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Genome-Wide Identification of the F-box Gene Family and Expression Analysis under Drought and Salt Stress in Barley

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Abstract: The *F-box* protein-encoding gene family plays an essential role in plant stress resistance. In present study, 126 non-redundant *F-box* genes were identified in barley (*Hordeum vulgare* L., *Hv*). The corresponding proteins contained 165–887 amino acid residues and all were amphiphilic, except 5 proteins. Phylogenetic analysis of F-box protein sequences in barley and stress-related F-box protein sequences in wheat and *Arabidopsis thaliana* (*At*) was used to classify barley *F-box* genes are divided into 9 subfamilies (A–I). A structure-based sequence alignment demonstrated that F-box proteins were highly conserved with a total of 10 conserved motifs. In total, 124 *F-box* genes were unevenly distributed on 7 chromosomes; another 2 genes have not been anchored yet. The gene structure analysis revealed high variability in the number of exons and introns in *F-box* genes. Comprehensive analysis of expression profiles and phylogenetic tree analysis, a total of 12 *F-box* genes that may be related to stress tolerance in barley were screened. Of the 12 detected *F-box* genes, 8 and 10 were upregulated after drought and salt stress treatments, respectively, using quantitative real-time polymerase chain reaction (qRT-PCR). This study is the first systematic analysis conducted on the *F-box* gene family in barley, which is of great importance for clarifying this family's bioinformatic characteristics and elucidating its function in barley stress resistance. These results will serve as a theoretical reference for subsequent research on molecular regulation mechanisms, genetic breeding, and improvement.

Keywords: Drought and salt stress; barley; expression analysis; *F-box* gene family; phylogenetic analysis

1 Introduction

The *F-box* gene family is one of the most abundant and functionally diverse families found in plants [1,2], which mainly degrades most alienated proteins through the ubiquitin proteasome pathway (UPP) in order to cope with adverse external stimuli, such as drought, saline-alkali, low temperature, and heavy metal stress. The F-box protein was first recognized by Kumar et al. [3] when they studied the WD (Trp-Asp) domain but was named after the F-box protein after the F-box domain was discovered by Bai et al.

[4]. The N-terminus of the F-box protein usually contains a polypeptide region of 40–50 amino acids, which is the binding site of Skp1 or Skp1 similar proteins in the SCF complex and mediates protein-protein interactions. However, these conserved amino acids are less in quantity [5]. The C-terminal domain specifically recognizes substrates and is used for classification of the F-box protein family. However, *F-box* genes with high structural similarity can identify the same type of substrate, and, thus, some F-box proteins serve similar functions.

With ongoing research of the F-box domain, an increasing number of *F-box* genes have been identified in plants, including 694 in *Arabidopsis thaliana* [6], 359 in corn [7], 285 in chickpeas [8], 972 in *Medicago truncatula* [9], and 26 in pears [10]. The function of *F-box* genes has also been heavily studied in important species, including *A. thaliana* [11], rice [12], and wheat [13]. The *F-box* gene family has been divided into 9 subfamilies: FBU (domain of unknown functions), FBL (leucine-rich repeats), FBK (Kelch repeats), FBA (F-box associated domain), FBD (F-box domain), FBT (Tubby domain), FBP (phloem protein 2, PP2), FBW (tryptophanaspartic acid 40), and FBO (other domains besides F-box) [7–10].

Drought and salinity are the main environmental stressors that severely restrict plant growth and sustainable agricultural development. Previous studies found that some *F-box* genes in *M. truncatula* contain drought-responsive cis-acting elements and respond to drought stress [14]. Additionally, the *TaFBA1* gene identified in wheat was upregulated in response to drought, high salt, and abscisic acid (ABA) stress, proving that *TaFBA1* is strongly resistant to stress [15]. Overexpression of *TaFBA1* in tobacco under salt stress promoted seed germination and root growth, and as exposure to stress increased over time, photosynthesis in transgenic plants became more stable than the wild type, which is conducive to mass accumulation [16]. The overexpression of *Triticum aestivum SKP1-like 1* (*TSK1*) in *A. thaliana* resulted in delayed seed germination and hypersensitivity to ABA [17]. Through a bioinformatics analysis and identification of the promoter and related drought-resistant elements of the *F-box* gene in *A. thaliana*, an *F-box* gene, $AtPP_2$-B_{11}, was downregulated under drought stress. Further research found that the $AtPP_2$-B_{11} protein interacted with a downstream drought response factor, LEA14, which affects plant drought resistance [18]. Jia et al. [19] found a total of 51 *F-box* genes that were differentially expressed under salt stress. Among them, 34 were upregulated and 17 were downregulated, indicating that *F-box* genes are involved salt stress response in soybeans [19]. *OsMsr9*, which contains an F-box domain, was overexpressed in rice and *A. thaliana*. These plants exhibited strong salt tolerance, and the expression of other genes related to salt tolerance was also enhanced, indicating that *OsMsr9* has a positive effect on enhancing salt tolerance [20]. Chen et al. [21] found that $GmSK_1$, an SKP_1 homolog in soybeans, was constitutively expressed in all tested tissues, especially the roots. $GmSK_1$-overexpressing lines exhibited a significant increase in root number and an obvious decrease in water loss [21]. These results indicated that the *F-box* gene family plays a different regulatory role in plant stress resistance.

Barley belongs to the Poaceae family and is a widely planted cereal crop [22], which is highly tolerant to drought and salt stress and is of great research importance. Completion of barley genome sequencing provided favorable conditions for barley genome identification. Since then, several gene families have been identified in barley by comparing homologous sequences from other plants. Research on the *SQUAMOSA PROMOTER BINDING PROTEIN* box (*SBP-box*) gene family revealed that there were 13 *SBP* genes unevenly distributed on 5 chromosomes in barley, which were divided into 5 evolutionary subfamilies according to their diverse structures [23]. Based on a genome-wide analysis of *Mitogen-activated protein kinase* (*MAPK*), a total of 20 *MAPKs*, 6 *MAPKK*, and 156 *MAPKKK* genes have been discovered in barley. A gene duplication analysis revealed that the amplification of *MAPK cascade* genes originated from tandem repeats [24]. Moreover, 45 WRKY proteins were identified in barley by using the protein sequences of different WRKY domains in *A. thaliana*. Further analysis revealed that HvWRKY proteins had high sequence similarity and expression pattern correlations with *A. thaliana* [25].

F-box proteins greatly affect plant growth, endogenous hormone signal transduction, and cell cycle regulation. Therefore, many studies have investigated the structure and function of F-box proteins in important plants, including rice, wheat, and *A. thaliana*, but only a few reports have explored the *F-box* gene family in barley. In this study, 126 *F-box* genes were identified by utilizing different bioinformatic methods, including gene structure analysis, chromosomal location, physicochemical properties, phylogenetic relationships, and tissue expression. Additionally, qRT-PCR was used to study the expression patterns of some family members under drought and salt stress. The combined analyses on the biological characteristics and expression patterns of the *F-box* gene family in barley will provide a theoretical reference in future applications for improving barley drought- and salt-tolerant varieties.

2 Materials and Methods

2.1 Plant Materials

The barley variety Morex was planted in the experimental field of the Hangzhou Normal University (Xiasha Campus) and selected as the materials. Morex seeds were cultured in a light culture incubator at 26°C under a 14/10 h light/dark photoperiod and photosynthetically activated radiation at 18,000 lx. Drought and salt stress experiments were performed at the two-leaf stage. A total of 3 groups were tested: (1) control (CK) (0); (2) salt stress (200 mmol·L^{-1} NaCl); (3) drought stress (20% PEG6000). Leaves were collected for total RNA extraction after 24 h of treatment. Duplicate samples were stored at –80°C in an ultra-low temperature refrigerator.

2.2 Total RNA Extraction and cDNA Synthesis

Total RNA was extracted from the barley leaves with the reference RNA plant extraction kit (AxyPrep). DNA contaminants were removed through DNase I treatment (RNase free). Total RNA was reverse-transcribed to generate cDNA using the Hifair™ II 1st Strand cDNA Synthesis Kit. cDNA products were stored at –20°C prior to qRT-PCR analysis.

2.3 Identification and Structural Analysis of F-box Family Genes

The barley protein candidate sequences containing the F-box conserved domain were obtained online using the default parameters of HMMER (https://www.ebi.ac.uk/Tools/hmmer/) ($E < 10^{-10}$) [26]. The HMMER profile of F-box domain (PF00646) was downloaded from the Pfam database (http://pfam.xfam.org/) [27]. First, the candidate sequences that were removed for being to short and redundant using CD-HIT (http://weizhong-lab.ucsd.edu/cdhit-web-server/cgi-bin/) were submitted to the NCBI Conserved Domains (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) [28] and SMART web server (http://smart.embl-Heidelberg.de/) to verify whether the F-box domain was included and removed the ones without the F-box domain [29]. Next, the incomplete reading frame and redundant sequences were removed manually, after which the F-box genes and protein sequences in barley were finally obtained. Molecular weights and theoretical isoelectric points of the barley F-box proteins were computed using Compute pI/Mw tool on the ExPASy server (https://web.expasy.org/compute_pi/). Subcellular localization prediction of the F-box proteins was predicted using WoLF PSORT (https://wolfpsort.hgc.jp/) [30]. F-box protein conserved motifs were analyzed in MEME online tool (http://memesuite.org/tools/meme) [31]. The exon/intron structure of F-box genes was analyzed using Gene Structure Display Server v2.0 (GSDS v2.0, http://gsds.cbi.pku.edu.cn/) (Center for Bioinformatics (CBI), Beijing, China) [32]. The location of F-box genes on the barley chromosomes were mapped by using Map Gene 2Chromosome v2 (MG2C, http://mg2c.iask.in/mg2c_v2.0/).

2.4 Phylogenetic Analysis of the F-box Gene Family

The identified full-length sequences of F-box proteins in barley and F-box proteins from *A. thaliana* and wheat with well-defined stress resistance from NCBI were aligned with the default settings of ClustalW.

A Neighbour-Joining (NJ) phylogenetic tree was constructed using p-distance model in MEGA v7.0 program (https://www.megasoftware.net/). Bootstrapping was performed with 1000 replications and other parameters remained default [33].

2.5 F-box Gene Expression Profile Mapping and Candidate Gene Selection

To analyze the tissue-specific expression patterns of HvFBXs, RNA-Seq data of fifteen developmental stages were downloaded from IPK (https://webblast.ipk-gater sleben.de/barley_ibsc/index.php) and the James Hutton Institute (https://ics.hutton.ac.uk/more xGenes/index.html) websites, respectively. The gene expression values are represented by fragments per kilobase of exon per million fragments mapped (FPKM). The developmental stages of expression analysis were as follows: 4-day embryos (EMB); roots from seedings (10 cm shoot stage) (ROO1); shoots from seedings (10 cm shoot stage) (LEA); developing inflorescences (INF2); developing tillers, 3rd internode (5 DAP) (NOD); developing grain (5 DAP) (CAR5); developing grain (15 DAP) (CAR15); etiolated seeding, dark cond (10 DAP) (ETI); inflorescences, lemma (42 DAP) (LEM); inflorescences, lodicule (42 DAP) (LOD); dissected inflorescences (42 DAP) (PAL); epidermal strips (28 DAP) (EPI); inflorescences, rachis (35 DAP) (RAC); roots(28 DAP) (ROO2); senescing leaves (56 DAP) (SEN). Then the FPKM values were used as the expression spectrum data source of F-box genes. After checking and sorting out, the tissue-specific expression profile was draw using the Multiple Experiment Viewer (MeV) (J. Craig Venter Institute, La Jolla, CA, USA), and the candidate genes were screened according to the expression amount [34].

2.6 F-box Gene Expression Analysis

At the two-leaf stage, hydroponic barley seedlings were exposed to drought and salt stress treatments for 24 h and subsequently used for RNA extraction. Then, the expression levels of 12 *F-box* genes were quantitatively analyzed. All primers selected for qRT-PCR were designed by Primer Premier v5.0 (PREMIER Biosoft, San Francisco, CA, USA) (Tab. 1). Relative expression was calculated using the $2^{-\Delta\Delta CT}$ Livak method [35]. Each sample was repeated 3 times. qRT-PCR data were analyzed using SigmaPlot v10.0 (SYSTAT, San Jose, CA, USA) and IBM SPSS Statistics v20 (IBM, Armonk, NY, USA) statistical software. ANOVA was used to test significance ($p < 0.05$ indicates significant difference, indicated by *; $p < 0.01$ indicates high significance, indicated by **; $p < 0.001$ indicates high significance, indicated by ***).

Table 1: Primers used for qRT-PCR amplification

Gene name	Primer	Forward primer sequence	Reverse primer sequence
HvFBX6	Fbox-1	5'CAATGTAGCCACTGCTTGACCG3'	3'ATAATGGCAAGTTCCTACTATCGG5'
HvFBX10	Fbox-2	5'CAACACGCCCTTCCTCTTCG3'	3'CCTGAGTACACCATCACGCAGT5'
HvFBX20	Fbox-3	5'CAGGTTCTCCGAAGGTGCT3'	3'TTGCGGGTTGAGTTGGTTG5'
HvFBX30	Fbox-4	5'TTACGCCAACCTGTAACCATT3'	3'GGATGGTGCCTTTCATTGGAG5'
HvFBX43	Fbox-5	5' AGCCTAGCACCAGGGACAAA3'	3' TCCTACGAGCGTGGACAGCA5'
HvFBX56	Fbox-6	5'CTATGGCATGACCAACAGCAT3'	3'TTCAAGCGACAAATAGCGTACT5'
HvFBX67	Fbox-7	5'CCTTAGAGCGTCCGTTTGC3'	3'ACCCGTTGGTGACTTTGCG5'
HvFBX75	Fbox-8	5'AAATCCTTGTTCTTCCGCCACT3'	3'TCCTGCTACGACGCTCACCT5'
HvFBX80	Fbox-9	5'TGGGCAGGGATTCGTGAGT3'	3'CAGGAAGGTCGCAGAAGCATA5'
HvFBX158	Fbox-10	5'AGTTTGGGTAGACTTGTCCCTT3'	3'ACTTTCCCTGTTTCCTTGAAGC5'
HvFBX137	Fbox-12	5' TTGTGCTTCGCTTCCTCCA3'	3' CACCAGCTTCTCGATTCCTAC5'
HvFBX152	Fbox-13	5'TATGACAGCGACGATGACGA3'	3'CACTCTTTTCTGAGGGAATGTG5'
HvActin	Actin	5'AAGCATGAAGATACAGGGAGTGTG3'	3'ACATGTTGGAGAAGGCTCTTATTTAAA5'

3 Results

3.1 Molecular Characteristics of F-box Genes

In this study, 126 F-box protein sequences were obtained after removing redundant sequences and secondary verification of the structure using CD-HIT, SMART, and NCBI Conserved Domains. These sequences were identified as *F-box* gene family members in barley and named HvFBXs. The physicochemical analysis revealed that the average number of amino acids was 420. The theoretical isoelectric point (pIs), relative molecular mass, and subcellular localization of *F-box* genes in barley were summarized in Appendix A. HvFBX61 contained the largest number of amino acids, encoded 819 amino acid residues, had a relative molecular mass of 91.29346 kDa, and a theoretical isoelectric point of 5.73. HvFBX21 contained the smallest number of amino acids and encoded 165 amino acid residues. The predicted molecular weight of HvFBX21 was 18.77672 kDa, with a theoretical isoelectric point 9.00. The isoelectric point of HvFBXs ranged from 4.54 to 11.62. The isoelectric point and relative molecular weight information of 6 F-box proteins were not provided as they contained unidentified amino acid residues. Of the 120 F-box proteins, 53 were acidic amino acids and 67 were basic amino acids. According to the principle of hydrophilicity index, amphoteric proteins ranged from –0.5 to 0.5 (GRAVY is a negative value for hydrophilicity and a positive value for hydrophobicity); only HvFBX17, HvFBX135, HvFBX149, HvFBX131, and HvFBX152 were hydrophilic proteins, and all others were amphoteric proteins. The subcellular localization of the 126 HvFBX proteins obtained in this study revealed that 39.68% of the total proteins were located in the Chloroplast, 24.60% were located in the Cytoplasm, and 18.25% were located in the Nucleus; a few proteins were localized in Mitochondria and Peroxisomes.

3.2 Identification and Phylogenetic Analysis of F-box Family Genes

NCBI database was used to search *F-box* genes related to plant stress resistance, and finally 1 wheat stress-resistant F-box protein sequence and 10 *A. thaliana* stress-resistant F-box protein sequences were selected according species affinities. In order to further screen the *F-box* genes associated with stress resistance in barley, 126 HvFBX protein sequences and 11 protein sequences mentioned above were used to construct a phylogenetic tree for subsequent analysis. The phylogenetic tree revealed that the *F-box* genes in barley, wheat and *A. thaliana* were divided into 9 subfamilies based on sequence similarity and named subfamilies A–I. Among them, subfamily D and I contained the largest number of *F-box* family members, accounting for 18.25% and 19.84%, respectively. However, subfamily B contained the smallest number of *F-box* family members, accounting for only 5.56% (Fig. 1). The anti-stress functional proteins from *A. thaliana* in subfamilies A and B were regarded as orthologs of barley [36–40]. Therefore, it is speculated that the genes in subfamilies A and B may serve anti-stress functions. Members of subfamily E were highly evolutionarily similar to *FBP7* (GenBank: BAE99994.1) from *A. thaliana* [41] and unnamed proteins (GenBank: AEL33721.1) from wheat [42]. Thus, it is speculated that genes in subfamily E possess high homology with *F-box* genes in *A. thaliana* and wheat.

3.3 Expression Analysis of F-box Family Genes and Target Gene Screening

Analysis of the gene expression patterns provided key information for gene function research. The expression profiles of the 126 F-box genes revealed that the expression of these genes was tissue-specific, and only a small number were highly expressed or not expressed in each period (Fig. 2). Based on the expression profile and phylogenetic tree analyses, it was predicted that 12 HvFBX genes differentially responded to stress during barley stress tolerance (Tab. 1). Among them, HvFBX6 was highly similar to *A. thaliana* TIR1 (GenBank: AAB69176.1) [43], MAX2 (GenBank: AAK97303.1) [44], EBF1 (GenBank: CAE75864.1) [39], and AFBA1 (GenBank: AQR57191.1) [40]. HvFBX10 was located in the same subfamily as *A. thaliana* FOA1 (GenBank: AEE75937.1) [45]. HvFBX158 was homologous to

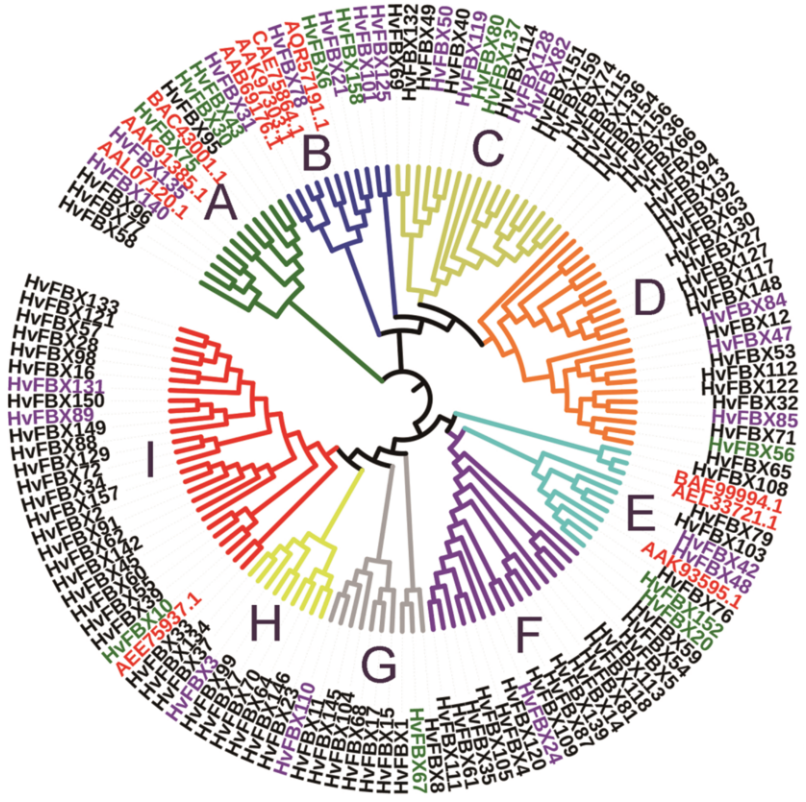


Figure 1: Phylogenetic tree constructed using the full-length F-box proteins sequences from *Hordeum vulgare* L. (HORVV), *Triticum aestivum* L. (WHEAT) and *A. thaliana* (ARATH) in MEGA v7.0. All F-box members were classified into nine groups (A–I) which differentiate by different colors. Proteins with high expression are in purple font. Genes corresponding to the green font protein are used to design primers. The red font represents proteins with clear anti-stress function in *A. thaliana* and wheat. F-box protein sequences of wheat and *A. thaliana* are detailed in Appendix B

TIR1 and AFBA1. HvFBX20 and HvFBX152 were homologous to PP2-B11 (GenBank: AAK93595.1) [46]. TIR1, MAX2, EBF1, AFBA1, FOA1, and PP2-B11 were all related to plant stress resistance. Therefore, it was predicted that HvFBX6, HvFBX10, HvFBX158, and HvFBX152 would crucially affect stress responses.

3.4 Chromosomal Mapping of F-box Family Genes

Based on the genome annotations, 124 of the 126 HvFBXs genes were anchored to 7 chromosomes with the largest number of genes detected on Chr5 and Chr6 (26), followed by 20 genes on Chr7, 19 on Chr3, and 9 on Chr1 and Chr4 (Fig. 3). Additionally, HvFBX103 and HvFBX139 were not anchored to the chromosome due to many unknown amino acid residues, and no clear localization information was obtained. These results suggested that F-box genes were non-randomly distributed on barley chromosomes, and most were concentrated on or near the end of the chromosomes (Fig. 3).

3.5 Conserved Domain Analysis of F-box Gene Family Members

Conservative analysis of HvFBX protein sequences revealed a total of 10 conserved motifs. However, HvFBX family members contained 1–6 motifs and all members with > 4 motifs gathered in subfamilies D and I, which confirmed the evolutionary tree results and indicated that the results had high reliability

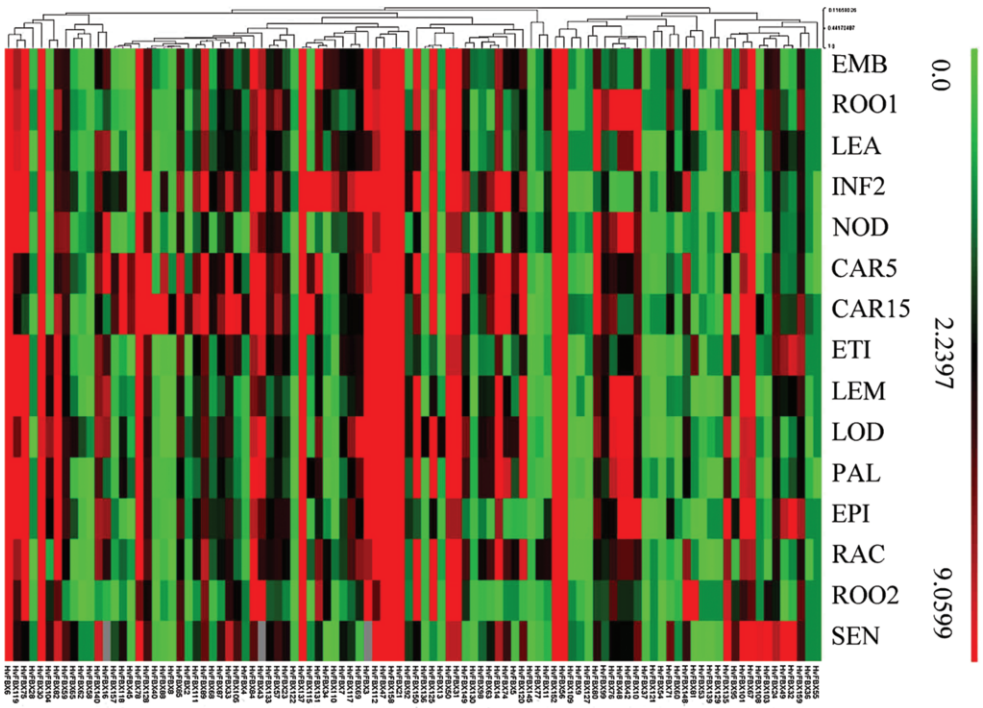


Figure 2: Expression profiles of *HvFBX* genes in different tissues and stages of development. Data were obtained from a publicly available database. Rows represent *HvFBX* members, while columns show different developmental stages and tissues. The expression level of *HvFBXs* [$\log_2$(FPKM + 1)] is shown by the intensity of color, where in red represents high expression, and green represents low expression. EMB, 4-day embryos; ROO1, Roots from seedings (10 cm shoot stage); LEA, Shoots from seedings (10 cm shoot stage); INF2, Developing inflorescences; NOD, Developing tillers, 3rd internode (5 DAP); CAR5, Developing grain (5 DAP); CAR15, Developing grain (15 DAP); ETI, Etiolated seeding, dark cond (10 DAP); LEM, inflorescences, lemma (42 DAP); LOD, inflorescences, lodicule (42 DAP); PAL, Dissected inflorescences (42 DAP); EPI, Epidermal strips (28 DAP); RAC, inflorescences, rachis (35 DAP); ROO2, Roots (28 DAP); SEN, Senescing leaves (56 DAP)

(Fig. 4). Additionally, all members contained Motif1 and -2 individually or together, but only a few proteins contained Motif5, -7, and -10, indicating that the occurrence frequency of different motifs greatly differed. With the exception of HvFBX65 located in subfamily E, Motif5-containing proteins were all distributed in subfamily D, Motif7-containing proteins were distributed in subfamily D, and Motif10-containing proteins were concentrated in subfamilies C and D. Compared to other subfamilies, the conserved sequences of subfamily D were considerably different; thus, it is speculated that stress resistance may be functionally diverse.

3.6 Analysis of the Exon and Intron Structures of F-box Gene Family Members

F-box genes in barley are highly variable in exon and intron numbers (Appendix C). The 126 HvFBX genes contained 1–11 exons and 107 (84.92%) contained 1–10 introns. The number of HvFBX genes with 1 exon accounted for 32.54% of the total. The number of HvFBX genes with 2 exons accounted for 31.75%. Of the 107 HvFBX genes, 105 (98.13%) contained < 6 introns.

Based on the evolutionary tree results, with the exception of HvFBX125 in subfamily B and HvFBX69 in subfamily C, the number of introns contained by subfamilies B–D ranged from 0 to 5, which originated

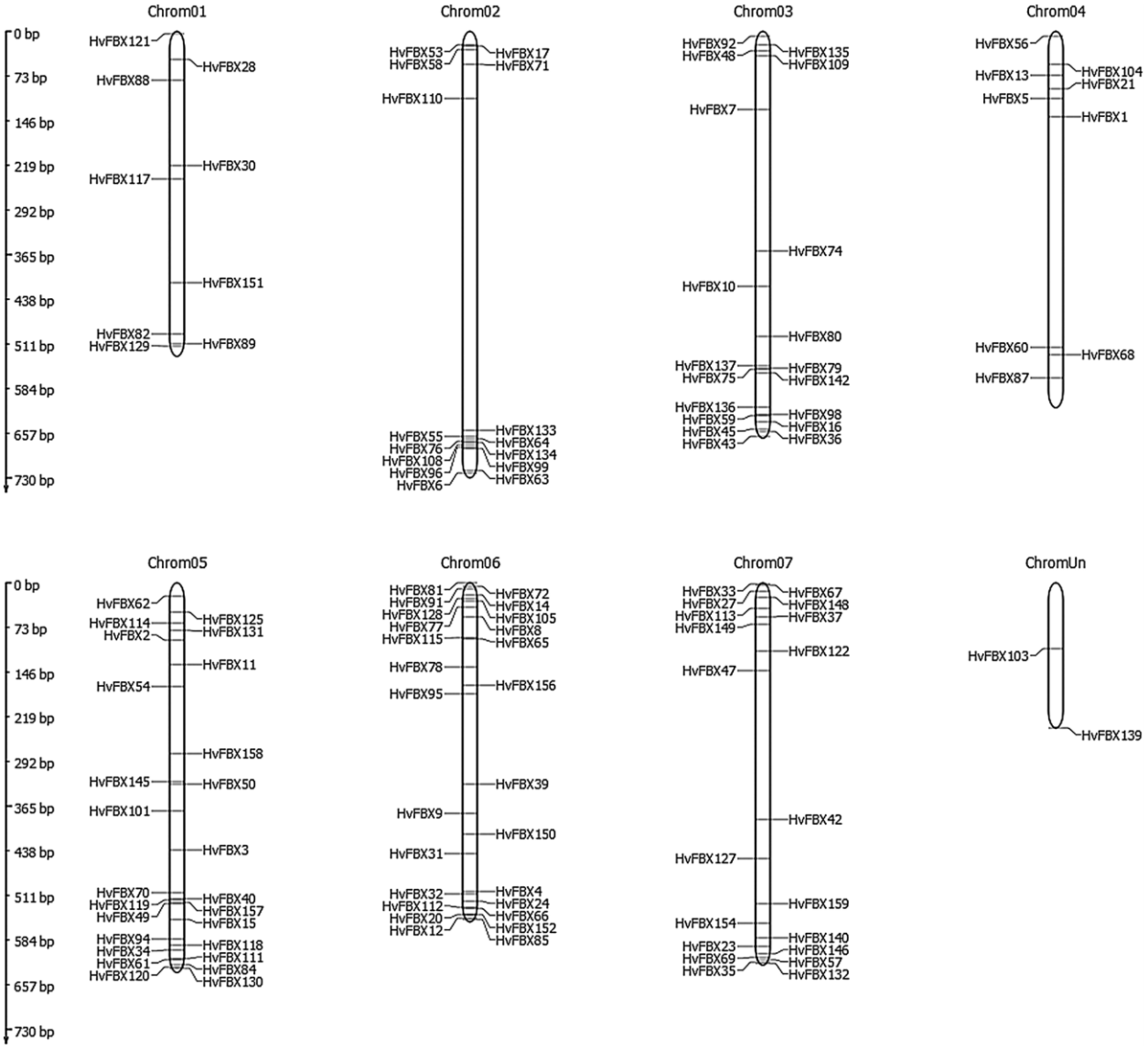
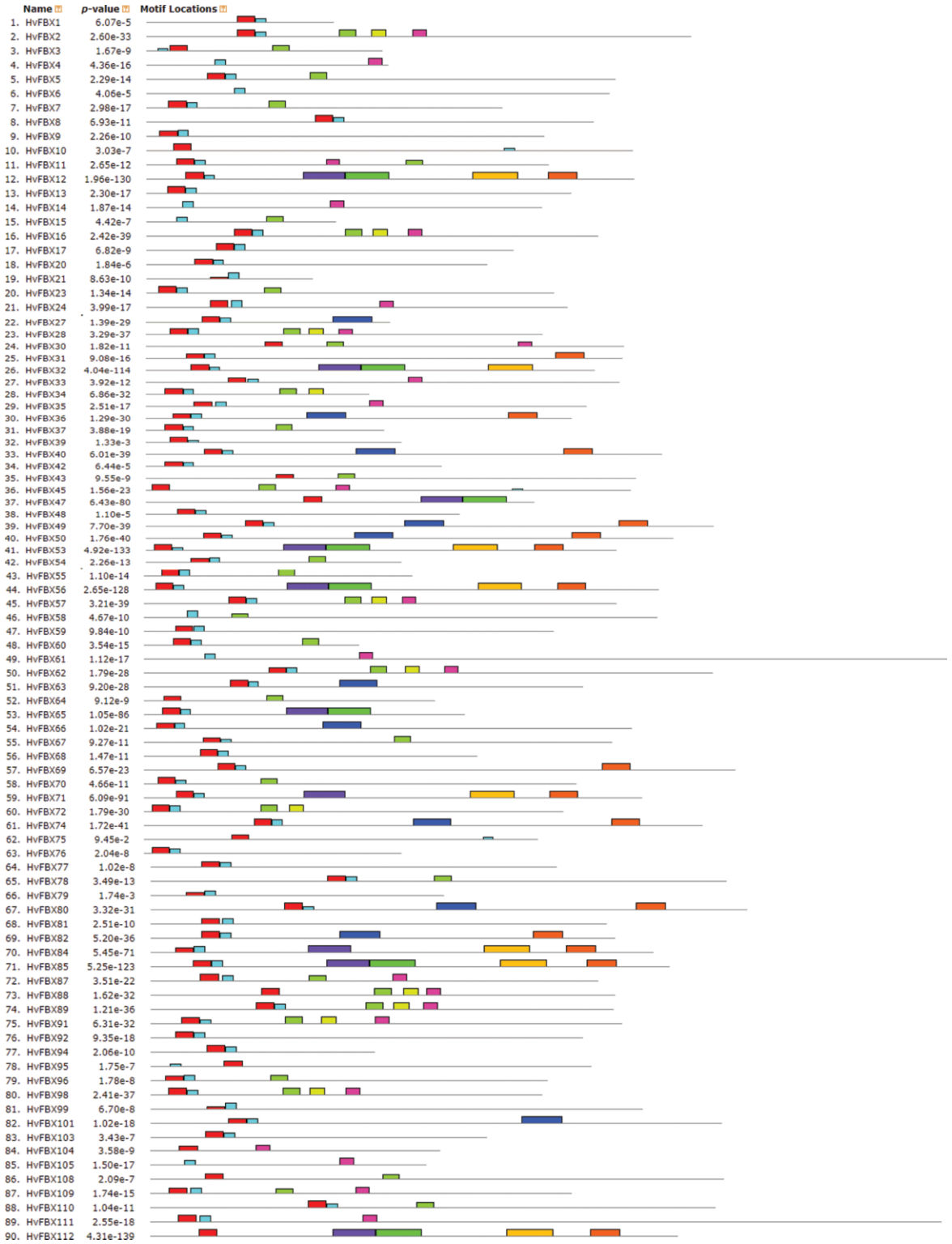


Figure 3: Distribution of *HvFBX* genes in barley chromosomes. The location of *HvFBX* gene family members in the recently release of barley cultivar 'Morex' genome database (*Hordeum vulgare*. IBSC_v2)

from the same branch; thus, the genetic structure of F-box genes in barley possesses evolutionary similarity. HvFBX125 in subfamily B contained 10 introns and 11 exons, and HvFBX69 in subfamily C contained 6 introns and 7 exons, indicating that some genes were located on the same branch, but there were still some differences in the number of introns and exons. Members of subfamily A also contained 0–5 introns and were closer to the subfamily B–D branch, indicating that the genetic structure of its members to the closer family was similar. Additionally, members of subfamilies E and G contained 0–2 introns, while members of subfamily H contained 1–4 introns.

3.7 Expression Analysis of F-box Genes under Drought and Salt Stress

The F-box proteins serve various functions, such as delaying plant senescence [47], regulating flowering [48], and responding to various abiotic stressors. In order to verify the diversity of screened genes, qRT-PCR


Name
p-value
Motif Locations
1. HvFBX1 6.07e-5
2. HvFBX2 2.60e-33
3. HvFBX3 1.67e-9
4. HvFBX4 4.36e-16
5. HvFBX5 2.29e-14
6. HvFBX6 4.06e-5
7. HvFBX7 2.98e-17
8. HvFBX8 6.93e-11
9. HvFBX9 2.26e-10
10. HvFBX10 3.03e-7
11. HvFBX11 2.65e-12
12. HvFBX12 1.96e-130
13. HvFBX13 2.30e-17
14. HvFBX14 1.87e-14
15. HvFBX15 4.42e-7
16. HvFBX16 2.42e-39
17. HvFBX17 6.82e-9
18. HvFBX20 1.84e-6
19. HvFBX21 8.63e-10
20. HvFBX23 1.34e-14
21. HvFBX24 3.99e-17
22. HvFBX27 1.39e-29
23. HvFBX28 3.29e-37
24. HvFBX30 1.82e-11
25. HvFBX31 9.08e-16
26. HvFBX32 4.04e-114
27. HvFBX33 3.92e-12
28. HvFBX34 6.86e-32
29. HvFBX35 2.51e-17
30. HvFBX36 1.29e-30
31. HvFBX37 3.88e-19
32. HvFBX39 1.33e-3
33. HvFBX40 6.01e-39
34. HvFBX42 6.44e-5
35. HvFBX43 9.55e-9
36. HvFBX45 1.56e-23
37. HvFBX47 6.43e-80
38. HvFBX48 1.10e-5
39. HvFBX49 7.70e-39
40. HvFBX50 1.76e-40
41. HvFBX53 4.92e-133
42. HvFBX54 2.26e-13
43. HvFBX55 1.10e-14
44. HvFBX56 2.65e-128
45. HvFBX57 3.21e-39
46. HvFBX58 4.67e-10
47. HvFBX59 9.84e-10
48. HvFBX60 3.54e-15
49. HvFBX61 1.12e-17
50. HvFBX62 1.79e-28
51. HvFBX63 9.20e-28
52. HvFBX64 9.12e-9
53. HvFBX65 1.05e-86
54. HvFBX66 1.02e-21
55. HvFBX67 9.27e-11
56. HvFBX68 1.47e-11
57. HvFBX69 6.57e-23
58. HvFBX70 4.66e-11
59. HvFBX71 6.09e-91
60. HvFBX72 1.79e-30
61. HvFBX74 1.72e-41
62. HvFBX75 9.45e-2
63. HvFBX76 2.04e-8
64. HvFBX77 1.02e-8
65. HvFBX78 3.49e-13
66. HvFBX79 1.74e-3
67. HvFBX80 3.32e-31
68. HvFBX81 2.51e-10
69. HvFBX82 5.20e-36
70. HvFBX84 5.45e-71
71. HvFBX85 5.25e-123
72. HvFBX87 3.51e-22
73. HvFBX88 1.62e-32
74. HvFBX89 1.21e-36
75. HvFBX91 6.31e-32
76. HvFBX92 9.35e-18
77. HvFBX94 2.06e-10
78. HvFBX95 1.75e-7
79. HvFBX96 1.78e-8
80. HvFBX98 2.41e-37
81. HvFBX99 6.70e-8
82. HvFBX101 1.02e-18
83. HvFBX103 3.43e-7
84. HvFBX104 3.58e-9
85. HvFBX105 1.50e-17
86. HvFBX108 2.09e-7
87. HvFBX109 1.74e-15
88. HvFBX110 1.04e-11
89. HvFBX111 2.55e-18
90. HvFBX112 4.31e-139

(continued)

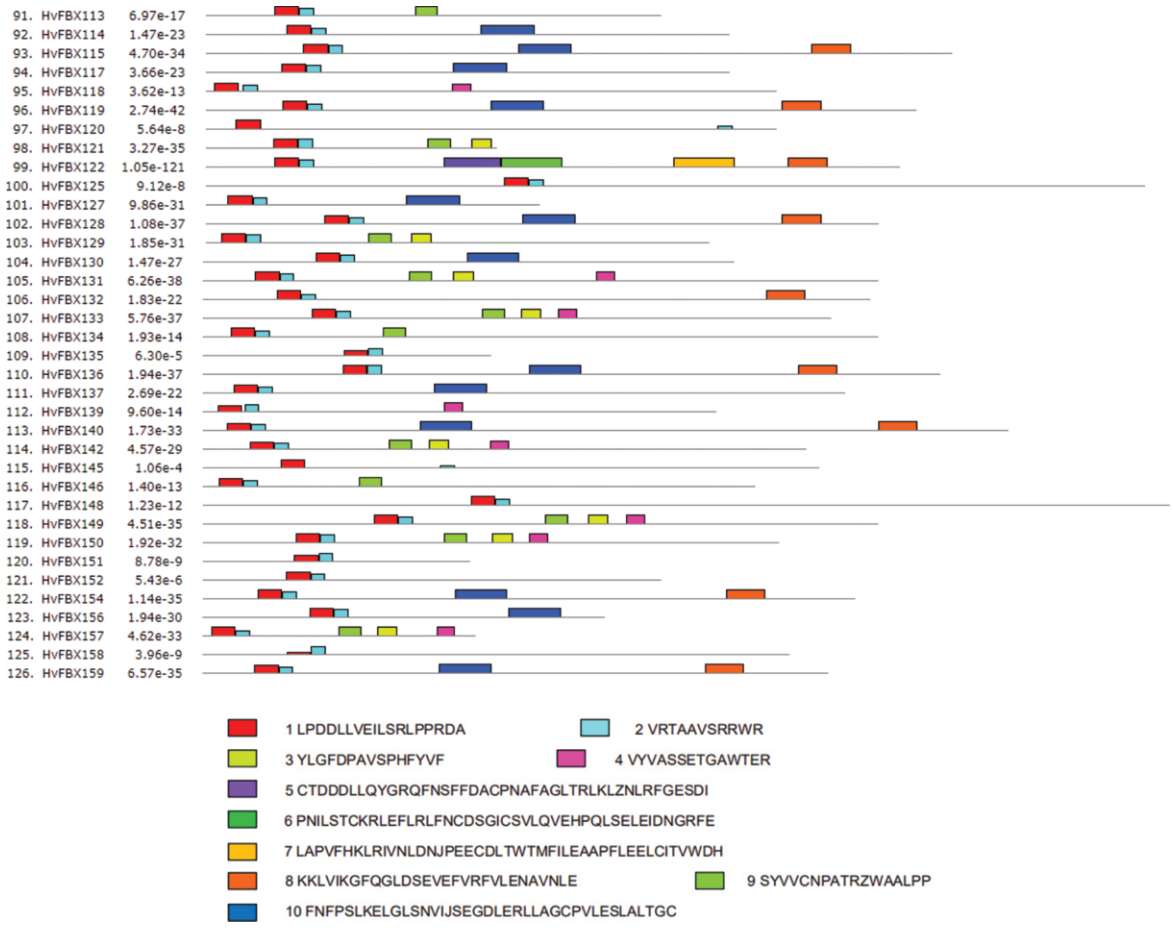


Figure 4: Motif distribution of F-box family in barley. Different conservative motifs are represented by boxes of 10 different colors

was used to determine the expression levels of 12 HvFBX genes (Tab. 1) at the seedlings stage under drought and salt stress treatments.

The qRT-PCR results of the 12 HvFBX gene expression patterns were highly consistent with the RNA-Seq results (Fig. 5). Under salt stress, 10 genes were upregulated and 2 were downregulated. Under drought stress, 8 genes were upregulated and 4 were downregulated. Some target genes were intensely induced by salt treatment; specifically, the expression of HvFBX75 increased by 96.72%, that of HvFBX20 and HvFBX158 increased by ~70%, that of HvFBX6, HvFBX10, and HvFBX56 increased by ~60%, and that of HvFBX152 decreased by 33.82%. Additionally, the expression of HvFBX10, HvFBX20, and HvFBX75 increased by 161.62%, 157.77%, and 140.00%, respectively, after drought stress, while that of HvFBX152 decreased by 39.66%. These 4 genes were clearly sensitive to drought, but the underlying molecular mechanism remains unknown. Combined with the phylogenetic analysis, these 12 genes were found to be distributed in subfamilies A–E and I, thus, it was speculated that genes in these 8 subfamilies were related to barley drought and salt tolerance.

In summary, 10 upregulated genes under salt stress and 8 upregulated genes under drought stress were identified (Fig. 5). The expression of HvFBX158 significantly increased after salt (ANOVA, $p = 0.042$, df = 2) and drought (ANOVA, $p = 0.025$, df = 2) stress, and that of HvFBX152 significantly decreased

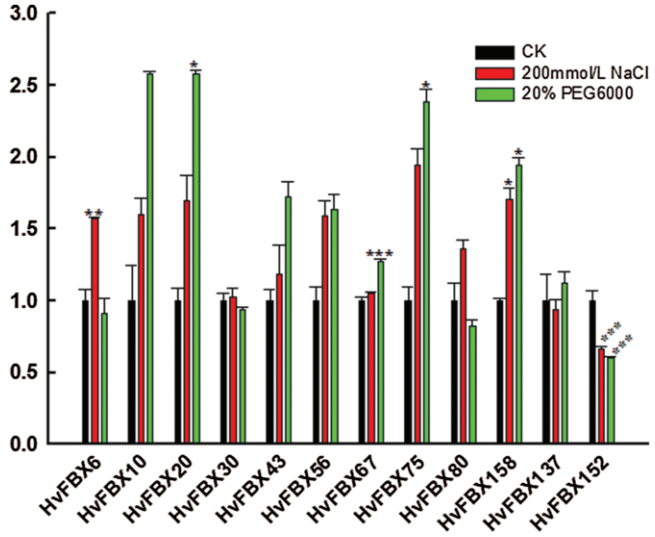


Figure 5: Relative expression analysis of 12 *F-box* family genes under stress in barley. Different treatments are represented by 3 different colors, columns in black represent CK, columns in red represent 200 mmol·L^{-1} NaCl and columns in green represent 20% PEG6000. ANOVA was used to test significance. * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$. Error bars represent standard deviation

after salt (ANOVA, $p = 3.6 \times 10^{-5}$, df = 2) and drought (ANOVA, $p = 1.1 \times 10^{-5}$, df = 2) stress. Thus, these 2 genes may play influential roles in barley drought and salt tolerance. Based on the above results, members of subfamilies A–E and I are speculated to exert an enormous effect on barley drought and salt stress response, thereby providing a basis for further research.

4 Discussion

The F-box gene family possesses a large number of members that exist in various eukaryotes from nematodes to humans. Previous studies found that model organisms, Drosophila and *Caenorhabditis elegans*, contained 22 and 326 F-box genes [5], respectively. Humans possess 68 F-box genes [49], which is close to the 74 F-box genes identified in mice [49], which indicates the similarity between these 2 species at the genomic level. The identification of F-box gene families in multiple species has been completed, including 359 in corn [7] and 687 in rice [50], reflecting the large Gramineae F-box gene family.

In this study, 126 non-redundant HvFBX genes were identified in barley. The phylogenetic analysis revealed that the genes divided into 9 subfamilies. Kelch repeats were found in HvFBX95, HvFBX30, and HvFBX43 of subfamily A and HvFBX67, HvFBX104, and HvFBX145 of subfamily G; the remaining family members contained FBOX or FBD domains. Kelch possesses a specific domain in plant F-box proteins. A previous study indicated that a few characterized wheat FBKs were involved in heat stress tolerance [42]. HvFBX101 of subfamily B and HvFBX137 of subfamily C contained LRR domains, which are Leu repeat units. These LRR domains play an important role in protein-ligand and protein-protein interactions and are involved in plant immune responses [51]. COI1, an F-box protein with an LRR domain in *A. thaliana*, plays a fundamental role in response to jasmonates (JA), which regulate plant root growth, pollen fertility, wounding and healing, and defense against pathogens and insects [52]. Wang et al. [53] isolated an F-box protein, GmCOI1, with an LRR domain from soybeans, which also mediates JA-regulated plant defense and fertility. Thus, it is speculated that HvFBX101 and HvFBX137 are related to plant signaling and resistance in biological stress [54]. In this study, 38 family members were found in subfamilies D–F. The phylogenetic tree results revealed that they only contained

an FBOX domain. HvFBX134 contained an FBA domain, while the other 8 members of subfamily H only contained an FBOX domain. FBA specifically recognizes the target protein and plays an important role in plant 26S proteasome-mediated ubiquitination of proteins. In subfamily I, only HvFBX131 contained an FBA domain, and the remaining members only contained an FBOX domain. Therefore, compared to other subfamilies, members of subfamilies A–C and G–I with special domains were more likely to play important roles in plant signal transduction and coping with various stressors.

Gene replication events provide more choice sites for evolution, leading to several gene family members, which primarily contribute to gene family expansion and genetic novelty. Some plausible explanations may account for the large number of F-box genes members. First, whole genome duplication (WGD) is universal in plant genomes [55], which has doubled the entire nuclear gene pool and produced a large number of supergene families, such as the F-box gene family. Second, in addition to several WGD events that occurred in the common ancestor of angiosperms, some additional lineage-specific (LS)-WGDs were found in plants, including *A. thaliana*, corn, soybeans, poplar, and pears. LS-WGDs make new repeat genes in F-box gene families of different species, which differentiate in size [10]. Paterson et al. [56,57] found that Poaceae had a genome-wide doubling event before differentiation. In this study, analysis of the phylogenetic tree results revealed that HvFBX140, HvFBX135, and HvFBX75 of subfamily A, HvFBX31 and HvFBX78 of subfamily B, HvFBX108 of subfamily E, and HvFBX33 of subfamily I were also found in *A. thaliana* and have orthologs. However, HvFBX76, HvFBX152, and HvFBX20 of subfamily E all have paralogs in *A. thaliana*. Therefore, it can be assumed that the genes corresponding to the above 10 F-box proteins differentiated relatively early. Additionally, HvFBX95 of subfamily A was likely to have branch-specific repetitive events after differentiation from barley, which in turn produced HvFBX30 and HvFBX43 that are highly similar in protein sequence, gene structure, and expression. Such events are common in the F-box gene family in barley; thus, it can be speculated that among the 126 HvFBXs identified, gene duplication had an effect on the amplification and evolution of this gene family, which eventually lead to F-box gene diversity in terms of quantity, structure, and function in barley.

F-box proteins are widely involved in plant stress resistance. The homology analysis of F-box proteins revealed that some of these proteins served unclear functions and were more conducive for exploring the potential function of barley F-box proteins. In this study, protein sequences corresponding to the 11 F-box genes and 126 F-box protein sequences were used to construct a phylogenetic tree (Fig. 1). By comprehensively analyzing the results of the evolutionary tree and the expression profile, 12 genes were screened that exhibited high expression levels and may be related to stress responses (Tab. 1). The qRT-PCR results revealed that the expression of HvFBX152 decreased significantly, while the expression of HvFBX20 and HvFBX158 increased after salt and drought stress treatments. HvFBX152 and HvFBX20 of subfamily E are homologous to the drought-resistant and salt-tolerant-related gene, AtPP2-B11, which encodes an SCF E3 ligase that responds to drought stress as a negative regulator and to salt stress by regulating gene expression [46]. HvFBX158 of subfamily B is homologous to the salt stress-related gene, TIR1 [43], and the drought and salt tolerance-related gene, AFBA1 [40]. HvFBX6 is homologous to TIR1 and AFBA1 and was upregulated by salt stress and downregulated by drought stress in this study. The above results indicate that the changes in expression after adversity control were in line with theoretical expectations and that the evolutionary tree results have high credibility.

This study revealed that HvFBXs could respond to two different stress, which suggested that HvFBXs might not only improve salt tolerance but also drought tolerance. But the diverse expression level also been observed. For example, HvFBX6, HvFBX10, HvFBX20, HvFBX56, HvFBX75, HvFBX80 and HvFBX158 were more sensitive to salt than HvFBX30, HvFBX43 and HvFBX67 among 10 upregulated genes after salt stress. Meanwhile, HvFBXs in leaf tissues were also sensitive to drought. For example, HvFBX10, HvFBX20, HvFBX75 and HvFBX158 were more sensitive to drought than HvFBX43, HvFBX56,

HvFBX67 and HvFBX137 among 8 upregulated genes after salt stress. Salt stress causes barley cell membrane damage, weakens photosynthesis, and leads to nutrient and water deficiency. It often manifests as physiological drought, and drought stress causes similar damage to barley. Therefore, the changes in gene expression caused by physiological and environmental drought may have certain similarities. According to the results of this study, HvFBX10, HvFBX20, HvFBX43, HvFBX56, HvFBX67, HvFBX75, HvFBX158 and HvFBX152 changed in the same direction after subjugation to drought and salt stress; therefore, it can be speculated that there is a certain relationship between the internal response mechanisms of these 8 genes to stress.

5 Conclusions

This study conducted bioinformatics research in various aspects on the *F-box* gene family in barley. Results revealed its origin, amplification, evolution, and possible functional differentiation, as well as screened out 12 highly expressed genes and their expression levels after stress. Among the 12 target genes, 10 genes were upregulated and 2 genes were downregulated after salt stress. The genes whose expression was upregulated were *HvFBX6*, *HvFBX10*, *HvFBX20*, *HvFBX30*, *HvFBX43*, *HvFBX56*, *HvFBX67*, *HvFBX75*, *HvFBX80*, and *HvFBX158*. However, among the 12 target genes, 8 genes were upregulated and 4 genes were downregulated after drought stress. The genes whose expression was downregulated were *HvFBX10*, *HvFBX20*, *HvFBX43*, *HvFBX56*, *HvFBX67*, *HvFBX75*, *HvFBX158*, and *HvFBX137*. This study is the first to confirm that *HvHBX152* and *HvHBX158* play important roles in drought resistance and salt tolerance, providing a theoretical basis for future in-depth research on related genes and applications aimed at improving barley varieties.

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Appendix

Appendix A: Physicochemical properties and subcellular localization of F-box proteins in barley

Gene name	Protein number	ORF (aa)	MW(kD)	PI	Hydropphilicity index	Subcellular localization
HvFBX1	A0A287NKR7	186	20.23809	7.31	−0.431	Chloroplast
HvFBX2	A0A287QL82	540	62.37077	5.43	−0.366	Endoplasmic reticulum
HvFBX3	M0VNK7	234	26.08521	8.90	−0.069	Cytoplasm
HvFBX4	A0A287USN2	240	26.27199	6.16	−0.071	Cytoplasm
HvFBX5	F2EGG2	465	52.47029	5.32	−0.107	Chloroplast
HvFBX6	A0A287JST9	459	51.01019	9.43	−0.432	Chloroplast
HvFBX7	A0A287KKK8	353	39.43257	9.03	−0.096	Cytoplasm
HvFBX8	A0A287TJ68	443	49.3797	8.97	−0.361	Chloroplast
HvFBX9	A0A287UBU5	394	44.79432	5.91	−0.242	Cytoplasm
HvFBX10	A0A287L6S0	482	52.7286	9.48	0.107	vacuole
HvFBX11	A0A287QPT9	399	Unknown	Unknown	−0.046	Chloroplast
HvFBX12	A0A287V6T5	483	54.78763	6.35	−0.037	Nuclear
HvFBX13	M0VV39	421	47.68685	6.10	−0.019	Cytoplasm
HvFBX14	A0A287TB53	392	43.90962	8.62	−0.053	vacuole
HvFBX15	A0A287S555	188	21.24054	10.18	−0.374	Nuclear
HvFBX16	A0A287MKW8	448	50.49253	7.05	−0.198	Chloroplast
HvFBX17	A0A287H369	364	Unknown	Unknown	−0.746	Nuclear
HvFBX20	A0A287V4K6	338	37.03785	5.81	−0.358	Chloroplast
HvFBX21	M0UQC5	165	18.77672	9.00	−0.155	Chloroplast
HvFBX23	M0XU49	404	45.98427	9.24	−0.197	Nuclear
HvFBX24	A0A287UXJ0	417	46.19238	8.37	−0.012	Chloroplast
HvFBX27	A0A287VHQ3	243	27.45253	5.58	−0.031	Nuclear
HvFBX28	M0VG93	394	45.29275	5.80	−0.181	Mitochondrial
HvFBX30	A0A287F439	475	53.5457	9.36	−0.329	Chloroplast
HvFBX31	A0A287UI08	474	51.87319	9.31	0.071	Nuclear
HvFBX32	A0A287UTB3	446	51.20449	8.82	−0.128	Mitochondrial
HvFBX33	M0Z765	471	52.06739	5.29	0.086	Cytoplasm
HvFBX34	A0A287SPI6	222	25.46952	8.02	−0.096	Nuclear
HvFBX35	F2EB74	438	48.27137	6.62	−0.061	Chloroplast

(Continued)

Appendix A (continued).

Gene name	Protein number	ORF (aa)	MW(kD)	PI	Hydropphilicity index	Subcellular localization
HvFBX36	A0A287MQU5	423	48.1579	5.29	0.01	Cytoplasm
HvFBX37	A0A287VWF8	237	25.92117	8.73	0.028	Chloroplast
HvFBX39	A0A287U7W7	254	27.17371	11.62	−0.004	peroxisome
HvFBX40	M0X9T5	513	57.83129	8.99	−0.062	Mitochondrial
HvFBX42	A0A287WV14	294	32.01235	5.69	−0.142	Cytoplasm
HvFBX43	A0A287MU03	487	55.42109	9.97	−0.385	Chloroplast
HvFBX45	A0A287MPP2	482	54.52433	5.19	−0.101	Cytoplasm
HvFBX47	A0A287WC43	386	43.34022	10.10	−0.457	Nuclear
HvFBX48	M0XYB1	312	33.33863	5.50	−0.153	Endoplasmic reticulum
HvFBX49	M0VAD6	564	63.94444	8.24	−0.309	Nuclear
HvFBX50	M0VG42	524	58.67722	9.05	−0.143	Chloroplast
HvFBX53	A0A287H2G0	468	52.87857	7.22	−0.024	Cytoplasm
HvFBX54	A0A287QS59	254	Unknown	Unknown	−0.115	Chloroplast
HvFBX55	M0W316	273	30.05418	6.59	−0.328	Cytoplasm
HvFBX56	M0XT08	524	59.05885	5.54	−0.018	Cytoplasm
HvFBX57	A0A287XZH9	481	53.41613	8.11	−0.122	Chloroplast
HvFBX58	A0A287H519	523	58.86852	4.54	−0.42	Cytoplasm
HvFBX59	M0WNJ8	417	45.69862	8.60	0.035	Mitochondrial
HvFBX60	M0V3B4	219	24.85779	6.50	−0.176	Cytoplasm
HvFBX61	A0A287SV44	819	91.29346	5.73	−0.299	Chloroplast
HvFBX62	A0A287QBF1	579	65.79676	6.30	−0.445	Chloroplast
HvFBX63	A0A287JRA6	447	49.93917	8.83	−0.014	Chloroplast
HvFBX64	M0UEE7	296	33.48446	6.55	−0.17	Peroxisome
HvFBX65	A0A287TMQ0	327	37.39692	6.41	−0.109	Cytoplasm
HvFBX66	A0A287V179	497	56.23614	7.30	−0.327	Nuclear
HvFBX67	A0A287VDC8	477	51.86974	8.59	−0.075	Chloroplast
HvFBX68	A0A287PFQ9	339	37.37012	5.36	0.058	Mitochondrial
HvFBX69	A0A287XXL8	602	68.08612	8.91	−0.142	Nuclear
HvFBX70	A0A287RQT8	440	49.89437	8.88	−0.281	Chloroplast
HvFBX71	A0A287HAL1	507	58.61449	9.22	−0.166	Mitochondrial
HvFBX72	A0A287T4W1	427	49.93148	7.54	−0.327	Chloroplast
HvFBX74	A0A287L1T0	569	62.26423	9.35	0.009	Cytoplasm
HvFBX75	A0A287LTX2	401	45.13744	8.59	−0.425	Chloroplast

Appendix A (continued).

Gene name	Protein number	ORF (aa)	MW(kD)	PI	Hydropphilicity index	Subcellular localization
HvFBX76	A0A287JA66	262	28.69766	5.11	−0.18	Nuclear
HvFBX77	A0A287TGA1	393	42.78684	9.17	0.118	Chloroplast
HvFBX78	A0A287TU38	557	62.07352	9.14	−0.121	Chloroplast
HvFBX79	A0A287LTG2	284	29.99721	8.11	0.014	Cytoplasm
HvFBX80	A0A287LIH4	577	63.35826	8.64	−0.032	Chloroplast
HvFBX81	A0A287T3M1	441	50.76691	8.58	−0.036	Plasmic
HvFBX82	A0A287GD44	449	49.97483	8.64	−0.009	Chloroplast
HvFBX84	M0XHC6	486	55.50842	5.44	0.032	Cytoplasm
HvFBX85	M0XPR2	502	56.8948	8.62	−0.167	Cytoplasm
HvFBX87	A0A287PP75	433	47.85675	5.90	−0.181	Chloroplast
HvFBX88	A0A287EUT7	449	49.92559	5.34	−0.375	Nuclear
HvFBX89	A0A287GL42	448	49.29083	6.33	−0.389	Vacuole
HvFBX91	A0A287T7C4	456	51.89818	5.97	−0.245	Chloroplast
HvFBX92	M0X5I7	418	47.61486	6.17	−0.025	Nuclear
HvFBX94	A0A287SHU6	217	23.76635	8.47	−0.066	Cytoplasm
HvFBX95	F2DCG6	426	46.43829	9.88	−0.195	Chloroplast
HvFBX96	A0A287J677	384	Unknown	Unknown	−0.146	Cytoplasm
HvFBX98	M0UW06	379	42.97462	5.57	−0.219	Nuclear
HvFBX99	M0Y0V7	476	52.98308	8.18	−0.008	Chloroplast
HvFBX101	A0A287R6E0	552	58.33454	9.07	−0.022	Chloroplast
HvFBX103	A0A287E6S3	325	36.47627	5.54	−0.399	Cytoplasm
HvFBX104	A0A287NBU7	280	30.29793	5.40	−0.392	Cytoplasm
HvFBX105	M0XDF0	267	29.781	6.03	−0.239	Cytoplasm
HvFBX108	A0A287JCQ8	554	62.56645	6.33	−0.259	Chloroplast
HvFBX109	A0A287K9K7	407	45.35702	7.33	−0.121	Peroxisome
HvFBX110	A0A287HK80	546	58.18804	5.75	−0.182	Chloroplast
HvFBX111	A0A287SUD5	764	83.86995	5.43	−0.275	Mitochondrial
HvFBX112	A0A287V0N8	509	57.79973	8.01	−0.257	Nuclear
HvFBX113	A0A287VRW1	331	37.17215	5.90	0.099	Chloroplast
HvFBX114	A0A287QI34	381	42.90887	8.05	−0.057	Chloroplast
HvFBX115	A0A287TN22	543	58.76303	7.14	0.07	Chloroplast
HvFBX117	A0A287F5I6	381	42.77919	5.97	−0.34	Peroxisome
HvFBX118	A0A287SL94	415	46.41936	5.10	−0.256	Cytoplasm
HvFBX119	M0VX36	517	58.15014	7.79	−0.081	Nuclear

(Continued)

Appendix A (continued).

Gene name	Protein number	ORF (aa)	MW(kD)	PI	Hydropphilicity index	Subcellular localization
HvFBX120	A0A287SZN3	415	46.92284	5.60	−0.137	Plasmic
HvFBX121	A0A287EGE6	212	23.68916	8.41	−0.238	Chloroplast
HvFBX122	A0A287W7C1	505	57.78586	8.14	−0.268	Nuclear
HvFBX125	A0A287QEZ5	683	73.09063	9.20	−0.111	Chloroplast
HvFBX127	A0A287X1K4	243	Unknown	Unknown	0.092	Cytoplasm
HvFBX128	A0A287TDD0	489	54.41072	8.70	−0.133	Chloroplast
HvFBX129	M0W5I2	366	41.56554	5.72	−0.038	Cytoplasm
HvFBX130	A0A287T002	394	43.99825	8.84	0.088	Vacuole
HvFBX131	A0A287QJQ1	501	57.22944	5.67	−0.543	Chloroplast
HvFBX132	M0WKD1	495	56.33528	9.00	−0.146	Chloroplast
HvFBX133	A0A287J4A7	466	51.22151	6.20	−0.12	Nuclear
HvFBX134	A0A287JBI2	501	55.54399	8.92	−0.092	Chloroplast
HvFBX135	A0A287K352	214	23.68892	9.56	−0.541	Chloroplast
HvFBX136	A0A287MCM9	547	61.21005	8.59	−0.01	Mitochondrial
HvFBX137	M0UKT8	477	55.11311	4.87	−0.247	Nuclear
HvFBX139	A0A287EE09	381	42.22257	6.36	0.002	Nuclear
HvFBX140	A0A287XLY6	598	67.6066	5.05	−0.297	Nuclear
HvFBX142	A0A287LVY1	448	51.11435	5.75	−0.143	Cytoplasm
HvFBX145	A0A287QZT7	457	47.20054	9.76	0.276	Chloroplast
HvFBX146	M0XTT7	410	46.23245	8.70	−0.167	Peroxisome
HvFBX148	A0A287VLM6	717	79.84761	7.77	−0.459	Chloroplast
HvFBX149	A0A287VZL9	501	56.90174	8.63	−0.542	Nuclear
HvFBX150	A0A287UFS6	428	47.7734	6.26	−0.326	Mitochondrial
HvFBX151	A0A287FTA6	198	22.54992	5.52	0.056	Cytoplasm
HvFBX152	A0A287V4L8	340	37.57096	4.93	−0.554	Chloroplast
HvFBX154	A0A287XFN5	484	Unknown	Unknown	0.131	Cytoplasm
HvFBX156	A0A287TX71	301	32.7752	9.36	0.192	Chloroplast
HvFBX157	A0A287RUQ6	204	22.65514	4.85	0.085	Cytoplasm
HvFBX158	A0A287QWV6	439	48.97386	9.39	−0.362	Chloroplast
HvFBX159	A0A287XA02	468	51.96919	7.29	0.007	Chloroplast

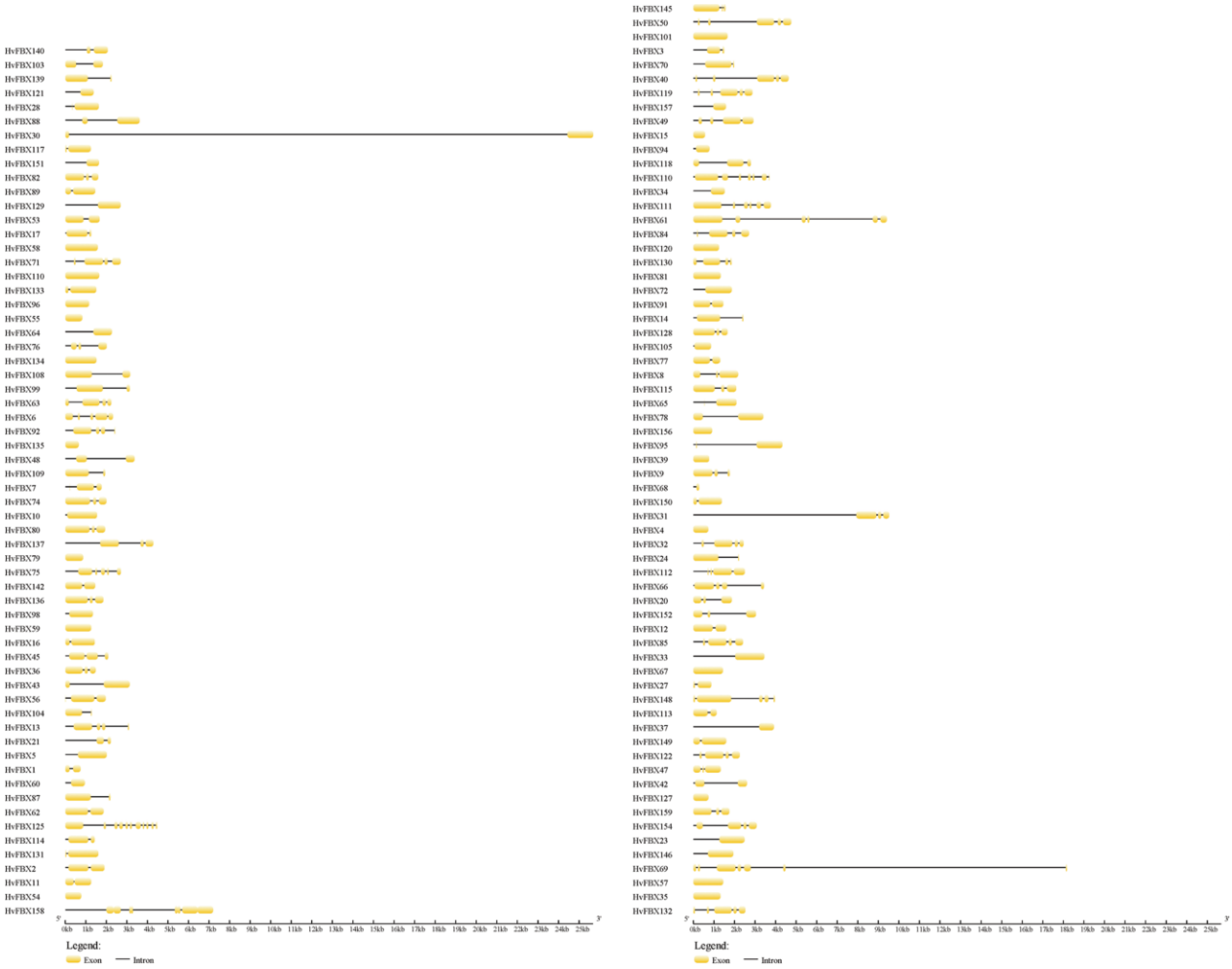
Appendix B: F-box protein sequences in wheat and *A. thaliana*

GenBank	Protein sequence
BAC43001.1	MALGKKRIVTQKPNLRQRRDVDNGGLGLGLEFVQYKRGFGRKRILISSGDEMEDSIFTSPVGKKLCDDKT TSVAEGQSRELEDLPLDILVRIICGVEHEDLKQLFHVSKTIREATMIAKQSHFAYSTPRKTSVFHHGRFG WDKPFDVEDDDEEIEAPGAPLQKRYRLSRINRNKDDSGVSVALFH
AEL33721.1	MEEHREERCWEDLLPDALGLVFRNLSLQEMLTVVPRVCKSWSRVVSGPYCWQEIDIQEWSQQQNKPDQLT RMVHTLVTRSGDSFRRISVSGLPNDSLFTFIANHARSLKTLELPRSEISDCIVEDVAQRLSNVTFLDVSS CTKIGARALEAFGKNCKSLVGLRRVMHPIDVAGEVCQHDEARAIACSMPKLRHLEIGYMLIATNAVVEIA SRCRDLSFLDLRGCWGVDDKLLQDKYPGLKVLGPRVDDCYENSFLEECSDDSDDDSIYSWEEFMDDEDYF AAAGSDEDEEALWADGHALEGLEVRFYGGGFGEGAFAGIDWPESP
CAE75864.1	MSQIFSFAGENDFYRRGAIYPNPKDASLLLSLGSFADVYFPPSKRSRVVAPTIFSAFEKKPVSIDVLPDE CLFEIFRRLSGPQERSACAFVSKQWLTLVSSIRQKEIDVPSKITEDGDDCEGCLSRSLDGKKATDVRLAA IAVGTAGRGGLGKLSIRGSNSAKVSDLGLRSIGRSCPSLGSLSLWNVSTITDNGLLEIAEGCAQLEKLEL NRCSTITDKGLVAIAKSCPNLTELTLEACSRIGDEGLLAIARSCSKLKSVSIKNCPLVRDQGIASLLSNT TCSLAKLKLQMLNVTDVSLAVVGHYGLSITDLVLAGLSHVSEKGFWVMGNGVGLQKLNSLTITACQGVTD MGLESVGKGCPNMKKAIISKSPLLSDNGLVSFAKASLSLESLQLEECHRVTQFGFFGSLLNCGEKLKAFS LVNCLSIRDLTTGLPASSHCSALRSLSIRNCPGFGDANLAAIGKLCPQLEDIDLCGLKGITESGFLHLIQ SSLVKINFSGCSNLTDRVISAITARNGWTLEVLNIDGCSNITDASLVSIAANCQILSDLDISKCAISDSG IQALASSDKLKLQILSVAGCSMVTDKSLPAIVGLGSTLLGLNLQQCRSISNSTVDFLVERLYKCDILS
BAE99994.1	MTSDALTIPSELESALRLRTVQYFITKRPWLDLYGVHVRPVPPFGSTSRKPHFDPALIHRCLPDELLFEV FARMMPYDLGRASCVCRKWRYTVRNPMFWRNACLKAWQTAGVIENYKILQSKYDGSWRKMWLLRSRVRTD GLYVSRNTYIRAGIAEWKITNPVHIVCYYRYIRFYPSGRFLYKNSSQKLKDVAKYMNFKASKSENLYKGT YTLSMSDDKIEAAVLYPGTRPTVLRIRLRLRGTAIGANNRMDLLSLVTSGVNDEEISSTEEDILGLVEGW EDDETHNPDIPAVSHKRGMTAFVFVPFEEVDESVLNLPPEKMDYYVTG
AAK97303.1	MASTTLSDLPDVILSTISSLVSDSRARNSLSLVSHKFLALERSTRSHLTIRGNARDLSLVPDCFRSISHL DLSFLSPWGHTLLASLPIDHQNLLALRLKFCFPFVESLNVYTRSPSSLELLLPQWPRIRHIKLLRWHQRA SQIPTGGDFVPIFEHCGGFLESLDLSNFYHWTEDLPPVLLRYADVAARLTRLDLLTASFTEGYKSSEIVS ITKSCPNLKTFRVACTFDPRYFEFVGDETLSAVATSSPKLTLLHMVDTASLANPRAIPGTEAGDSAVTAG TLIEVFSGLPNLEELVLDVGKDVKHSGVALEALNSKCKKLRVLKLGQFQGVCSATEWRRLDGVALCGGLQ SLSIKNSGDLTDMGLVAIGRGCCKLTTFEIQGCENVTVDGLRTMVSLRSKTLTDVRISCCKNLDTAASLK AIEPICDRIKRLHIDCVWSGSEDEEVEGRVETSEADHEEEDDGYERSQKRCKYSFEEEHCSTSDVNGFCS EDRVWEKLEYLSLWINVGEFLTPLPMTGLDDCPNLEEIRIKIEGDCRGKRRPAEPEFGLSCLALYPKLSK MQLDCGDTIGFALTAPPMQMDLSLWERFFLTGIGSLSLSELDYWPPQDRDVNQRSLSLPGAGLLQECLTL RKLFIHGTAHEHFMNFLLRIPNLRDVQLRADYYPAPENDMSTEMRVGSCSRFEDQLNSRNIID
AAK93595.1	MNNLPEDCIAKILSLTTPLDVCRLSAVSSIFRSAAGSDDVWNHFLPADFPAGFAAPAGLPTRKQLFFSLV DNPLLINGTLLSFSLERKSGNKCYMMAARALNIVWGHEQRYWHWISLPNTRFGEVAELIMVWWLEITGKI NITLLSDDTLYAAYFVFKWNHSPYGFRQPVETSLVLADTESTDNVVQPSMISLMQDSGGEEGQSPVLRRD GWYEVELGQFFKRRGDLGEIEMSLKETKGPYEKKGLIVYGIEIRPVP
AAK91385.1	MNSQACLLLQKQLKDLCKHPVDGFSAGLVDEKNIFEWSVTIIGPPDTLYEGGFFYAIMSFPQNYPNSPPT VRFTSDIWHPNVYPDGRVCISILHPPGDDPSGYELASERWTPVHTVESIMLSIISMLSGPNDESPANVEA AKEWREKRDEFKKKVSRCVRKSQEMF
AEE75937.1	MTKISDLPRDLAEEVLSRVPVTYLRAIRFTCKKWNTLTKRRSFTKKLIGQEKAEAKVKEFHAIMTLNSRL HLMSVNLDGIHKDENVESSIKQKGKLISLTVADPDRIVISQVYHCDGLLLCITNEINSRLVVWNPYSGQT RWIEPRTSYREWDIYALGYESKNNAKRSYKILRYLDAYEDMGDMSVEPRTRVCEFEIYSLDTNSWKVIEV TTDWDLCFLHRGVTLKGNTYWFAREKIPPPPRERVIEDIPLGEAEINVEIPSFLLCFDFTIEKFGSRLPL PFRPCVDDTITLSSVREEKLAVLYQRWDITWTGIWISNKIEPNAVSWSKLFFPMGRIRPLEAASGTFFVD EENKLVVLFDKGESILNPTRNTAYIVGEDGYIKPVDLGESVHKYCFPLACSYVPSSVQI

(Continued)

Appendix B (continued).

GenBank	Protein sequence
AAL07120.1	MLLEGRFSVVQMNTNRRLKFNQPSRLPNSGKSGIENERVLVLVFESISWDIHTLCTIASLSRRFCAIARR ILWRRLCVNRAPGMVAALSGEDPSGRIDGGWHALAKLMFFCGGGESTRYFNLSQPTSGHFACESRFSKTS GRFFLPKNCRRDLLYMSDPCEHQAVGGDEHLGVFRGVFREFMRSKTRECLVRRQAALEEKVRCPYCGGRV WSMTAARLVPKSAARRLGSREGGLEFFVCVNGHLHGTCWLIPLSSEEEDNGEDDDNSDGSVI
AAB69176.1	MQKRIALSFPEEVLEHVFSFIQLDKDRNSVSLVCKSWYEIERWCRRKVFIGNCYAVSPATVIRRFPKVRS VELKGKPHFADFNLVPDGWGGYVYPWIEAMSSSYTWLEEIRLKRMVVTDDCLELIAKSFKNFKVLVLSSC EGFSTDGLAAIAATCRNLKELDLRESDVDDVSGHWLSHFPDTYTSLVSLNISCLASEVSFSALERLVTRC PNLKSLKLNRAVPLEKLATLLQRAPQLEELGTGGYTAEVRPDVYSGLSVALSGCKELRCLSGFWDAVPAY LPAVYSVCSRLTTLNLSYATVQSYDLVKLLCQCPKLQRLWVLDYIEDAGLEVLASTCKDLRELRVFPSEP FVMEPNVALTEQGLVSVSMGCPKLESVLYFCRQMTNAALITIARNRPNMTRFRLCIIEPKAPDYLTLEPL DIGFGAIVEHCKDLRRLSLSGLLTDKVFEYIGTYAKKMEMLSVAFAGDSDLGMHHVLSGCDSLRKLEIRD CPFGDKALLANASKLETMRSLWMSSCSVSFGACKLLGQKMPKLNVEVIDERGAPDSRPESCPVERVFIYR TVAGPRFDMPGFVWNMDQDSTMRFSRQIITTNGL
AQR57191.1	MVSRSREDYFNPDLKHLTTLVLGSSSSVTIPTPWEKDKEKEKEKEKEDEEFFLVSFDSCDGLVCLYKYWK SGYVVNPTTRWYRPLPLSQLQQLLISLGRSVFELGYTVCDIGFGKDKITGTYKPVWLYNSLEIGLENATT CEVFDFNTNAWRYVSPTAPYREETKILSFDLHTETFRVVSKAPFTNVKAFDIVMCNLGNRLCVSEKNWPN QVIWSFNSGNKTWHKMFSINLDVTSHWFGNHIAAVMPLALFYEKKKKKKLLFYCRVRSRTLMVYDPETES YDVAFNDYSIGYPLCYFQSLISIS



Appendix C: Intron-exon structures of the F-box family genes in barley. The intron-exon structures were examined using the GSDS v2.0 online tool. The exons and introns are indicated by yellow boxes and black lines, respectively.