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Research Article

Investigating *TIFY* Genes for Salt Stress Adaptation in Quinoa (*Chenopodium quinoa* Willd.): A Genome-Wide Approach

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a nutritious grain with high protein, fiber, vitamin, and mineral content, offering high economic value due to its superior yield potential. The TIFY family, including TIFY, Jas, and GATA motifs, is crucial in plant defense mechanisms and response to stressors. Many plant species have studied the *TIFY* gene family, but quinoa has not yet undergone such a study. This study identified 16 *Cq-TIFY* genes in the quinoa genome, designated as *Cq-TIFY-1* to *Cq-TIFY-16*, and characterized their structural and functional properties through bioinformatics analyses. The Cq-TIFY proteins in quinoa have molecular weights varied from 19.99 to 48.59 kDa, amino acid numbers varied from 189 to 450, and theoretical isoelectric points varied from 4.84 to 10.1. The results of phylogenetic tree analysis indicated that these *TIFY* genes fall into three classes. The diverse classes of TIFY family membership were generally found to have similar gene structures. Seven segmental duplicated genes have been identified in quinoa, and subsequent Ka/Ks analysis shows that all are exposed to the purifying selection evolutionary process. Synteny analyses of *TIFY* genes in *Chenopodium quinoa*, *Arabidopsis thaliana*, and *Spinacia oleracea* plants revealed a relationship between these three plants regarding *TIFY* genes. Promoter analysis highlighted the presence of stress-responsive and hormone-related cis-acting elements. RNAseq data was utilized to investigate the expression profiles of *Cq-TIFY* genes in root and shoot tissue at salt conditions. Expression profiling revealed tissue-specific responses to salt stress, with significant upregulation in roots and shoots, indicating their functional role in salt tolerance pathways. This research elucidates the *TIFY* gene family in quinoa, establishing a basis for subsequent investigations into its functional activities and serving as a resource for developing stress-tolerant cultivars via breeding or genetic engineering.

Keywords: Abiotic stress, cis-regulatory element, JAZ domain, phylogenetic analysis, RNAseq.

Kinoa'da Tuz Stresi Adaptasyonu için *TIFY* Genlerinin Araştırılması: Genom Çapında Yaklaşım

Öz

Kinoa (*Chenopodium quinoa* Willd.), yüksek protein, lif, vitamin ve mineral içeriğine sahip besleyici bir tahıl olup, yüksek verimi nedeniyle ekonomik değeri de yüksektir. TIFY ailesi, TIFY, Jas ve GATA motiflerini içeren bitkilerin savunma mekanizmalarında ve stres faktörlerine karşı verdikleri yanıtta önemli rol oynayan bir gen ailesidir. *TIFY* gen ailesi birçok bitki türünde araştırılmış olmasına rağmen, kinoada henüz incelenmemiştir. Bu çalışmada, 16 *Cq-TIFY* geni tanımlanmış, bu genler Cq-TIFY-1'den Cq-TIFY-16'ya kadar numaralandırılarak yapısal ve işlevsel özellikleri karakterize edilmiştir. Tanımlanan Cq-TIFY proteinlerinin moleküler ağırlıkları 19,99 ile 48,59 kDa, amino asit sayıları 189 ile 450, teorik izoelektrik noktaları ise 4,84 ile 10,1 arasında değişmektedir. Filogenetik analiz sonuçlarına göre, *TIFY* genlerinin üç sınıfa ayrıldığı belirlenmiştir. Gen ailesinin farklı sınıflarındaki üyelerin gen yapılarının genellikle benzer olduğu belirlenmiştir. Kinoa'da yedi segmental duplikasyon geçirmiş gen tanımlanmış olup, Ka/Ks analizi bu genlerin evrimsel süreçte arındırıcı (negatif) seçilime maruz kaldığını göstermiştir. *Chenopodium quinoa*, *Arabidopsis thaliana* ve *Spinacia oleracea* türleri arasındaki *TIFY* genlerinin sinteni analizi, bu üç bitki arasında *TIFY* genleri açısından bir ilişki olduğunu ortaya koymuştur. Promotör analizi sonucunda, *TIFY* genlerinde strese duyarlı ve hormonla ilişkili cis-elementlerin varlığı ortaya çıkarılmıştır. Araştırmada, RNA-seq verileri, tuz stres koşulları altında kök ve sürgün dokularında *Cq-TIFY* genlerinin ifade modellerini incelemek için kullanılmıştır. Genlerin tuz stresi altındaki ifade profili köklerde ve sürgünlerde dokuya özgü olarak farklılık göstermiş ve ifadelerinde anlamlı bir artış belirlenmiştir. Bu sonuç, genlerin tuz toleransı mekanizmalarında rol oynayabileceğini düşündürmüştür. Bu araştırma, kinoadaki *TIFY* gen ailesini açığa çıkararak fonksiyonel görevlerine yönelik sonraki araştırmalar için bir temel oluşturmakta ve ıslah veya genetik mühendisliği yoluyla strese dayanıklı çeşitler geliştirmek için bir kaynak görevi görmektedir.

Anahtar Kelimeler: Abiyotik stres, cis-düzenleyici element, JAZ domain, filogenetik analiz, RNAseq.

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1. Introduction

Plants are concurrently facing several biotic and abiotic stressors, such as heat, salt, osmotic stress, drought, and infection by pathogens and viruses [1-3]. These factors significantly impact agricultural productivity, potentially reducing it by at least 50% of crop yields [4]. Salinity is considered one of the most detrimental abiotic stress factors, impacting nearly 25% of arable lands and reducing global agricultural productivity by one-third [5]. Salinity impacts plants through osmotic stress, nutrient imbalance, ion toxicity, and oxidative damage, resulting in reduced growth, impaired photosynthesis, and a lower crop yield [6-10].

Plants mitigate salt stress through a coordinated activation of stress-responsive genes, protective proteins, and metabolite accumulation [11]. Salinity-responsive genes in plants fall into two main groups: the first includes genes for protein channels, transporters, detoxifying enzymes, protease inhibitors, and other proteins aiding in osmotic balance and stress tolerance, while the second comprises regulatory genes such as transcription factors (TFs) and protein kinases, which modulate downstream gene expression profiles in response to stress signals [12]. TFs, referred to as trans-acting factors, are critical elements of signal transduction pathways induced by abiotic stress [13].

The *TIFY* gene family, unique to plants, encodes transcription factors involved in development, reproduction, secondary metabolism, defense, and stress adaptation [14, 15]. The first characterized member is the AT4G24470 gene in *Arabidopsis thaliana*, also known as ZIM (zinc finger protein expressed in the inflorescence meristem) due to its C2C2-GATA zinc-finger structure [16]. Within the TIFY domain, the *TIFY* gene family is characterized by the presence of 36 highly conserved amino acid sequences (TIF[F/Y] XG) [17]. The *TIFY* gene family is divided into four phylogenetic subfamilies: TIFY, ZIM/ZML (ZIM-like), PPD (PEAPOD), and JAZ (jasmonate-ZIM-domain), all of which contain a conserved TIFY domain [18-20]. The TIFY subfamily exclusively features the TIFY (TIF[F/Y] XG) domain [21]. ZML subfamily proteins contain a C2C2-GATA zinc finger domain facilitating DNA binding and a CCT (CONSTANS/CO-like/TOC1) domain enabling protein-protein interactions [22]. PPD subfamily proteins possess a PPD domain in the N-terminals and a revised Jas motif in the C-terminals instead of the conserved proline–tyrosine [23]. JAZs are the largest TIFY gene subfamily and have two conserved domains: TIFY and JA-associated (Jas, CCT-2) [24]. Moreover, there has been a strong association between the *TIFY* gene family and reactions to both biotic and abiotic stress [25].

Hormones in plants play a crucial role in stress responses; they include jasmonic acid (JA), abscisic acid (ABA), ethylene (ET), and salicylic acid (SA) [26]. The *TIFY* gene family, especially the JAZ subfamily, is of great interest as it shows a vital role in various biological processes [14]. Although the *TIFY* gene family investigated genome-wide has been described as functioning in stress factors some plant species, such as alfalfa (*Medicago sativa* L.) [27], pepper (*Capsicum annuum* L.) [28], tobacco (*Nicotiana tabacum*) [29], wheat (*Triticum aestivum* L.) [30], Tartary buckwheat (*Fagopyrum tataricum*) [31], soybean (*Glycine max*) [32],

no studies have been achieved on the stress tolerance of *TIFY* genes in quinoa (*Chenopodium quinoa* Willd.).

Quinoa is a halophytic pseudocereal crop that originated in South America's Andes. It frequently grows on plateaus higher than 4500 meters [33]. International nutritionists have called quinoa the “golden grain” and “superfood” because of its high protein content, essential amino acids, minerals, enhanced vitamins, unsaturated fatty acids, dietary fiber, and gluten-free status [34]. Quinoa’s remarkable resilience to harsh conditions like soil salinity, drought, and frost makes it ideal for expanding into marginal lands and identifying genes that enhance stress tolerance [35]. Furthermore, the quinoa genome release laid the framework for future quinoa breeding and genetic improvement efforts [36].

This study performed comparative bioinformatics analyses, including phylogenetic tree construction, gene structure, collinearity analysis, conserved motifs, protein-protein interactions, chromosomal locations, and cis-acting element analysis. Studying the quinoa genome can enhance our understanding of polyploidy’s role in stress resilience, as quinoa’s complex genome structure may provide it with unique plasticity to cope with harsh environmental factors. The responses of sixteen *Cq-TIFY* genes to salt stress were also studied using in silico gene expression. Exploring the role of the *TIFY* gene family in quinoa is vital for advancing the breeding and development of Chenopodium germplasm sources, paving the way for innovative agricultural solutions. Moreover, this information is crucial for breeding strategies to increase resilience in staple crops, which are increasingly exposed to abiotic stresses due to climate change and soil degradation.

2. Material and Methods

2.1. Determination and Characterization of *Cq-TIFY* Genes in the Quinoa Genome

TIFY gene sequences in the *Chenopodium quinoa* genome were retrieved from Phytozome v13 using the Pfam ID (PF06200) [37] received from the Pfam database. The Phytozome v13 database was used for BLASTP and Hidden Markov Model (HMM) screening to detect all putative TIFY protein sequences with this accession number in *Chenopodium quinoa*, *Arabidopsis thaliana*, and *Spinacia oleracea*. The presence of the TIFY domain in the extracted sequences was evaluated using the HMMER database. The ProtParam tool (https://web.expasy.org/protparam/) was used to estimate the amino acid (aa) number, molecular weight (MW), and isoelectric point (pI) of the determined TIFY proteins.

2.2. Sequence Alignment and Phylogenetic Tree Analysis

The Multiple Sequence Alignment with ClustalW tool [38] was employed to align the protein sequences of the *TIFY* gene family members in the genomes of *C. quinoa*, *A. thaliana*, and *S. oleracea* species**.** The MEGA v11 program [39] was enjoyed in creating the phylogenetic tree using the neighbor-joining (NJ) method with 1000 bootstrap replicates. The phylogenetic tree was envisioned using the Interactive Tree of Life (iTOL) v6 interface [40].

2.3. Identification of the Structure, Physical Location, Conserved Region Motifs, and Gene Duplications of *Cq-TIFY* Genes

The Gene Structure Display Server v2.0 (GSDS) [41] was utilized to identify and visualize the intron and exon regions of Cq-TIFY and provide detailed insights into their gene structures. Genomic and coding DNA sequences (CDS) were utilized to estimate *Cq-TIFY* gene positions. The chromosomal positions of *Cq-TIFY* genes were acquired from the Phytozome database v13. The TBtools program was used to plot all quinoa chromosomes where *Cq-TIFY* genes are located [42]. Following the annotation of *Cq-TIFY* genes on chromosomes and their representation using Circos [43], a syntenic map was generated with TBtools. Conserved motifs in Cq-TIFY proteins were identified using MEME Suite v5.5.7 (https://meme-suite.org/meme/) [44]. In the MEME Suite tool, the width parameters are configured with a minimum of 6 and a maximum of 50, the upper limit for motifs is established at 10, motif regions range from 2 to 600, and the region dependency allows for any number of repeats (ANR). Gene duplication events were determined with the Basic Ka/Ks Calculator (NG) tool in the TBtools. Nonhomologous (Ka), homologous (Ks), and homologous to nonhomologous rations (Ka/Ks) between binary pairs of *Cq-TIFY* genes were determined via the basic Ka/Ks calculator algorithm in the TBtools [45]. The formula T=Ks/2λ, where λ represents (6.56x10-9), was employed to determine the timing of duplication and separation of each *Cq-TIFY* gene [46].

2.4. Promoter Analyses and Subcellular Localization of Cq-TIFY proteins

The cis-regulatory elements (CREs) of the 2000-bp promoter sequence of the *Cq-TIFY* gene family were investigated using the PlantCARE database [47]. The data was presented using the TBtools program. Using peptide sequences obtained from the Phytosome v13 database, the subcellular localization of all Cq-TIFY proteins was ascertained with the WoLF PSORT (https://wolfpsort.hgc.jp/) database [48].

2.5. Protein-Protein Interactions and Homology Modeling of TIFY Proteins in Quinoa

The STRING v12 (https://string-db.org/) online tool was used to assess the relevance of Cq-TIFY protein-protein interactions (PPIs) to biological processes and molecular functions [49]. Using previously acquired TIFY protein sequences, three-dimensional (3D) structures were modeled via Phyre2 v2 [50]. Protein models were visualized with a greater than 95% confidence level.

2.6. Synteny Analysis of TIFY Proteins

Protein sequences of *TIFY* gene orthologs found in *C. quinoa*, *A. thaliana*, and *S. oleracea* genomes were determined with the help of the Phytozome database v13. The homology and collinearity of TIFY genes in different species were further examined using the one-step MCScanX algorithm from TBtools. Moreover, the synteny map was drawn with the Multiple Synteny Plot function in the TBtools [51].

2.7. In-silico Gene Expression Analysis

Illumina RNA-seq data from the NCBI Sequence Reading Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra/) were used to analyze gene expression profiles under salt stress conditions. To find the relevant RNA-seq data, the accession numbers of control [SRR11050560 (root salt control) and SRR11050558 (shoot salt control)] and salt stress [SRR11050571 (salt-stressed root) and SRR11050565 (salt-stressed shoot)] were used. In silico gene expression analysis, log2-transformed RPKM (reads per kb per million mapped read) values were utilized. The One Matrix CIM from the CIMMiner algorithm (https://discover.nci.nih.gov/cimminer/oneMatrix.do) was utilized in the following step to create a heatmap.

3. Results and Discussion

3.1. Determination and Characterization of *Cq-TIFY* Genes

The Pfam database searched for the TIFY family members with Pfam entry number (PF06200) obtained in the quinoa genome using Phytozome database v13. The HMMER results revealed 16, 18, and 11 TIFY proteins in the genomes of *C. quinoa*, *A. thaliana*, and *S. oleracea,* respectively. Genes have been given new names ranging from Cq-TIFY-1 to Cq-TIFY-16 (Table 1). Previous genome-wide studies have identified 48 TIFY proteins in maize (*Zea mays*), 26 in tomato (*Solanum lycopersicum*), 38 in soybean (*Glycine max*), 84 in alfalfa (*Medicago sativa*) [27], 29 in peanut (*Arachis hypogaea*) [52], 17 in cucumber (*Cucumis sativus*) [37], and 22 in tea (*Camellia sinensis*) [53], 34 in *Artemisia argyi* [54], 21 in *Hordeum vulgare Morex* and 22 in *Hordeum vulgare Barke* [55].

The Cq-TIFY proteins in quinoa varied from 189 (in Cq-TIFY-7) to 450 (in Cq-TIFY-10) aa in length, with the MWs varied from 19.99 to 48.59 kDa in these TIFY proteins, respectively (Table 1). The pI of the quinoa TIFY proteins varied from 4.84 (in Cq-TIFY-4) to 10.1 (in Cq-TIFY-8) (Table 1). The instability index ranges between 34.45 (in Cq-TIFY-12) and 54.2 (in Cq-TIFY-1) (Table 1). The instability index showed the protein's stability (≤ 40 means it might be stable; >40 means it might be unstable) [56]. Table 1 shows that except for Cq-TIFY-12, other proteins were unstable. The results of subcellular localization indicated that the common of quinoa TIFY proteins are primarily localized in the nucleus, chloroplast, cytosol, and other organelles (Table 1). Subcellular localization analysis in *Dendrobium huoshanense* reveals that most DhTIFY proteins are distributed in the nucleus [57]. Prediction subcellular localization of the TIFY proteins in pineapple showed that most of them are in the nucleus, while FaJAZ9C is in the chloroplast and FaPPD1A/B/C/D is in the cytoplasm, nucleus, and cell membrane [58].

3.2. Phylogenetics Analysis of TIFY in *C. quinoa*, *A. thaliana*, and *S. oleracea*

Phylogenetic trees were constructed using the NJ method in MEGA 11, based on TIFY protein sequences from *C. quinoa* (16), *A. thaliana* (18), and *S. oleracea*. This research revealed that the 41 TIFY proteins from these species were divided into three clades: Clade A, Clade B and Clade C (Figure 1). The Clade A group contains 6 quinoa, 3 Arabidopsis, and 2 spinach TIFY

proteins. Clade B contains one TIFY protein from each species. Finally, the Clade C group includes 9 quinoa, 14 Arabidopsis, and 8 spinach TIFY proteins (Figure 1). Zheng et al. [59] indicate that the phylogenetic tree group of cassava determined 26 TIFY proteins, which were categorized into three clusters together with *Brachypodium distachy* (4), Arabidopsis (17), rice (19), *Populus trichocarpa* (22), *Brassica napus* (4), *Gossypium arboretum* (7), and *Vitis vinifera* (6).

3.3. The Gene Structure and Chromosomal Location of *Cq-TIFYs*

The structural variation of the quinoa *TIFY* genes was investigated using an intron-exon structure analysis. These *Cq-TIFY* genes have three to eleven exons, as shown in Figure 2. Three exons are present in both the *Cq-TIFY-9* and *Cq-TIFY-14* genes. The most exons (11) and intron (10) were found in the *Cq-TIFY-13* gene. The Cq-TIFY-4 gene was found to be intronsless. Some research further suggested that these intronless genes may be processed pseudogenes without a 5′ promoter region [19]. Prior investigation has demonstrated that the exon-intron structure can support phylogenetic groupings since this divergence usually establishes gene families [60]. Gene exon-intron organization revealed a substantial link between phylogeny and exon-intron structure, with genes with similar exon-intron structures such as *Cq-TIFY-9/Cq-TIFY-14* and *Cq-TIFY-8/Cq-TIFY-11* belonging to the same phylogenetic group (Figure 1 and Figure 2). The identified *Cq-TIFY* genes were found on the unidentified scaffold of the common quinoa genome (Figure 3). The scaffold with the highest number of genes is Cq_Scaffold_2716 (2 members); the other scaffolds have one member (Figure 3). 16 genes were found distributed over 15 scaffolds. Zhao et al. [61] reported that 38 *TIFY* genes were situated on 13 chromosomes, while the other 12 genes were identified on nine scaffolds in cotton.

3.4. Conserved Region Motifs and Gene Duplications of *Cq-TIFY* Genes

The MEME software was utilized to investigate the motifs of *Cq-TIFY* gen family, identifying ten motifs called Motif 1 to Motif 10. The length of the find-out motifs varied from 24 (Motif 2) to 50 (Motif 6, 8, and 10) amino acids (Table 2). The number of identified motifs varies between 1 and 6. Cq-TIFY-2 has only one conserved motif, while Cq-TIFY-4, Cq-TIFY-6, and Cq-TIFY-13 have the highest number of motifs, each with six conserved motifs (Figure 4). Additionally, conserved domains in quinoa TIFYs were assessed using the InterPro web server, and the presence of the TIFY domain, CCT domain, Zinc finger, and GATA (Znf_GATA) domain was determined among the ten conserved motifs found (Table 2). Motif 1 (TIFY domain) is existent in all genes, while Motif-2 (CCT domain) is only absent in the *Cq-TIFY-2* gene (Figure 4). Tao et al. [15] identified ten conserved motifs in kiwifruit. Among the conserved motifs, only Motif 1 was present in all kiwifruit TIFY members, but Motif 2 was found in most. Moreover, also identified seven possible conserved domains: the TIFY domain, CCT domain, Dynamin_M domain, GED domain, GATA domain, Jas_motif domain (CCT_2), and Transp_inhibit domain. In another genome-wide study on the TIFY gene, it was determined that the conserved motifs include the TIFY motif, CCT domain, GATA zinc finger domain, N-terminus of the AS/CCT domain, C-terminus of the Jas domain, and PPD motif domains [23].

Various forms of gene duplication, involving tandem, segmental, and whole genome duplication, facilitate the expansion of gene families and the diversification of numerous species [56]. Tandem duplications involve two or more identical genes on the same chromosome, while segmental duplications involve genes on distinct chromosomes [62]. Seven segmental duplications were detected between the *Cq-TIFY-6*/*Cq-TIFY-1*, *Cq-TIFY-16*/*Cq-TIFY-3*, *Cq-TIFY-13*/*Cq-TIFY-4*, *Cq-TIFY-8*/*Cq-TIFY-9*, *Cq-TIFY-14*/*Cq-TIFY-9*, *Cq-TIFY-12*/*Cq-TIFY-10*, and *Cq-TIFY-8*/*Cq-TIFY-11* genes in quinoa. However, tandem duplication was not identified in the Cq-TIFY family. Segmental duplications events of TIFY genes have also appeared in rice [63], maize [64], *Populus trichocarpa* [65], and *Betula platyphylla* [66]. The date of duplication events was estimated using Ka and Ks values and Ka/Ks ratios (Table 3). When Ka/Ks is greater than 1, it points out positive selection, purifying selection when less than 1, and natural selection in duplication circumstances when equal to 1 [67]. The *Cq-TIFY* genes evolved under purifying selection because all duplicate gene pairs had Ka/Ks ratios below 1.0. Segmental duplications of the *TIFY* genes in *C. quinoa* appeared from 5.51 million years ago (MYA) (Cq-TIFY-16/Cq-TIFY-3) to 15.76 MYA (Cq-TIFY-6/Cq-TIFY-1) with a mean of 8.59 MYA (Table 3). Huang et al. [68] reported that the duplication events of *PeTIFYs* may have arisen around 16.7 MYA.

3.5. Promoter Analyses of Cq-TIFY Proteins

Understanding cis-acting elements is essential for regulating biological processes like hormone synthesis and abiotic stress responses [69]. To identify potential expression regulation patterns of quinoa *TIFY* genes, cis-acting elements in promoter regions were predicted using sequences from 2000 bp in the 5′ upstream region of *TIFY* genes family members. The promoter sequences of the quinoa *TIFY* genes contained numerous possible cis-acting elements involved in stress response, phytohormone synthesis, anaerobic response, and plant growth and development (Figure 5). The interaction between cis-regulatory elements and transcription factors controls plant response to abiotic stressors [45]. The elements that were shown to be associated with environmental stress included MYB, MYC, and MBS (drought-related regulatory), MRE, ACE, AE-box, Sp1 and 3-AF1 binding site (light responsiveness), STRE and TC-rich repeats (stress-responsive element), As-1-type (response to xenobiotic chemical stress), WRE3 (high-temperature elements), LTR, (low-temperature responsiveness) and DRE (low-temperature and salt stresses) (Figure 5). Lv. et al. [66] show that *TIFY* genes had numerous cis-acting elements, including the ABRE, GARE-motif, MBS, TCA element, TC-rich repeats, TGACG motif, and WUN-motif, which were linked to various hormones and stress.

The phytohormone-related cis-acting elements are mainly involved in P-box and GARE- motif (gibberellin responsiveness), TCA-element (salicylic acid responsiveness), TGA- element and AuxRR-core (auxin-responsive element), ABRE3a and ABRE4 (abscisic acid-responsive elements) and ERE (ethylene-responsive element) (Figure 5). Li et al. [17] indicated that AS-1, AuxRR-core, GARE-motif, TGA-elements, and P-box (phytohormone regulatory elements), AACA and GCNA-motif (tissue and development-specific elements), and DRE1 GATA-motif, WUN-motif, and DRE core (stress-responsive elements) were present in most VcTIFY promoters in blueberry. In the growth and development category, 0_2-site (regulation of zein

metabolism) and anaerobic induction, ARE (regulatory anaerobic induction) are present (Figure 5). Li et al. [57] identified several commonly occurring cis elements, including ARE, MBS, and ABRE, in the promoter regions of *DhTIFY* genes in *Dendrobium huoshanense.* The variety and complexity of cis regularity elements in CqTIFY promoters may be an evolutionary response to the changing conditions *Chenopodium quinoa* faces.

3.6. Protein-Protein Interactions and Homology Modeling of TIFY Proteins in Quinoa

Protein-protein interactions (PPIs) will link unidentified functional proteins into interaction networks, enhancing our comprehension of protein biology. PIPs between Cq-TIFY and other proteins were explored utilizing the STRING database. Cq-TIFY-2, Cq-TIFY-12, and Cq-TIFY-13 proteins did not interact with any proteins. It has been determined that the Cq-TIFY-7, Cq-TIFY-11, and Cq-TIFY-16 proteins interact with each other and with other proteins. Other proteins include other than Cq-TIFY, Jas domain-containing protein (SOVF_147170), uncharacterized protein (SOVF_063310 and SOVF_098320), bHLH-MYC_N domain-containing protein (SOVF_125950), and BHLH domain-containing protein (SOVF_089090) (Figure 6). According to the results of PIP analysis, Cq-TIFY-7, Cq-TIFY-11, Cq-TIFY-14, and Cq-TIFY-15 proteins regulate the jasmonic acid-mediated signaling pathway, response to wounding, and defense response (Figure 6). PIPs between MsTIFY proteins, nuclear-localized proteins, DNA-binding family proteins MYC, and jasmonic acid-mediated plant-resistant responses were observed [27]. Zhao et al. [31] elucidated the correlation between the TIFY-mediated jasmonic acid signaling system and buckwheat's tolerance to abiotic stressors by in vivo PIP research.

Visually interpretable 3D models of Cq-TIFY proteins were created by the Phyre2 program. These models of the identified 16 Cq-TIFY proteins are illustrated in Figure 6. 3D structures demonstrated in all examined proteins, with a characteristic frame of β-strand and parallel α-helixes (Figure 7). Additionally, TM helix structures were detected in Cq-TIFY-10 and Cq-TIFY-15 proteins. Among them, the α-helixes were the most common structure in *C. quinoa* TIFY amino acid sequences, accounting for a range of 5% (Cq-TIFY-10) to 28% (Cq-TIFY-7), followed by β-strand, which ranged from 2 (Cq-TIFY-5 and Cq-TIFY-13) to 8% (Cq-TIFY-10), and the TM helix, which accounts for 4% (Cq-TIFY-10 and Cq-TIFY-15) (Table 4). Heidari et al. [21] showed that the TIFY proteins have a structure mainly consisting of β-sheets and parallel α-helixes, demonstrating the existence of the conserved TIFY domain.

3.7. Synteny Analysis of TIFY Proteins

The synteny analysis using MCScanX examined duplicated genes among *C. quinoa* genes and their interconnections. In the synteny analysis of *TIFY* genes within *C. quinoa*, duplicate genes exist on all chromosomes except Cq-TIFY-2, Cq-TIFY-5, Cq-TIFY-7, and Cq-TIFY-15 (Figure 8). Moreover, to search for homologs, colinearity correlations were examined between the *TIFY* genes of quinoa and similar genes from two different species (*A. thaliana* and *S. oleracea*). The analysis revealed that *C. quinoa* and *A. thaliana* genomes contain 11 orthologous gene pairs (Figure 9), while *C. quinoa* and *S. oleracea* contain nine orthologous gene pairs (Figure 10). Previous research has revealed that *TIFY* genes show orthology in plants such as *Actinidia*

eriantha, *Arabidopsis thaliana*, *Camellia sinensis*, *Solanum lycopersicum*, *Oryza sativa*, and *Vitis vinifera* [15].

3.8. In-silico Gene Expression Analysis

The expression profiles of *Cq-TIFY* genes in quinoa plants' root and shoot tissues were analyzed using SRA data under salinity stress. Figure 11 presents the results of the gene expression analysis. *Cq-TIFY* genes show significant expression levels between roots and shoots. When the effects of salt treatments on the expression levels in root tissue were examined, *Cq-TIFY-3, Cq-TIFY-7, Cq-TIFY-8, Cq-TIFY-9, Cq-TIFY-11, Cq-TIFY-14,* and *Cq-TIFY-16 increased, and Cq-TIFY-1, Cq-TIFY-2, Cq-TIFY-4, Cq-TIFY-5, Cq-TIFY-6, Cq-TIFY-10, Cq-TIFY-12,* and *Cq-TIFY-13* decreased. No significant expression difference was observed in *Cq-TIFY-15.* Analysis of shoot tissue revealed a notable upregulation of the Cq-TIFY-8 gene in salt-exposed shoots relative to control shoots, indicating its potential involvement in the stress response of shoot tissues. The expression changes of *Cq-TIFY-1, Cq-TIFY-2, Cq-TIFY-5, Cq-TIFY-7, Cq-TIFY-10, Cq-TIFY-11, Cq-TIFY-12, Cq-TIFY-14*, and *Cq-TIFY-15* genes in shoot tissues compared to control were not significant. Activation during salt stress shows that *TIFY* genes may be engaged in signal transduction pathways or metabolic processes needed for stress adaptation [21, 32]. Ebel et al. [70] In wheat, *TdTIFY* genes (except TdTIFY10c, which was slightly induced) were induced by salt stress, but their expression was restored after six hours. In addition, the most strongly expressed gene was *TdTIFY11a*. *ZmTIFY16* gene in maize displayed high expression in young and mature leaves and was highly induced by abscisic acid, dehydration, drought, low temperature (4ºC), and salt [71]. The *BrTIFY* JAZs genes were activated in *Brassica rapa* in response to ABA, Fusarium, salt, drought, and SA treatments [72]. The TIFY gene family, which includes transcriptional regulators such as the JAZ domain proteins, is very important for how plants react to environmental stresses, like salt stress [73]. In line with the results of the cis-acting analysis, *TIFY* genes, due to their association with hormonal signaling pathways, may enable a coordinated response that integrates multiple signals, enabling the plant to adapt more effectively to complex environmental challenges.

4. Conclusion

16 *TIFY* genes were characterized in *C. quinoa* through genome-wide analysis. Expression profiling identified specific *Cq-TIFY* genes upregulated in root and shoot tissues under salt stress, indicating their role in stress adaptation. Promoter analysis revealed stress- and hormone-responsive cis-acting elements, while protein-protein interactions indicated their role in jasmonic acid signaling pathways, essential for abiotic stress resilience. The study's findings will guide future research and breeding on *C. quinoa*, providing a foundation for further functional research and understanding.

Ethics in Publishing

This study presents no ethical concerns related to its publication.

Author Contributions

Esma Yiğider: Authored the manuscript, conducted experimental studies, and interpreted the conclusions.

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