Genome-wide identification of the *FAD* gene family in barley and gene expression under saline stress (#89720)

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Genome-wide identification of the *FAD* gene family in barley and gene expression under saline stress

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Barley is the most salt-tolerant cereal crop. Fatty acid desaturase (*FAD*) gene family plays an essential role in plant growth, development, and defense by catalyzing the formation of double bonds in saturated or unsaturated fatty acids. In this study, we identified 24 *HvFAD* genes in barley (cv. Morex) genome and categorized them into five subfamilies based on their amino acid sequence similarity. We analyzed the genes for their chromosomal location, protein molecular weight, theoretical isoelectric point, conserved motifs, gene structure, phylogeny, and cis-acting regulatory elements. Highly expressed genes under salt treatment were selected for quantitative experiments, and significantly upregulated genes under salt and alkali stress were analyzed for their subcellular localization. The barley desaturase genes are distributed on six out of the seven chromosomes and phylogenetically divided into five subfamilies, which correspond to *Arabidopsis* counterparts *FAB2*, *FAD4*, *SLD*/*DES*, *FAD2*/*FAD6*, and *FAD3*/*FAD7*/*FAD8*. The gene structures and motif components were found to be evolutionarily conserved within the same subfamily. Quantitative analysis revealed that *FADs* were expressed in all tissues of barley, including roots, stems, leaves, and tillers, with increased expression after salt treatment. This study provides valuable insights for the functional validation of salt-tolerant genes in barley.

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Abstract

Barley is the most salt-tolerant cereal crop. Fatty acid desaturase (*FAD*) gene family plays an essential role in plant growth, development, and defense by catalyzing the formation of double bonds in saturated or unsaturated fatty acids. In this study, we identified 24 *HvFAD* genes in barley (cv. Morex) genome and categorized them into five subfamilies based on their amino acid sequence similarity. We analyzed the genes for their chromosomal location, protein molecular weight, theoretical isoelectric point, conserved motifs, gene structure, phylogeny, and cis-acting regulatory elements. Highly expressed genes under salt treatment were selected for quantitative experiments, and significantly upregulated genes under salt and alkali stress were analyzed for their subcellular localization. The barley desaturase genes are distributed on six out of the seven chromosomes and phylogenetically divided into five subfamilies, which correspond to *Arabidopsis* counterparts *FAB2*, *FAD4*, *SLD*/*DES*, *FAD2*/*FAD6*, and *FAD3*/*FAD7*/*FAD8*. The gene structures and motif components were found to be evolutionarily conserved within the same subfamily. Quantitative analysis revealed that *FADs* were expressed in all tissues of barley, including roots, stems, leaves, and tillers, with increased expression after salt treatment. This study provides valuable insights for the functional validation of salt-tolerant genes in barley.

Introduction

Fatty acid desaturases (*FADs*) are pivotal in determining plant stress tolerance. They introduce double bonds into FA hydrocarbon chains, metamorphosing saturated fatty acids into their unsaturated counterparts. Crucially, *FADs* orchestrate the synthesis and balance of unsaturated fatty acids, ensuring the fluidity and integrity of cellular membranes. *FADs* within analogous

subfamilies exhibit remarkably preserved amino acid sequences. (Chen et al. 2019). For example, *FAD2* inherently possesses two enduring histidine motifs, whereas membrane-affiliated *FADs* boast three such motifs. In advanced flora, *FADs* are delineated by their solubility into two distinct categories: soluble desaturases and membrane integrins (Dehghan et al. 2014). Those tethered to membranes are further stratified into four nuanced subfamilies, predicated on their designated roles, encompassing *FAD4*, *FAD2/FAD6*, *FAD3/FAD7/FAD8*, and *ADS/SLD/DES* (Saini et al. 2019). Notably, the stearoyl ACP desaturase (*SAD*) remains an exclusive *FAD,* ensconced within the plastid matrix of evolved plants. All alternative desaturases are in the integrin category and are anchored to the endoplasmic reticulum and plastid membranes. The elucidation of myriad plant genomes has unveiled a repository for comprehensive assessments of *FAD* gene families across diverse plant species. For instance, the cucumber (*Cucumis sativus*) genome encompasses 23 integral *FAD* genes (Dong et al. 2016), in contrast to the soybean's (*Glycine max*) 29 and, the rice's (Oryza sativa L) 20 (Chen et al. 2019). Concurrently, research has illuminated the instrumental role of the *ACC1* gene in modulating the de novo synthesis of fatty acids via Acyl-CoA carboxylase (Hao et al. 2019). Probing its subcellular presence reveals *ACC1*'s predominant disposition within chloroplasts, and its tissue expression apex is predominantly witnessed in seeds and foliage.

Plant *FADs* are reputedly instrumental in fortifying plants against both abiotic and biotic adversities, including thermal extremities, desiccation, salinity, trauma, and pathogenic onslaughts (Saini and Kumar et al. 2019). These entities further facilitate the modulation of phytohormones, like jasmonic acid, salicylic acid, and abscisic acid. In soybean studies, the

augmented expression of *GmFAD3A* conspicuously bolstered the plant's resilience to cold(Wang et al. 2019). Analogously, in tomatoes, the contrarian overexpression of *LeFAD3* markedly optimized their photosynthetic prowess(Wang et al. 2010). It has been ascertained that the heightened expression of *AtFAD2* accentuated germination and salinity endurance in *Arabidopsis* (Zhang et al. 2010), while the contrarian overexpression of *AtFAD7* rcur ed its tolerance to salinity and desiccation (Im et al. 2002). Additionally, the ectopic amplification of either *AtFAD3* or *AtFAD8* fortified osmotic stress defiance in tobacco (Zhang et al. 2005). Within the rice realm, the upregulated expression of *OsFAD7* and *OsFAD8* undermined resistance to Phytophthora infestans (Yara et al. 2007). Moreover, the surging expression of *TaSSI2* in *Arabidopsis thaliana* fortified its defense against the incursion of powdery mildew fungi (Song et al. 2013). Conversely, the muting of *OsSSI2* notably enhanced resistance to rice blast and leaf blight (Jiang et al. 2009).

Land salinization on a global scale poses a formidable challenge, inhibiting the quintessential growth and maturation of plants and culminating in a diminution of global agricultural yield averages. Among the pantheon of ancient crops, barley emerges eminent, attributed to its robust resilience against salinity, alkalinity, and other stresses, rendering it an exemplary subject for the scrutiny and enhancement of saline and alkaline-tolerant crops. Within this exploration, we discerned 24 gene entities from the barley genome pertinent to the *FADs* lineage through a sophisticated bioinformatic methodology. These genes were christened in accordance with their chromosomal domiciles. Employing phylogenetic analysis, these genes were stratified into five subfamilies: *FAB2*, *FAD4*, *ADS*/*SLD*/*DES*, *FAD2*/*FAD6*, and

FAD3/*FAD7*/*FAD8*. We conducted a comprehensive analysis on the gene structure, conserved motifs, chromosomal localization, covariance analysis, cis-acting elements in the promoters of *HvFADs*, and protein-protein interaction networks of *HvFADs* family members. Subsequently, we selected several members of the *HvFADs* gene family to undergo quantitative experiments on barley that was subjected to salt and alkali stress treatments. Based on the expression results, we analyzed genes that exhibited significant expression changes under salt and alkali stress for their subcellular localization.

Materials and methods

Identification and characterization of *FAD* family members in the Hordeum vulgare genome.

To effectively identify the candidate *FAD* family members of *Hordeum vulgare* cv. Morex genome, candidate protein sequences were downloaded from the Pfam protein family database (http://Pfam.xfam.org/) corresponding to the structural domains of FA_desaturatase (PF00487), FA_desatase 2 (PF03405), and TMEM189 (PF10520) were analyzed. Markov model (HMM) files against barley (*Hordeum vulgare*) were then used to confirm the *HvFAD* of each putative *HvFADs* using the SMART database (http://SMART.embl-heidelberg.de/). tBtools software was used to analyze the physicochemical properties, transmembrane structural domains, and signaling peptides of the members of *HvFAD*.

Chromosomal distribution of *FAD* genes.

113 The location of *HvFAD* members on chromosomes was extracted from the genome
114 annotation files, the density of the whole chromosome was determined, and visual
115 analysis was performed using TBtools software (Chen et al. 2020). Only chromosomes
116 containing *HvFAD* gene family members are displayed,analysis of conserved motifs
117 and domains. Conserved motif analysis of the FAD protein sequences was performed
118 using the classical motif discovery model of MEME-suite 5.3.3 (Bailey et al. 2006).
119 The parameter of the optimum motif was set from 6 to 200, and the maximum number
120 of motifs was set to 10. Conserved domain analysis was performed using TBtools
121 (Chen et al. 2020).

122 **Collinearity analysis.**

123 To investigate the mechanisms mediating *FAD* gene family evolution, MCScanX
124 (Wang et al., 2012) was used to analyze tandem, proximal, and dispersed duplications.
125 Collinearity analysis of *FAD* in the *Hordeum vulgare* genome was conducted locally
126 using the TBtools software package (Chen et al. 2020) to map the genes to
127 chromosomal locations (Xie et al. 2018). In the course of this research, collinearity
128 attributed to segmental duplication events, and tandem duplicates resulting from gene
129 duplication events were discerned within our data. Upon examining the alignment
130 results, if a duo of alignment sequences presented a chromosomal connection, the
131 MCScanX algorithm inferred that one sequence emerged due to the duplication of its
132 counterpart, signifying a gene duplication occurrence. In addition, genomic clusters
133 were evident, and the MCScanX software substantiated the notion that a consortium of

multiple sequence pairs originated from the duplication of another distinct group, indicating a single fragment duplication incident.

Phylogenetic analysis.

The phylogenetic relationship between FAD proteins in jasmine, *Arabidopsis*, and rice has been investigated (Diao et al. 2020). A phylogenetic four of *Hordeum vulgare*, *Glycine max*, rice and *Arabidopsis* proteins was constructed using the neighbor-joining method. The coding sequences of the full-length genes were aligned by multiple gene alignment using fast Fourier transform (MAFFT) (Kazutaka et al. 2019). Comparison of protein sequences and generation of phylogenetic trees were performed using MEGA7 software (Arizona State University, Tempe, AZ, USA) with 1000 bootstrap replicates. The FAD protein sequences of *Arabidopsis* and rice were downloaded from the *Arabidopsis* (Philippe et al. 2012) and rice (Kawahara et al. 2013) databases for the construction of a phylogenetic tree, and figures were generated using the iTOL online website (Letunic and Bork 2019).

Protein-protein interaction (PPI) network.

To predict the interactions between *HvFAD* proteins, FAD protein sequences were submitted to STRING V10 (Szklarczyk et al. 2015). The PPI networks were then visualized using Cytoscape V3.7.2 (Shannon et al. 2003).

Plant material, treatment, and quantitative real-time polymerase chain reaction (qRT-PCR) Analysis.

Full-length *HvFAD* cDNA clones were grown in a light incubator until two leaves and one heart, and then the variety golden was selected. After growing to two leaves and one heart, the healthy barley variety golden was selected and the plants were subjected to 0h, 3h, 12h and 24h saline treatment, and the leaves of the healthy barley variety golden were collected for RNA extraction. Similarly, roots, stems, leaves and tiller tissues of healthy barley plants of variety golden were collected at the time of two leaves and one heart for RNA extraction. A RNA quality and concentration were measured using NanoDrop 2000 UV spectrophotometer. First-strand cDNA was synthesized using the Novizen Reverse Transcription Kit according to the manufacturer's protocol. cDNA was homogeneously diluted to 100 ng/μl and used as a template for the quantitative reverse transcription-polymerase chain reaction (qRT-PCR) reaction. Gene-specific primers for RT-PCR and qRT-PCR were designed using NCBI. Five genes belonging to four subfamilies were selected to analyze the expression of the *FAD* family in different tissues.

The PCR program was as follows: 94 °C for 2 min; 39 cycles of 95 °C for 5 s, 60 °C for 30 s; 95 °C for 5 s, 65 °C for 5 s, and 95 °C for 5 s. The PCR program was performed as follows.

Gene Expression Analysis™ Real-Time PCR Detection System (Bio-Rad) and Taq Pro Universal SYBR qPCR Master Mix (Q712-03) Master Kit (Novozymes) were used for gene expression analysis by quantitative T-PCR qRT-PCR experiments using FX96 Touch. Relative fold differences were calculated based on the comparative cycle

threshold (2-ΔΔct) using Tublin (F:AGTGTCCTGTCCACCCACTC; R:AGCATGAAGTGGATCCTTGG) as the reference gene. The PCR mixture (20 μL) consisted of 10 μL of 2× Taq Pro Universal SYBR qPCR Master Mix (Novozymes), 10 μM of each primer, 100 ng cDNA template, and nuclease-free water. The PCR program was as follows: 94°C for 2 min; 39 cycles of 95°C for 5 s, 60°C for 30 s; 95°C for 5 s, 65°C for 5 s, and 95°C for 5 s.

Transient expression of *HvFAD14*.

Subcellular localization of barley protoplasts was analyzed using a transient transformation system. A full-length open reading frame (ORF) fragment (1156 bp) of *HvFAD14* was constructed with GFP empty as a new GFP vector (*HvFAD14*-GFP). The newly constructed vector was transformed into Fast-T1 chemoreceptor cells. After that, the cells were harvested by shaking the bacteria and centrifugation for bulk plasmid extraction. Protoplasts were extracted from barley yellowing seedling leaves using cellulase and dissociative enzyme solutions, and the reconstructed GFP plasmid was transformed into protoplasts by mmg solution using 40% PEG. After 16 h of dark expression, the plasm were observed using a Leica Leica-fluorescence focusing microscope.

Results

Identification and analysis of *HvFADs* genes in barley

We identified a total of 24 *HvFADs* genes from the barley genome. These genes were named based on their chromosomal locations and phylogenetic analysis, resulting in names ranging from *HvFAD1* to *HvFAD24*. Details of these 24 genes (gene ID, gene name, protein amino

196 acid length and basic data of the derived peptides) are provided in Table 1. The coding
197 sequence lengths of these 24 *HvFADs* genes varied from 987 bp to 1329 bp. e lengths of the
198 amino acid sequences encoded by these genes ranged from 329 to 469 amino acids. Additionally,
199 the predicted molecular weights (MW) of these proteins ranged from 37,724.61 Da (*HvFAD9*) to
200 52,548.61 Da (*HvFAD18*), while the theoretical isoelectric points (pI) ranged from 6.04 to 9.37
201 (Table 1).

202 **Chromosomal localization of *HvFADs* genes in barley**

203 The chromosomal locations of *HvFADs* were precisely identified based on available
204 genome sequences. The distribution of the *FAD* gene on 7 barley chromosomes was not
205 uniform. *HvFAD1* to *HvFAD9* are located on chromosome 2H (Chr2H), *HvFAD10* to *HvFAD13*
206 on chromosome 3H (chr3H), *HvFAD14* to *HvFAD15* on chromosome 4H (chr4H), *HvFAD16* to
207 *HvFAD20* on chromosome 5H (chr5H), *HvFAD21* to *HvFAD23* are located on chromosome
208 chr6H, *HvFAD24* is located on chromosome 7H (chr7H), chromosome 1H (chr1H) does not
209 contain *HvFAD* (Figure. 1) .

210 **Phylogenetic relationship analysis of HvFADs proteins**

211 In an endeavor to unravel the phylogenetic intricacies among *HvFADs*, we performed a
212 phylogenetic tree analysis of adjacent linkages using *FADs* from wheat (68), soybean (27),
213 *Arabidopsis thaliana* (27), and rice (18). we noted, as depicted in Figure 2, that these *FADs* can
214 be delineated into five distinct subfamilies: *FAB2*, *FAD4*, *FAD3*/*FAD7*/*FAD8*, *FAD2*/*FAD6*, and
215 *DES*/*SLD*. Among these subfamilies, the *FAB2* subfamily has 14 genes for *HvFADs*
216 (*FAD1,FAD2,FAD3,FAD4,FAD5,FAD8,FAD10,FAD11,FAD12,FAD13,FAD16,FAD19,FA*
217 *D20,FAD24*), the *FAD3*/*FAD7*/*FAD8* subfamily has four genes for *HvFADs*
218 (*FAD6,FAD14,FAD15,FAD17*), the *FAD2*/*FAD6* subfamily has four *HvFAD* genes

(*FAD7,FAD21,FAD22,FAD23*), and the *DES/SLD* subfamily has two *HvFAD* genes (*FAD9,FAD18*). Furthermore, the *FAD4* subfamily contains no *HvFAD* genes (Figure.2).

Analysis of *HvFADs* gene structure and conserved motifs

we noted, as depicted in Figure 3, except for *HvFAD5, HvFAD10, HvFAD18*, *HvFAD22*, and *HvFAD23*, which have no introns, the other 19 *HvFAD* genes consist of both introns and exons. The number of introns ranges from 1 to 9, and the number of exons ranges from 1 to 10. Among them, the *HvFAD7* gene has the highest number of introns and exons, with 9 introns and 10 exons. *HvFAD1*, *HvFAD3*, *HvFAD4*, *HvFAD24*, *HvFAD19*, *HvFAD11*, *HvFAD9*, and *HvFAD21* each have 1 intron and 2 exons. *HvFAD2*, *HvFAD12*, *HvFAD13*, *HvFAD8*, *HvFAD16*, and *HvFAD20* each have 2 introns and 3 exons. *HvFAD6*, *HvFAD14*, *HvFAD15*, and *HvFAD17* each have 7 introns and 8 exons.

To investigate the distribution of these *HvFAD* motifs, 10 motifs were identified in barley. Conserved motif analysis of UEV genes using MEME software revealed that *HvFAD1*, *HvFAD3*, *HvFAD4*, *HvFAD5*, *HvFAD24*, *HvFAD12*, *HvFAD19*, *HvFAD11*, *HvFAD13*, *HvFAD8*, *HvFAD16*, and *HvFAD20* in the FAB2 subfamily have seven identical motifs (motif1, motif2, motif3, motif4, motif5, motif7, motif10). Additionally, *HvFAD2* and *HvFAD1* have six identical motifs (motif1, motif2, motif3, motif5, motif7, motif10). *HvFAD10* and *HvFAD1* have five identical motifs (motif1, motif2, motif3, motif5, motif10). *HvFAD9*, *HvFAD18*, and *HvFAD7* in the *DES/SLD* subfamily have 2 identical motifs (motif6, motif8). Lastly, *HvFAD21*, *HvFAD22*, *HvFAD23*, *HvFAD6*, *HvFAD15*, *HvFAD14*, and *HvFAD17* in the FAD2/FAD6 subfamily and FAD3/FAD7/FAD8 subfamily have 3 identical motifs (motif6, motif8, motif9) (Figure. 3).

Chromosomal localization and covariance analysis of HvFADs genes

Intriguingly, *HvFADs* genes manifested a sporadic and non-uniform distribution across all barley chromosomes, with chromosome 1H (Chr1H) being the sole exception. Chromosome 2H (Chr2H) contained the most *HvFADs* (nine members), followed by five *HvFADs* genes on chromosome 5H (Chr5H), four *HvFADs* genes on chromosome 3H (Chr3H), three *HvFADs* genes on chromosome 6H (Chr6H), 2 *HvFADs* genes on Chr4H, and 1 *HvFAD* gene on chromosome 7H (Chr7H). Covariance analysis was performed to further investigate gene duplication events in the *HvFADs* family. Our data revealed a single pair of segmentally duplicated genes. (Figure. 4).

Analysis of cis-acting elements in *HvFADs* promoters

A comprehensive investigation was undertaken to elucidate the cis-regulatory elements present in the promoter domains of *HvFADs*. The discerned cis-acting components within these promoters were categorized into four principal groups: light-responsive, hormone-responsive, stress-responsive elements, and elements integral to plant growth and maturation, as depicted in Figure 5. Light-responsive elements permeated all *HvFADs* promoters, prominently featuring the Sp1 element with 148 occurrences. It is worth noting, however, that the promoters of *HvFAD11*, *HvFAD23*, and *HvFAD24* were devoid of this particular element.

In the realm of hormone-responsive elements, the methyl jasmonate (MeJA) response element emerged as predominant with 118 instances, closely succeeded by the abscisic acid (ABA) response element at 113 instances. All *HvFADs* promoters, with the exception of HvFAD8, encompassed ABA response motifs (specifically ABRE and DRE1). The cis-regulatory motifs associated with the MeJA response, including the TGACG and CGTCA patterns, were manifest in 20 *HvFADs* promoters. Diving deeper, varied numbers of *HvFADs*, namely 4, 6, 13, and 12, bore promoters featuring growth hormone, ethylene, SA, and gibberellin response motifs, respectively. Moreover, a minimum of two hormone-responsive motifs could be ascertained in all

HvFADs promoters. Notably, *HvFAD4* and *HvFAD5* promoters were characterized by the inclusion of all the previously mentioned hormone-responsive motifs.

Turning our attention to stress-responsive motifs, the drought response elements, specifically MYC, as-1, and MBS, exhibited a conspicuous presence with a tally of 167. Nonetheless, the MYC element was absent in the promoters of *HvFAD11* and *HvFAD16*, but was a staple in the remaining 22 *HvFADs* promoters. The as-1 motif had a broad distribution, only being excluded from the promoters of *HvFAD9*, *HvFAD13*, *HvFAD17*, and *HvFAD24*. A more restricted presence was noted for MBS elements, featured only in 16 *HvFADs* promoters. Additionally, defense and stress response motifs, encompassing STRE, CARE, and TCrich repeat sequences, were prominent, totaling 77, with STRE patterns found in 18 promoters, CARE in a singular promoter, and TCrich repeat sequences in 6 promoters. A myriad of other elements related to drought, trauma, pathogens (WRE3, box, and WUN motifs), defense and stress, temperature fluctuations, dehydration (DRE core), anaerobic induction (ARE), and hypoxia-specific induction (GC motif) were distributed among 24, 21, 19, 11, 7, 16, and 9 *HvFADs*, respectively, as illustrated in Figure 5.

Analysis of protein-protein interaction network of *HvFADs* family members

As shown in Figure. 6, members of *HvFADs* interacted with each other. The highest interaction coefficient was found between *HvFAD7* and *HvFAD17*. There were interactions between *HvFAD7* and *HvFAD17*, *HvFAD20*, *HvFAD23*, and *HvFAD5*. There were also interactions between *HvFAD9* and *HvFAD18*, *HvFAD24*, *TCS10B*, *TCS10A*, and *ATCES1*. Additionally, there were interactions between *HvFAD11* and *FATB*, *HvFAD12* and *FATB*, *HvFAD17* and *FAD5*, *HvFAD7* and *HvFAD20*, *HvFAD18* and *TCS10B*, *TCS10A*, *HvFAD9* and *HvFAD23*, *FAD5*,

HvFAD19 and *HvFAD23*, *FATB*, *HvFAD20* and *HvFAD17*, *FAD5*, *HvFAD23*, *FATB*, and *HvFAD7*. Finally, *HvFAD24* interacted with *HvFAD9*, *HvFAD23*, and *FATB* (Figure. 6).

qRT-PCR expression analysis of *HvFADs* genes under different stresses

HvFADs genes have been reported to function under many abiotic and biotic stresses (Saini and Kumar et al. 2019). In this study, qRT-PCR was utilized to detect changes in the expression of *HvFADs* genes under saline and alkaline stress conditions. Five genes were selected for real-time quantitative analysis: two genes from the *FAB2* subfamily, two genes from the *FAD3*/*FAD7*/*FAD8* subfamily, and one gene from the *FAD2*/*FAD6* subfamily for qRT-PCR analysis. The results, as shown in Figure. 7, demonstrated that the expression of *HvFAD8*, *HvFAD7*, *HvFAD13*, *HvFAD14*, and *HvFAD15* was down-regulated at the beginning of salt treatment. However, with an increase in the duration of salt treatment, their expression gradually increased. At 24 hours of salt treatment, the expression of *HvFAD8*, *HvFAD7*, *HvFAD13*, *HvFAD14*, and *HvFAD15* reached their highest levels, showing a fold increase of 1.1 to 1.5, respectively (Figure. 7).

The results showed that *HvFAD13* was down-regulated at all observed treatment times in the alkali treatment. *HvFAD8* was up-regulated at the beginning of the alkali treatment, but the expression gradually decreased as the treatment time extended, with the highest expression at 12 hours. *HvFAD7* was down-regulated initially in the alkali treatment, but as the treatment time extended, the expression first increased and then decreased, with the highest expression at 12 hours. *HvFAD15* was down-regulated at the beginning of the alkali treatment, but the expression first increased and then decreased as the treatment time extended, with the highest expression at 12 hours. *HvFAD14* was up-regulated at all observed treatment times in the alkali treatment, with the highest expression at 12 hours. *HvFAD15* was up-regulated at all observed treatment times in the alkali treatment, with the highest expression at 3 hours. *HvFAD15* was up-regulated at all

observed treatment times in the alkali treatment, with the highest expression at 3 hours (Figure. 8).

Organ-specific expression of barley *HvFADs* genes

In the present study, all five genes were expressed in all the tested tissues. *HvFAD14* was expressed in all four tissues - root, stem, leaf, and tiller, with higher expression in roots and lower expression in leaves, stems, and tillers. *HvFAD13* had the highest expression in leaves, with lower expression in stems, roots, and tillers, and the lowest expression in roots. *HvFAD8* showed the highest expression in tillers, with higher expression in leaves and stems and the lowest expression in roots. *HvFAD15* had the highest expression in stems, higher expression in leaves, lower expression in roots, and the lowest expression in tillers. *HvFAD7* had the highest expression in leaves, lower expression in stems, and almost no expression in tillers and roots (Figure. 9).

Localization analysis of *HvFAD14* proteins

The localization of *HvFAD14* proteins was distributed in the endoplasmic reticulum compared to the control (Figure. 10).

Discussion

In contemporary botanical research, the *FADs* gene family has emerged as a subject of considerable interest, being recognized and characterized across an extensive array of plant species, each exhibiting a distinct number of family members. Within the ambit of this study, we delineated 24 distinct *FADs* genes resident within the Hordeum vulgare genome. These *HvFADs* genes can be taxonomically stratified into five notable subfamilies: *FAB2*, *FAD4*, *SLD/DES*, *FAD2/FAD6*, and *FAD3/FAD7/FAD8*. Intriguingly, this classification exhibits a congruence with

the taxonomy of rice *FADs* (Paterson et al. 2009). We did not find *ADS* members in barley, as well as in rice and wheat. Previous research on the banana *FAD* family also did not show the presence of *ADS* members (Cheng et al. 2022). However, soybean and *Arabidopsis* contained *ADS* members, suggesting that the *ADS* subfamily appeared after the divergence of monocotyledons and dicotyledons (Zhang et al. 2021). The phylogenetic tree and cluster types of FADs proteins in wheat are consistent with other plants, such as sunflower (Celik Altunoglu et al. 2018), cucumber (Dong et al. 2016), and soybean (Chi et al. 2011). The similar amino acid composition of each cluster indicates that the phylogenetic distribution of wheat FADs proteins is associated with their motif content. All members of the *FAB2* subfamily possess motifs 1, 2, 3, 4, 5, 7, and 10, with the exception *FAD10*, which lacks motif 7, and *FAD2*, which lacks motif 4. All members of the *FAD3/FAD7/FAD8* subfamily contain motifs 6, 8, and 9. Similarly, all members of the *FAD2/FAD6* subfamily possess motifs 6, 8, and 9, except for *FAD7*, which does not have motif 9. All members of the *DES/SLD* subfamily contain motifs 6 and 8. Notably, the protein sequences of the *SLD/DES, FAD2/FAD6*, and *FAD3/FAD7/FAD8* subfamilies are highly similar, indicating a closer relationship between these subfamilies compared to the *FAB2* subfamily. Moreover, members of *HvFADs* in the same subfamily exhibit similar intron/exon structures and intron phases, as well as similar motif compositions in their encoded proteins. Similar results were also observed in wheat and rice, indicating high conservation of the *FADs* gene family. The study of promoter regions helps in understanding gene interactions and functions. Transcription factors assume a paramount role in orchestrating signaling cascades in tandem with abiotic stresses. Functionally, these molecular regulators affix themselves to the

352 promoter regions of target genes, mediating either their activation or repression (Lindemose et al.
353 2013). The presence of TGACG and CGTCA motifs in genes is associated with a response to
354 methyl jasmonate (Dong et al. 2013). In a nuanced exploration of regulatory elements, the
355 ABRE and MBS motifs emerged as instrumental in modulating responses to abscisic acid (ABA)
356 and drought conditions, respectively. Jasmonates, on the other hand, underpin a myriad of
357 physiological processes, encompassing seed germination, cellular senescence, and responses to
358 both biotic and abiotic stresses. The ABRE motif, characterized by the TACGGTC sequence,
359 becomes invigorated in the presence of ABA, catalyzing plant resilience against drought and
360 salinity adversities.

361 Gene duplication is an essential process in the evolution of various organisms, allowing for
362 the development of new structures and functions. Whole genome doubling (WGD) is a
363 significant evolutionary event that occurs in plants, animals, and fungi and leads to the
364 generation of numerous duplicated genes simultaneously. When analyzing covariance, it was
365 discovered that a pair of genes had undergone segmental duplication. Out of the 24 desaturase
366 genes examined, 11 of them exhibited tandem duplications, while 2 genes displayed segmental
367 duplications. The tandemly duplicated genes were located on chromosomes 2, 3, and 6. These
368 findings indicate that gene duplication events play a role in the expansion of the barley
369 desaturase family.

370 Delving deeper into specific gene promoters, the oil palm's *EgFAD8* promoter is replete
371 with a gamut of stress-responsive and phototropic elements (Cao et al. 2016). This gene's
372 expression exhibited a pronounced affinity towards low-temperature and reduced light conditions.

373 Furthermore, the transcriptomic dynamics of the Kale-type oilseed rape *BnFAD2-C5* revealed
374 upregulation in response to SA and JA stimuli. Notably, distinct SA-responsive and
375 JA-responsive elements are resident within specified regions of its promoter (Liu et al. 2016).
376 Moreover, the promoters of both *BnFAD2A5-1* and Sesame *SeFAD2* also incorporate
377 ABA-responsive cis-elements (ABRE), with their gene expression being inducible by ABA
378 (Xiao et al. 2014). Our investigation spotlighted an abundance of hormones (e.g., MeJA, ABA,
379 SA) and stress-related (e.g., drought, trauma, low temperature) cis-acting elements within the
380 *HvFADs* promoter regions. The prolific presence of such cis-acting elements, encompassing light,
381 hormonal, stress, and developmental responses, intimates a potential broad-spectrum expression
382 of *HvFADs* in response to diverse hormones and abiotic stresses. While our findings resonate
383 with extant literature, it's pivotal to acknowledge that the mere presence of a cis-acting element
384 doesn't invariably translate to gene expression under corresponding stresses or hormonal cues.
385 This paradox can be attributed to the intricate intricacies governing gene expression and the
386 inherent limitations of computational tools in precisely predicting promoter cis-elements (Liu et
387 al. 2014). Consequently, empirical methodologies, such as qRT-PCR, remain indispensable for
388 the accurate discernment of functional regulatory elements within *HvFADs* promoters.

389 Analysis of tissue expression data of *HvFADs* family genes showed that the homologous
390 genes exhibited similar expression patterns, indicating their functional conservation (Le et al.
391 2012). qRT-PCR results indicated that *HvFADs* family genes were involved in the
regulation of 392 abiotic stress in barley under salt stress and alkali stress; in particular, by
analyzing the
393 expression of the *HvFADs* family genes in the two sets of control materials, *HvFAD14*,,

394 *HvFAD15* and *HvFAD7* play crucial roles in controlling salt stress and alkali stress in barley.
395 This result is inextricably linked to their genetic structure. Tissue-specific expression profiles
396 illustrate the identification of specific genes and their particular roles at specific developmental
397 stages.

398 **Conclusions**

399 In this study, we identified 24 *FADs* family genes from the barley genome using bioinformatics
400 methods. Phylogenetic analysis revealed that *HvFADs* can be categorized into five subfamilies:
401 *FAB2*, *FAD4*, *ADS/SLD/DES, FAD2/FAD6*, and *FAD3/FAD7/FAD8*. Gene structure and conserved
402 motif analysis demonstrated that these genes were conserved within their respective subfamily
403 Covariance analysis identified a total of one pair of segmentally duplicated genes. Conclusively,
404 our analysis infers that the *HvFADs* gene expression may be modulated by an array of
405 determinants, including but not limited to, hormones, external stresses, and transcription factors.
406 Moreover, protein-protein interaction network analysis indicated that members of *HvFADs*
407 interact with each other. Quantitative results showed that *FADs* were expressed in all tissues of
408 barley roots, stems, leaves, and tillers, with increased expression after salt treatment. Notably,
409 alkali treatment led to more pronounced changes in *FADs* expression.
410

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References

Chen C, Yang J, Tong H, Li T, Wang L, Chen H. 2019. Genome-wide analysis of fatty acid desaturase genes in rice (Oryza sativa L.). *Scientific Reports* **9(1)**:1-11.

Nayeri FD, Yarizade K. 2014. Bioinformatics study of delta-12 fatty acid desaturase 2 (*FAD2*) gene in oilseeds. *Molecular biology reports* **41**:5077-5087.

Saini R, Kumar S. 2019. Genome-wide identification, characterization and in-silico profiling of genes encoding *FAD* (fatty acid desaturase) proteins in chickpea (Cicer arietinum L.). *Plant Gene* **18**:100180.

Xue Y, Chen B, Wang R, Win A N, Li J, Chai Y. 2018. Genome-wide survey and characterization of fatty acid desaturase gene family in Brassica napus and its parental species. *Applied biochemistry and biotechnology* **184**:582-598.

Juan DC, Ning C, Gang ZZ, Mao SQ. 2016. Characterization of the Fatty Acid Desaturase Genes in Cucumber: Structure, Phylogeny, and Expression Patterns. *PloS one* **11(3)**:e0149917.

yuan CX, li YQ, du LY, yan WJ, fen ZQ, juan PL, na CM, nan HY, lin YS. 2013. Genomic level analysis of soybean fatty acid desaturatic enzyme gene(in Chinese). Proceedings of the Seventh Congress and Annual Academic Meeting of the Oil Crops Professional Committee of the Chinese Crop *Society* **2013:**240-240.

Chen C, Yang J, Tong H, Li T, Wang L, Chen, H. 2019. Genome-wide analysis of fatty acid

desaturase genes in rice (Oryza sativa L.). *Scientific Reports* **9(1)**:1-11.

Hao L, Qing L, fen LH, bin HY, ping CX, Qiang LX, xiong LS. 2019. Cloning and expression analysis of peanut acyl-CoA carboxylase gene ACC1(in Chinese). Shandong Agricultural *Sciences* **51(9)**:21-27.

Saini R, Kumar S. 2019. Genome-wide identification, characterization and in-silico profiling of genes encoding FAD (fatty acid desaturase) proteins in chickpea (Cicer arietinum L.). *Plant Gene* **18**:100180.

Wang X, Yu C, Liu Y, Yang L, Li Y, Yao W, Peng X. 2019. GmFAD3A, a ω-3 fatty acid desaturase gene, enhances cold tolerance and seed germination rate under low temperature in rice. *Internationaljournal ofmolecular sciences* **20(15)**:3796.

Wang HS, Yu C, Tang XF, Wang LY, Dong XC, Meng QW. 2010. Antisense-mediated depletion of tomato endoplasmic reticulum omega-3 fatty acid desaturase enhances thermal tolerance. *Journal of integrative plant biology* **52(6)**:568-577.

Zhang J, Liu H, Sun J, Li B, Zhu Q, Chen S, Zhang H. 2012. Arabidopsis fatty acid desaturase FAD2 is required for salt tolerance during seed germination and early seedling growth. *PloS one* **7(1)**:e30355.

Im YJ, Han O, Chung GC, Cho BH. 2002. Antisense expression of an Arabidopsis omega-3 fatty acid desaturase gene reduces salt/drought tolerance in transgenic tobacco plants. *Molecules and cells* **13(2)**:264-271.

Zhang M, Barg R, Yin M, Gueta-Dahan Y, Frenkel AL, Salts Y, Hayyim GB. 2005. Modulated fatty acid desaturation via overexpression of two distinct ω-3 desaturases

differentially alters tolerance to various abiotic stresses in transgenic tobacco cells and plants. *The Plant Journal* **44(3)**:361-371.

Yara A, Yaeno T, Hasegawa M, Seto H, Montillet JL, Kusumi K, Iba, K. 2007. Disease resistance against Magnaporthe grisea is enhanced in transgenic rice with suppression of ω-3 fatty acid desaturases. *Plant and cellphysiology* **48(9):**1263- 1274.

Song N, Hu Z, Li Y, Li C, Peng F, Yao Y, Sun Q. 2013. Overexpression of a wheat stearoyl-ACP desaturase (SACPD) gene TaSSI2 in Arabidopsis ssi2 mutant compromise its resistance to powdery mildew. *Gene* **524(2)**:220-227.

Jiang CJ, Shimono M, Maeda S, Inoue H, Mori M, Hasegawa M, Takatsuji H. 2009. Suppression of the rice fatty-acid desaturase gene OsSSI2 enhances resistance to blast and leaf blight diseases in rice. *Molecular plant-microbe interactions* **22(7)**:820-829.

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. 2020. TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Molecular plant* **13(8)**:1194- 1202.

Bailey TL, Williams N, Misleh C, Li WW. 2006. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic acids research* **34(2)**: W369-W373.

Wang Y, Tang H, DeBarry JD, Tan X, Li J, Wang X, Paterson AH. 2012. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic acids research* **40(7)**:e49-e49.

Xie T, Chen C, Li C, Liu J, Liu C, He Y. 2018. Genome-wide investigation of WRKY gene family in pineapple: evolution and expression profiles during development and stress. *BMC*

genomics **19:**1-18.

Diao D, Hu X, Guan D, Wang W, Yang H, Liu, Y. 2020. Genome-wide identification of the ARF (auxin response factor) gene family in peach and their expression analysis. *Molecular Biology Reports* **47**:4331-4344.

Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics* **20(4)**:1160-1166.

Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C, Sasidharan R, Huala E. 2012. The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. *Nucleic acids research* **40(1)**:1202-1210.

Kawahara Y, Bastide MdL, Hamilton JP, Kanamori H, McCombie WR, Ouyang S, Matsumoto T. 2013. Improvement of the Oryza sativa Nipponbare reference genome using next generation sequence and optical map data. *Rice* **6**:1-10.

Letunic I, Bork P. 2019. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic acids research* **47(1)**:256-259.

Szklarczyk D, FranceschiniA, Wyder S, Forslund K, Heller D Cepas, JH, Mering CV. 2015. STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic acids research* **43(1)**:447-452.

Shannon P, MarkielA, Ozier O, Baliga NS, Wang JT, Ramage D, Ideker T. 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome research* **13(11)**:2498-2504.

Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Gundlach H, Rokhsar DS. 2009. The Sorghum bicolor genome and the diversification of grasses. *Nature* **457(7229)**:551-556.

Cheng C, Liu F, Sun X, Wang B, Liu J, Ni X, Lü P. 2022. Genome-wide identification of FAD gene family and their contributions to the temperature stresses and mutualistic and parasitic fungi colonization responses in banana. *International Journal of Biological Macromolecules* **204:**661-676.

Zhang B, Xia P, Yu H, Li W, Chai W, Liang Z. 2021. Based on the whole genome clarified the evolution and expression process of fatty acid desaturase genes in three soybeans. *International Journal of Biological Macromolecules* **182:**1966-1980.

Altunoglu YC, Unel NM, Baloglu MC,Can THUF, Cetinkaya R. 2018. Comparative identification and evolutionary relationship of fatty acid desaturase (FAD) genes in some oil crops: the sunflower model for evaluation of gene expression pattern under drought stress. *Biotechnology & Biotechnological Equipment 32*(4):846-857.

Dong CJ, Cao N, Zhang ZG, Shang QM. 2016. Characterization of the fatty acid desaturase genes in cucumber: structure, phylogeny, and expression patterns. *PLoS One* **11(3)**:e0149917.

Chi X, Yang Q, Lu Y, Wang J, Zhang Q, Pan L, Yu S. 2011. Genome-wide analysis of fatty acid desaturases in soybean (Glycine max). *Plant molecular biology reporter* **29:**769-783.

Lindemose S, O'Shea C, Jensen MK, Skriver K. 2013. Structure, function and networks of transcription factors involved in abiotic stress responses *International journal of molecular sciences* **14(3)**:5842-5878.

Dong CJ, Shang QM. 2013. Genome-wide characterization of phenylalanine ammonia-lyase gene family in watermelon (Citrullus lanatus). *Planta* **238**:35-49.

Cao J, Li M, Chen J, Liu P, Li Z. 2016. Effects of MeJA on Arabidopsis metabolome under endogenous JA deficiency. *Scientific Reports* **6(1)**:37674.

Liu F, Wang G, Liu R, Guan C. 2016. The promoter of fatty acid desaturase on chromosome C5 in Brassica napus drives high-level expression in seeds. *Plant Biotechnology Reports* **10**:369-381.

Xiao G, Zhang ZQ, Yin CF, Liu RY, Wu XM, Tan TL, Guan CY. 2014. Characterization of the promoter and 5′-UTR intron of oleic acid desaturase (FAD2) gene in Brassica napus. *Gene* ***545*(1)**:45-55.

Liu Z, Zhang M, Kong L, Lv Y, Zou M, Lu G, Yu X. 2014. Genome-wide identification, phylogeny, duplication, and expression analyses of two-component system genes in Chinese cabbage (Brassica rapa ssp. pekinensis). *DNA research* **21(4)**:379-396.

Le DT, Nishiyama R, Watanabe Y, Vankova R, Tanaka M, Seki M, Tran LSP. 2012. Identification and expression analysis of cytokinin metabolic genes in soybean under normal and drought conditions in relation to cytokinin levels. *Plos one* **(8)**

Figure 1

Chromosomal mapping of *FAD* genes in *Hordeum vulgare*.

The phylogenetic tree was constructed usingthe neighbor-joining method in MEGA 7 with the conserved FAD domains of eggplant FAD proteins.The different groups were indicated with colors.

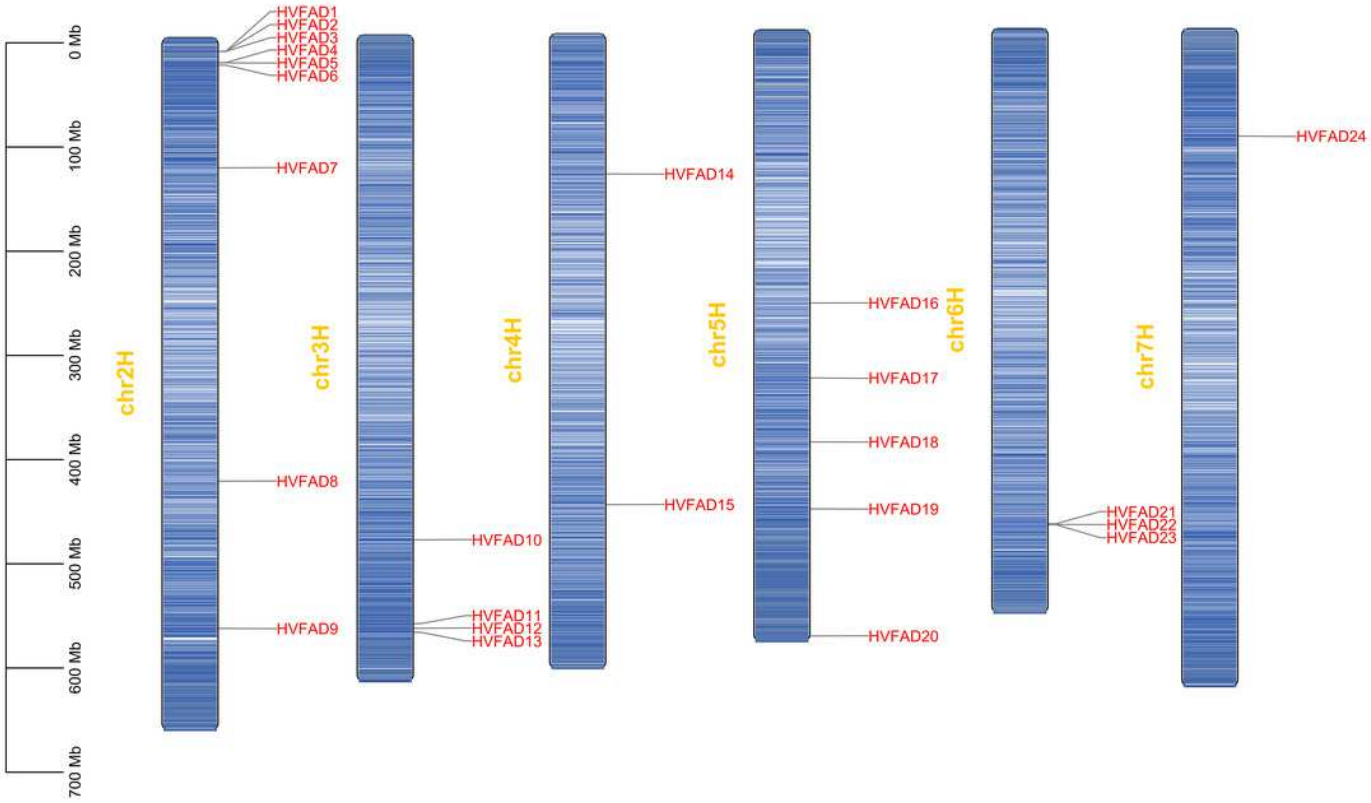

Figure 2

Phylogenetic tree for *Hordeum vulgare*, *Glycine max* L.□rice and *Arabidopsis FAD* proteins.

The phylogenetic tree was constructed usir□e neighbor-joining method in MEGA 7 with the conserved FAD domains of eggplant FAD proteins.The different groups were indicated with colors.

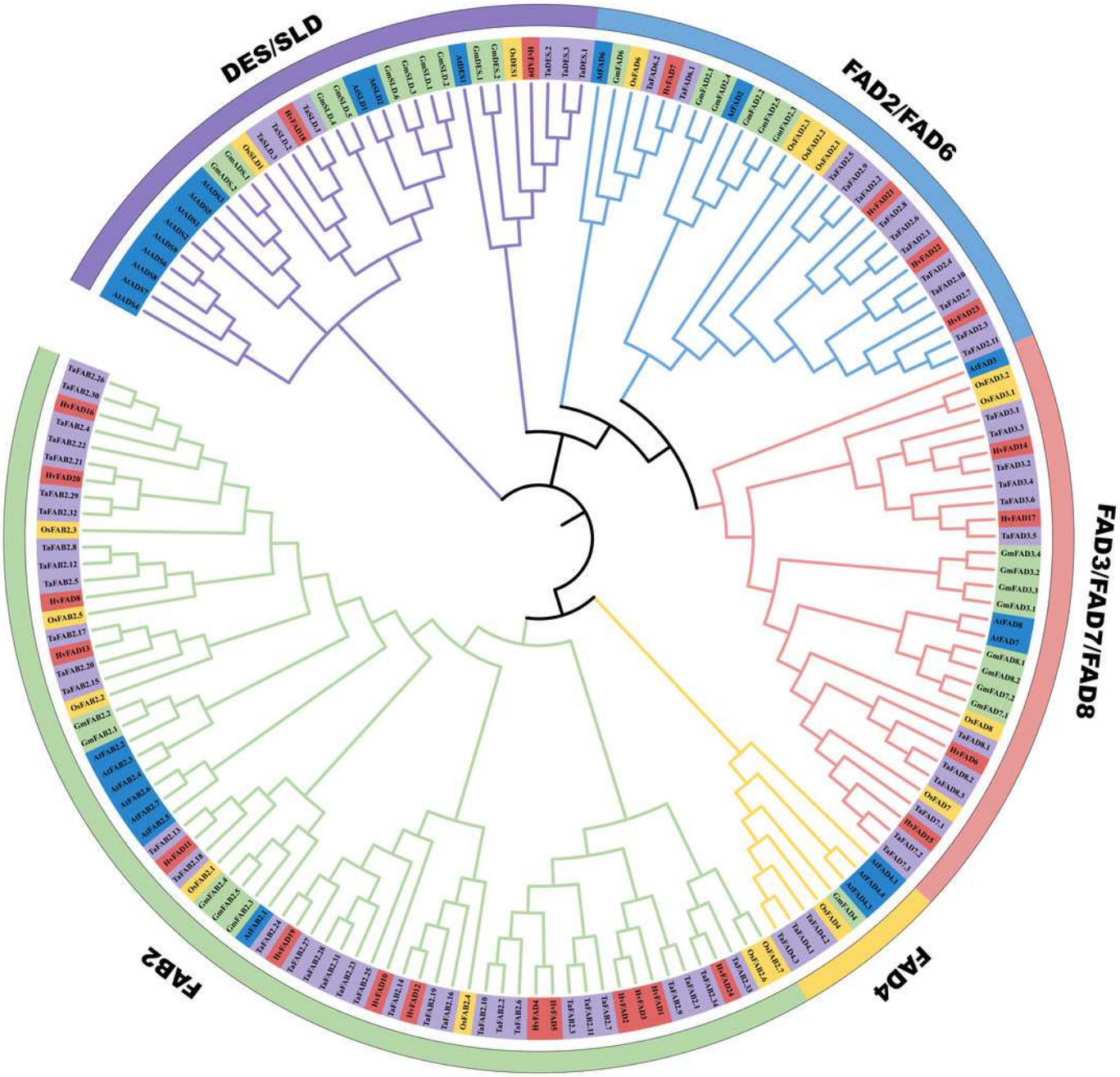
DES/SLD
FAD2/FAD6
FAD3/FAD7/FAD8
FAD4
FAB2

Figure 3

Gene structure and conserved motif analysis of the *FAD* family from *Hordeum vulgare*.

a Conserved motif of *HVFAD* family; b Gene structure of *HvFAD* family;Gene structure diagram of *HvFAD* genes. Exons were represented by green boxes and introns by black lines. The sizes of exons and introns could be estimated using the scale at bottom.

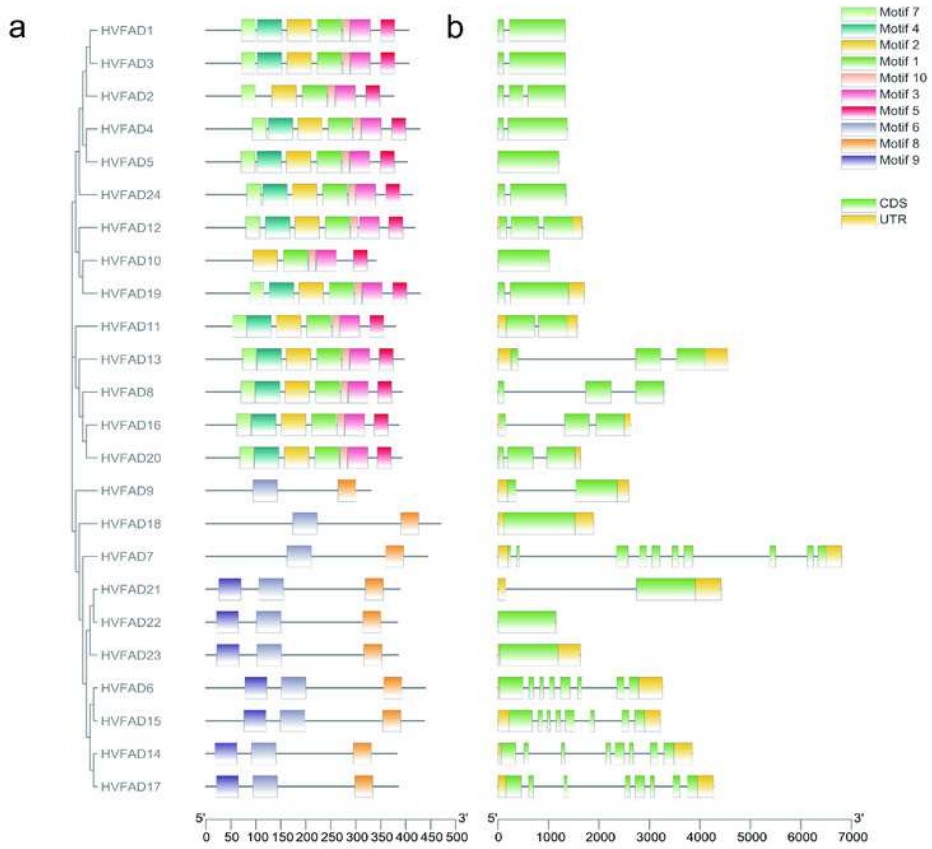

Figure 4

Chromosomal location and collinearity analysis of *HvFAD* family genes.

Respective chromosome numbers are indicated at the center of each arc. The scale on the arc is in megabases (Mb). The segmental duplication genes are connected by straight line in color.

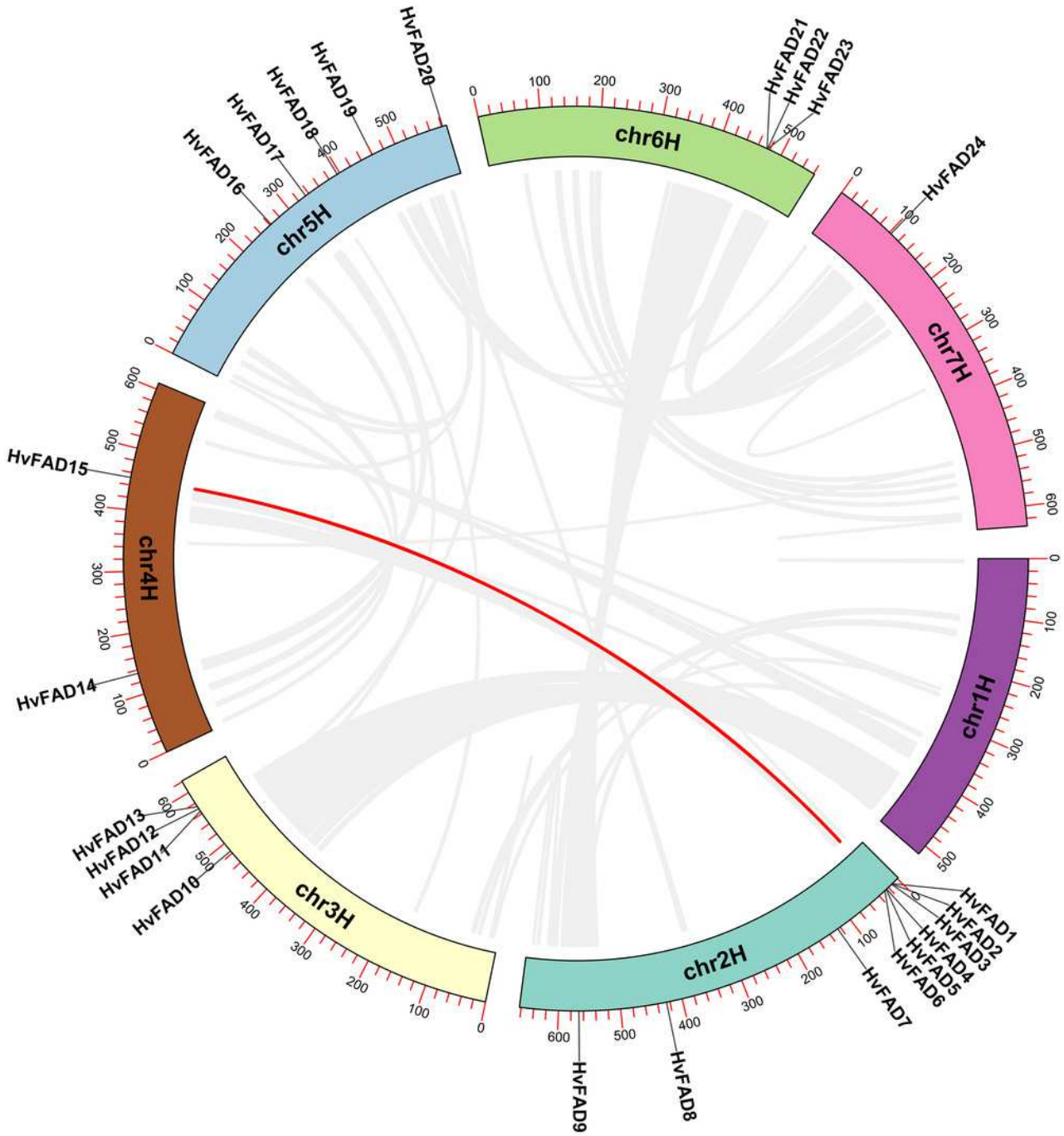
chr1H
chr2H
chr3H
chr4H
chr5H
chr6H
chr7H
HvFAD1
HvFAD2
HvFAD3
HvFAD4
HvFAD5
HvFAD6
HvFAD7
HvFAD8
HvFAD9
HvFAD10
HvFAD11
HvFAD12
HvFAD13
HvFAD14
HvFAD15
HvFAD16
HvFAD17
HvFAD18
HvFAD19
HvFAD20
HvFAD21
HvFAD22
HvFAD23
HvFAD24

Figure 5

The distribution of cis-acting elements in promoters of *HvFAD* gene family members.

The colors indicate expression intensity (red, high expression; white, low expression).

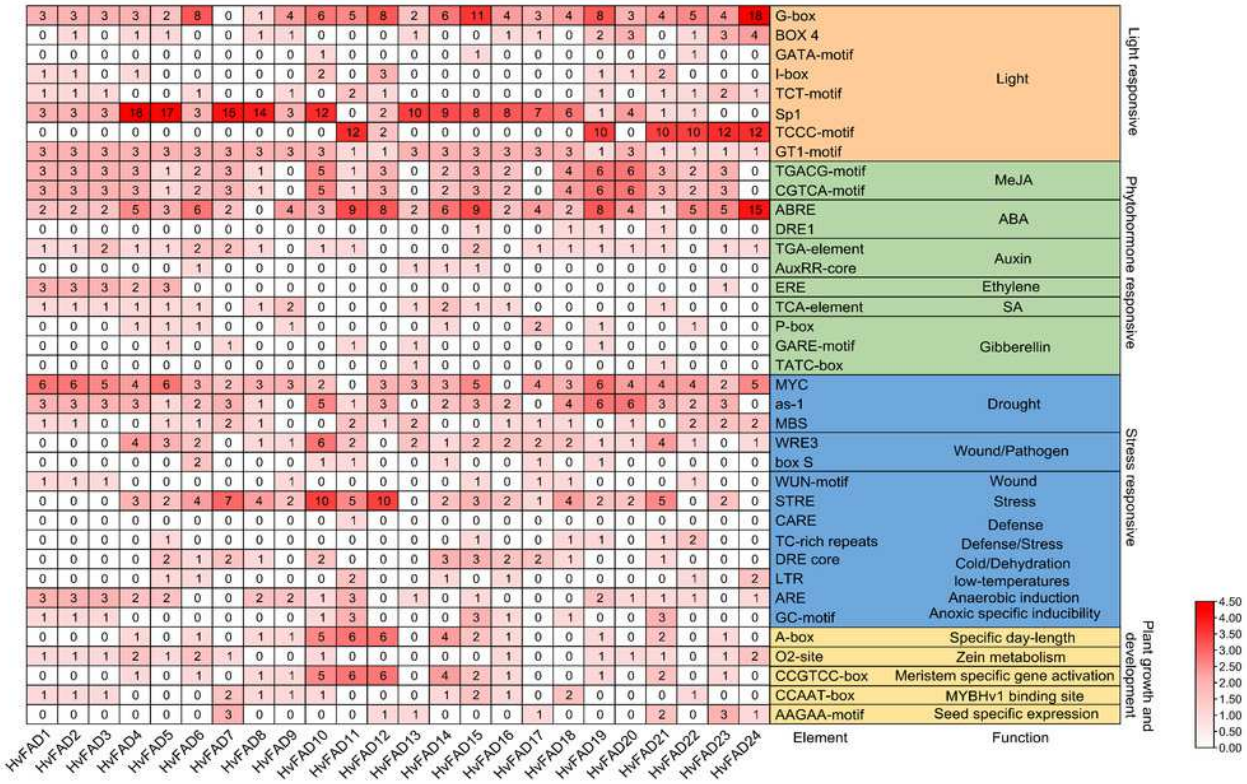

Figure 6

A protein-protein interaction network for *HvFADs* based on their orthologs in *Arabidopsis*.

HvFAD proteins were shown in brackets with *Arabidopsis* orthologs.

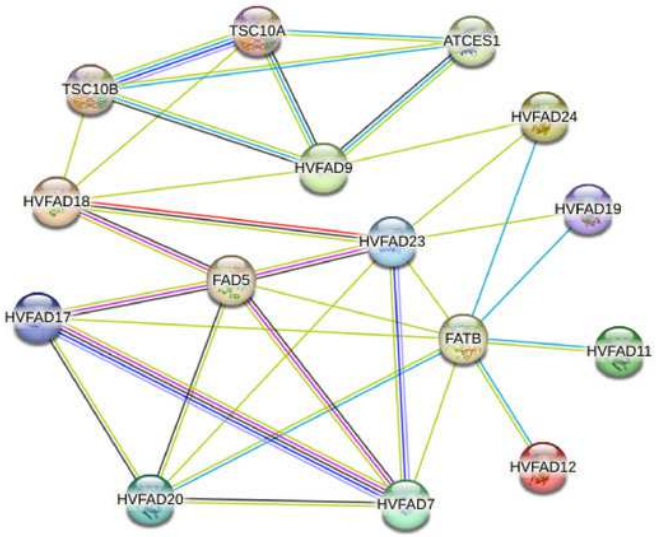

Figure 7

Expression analysis of HvFAD under NaCl treatment and characterization of its function in *Hordeum vulgare*.

HvFAD was induced at expression levels of 0, 3, 12 and 24 h by 200 mM NaCl. Bars represent the mean values of three replicates ± standard deviation (SD).

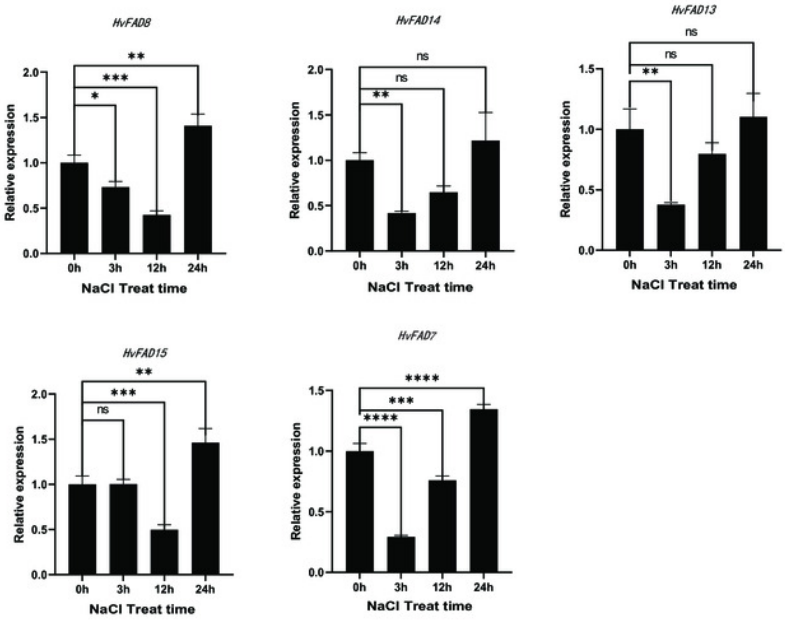

Figure 8

Expression analysis of HvFAD under NaHCO3 treatment and characterization of its function in *Hordeum vulgare*.

HvFAD was induced at expression levels of 0, 3, 12 and 24 h by 200 mM NaHCO3. Bars represent the mean values of three replicates ± standard deviation (SD).

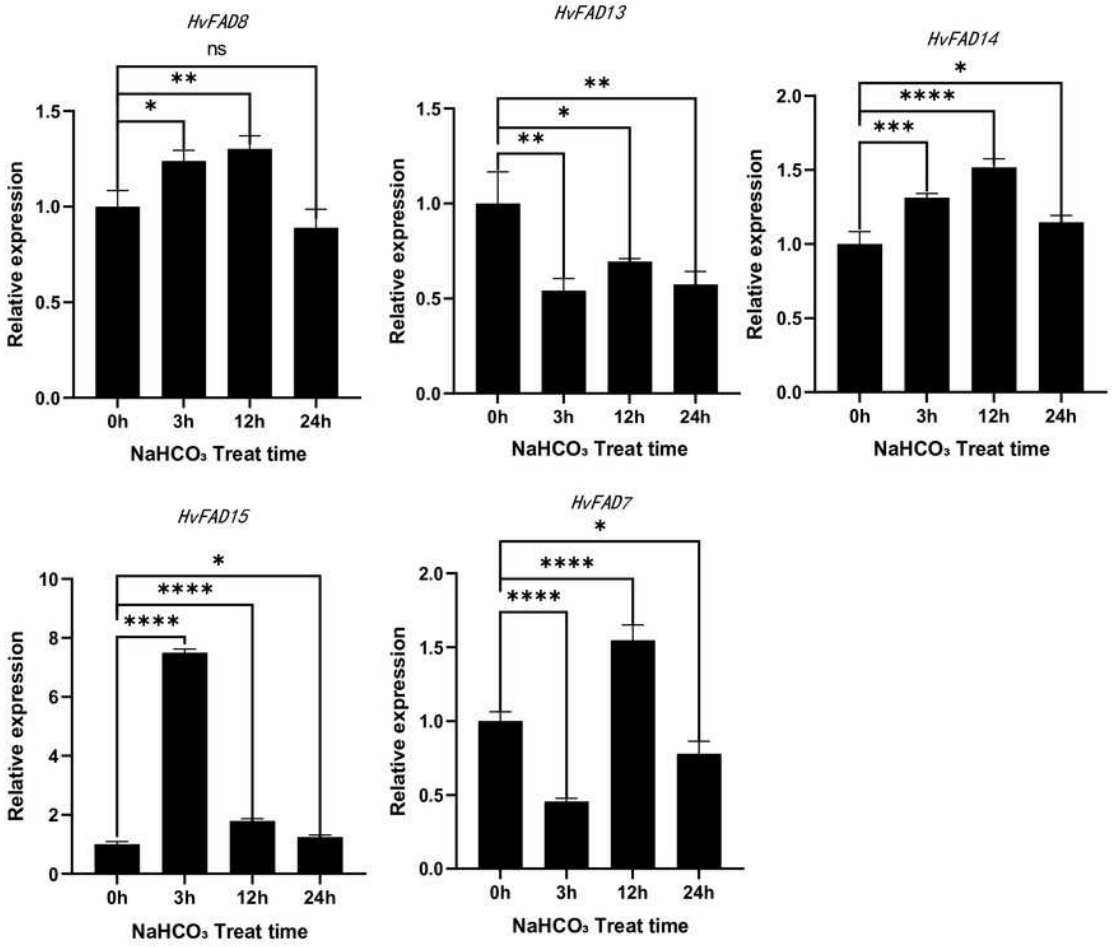
HvFAD8
ns
**
*
Relative expression
1.5
1.0
0.5
0.0
0h
3h
12h
24h
NaHCO₃ Treat time
HvFAD13
**
*
**
Relative expression
1.5
1.0
0.5
0.0
0h
3h
12h
24h
NaHCO₃ Treat time
HvFAD14
*
****
***
Relative expression
2.0
1.5
1.0
0.5
0.0
0h
3h
12h
24h
NaHCO₃ Treat time
HvFAD15
*
****
****
Relative expression
10
8
6
4
2
0
0h
3h
12h
24h
NaHCO₃ Treat time
HvFAD7
*
****
****
Relative expression
2.0
1.5
1.0
0.5
0.0
0h
3h
12h
24h
NaHCO₃ Treat time

Figure 9

Expression analysis of HvFAD genes in different tissue (root, stem, leaf, and tiller) of Hordeum vulgare.

The expression level in leaf tissue is used as a reference for relative expression. Bars represent the mean values of three replicates ± standard deviation (SD).

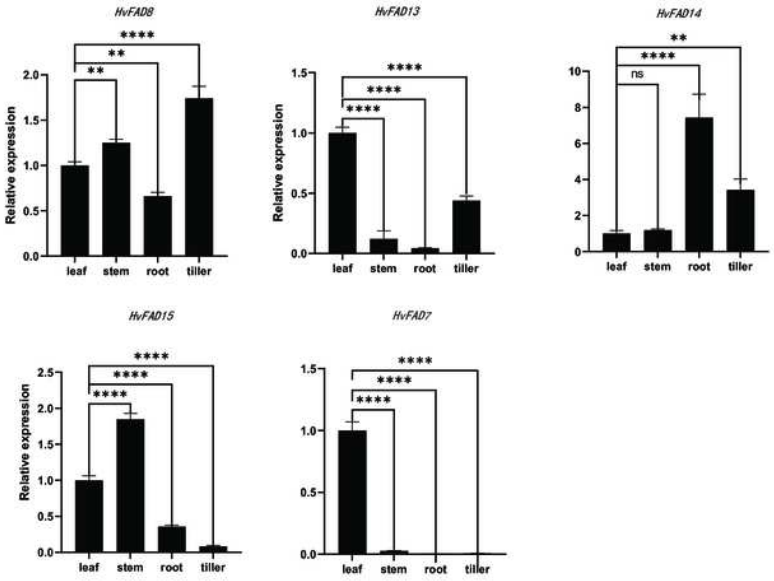

Figure 10

Subcellular localization of *HvFAD14* in Barley protoplast.

The scale represents 5 μm. “GFP”: green fluorescent protein signal, “Bright-field”: bright field image, “Merged”: merged set of images

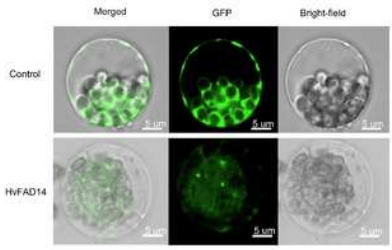

Table 1(on next page)

Table 1 Basic information for the *Hordeum vulgare FAD* gene family members.

Vertical columns indicate:Gene name;Gene ID;CDS;Number of Amino Acid;Molecular Weight;Theoretical;Instability Index;Aliphatic Index;Grand Average of Hydropathicity.

Gene_name	Gene_ID	CDS/ bp	Number of Amino Acid/ aa	Molecular Weight/ Da	Theoretical_pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity
HvFAD1	HORVU.MOREX.r3.2HG0101440.1	1215	405	44883.21	7.19	42.7	84.96	-0.241
HvFAD2	HORVU.MOREX.r3.2HG0101470.1	1125	375	41677.74	8.68	41.94	87.33	-0.257
HvFAD3	HORVU.MOREX.r3.2HG0101500.1	1215	405	44883.21	7.19	42.7	84.96	-0.241
HvFAD4	HORVU.MOREX.r3.2HG0106740.1	1281	427	47347	8.47	38.72	85.55	-0.267
HvFAD5	HORVU.MOREX.r3.2HG0106840.1	1206	402	44563	8.4	39.14	85.55	-0.236
HvFAD6	HORVU.MOREX.r3.2HG0108020.1	1314	438	48622.66	8.89	44.32	84.27	-0.157
HvFAD7	HORVU.MOREX.r3.2HG0129520.1	1329	443	51231.58	9.37	51.02	86.75	-0.115
HvFAD8	HORVU.MOREX.r3.2HG0161410.1	1176	392	44559.57	6.04	49.19	73.44	-0.49
HvFAD9	HORVU.MOREX.r3.2HG0183780.1	987	329	37724.61	8.79	46.99	86.57	-0.086
HvFAD10	HORVU.MOREX.r3.3HG0288610.1	1017	339	38752.42	9.03	43.19	76.31	-0.297
HvFAD11	HORVU.MOREX.r3.3HG0307490.1	1134	378	42389.33	6.24	41.04	79.29	-0.324
HvFAD12	HORVU.MOREX.r3.3HG0309010.1	1251	417	45352.59	6.87	42.75	78.01	-0.177

HvFAD13	HORVU.MOREX.r3.3HG0310210.1	1185	395	44530.92	6.65	36.32	78.61	-0.409
HvFAD14	HORVU.MOREX.r3.4HG0354970.1	1143	381	43631.91	8.48	44.31	84.93	-0.201
HvFAD15	HORVU.MOREX.r3.4HG0384980.1	1308	436	48836.08	9.2	45.27	86.17	-0.215
HvFAD16	HORVU.MOREX.r3.5HG0457600.1	1155	385	43660.82	5.97	44.8	74.75	-0.381
HvFAD17	HORVU.MOREX.r3.5HG0466020.1	1152	384	43943.35	8.22	43.96	85.03	-0.217
HvFAD18	HORVU.MOREX.r3.5HG0474290.1	1407	469	52548.61	8.45	35.2	90.72	0.135
HvFAD19	HORVU.MOREX.r3.5HG0486420.1	1284	428	47617.36	7.59	46.27	73.93	-0.306
HvFAD20	HORVU.MOREX.r3.5HG0535350.1	1173	391	44667.93	6.05	43.19	80.36	-0.405
HvFAD21	HORVU.MOREX.r3.6HG0609520.1	1161	387	44334.18	8.44	34.6	94.47	-0.02
HvFAD22	HORVU.MOREX.r3.6HG0609610.1	1146	382	43871.95	8.39	30.99	92.09	0.075
HvFAD23	HORVU.MOREX.r3.6HG0609630.1	1152	384	44743.92	8.19	39.75	97.47	0.017
HvFAD24	HORVU.MOREX.r3.7HG0668240.1	1236	412	45390.46	6.87	51.48	72.91	-0.344

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