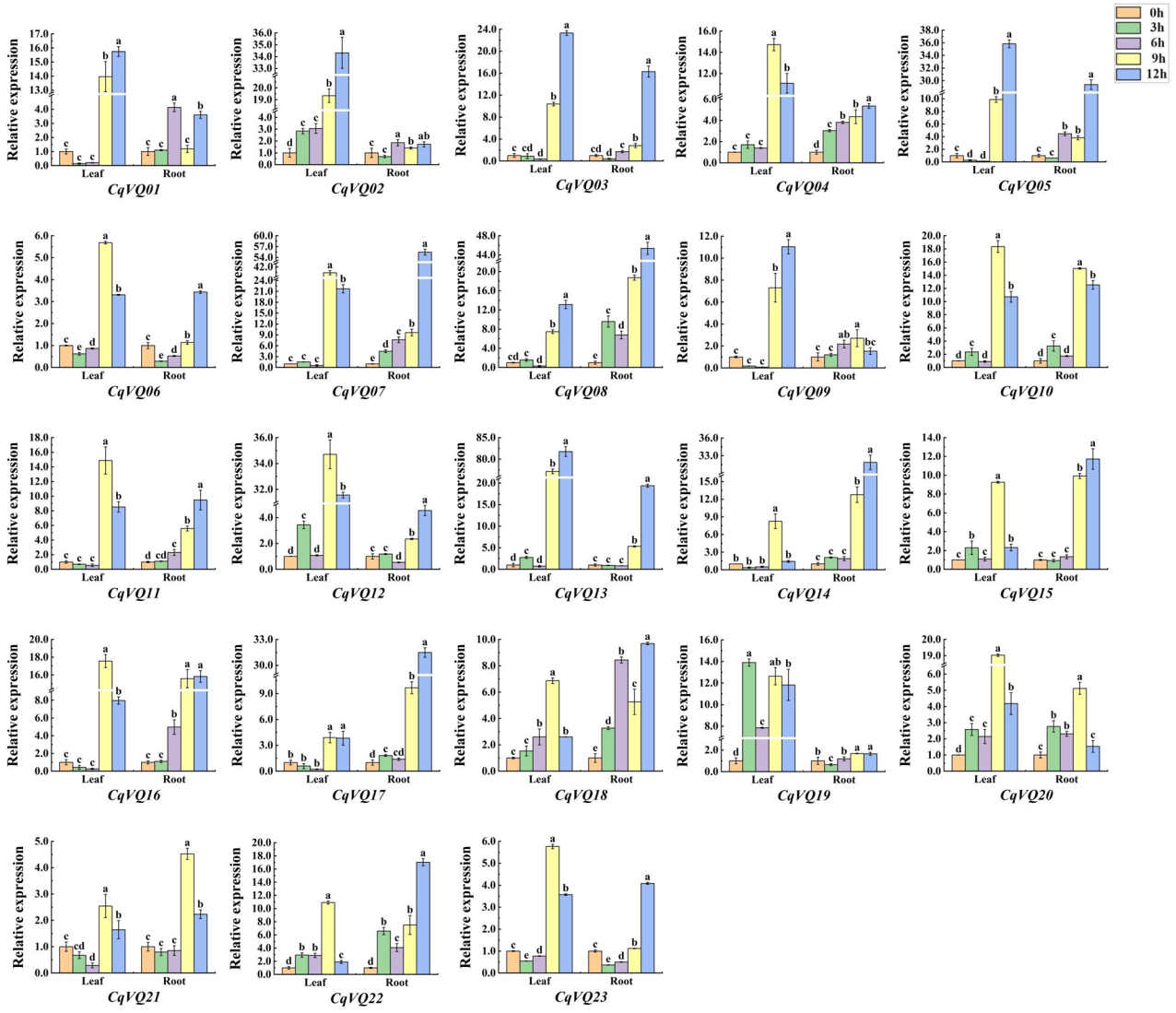

Fig. 7 Comparison of *VQ* gene expression in root tissue and leaf tissue of quinoa after 20% PEG6000 drought treatment for different times. Note: Different lowercase letters (a,b,c,d,e) represented significance at the 0.05 level ($p < 0.05$), the data were presented as mean ± SE, and the vertical line represented standard error

Discussion

Currently, the most effective way to alleviate drought stress is to use molecular means to mine plant resistance genes, and the important role of transcriptional regulatory factors in plant responses to stressful environmental stresses provides theoretical support for plant stress resistance breeding. VQ, a plant-specific transcriptional regulatory assistant factor, plays an important role in plant growth and development and stress responses. As research progresses, the function and mechanism of VQ protein will become clearer. *VQ* genes have been isolated and studied in various plants, including Arabidopsis, rice, soybeans, grapes, and maize. Although the VQ gene family has been extensively studied in many species, there have been no reports of it in quinoa. In our research, 23 *CqVQ* genes were identified in quinoa, and the structure and functional framework of *CqVQ* were established, providing a solid theoretical basis for future functional studies.

The systematic analysis not only elucidated the evolutionary process of gene pairs but also helped identify specific orthologous and paralogous *VQ* genes in various species. Based on our results, we constructed phylogenetic trees of VQ proteins in *Arabidopsis thaliana*, *Chenopodium quinoa*, *Brassica rapa*, and *Spinacia oleracea* (Fig. 1). The results showed that *Chenopodium quinoa* is closely related to *Spinacia oleracea*, which might be because *Chenopodium quinoa* and *Spinacia oleracea*, as members of the same genus in the family Chenopodiaceae, had formed close evolutionary relationships over a long evolutionary period. We identified 7 pairs of

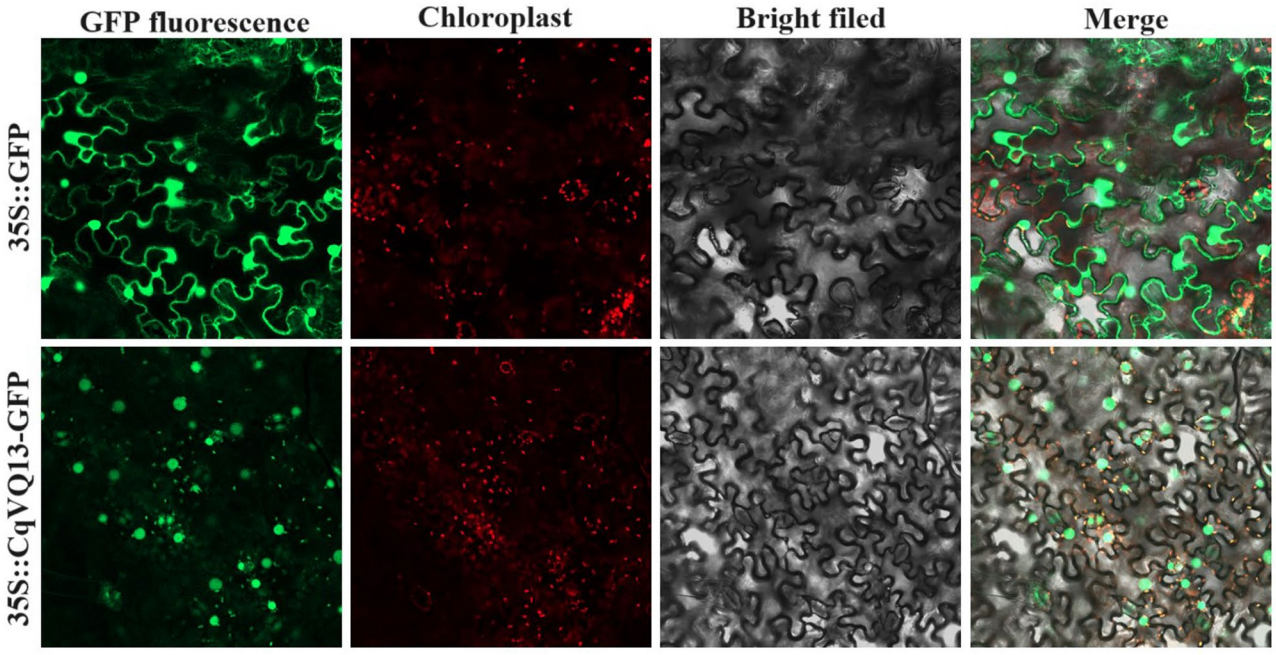

Fig. 8 Subcellular localization of CqVQ13 protein

orthologous genes between CqVQ and SoVQ proteins, suggesting that they may have originated from a common ancestral gene; moreover, we identified 7 pairs of paralogous genes in CqVQ proteins, confirming that quinoa had undergone gene duplication events. Genetic duplication plays an important role in expanding gene content and diversifying gene functions, helping plants better adapt to changing environments [46]. It is well known that plant genomes mainly expand through fragmental duplication and tandem duplication [47, 48]. Previous studies have shown that fragment duplication is the main mechanism for the expansion of VQ gene families in many angiosperms, with nine fragment duplication events reported in the *VQ* gene of Phyllostachys [49], 32 fragment duplication events reported in the *VQ* genes of P. Bret Schneider and P. communis [50], and nine fragment duplication events reported in the *VQ* gene of *Cucumis melo* [51]. Similarly, we identified three fragment duplication events in the *CqVQ* gene of *Chenopodium quinoa* (Table 1), but no tandem duplication was detected, further confirming that fragment duplication is the main mechanism for the expansion of VQ gene families in many angiosperms [52]. Moreover, in the VQ gene family of *Medicago sativa*, six pairs of genes originated from fragment duplication, and four pairs of genes underwent tandem duplication [53]. Most of the *VQ* genes in poplar are still located in conserved positions on segmental repetitive blocks [54], however, previous researchers did not find any repeat events in the genes containing VQ motifs in grapes [13], these results suggested that the evolution of the gene family containing VQ motifs in different species is different [50]. To study whether the Darwinian positive selection is related to the divergence of *VQ* genes after replication, the Ka and Ks of three paralogs were calculated. Because of the ratio of Ka/Ks (< 1) in several pairs of genes in the present study of hetero-hexaploid quinoa, we hypothesized that the VQ gene family of quinoa has undergone strong purifying selection pressure. Furthermore, the estimated duplication events of these three genes occurred between 7 and 13.5 MYA.

The structure of genes is important for determining the relationship between gene evolution and functional differences among members of a multigene family [55]. Many studies have hypothesized that in the long-term evolution of higher plants, the *VQ* genes have lost their introns [11] and that the fewer introns a gene has, the greater its ability to adapt to the external environment [56]. This has been confirmed in *Arabidopsis thaliana*, *Glycine max*, *Brassica rapa*, and *Coix lacryma-jobi* [10, 57–59], and in our study, based on gene structure analysis, we found that 60.8% (14/23) of the *VQ* genes in quinoa lack introns (Fig. 3), which is consistent with the results of studies on the evolution of *VQ* genes in higher plants. However, it is worth noting that the moss Physcomitrella, which is considered a lower plant, has only 7 *VQ* genes, of which 6 (6/7) lack introns. Therefore, explaining the loss of introns from an evolutionary perspective may require further research. In summary, the identification of the gene structure of *VQ* genes has enriched our understanding of the evolution of introns in the plant kingdom [52]. The MEME online tool can

identify different conserved motifs in each *VQ* gene. In our research, *CqVQ* genes in the same branch had similar structures and motif compositions (Fig. 2), revealing that these *VQ* genes may have similar functions. Moreover, Motif 1 is common in all quinoa *VQ* genes, and the same phenomenon was found in poplar, bamboo, and tea, suggesting that Motif 1 may confer specific functions to *VQ* genes [9, 49, 54]. Furthermore, protein secondary structure prediction indicates that α-helices were found in the VQ domain (Table S5), suggesting that the VQ domain can affect protein-protein interactions [60]. The protein interaction network diagram can intuitively predict the interaction relationships between proteins. The *VQ* gene, as a key transcriptional regulation auxiliary factor, has particularly attracted attention for its interaction with the WRKY transcription factor [13]. Research has shown that rice will coordinate the interaction between VQ and WRKY proteins to resist pathogen infection during different infections [61]. AtVQ20 can interact with WRKY2 and WRKY34 to regulate pollen development [22]. Furthermore, 21 of the 25 soybean VQ proteins interact with WRKY proteins, further proving that the *VQ* gene is an important interacting partner of the WRKY transcription factor [62]. Our results show, we predicted that all six *CqVQ* genes interact with multiple WRKY transcription factors (Fig. 6A). Therefore, we hypothesized that the function of CqVQ proteins in quinoa might also depend on their interaction with WRKY transcription factors, but the specific regulatory interaction mechanism remains to be explored. Cis-regulatory elements play a role in controlling the transcriptional regulation of genes involved in various biological processes and significantly affect gene function [63]. The findings indicate, we screened the upstream regions of *CqVQ* genes to identify cis-elements. Multiple cis-regulatory elements, such as GARE, MBS, TGA, ABRE, MRE, MSA, LTR, ARE, and WUN, involved in plant hormone, non-biological, and biological stress-related signaling pathways, have been identified in the promoter region of VQ (Fig. 5). In general, the cis-regulatory elements identified here help to understand their roles in various developmental stages and stress response mechanisms related to various stressors [26, 64–66].

Previous studies have shown that *VQ* genes are involved in regulating plant responses to non-biotic stressors such as drought, with the expression level of the *HaVQ* gene in sunflower (50%) being induced more than fivefold after drought stress [67], the *OsVQ2*, *OsVQ16*, and *OsVQ20* genes being dramatically induced by drought in rice [15], and the homologous genes in maize, *ZmVQ01*, *ZmVQ13*, and *ZmVQ21*, also being so [18]. As demonstrated in our research, we found that most of the *VQ* genes in quinoa were significantly upregulated in leaf and root tissue structures due to PEG stress, with *CqVQ13* reaching peak expression levels in both leaf and root tissue structures at 9 h and 12 h (Fig. 7). This suggests that *VQ* genes also participate in the response to drought stress in quinoa. However, previous studies have also shown that some upregulated *VQ* genes may have a negative impact on resistance to non-biotic stressors: Salt stress can induce the expression of *AtVQ9*, but overexpression of *AtVQ9* makes the plant hypersensitive to salt stress [68]. In addition, *AtVQ15* is induced by dehydration and high salt concentration, and its overexpression is associated with increased sensitivity to salt and mannitol stress during seed germination and seedling growth [58]. Therefore, we cannot directly conclude whether a gene is positively or negatively regulated by the plant in response to non-biological stress based solely on its up or down-regulated expression after the stress. Specific gene functions still need to be validated, but the research results will help select candidate genes for further in-depth study of the role of VQ proteins in plant non-biological stress responses. Furthermore, previous studies have shown that most VQ proteins in plants are located in the nucleus of the cell. Interestingly, although BjuVQ protein is mainly located in the nucleus, it also has a portion located in the cytoplasm; some members of the CsVQ and ClVQ protein families may target both the nucleus and the chloroplast; meanwhile, some ClVQ and AtVQ proteins have been found to be present in the mitochondria, while AtVQ and OsVQ proteins are also distributed in the cytoplasm [9, 15, 69]. This study reveals that, most of the CqVQ proteins were found to be located in the nucleus, but the CqVQ20 protein was also found to be located in the cell membrane and cytoplasm in addition to the nucleus, and the CqVQ23 protein was found to be located in the chloroplast (Table S2). These results suggest that VQ proteins play different functions in different cellular locations, and also suggest that these genes may have functions other than transcription factors [59, 70]. Further selection of the *CqVQ13* gene for subcellular localization vector construction showed that it was localized in the nucleus (Fig. 8), consistent with the research result that transcription factors mainly function in the nucleus of the cell [71]. In summary, all these results indicate that *CqVQ* genes play an important role in the non-biological stress response of plants, although the specific regulatory mechanism of *CqVQ* gene function is still unclear and will require further research.

Conclusion

Our analysis shows that, 23 quinoa VQ gene family members were systematically analyzed, including basic physicochemical properties, chromosome location, phylogenetic tree analysis, and gene duplication analysis. The analysis of cis-acting elements showed that the promoter region contained 52 cis-acting elements, especially in the

response to abscisic acid and drought stress. Notably, the *CqVQ13* gene showed a significant increase in expression level under drought stress by combining transcriptome gene expression profiling and qRT-PCR. Furthermore, subcellular localization revealed that the *CqVQ13* gene was localized to the nucleus. In conclusion, this study identified the VQ gene family in quinoa for the first time and screened the drought response candidate gene *CqVQ13* in quinoa, which provided a reference for further elucidating the potential molecular mechanisms of VQ family members in response to drought stress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11313-6.

Supplementary Material 1: Table S1. RT-qPCR primer sequences for VQ genes in quinoa. Table S2. Basic characteristics of VQ genes in quinoa. Table S3. qRT-PCR primer sequences for CqVQ13 gene. Table S4. The values of VQ genes in 22 tissues and 5 treatments were downloaded from NCBI. Table S5. Analysis of secondary structure of VQ protein in quinoa.

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Author contributions

This work was conceived by D.F.Z., X.L.Z., X.F.D., X.H.W., X.W., and B.Q.W., conducted bioinformatics analysis. D.F.Z., X.L.Z., X.F.D., and B.Q.W., analyzed the experimental data. The first draft was written by D.F.Z., X.L.Z, X.W., and B.Q.W. Refinement of the final draft was done by D.F.Z., X.L.Z., and X.H.W. All authors approved the final manuscript. All authors have reviewed the manuscript.

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Data availability

The reference genome assembly used for data analysis was obtained from the National Center for Biotechnology Information (NCBI) BioProject PRJNA675125 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA675125). The raw transcriptome data generated and analyzed in this study deposited in SRA of the NCBI under accession number PRJNA394651 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA394651) and PRJNA306026 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA306026). The datasets analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

The authors declare that all the experimental research studies on plants, including the collection of plant material, were carried out by relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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