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Acyl-CoA-binding protein (*ACBP*) genes involvement in response to abiotic stress and exogenous hormone application in barley (*Hordeum vulgare* L.)

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Abstract

Background Acyl-CoA-Binding proteins (ACBPs) function as coenzyme A transporters and play important roles in regulating plant growth and development in response to abiotic stress and phytohormones, as well as in membrane repair. To date, the *ACBP* family has not been a comprehensively characterized in barley (*Hordeum vulgare* L.).

Results Eight ACBP genes were identified in the barley genome and named as *HvACBP1–8*. The analysis of the proteins structure and promoter elements of *HvACBP* suggested its potential functions in plant growth, development, and stress response. These *HvACBPs* are expressed in specific tissues and organs following induction by abiotic stressors such as drought, salinity, UV-B exposure, temperature extremes, and exposure to exogenous phytohormones. The HvACBP7 and HvACBP8 amino acid sequences were conserved during the domestication of Tibetan Qingke barley.

Conclusions Acyl-CoA-binding proteins may play important roles in barley growth and environmental adaptation. This study provides foundation for further analyses of the biological functions of HvACBPs in the barley stress response.

Keywords Barley stress response, Acyl-CoA-binding protein (ACBP), Gene expression pattern

Background

Lipids are essential components of cell membranes and play pivotal roles in the cellular framework [1–3]. They provide energy for intracellular activity and sites for physiological processes such as photosynthesis, signal recognition, and transmission. They also act as signaling molecules in growth, development, and stress responses [4–6]. Key proteins involved in non-vesicular lipid transportation include lipid transfer proteins, ATP-binding cassette transporters, and acyl-coenzyme A-binding proteins (ACBPs) [7]. Compared with lipid transfer proteins and ATP-binding cassette transporters, ACBPs exhibit a wider range of lipid capabilities, allowing for broader

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subcellular localization. In addition, they can interact with long acyl-coenzyme A (acyl-CoA) moieties [8].

ACBPs are classified into four subfamilies (Class I–IV) based on their functional domains and relative molecular masses. Class I (small ACBPs) consists of low-molecular-weight ACBPs with only one ACBP domain. Class II (ANK-ACBPs) consists of higher-molecular-weight ACBPs and N-terminal ACBP domains coupled with C-terminal ankyrin domains [9, 10]. Class III (large ACBPs) includes various higher-molecular-weight ACBPs with one ACBP domain [11]. Class IV (Kelch-ACBPs) comprises higher-molecular-weight ACBPs with N-terminal ACBP domains and multiple C-terminal Kelch structural domains [12, 13].

As with other lipid transport proteins, the functions of ACBPs are influenced by their subcellular localization. All Class I ACBPs, including AtACBP6 [14], OsACBP1–3 [15], ChACBP1 [16], and HbACBP1 [9], are localized in the cytoplasm. Class II ACBPs, including AtACBP1/2 [17] and OsACBP4 [15], are localized in the cell membrane and endoplasmic reticulum, whereas HbACBP2 is localized in the endoplasmic reticulum [9]. The subcellular localization of Class III ACBPs is diverse: AtACBP2 is found in the peroxisomes, cell membranes, and endoplasmic reticulum around the Golgi apparatus complex [18]; OsACBP5 is localized in the peroxisomes [15]; and VfACBP3A and VfACBP3B are localized in the endoplasmic reticulum [19]. Among Class IV ACBPs, AtACBP4 and AtACBP5 are localized in the cytoplasm [20], whereas OsACBP6 is localized in the peroxisome [15].

In *Arabidopsis*, *AtACBP* expression varies widely among the different organs (roots, cotyledons, flowers, fruits, seedlings, and mature plants) [13]. *AtACBP1–2* expression is highest in the siliques and mature seeds [21–23], whereas *AtACBP3* is highly expressed in the pistils [11, 24, 25]. *AtACBP5* and *AtACBP6* are highly expressed in pollen, microspores, and trichome cells [2, 3, 14, 20, 26]. In rice, *ACBPs* are expressed in the roots, stems, leaves, and seeds, with the highest expression in the leaves and the lowest expression in the roots and stems [27, 28].

ACBPs have been studied for their roles in plant growth, development, and stress responses in model plants such as *Arabidopsis* and rice. In *Arabidopsis*, overexpression of *AtACBP6* enhances cold tolerance by upregulating the expression of phospholipase Dδ and increasing the levels of phosphatidic acid. AtACBP6 has binding affinity for various types of phosphatidylcholines, suggesting its role in phosphatidylcholine transport [14, 29]. *AtACBP6* knockout affects the seed oil content and lipid composition, leading to a reduced seed weight [30]. Similarly, in rice, overexpression of *OsACBP2* results in an increased grain size and oil content [31]. The expression of *OsACBP1–3* is suppressed under cold stress [27]. In *Arabidopsis*, *AtACBP1* and *AtACBP2* respond to cold, drought, hypoxia, and heavy metal stresses by maintaining a pool of membrane-associated acyl groups that are essential for early embryonic development [17, 20, 32, 33]. *AtACBP3* regulates autophagy-mediated leaf senescence and hypoxia responses, and its overexpression upregulates pathogenesis-related proteins and the salicylic acid pathway, thus resulting in improved pathogen resistance [25, 34]. Mutations in *AtACBP4–6* alter acyl-CoA expression, affecting both seed germination and pollen tube growth [2, 30]. *AtACBP4* responds to heavy metals, ethylene, methyl jasmonate (MeJA), and pathogen infections [35]. In rice, *OsACBP4* is responsive to salt and drought stress, and its overexpression significantly improves salt tolerance [27, 31]. *OsACBP5* overexpression enhances resistance to various pathogen types via the jasmonic acid and salicylic acid pathways [36, 37]. *OsACBP6* responds to physical damage, influences growth, participates in indole-3-butyric acid oxidation, and contributes to jasmonic acid biosynthesis [15, 27, 38]. In soybeans, *GmACBP3* and *GmACBP4* overexpression enhances lipoxygenase activity and salt tolerance [39]. These studies emphasize the critical role of ACBPs in plant biology, lipid metabolism, signaling pathways, and adaptive stress responses. However, there is a lack of research on the ACBP family of barley.

We comprehensively characterized the ACBP family in barley (*Hordeum vulgare* L.) and identified *HvACBPs* in barley using genome-wide bioinformatics analysis. Predicted the promoter elements to determine the changes in the expression of *HvACBPs* in response to abiotic stresses (temperature extremes, elevated salinity, drought, and UV exposure) and phytohormones indole-3-acetic acid (IAA), abscisic acid (ABA), and MeJA. This study provides a basis for the further in-depth exploration of the functional roles of *HvACBPs* to understand their complex functions in plant growth, development, and responses to environmental stimuli.

Results

Identification of barley ACBP gene family and phylogenetic and collinearity analysis

An extensive search for prior studies of ACBP in rice and *Arabidopsis* was conducted using public and private barley genomic and transcriptomic databases. Eight HvACBP genes were identified in the Morex reference genome. ACBP genes are distributed on all barley chromosomes, except for 3 H and 6 H. Additionally, *HvACBP3* and *HvACBP4* are tandem repeat genes on 2 H.

To analyze the evolutionary relationships of ACBP genes among different species, 59 ACBP protein sequences from seven crops were used to construct a phylogenetic tree. The genes were classified into the four ACBP Classes (Fig. 1a). Based on comparison with genes

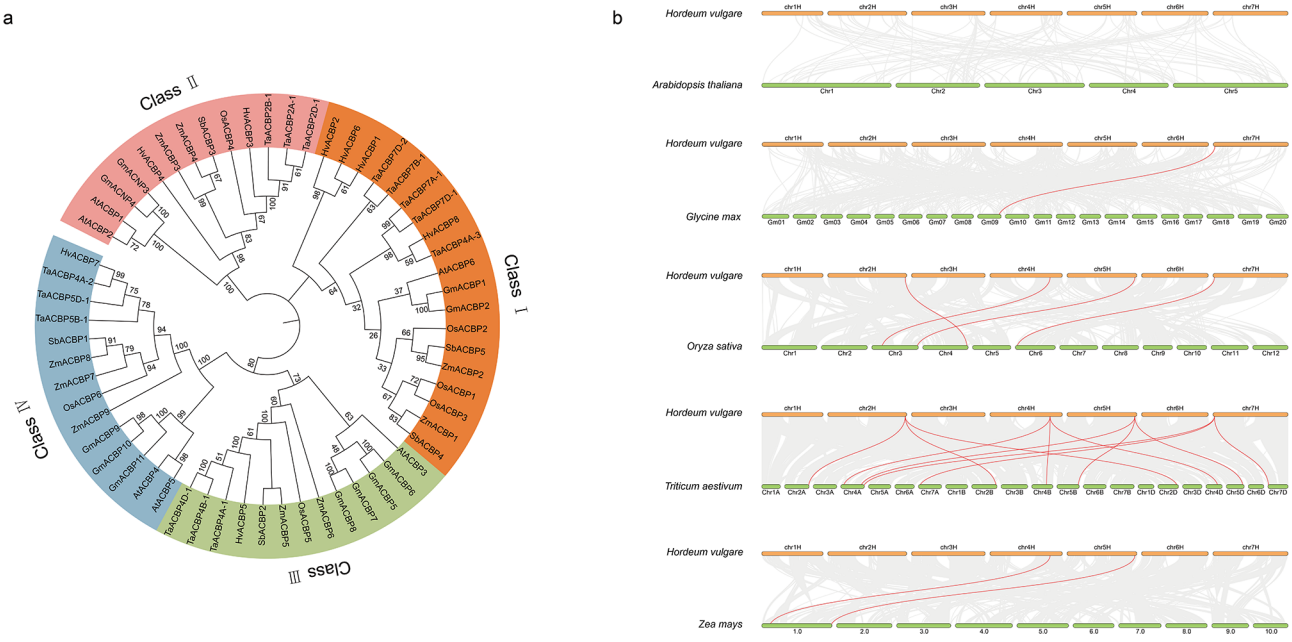

Fig. 1 Phylogenetic analysis and collinearity analyses of ACBP in *Arabidopsis thaliana*, *Glycine max*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*, *Sorghum bicolor*, and *Hordeum vulgare* (**a**) Phylogenetic tree of ACBP genes. The full-length sequence of the ACBP proteins was used for sequence alignment and phylogenetic analysis. Blocks of different colors indicate distinct subgroups, whereas in same color are indicated within a subgroup. (**b**) Collinear *ACBP* blocks in barley and related species. Gray lines in the background indicate collinear blocks within *Hordeum vulgare* and other plant genomes; collinear *ACBP* gene pairs are colored in red

in rice and *Arabidopsis*, *HvACBP1/2/6/8* were classified into Class I (small ACBPs), *HvACBP3/4* into Class II (ANK-ACBPs), *HvACBP5* into Class III (large ACBPs), and *HvACBP7* into Class IV (Kelch-ACBPs).

Collinearity analysis revealed the presence of collinear blocks in the barley and *Arabidopsis* genomes, although no collinear pairs of ACBP genes were identified. In contrast, comparisons of the barley, rice, and wheat genomes revealed four pairs of collinear ACBP genes (Fig. 1b).

Gene structure, conserved motif, and domain analysis

Gene structure analysis revealed that most of the genes contained upstream regulatory regions, and all contained exons, introns, and a conserved ACBP domain (Fig. 2a, b and d), with similar exon numbers and positions. The ACBP protein motifs and conserved domains were examined in *Arabidopsis*, rice, wheat, maize, and barley. The structural motifs of ACBPs proteins contained ten protein motifs (Motifs 1–10) of 0.21–50 amino acids in length. Motif 1 and Motif 2 are highly conserved in the ACBP protein family. Motif 9 was identified only in Class II, and Motifs 3–6 and 8 were only identified in Class IV (Fig. 2c). All ACBP proteins contained an ACBP domain. All ANK-ACBPs contained an ANKYR domain at the C-terminus, and all Kelch-ACBPs had an unequal number of Kelch domains.

HvACBP protein properties, secondary and tertiary structure

We analyzed the physical and chemical properties of HvACBP proteins. Their sizes ranged from 93 (HvACBP8) to 669 amino acids (HvACBP7), with molecular weights of 10.24 to 72.62 kDa (Table 1). The theoretical isoelectric point ranged from 4.23 (HvACBP5) to 6.22 (HvACBP1). Their instability index ranged from 30.54 (HvACBP2) to 52.97 (HvACBP3); those of HvACBP1, HvACBP3, HvACBP4, HvACBP5, and HvACBP7 exceeded 40, indicating that these proteins are unstable. Aliphatic index ranged from 66.33 (HvACBP1) to 84.07 (HvACBP5), suggesting relatively consistent thermal stability. The GRAVY (Grand average of hydropathicity) were negative, indicating that they are hydrophilic proteins (positive values indicate that proteins are more inclined towards hydrophobicity, whereas negative values indicate that proteins are more inclined towards hydrophilicity). The proteins were all predicted to localize in the cytoplasm.

Predictive analysis to predict HvACBP secondary and tertiary structures prediction revealed notable differences among the four gene Classes (Fig. 3). Class I ACBP proteins contained a structural motif characterized by 3–5 α-helical segments, accounting for 32.31–67.74% of the overall amino acid content (Table 1; Fig. 3b). HvACBP5 showed a similar structure to that of Class I. Class II HvACBPs exhibited more α-helical than random

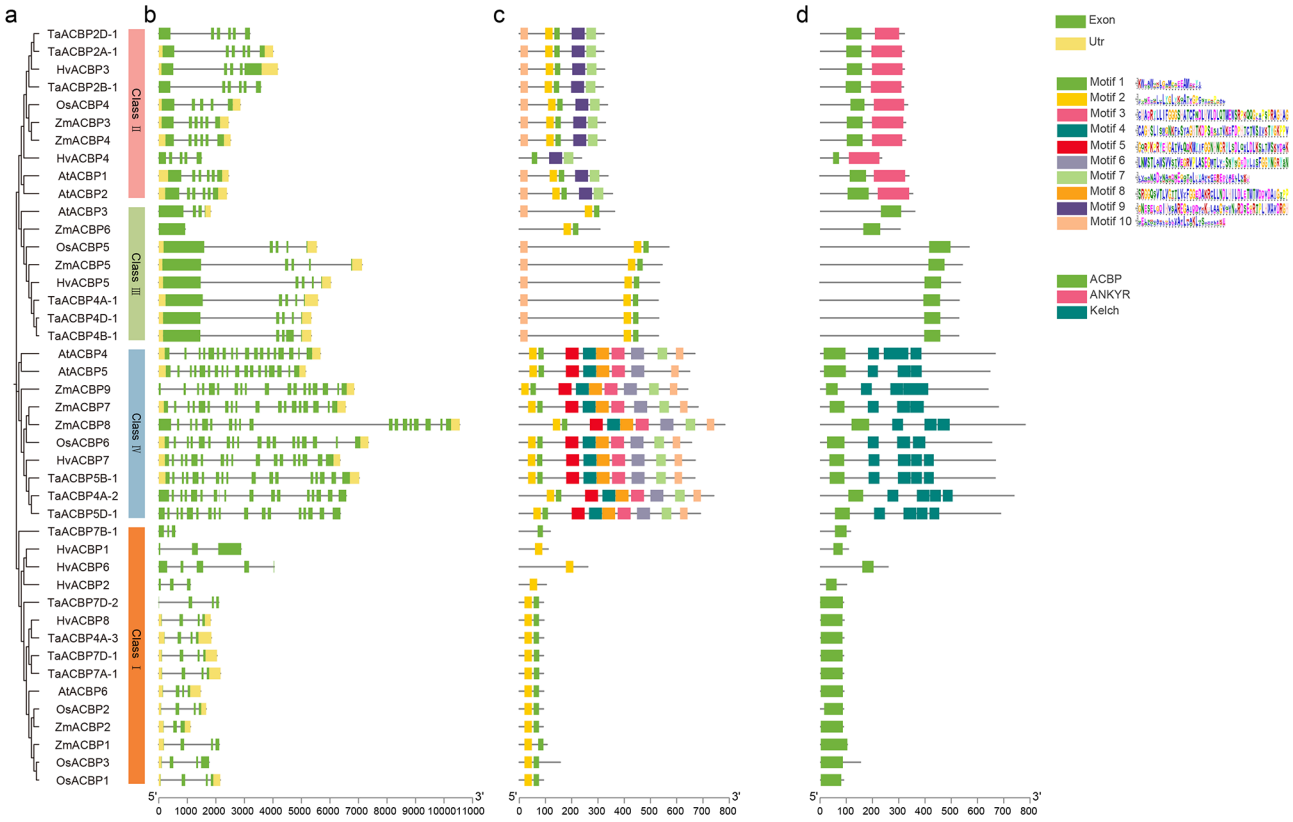

Fig. 2 Maximum likelihood phylogenetic structure and motifs of acyl-CoA-binding protein (ACBP) genes in *Hordeum vulgare* L. (**a**) Phylogenetic tree and *HvACBP* subfamilies, further divided into four groups. (**b**) HvACBP exon–intron organization. (**c**) ACBP motifs. (**d**) Ankyrin domains are conserved in the ANK-ACBP subfamily and the Kelch domain is conserved in the Kelch-ACBP subfamily

coil structures in terms of their total amino-acid composition. In contrast, for Class IV, most of the amino acids exhibited a random coil conformation. The Class IV tertiary structure showed substantial segments of β-fold conformations because of the prevalence of Kelch structures.

Cis-element analysis of HvACBP promoters

Cis-elements within eight HvACBP gene promoters were classified into three categories. The first category included elements closely associated with phytohormones, including auxins, MeJA, salicylic acid, ABA, gibberellin, and ethylene. The second category included elements closely associated with responses to environmental stressors, including drought, cold, wounding, anaerobic conditions, other stressors, and with defense, The third category included elements associated with plant growth and development, including meristem-specific and seed-specific expression, zein metabolism regulation, and transcription factor binding sites (specifically *MYB* and *MYC* transcription factors).

All eight *HvACBP* promoters contained elements associated with drought, light regulation, MYB binding, and stress responses; these elements participate in hormonal signaling, stress responses, and transcription factor binding. Our findings strongly suggest that *HvACBPs* influence hormonal signaling pathways and environmental responses, and regulate plant growth and development (Fig. 4).

Tissue-specific expression of *HvACBPs*

HvACBPs showed tissue-specific expression. *HvACBP6* expression was negligible in all the examined tissue types. *HvACBP2* expression was moderate in the ROO2, EPI, SEN, and EMB tissue types. *HvACBP1* was expressed predominantly during grain development, with minimal expression observed in the other tissue types. *HvACBP4* was few expressed in all detected tissues. Expression of the other four *HvACBPs* varied significantly within tissues.

HvACBP8 exhibited the highest expression across all tissue types, particularly in the young root, shoot, and grain tissues (LEA, ROO1, CAR5, and EMB). In all tissue types, *HvACBP3* and *HvACBP4* (in the ANK-ACBP subfamily) exhibited similar expression patterns, although *HvACBP3* expression substantially higher than that of *HvACBP4*, suggesting its importance in barley. Classes III and IV each included one member (*HvACBP5* and

Table 1 Properties of HvACBP proteins

Class	Number	Gene name		Number of amino acids	Theoretical pI	Molecular weight (kDa)	Instability index	Aliphatic index	Grand average of hydropathicity	Alpha helix (%)	Extended strand (%)	Beta turn (%)	Random coil (%)	Domain	Euk-mPLoc 2.0
Class I	HvACBP1	*HORVU.MOREX.r3.1HG0076680.1*		109	6.22	12.13	41.08	66.33	-0.189	0.3486	0.2661	0.0367	0.3486	ACBP	Cytoplasm
	HvACBP2	*HORVU.MOREX.r3.2HG0124500.1*		102	5.28	10.98	30.54	69.8	-0.089	0.2745	0.2745	0.1275	0.3235	ACBP	Cytoplasm
	HvACBP6	*HORVU.MOREX.r3.4HG0409130.1*		260	6.04	28.95	38.41	74.27	-0.369	0.3231	0.2154	0.0769	0.3846	ACBP	Cytoplasm
	HvACBP8	*HORVU.MOREX.r3.7HG0640790.1*		93	4.99	10.24	35.08	69.46	-0.566	0.6774	0.0215	0.0323	0.2688	ACBP	Cytoplasm
Class II	HvACBP3	*HORVU.MOREX.r3.2HG0213220.2*		324	4.4	34.61	52.97	78.43	-0.351	0.4414	0.0586	0.0802	0.4198	ACBP	Cytoplasm
	HvACBP4	*HORVU.MOREX.r3.2HG0213280.1*		236	4.6	26.19	42.2	83.47	-0.226	0.4195	0.1102	0.1017	0.3686	ACBP	Cytoplasm
Class III	HvACBP5	*HORVU.MOREX.r3.4HG0392690.1*		533	4.23	56.99	48.9	84.07	-0.417	0.5291	0.0657	0.0732	0.3321	ACBP	Cytoplasm
Class IV	HvACBP7	*HORVU.MOREX.r3.5HG0530170.1*		669	5	72.62	46.26	78.61	-0.418	0.3572	0.1659	0.0673	0.4096	ACBP	Cytoplasm

HvACBP7, respectively) that was abundantly expressed across all tissue types. The robust expression of *HvACBP3/4/5/7/8* across all barley tissues throughout the growth and developmental phases indicated the conservation and pivotal roles of *ACBP* genes in physiological processes in barley (Fig. 5).

HvACBP responses to hormonal stress induction

Bioinformatics analysis of the promoter elements revealed the presence of cis-regulatory elements for IAA, ABA, and MeJA in the upstream region of *HvACBP* (Fig. 4). The expression of *HvACBP1/6* was not detected. Treatment of barley seedlings with IAA, ABA, and MeJA induced *HvACBP* expression to varying degrees (Fig. 6f-h). Following IAA treatment, all genes, except for *HvACBP7*, exhibited robust upregulation at 1 and 3 h post-induction. However, at 6 h post-induction, *HvACBP* expression began to decline toward (or below) basal levels, ultimately returning to their baseline expression at 12–24 h post-induction (Fig. 6f).

Compared with the effects of ABA, MeJA caused longer and more sustained induction of *HvACBPs* (Fig. 6g, h). At 3 h post-treatment, the expression of *HvACBPs* was still elevated, although it had declined at 6, 12, and 24 h. In contrast, at 3 h post-ABA induction, *HvACBP* expression had reverted to pre-induction levels.

HvACBP expression under abiotic stress

We examined the regulation of *HvACBP* expression following exposure to abiotic stressors (salinity, drought, temperature variation, and UV radiation) (Fig. 6). *HvACBP1* and *HvACBP6* expression was not detected. Under salt stress, six of the *HvACBP* genes exhibited varying degrees of upregulation; in contrast, at 6 h, *HvACBP2* was slightly downregulated (Fig. 6a). *HvACBP4* exhibited significant upregulation (5.2-fold) after 6 h of salt stress, whereas *HvACBP3* exhibited a moderate response. HvACBP5 exhibited significant upregulation after 12 h of salt stress. Six *HvACBPs* exhibited rapid upregulation within 1 h of drought induction (Fig. 6b), although their expression declined over time. *HvACBP2* expression was lower than that at baseline after 6 and 12 h of drought stress.

HvACBP expression was differentially regulated under high and low temperature stress (Fig. 6c, d). Following exposure to 4 °C, *HvACBP* expression was rapidly upregulated, with *HvACBP4* exhibiting a remarkable 12-fold increase after 1 h. Heat stress induced significant upregulation of most of the *HvACBP*s, particulary for *HvACBP5*, which exhibited 20-fold upregulation after 24 h.

Six of the *HvACBPs* were differentially regulated in response to UV radiation, with upregulation at 1 h post-exposure (Fig. 6e), followed by a decline and subsequent increase to a peak at 12 h. *HvACBP2* and *HvACBP7*

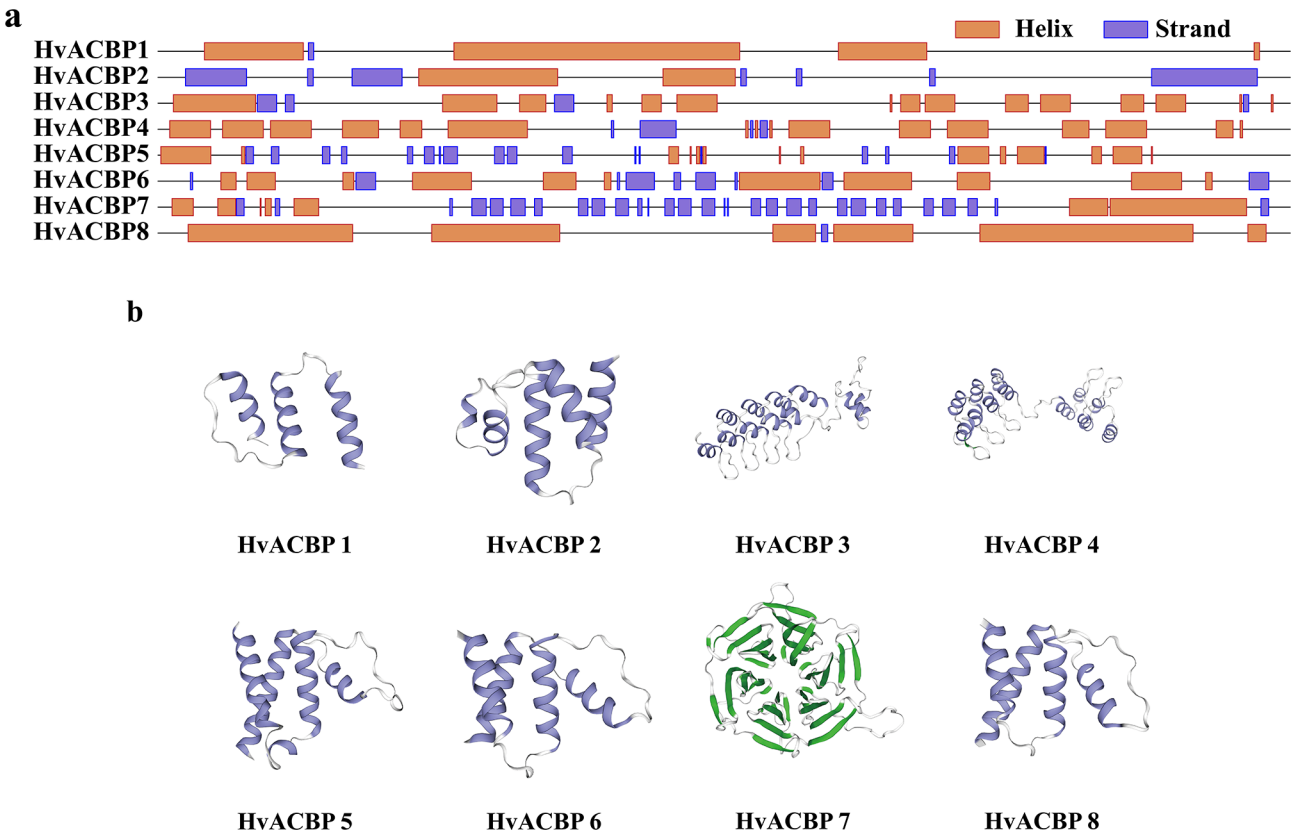

Fig. 3 Predicted secondary and tertiary structure of acyl-CoA-binding proteins (ACBPs) in *Hordeum vulgare* L. (**a**) HvACBP secondary structure. Orange block: α-helical conformation. Blue: random- coil structure. (**b**) HvACBP tertiary structure. Blue block: α-helical conformation. Green: β-fold conformation. White line: random-coil structure

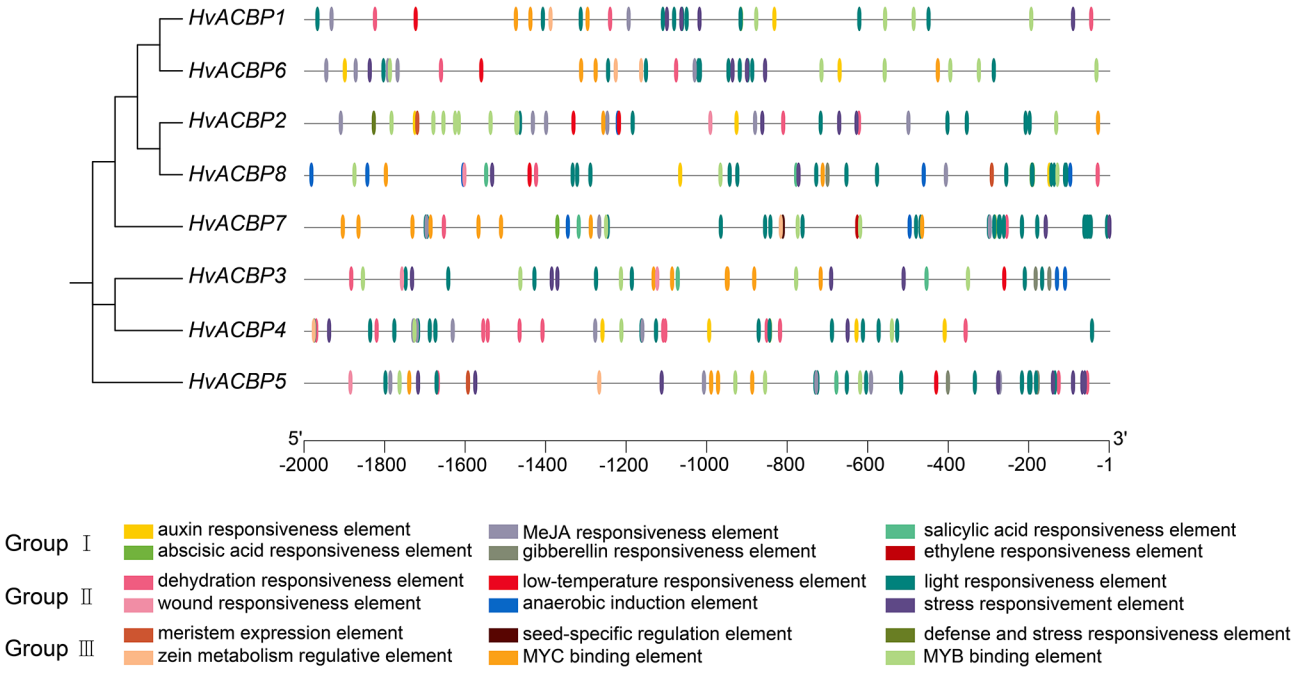

Fig. 4 Maximum likelihood phylogenetic trees and prediction of cis-acting elements in the acyl-CoA-binding protein gene (*ACBP*) promoters of *Hordeum vulgare* L. Two-kilobase pair promoter sequences were used to analyze hormone-related cis-elements, plant growth and development-related cis-elements, and stress-related elements. Different types of cis-elements are indicated by different colored symbols adjacent to their respective promoter

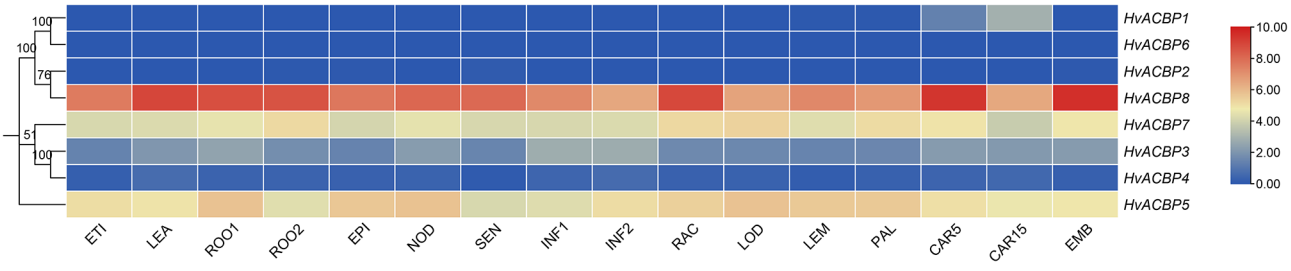

Fig. 5 Relative expression of acyl-CoA-binding protein genes (ACBPs) in different tissues of Hordeum vulgare L. Heat maps reflect the fragments per kilobase of transcript per million mapped fragments (FPKM) of *HvACBPs*. The color gradient from red to blue indicates high to low expression. ETI: seedlings were grown to 10 days after planting (dap) in the dark to isolate etiolated leaves. LEA: leaf tissue, 17 dap. ROO1: root tissue, 17 dap. ROO2: root tissue, 28 dap. EPI: epidermal strips were obtained from plant leaf tissue at 28 dap. NOD: third stem internode, 42 dap. SEN: senescing leaf, 56 dap. INF1: whole developing inflorescence tissue was sampled at 30 dap. INF2: whole developing inflorescence tissue, 50 dap. RAC: rachis, 35 dap. LOD: lodicule, 42 dap. LEM: lemma. PAL: palea. CAR5: development grain, 5 days post-anthesis(dpa). CAR15: development grain, 15 dpa. EMB: embryonic tissue, 4 d after germination of mature grain in Petri plates in the dark in the laboratory

showed the most significant responses to UV stress, with upregulation of 6.6- and 5.4-fold, respectively. These findings suggest that *HvACBPs* are crucial in mediating abiotic stress-tolerance responses.

Haplotype variations analysis of *HvACBP7* and *HvACBP8* between barley and Tibetan barley

To investigate the role of the ACBP genes in environmental adaptation in barley, we analyzed the haplotypes of ACBP members showing high expression levels. Ten haplotypes were identified in *HvACBP7*. Among them, Hap_10 was the most prevalent, present in 101 samples, whereas Hap_2 was the rarest, present in only 8 (Fig. 7; Table 2). Wild barley predominantly exhibited Hap_5 (23.7%) and Hap_1 (52.6%), whereas nearly all Tibetan barley samples exhibited Hap_7–10 (mostly HAP_10, 80.4%). Haplotype network analysis revealed a distant genetic relationship between Hap_7–10 and Hap_1–2, indicating that *HvACBP7* gathered in some new haplotypes during the domestication of Tibetan barley. Protein sequences analysis (Additional Data_2) revealed a substitution event (replacement of tyrosine with phenylalanine) in Hap_10 at amino acid 199 of HvACBP7.

Nine haplotypes were identified in *HvACBP8* (Supplementary Data 3). Hap_9, Hap_8, and Hap_6 were the dominate haplotypes. Among them, Tibetan barley mainly contained Hap_6, Hap7, Hap_8, and Hap_9. Remarkably, wild barley had a higher distribution proportion of these haplotypes. Protein sequence analysis revealed no amino acid variations between haplotypes, suggesting that the function of HvACBP8 is highly conserved in function.

Discussion

ACBPs are structurally conserved and colinear in gramineous crops

The functions of ACBPs have been extensively studied in higher plants, including *Arabidopsis*, rice [27], wheat [40], rapeseed [41], maize [42], and soybeans [43], but not in barley. Here, we comprehensively characterized *ACBP* sequences in barley, by comparing them with those of *Arabidopsis*, rice, maize, wheat, soybeans, and sorghum. All taxa were contained all four *ACBP* subfamilies, which shared a common ACBP structural domain and had a similar exon number and position, consistent with prior findings for maize [42]. This finding suggests that *ACBP* was conserved during plant evolution. Within subfamilies, different plants contain varying numbers of members, which may be related to genome size and gene copy number during evolution, such as the tandem repeats of *HvACBP3* and *HvACBP4*.

In addition, although the four subfamilies were conserved, and the collinearity of *ACBP* between the dicotyledonous plants, *Arabidopsis* and soybean, and barley was lower than that between monocotyledonous plants. This result suggests that, although homologous *ACBPs* with identical functional structural domains are common across plant species and that the four subfamilies evolved before the divergence of monocotyledonous and dicotyledonous plants, *ACBP* sequences widely varied during plant diversification, further indicating their importance and conserved roles, as well as highlighting the functional divergence among HvACBPs.

ACBP regulates responses to exogenous hormones and modulates plant growth and development

ACBPs perform critical roles in plant growth and development, as indicated by their tissue-specific expression and involvement in diverse phytohormone pathways [13, 44].

Previous studies revealed functional redundancy among *ACBP* members. For example, *AtACBP1* and *AtACBP2* have similar expression patterns, with the loss of one gene leading to compensatory expression of the other gene. The double mutant of *atacbp1* and *atacbp2* can cause embryonic death in seeds [45]. *HvACBP3* and

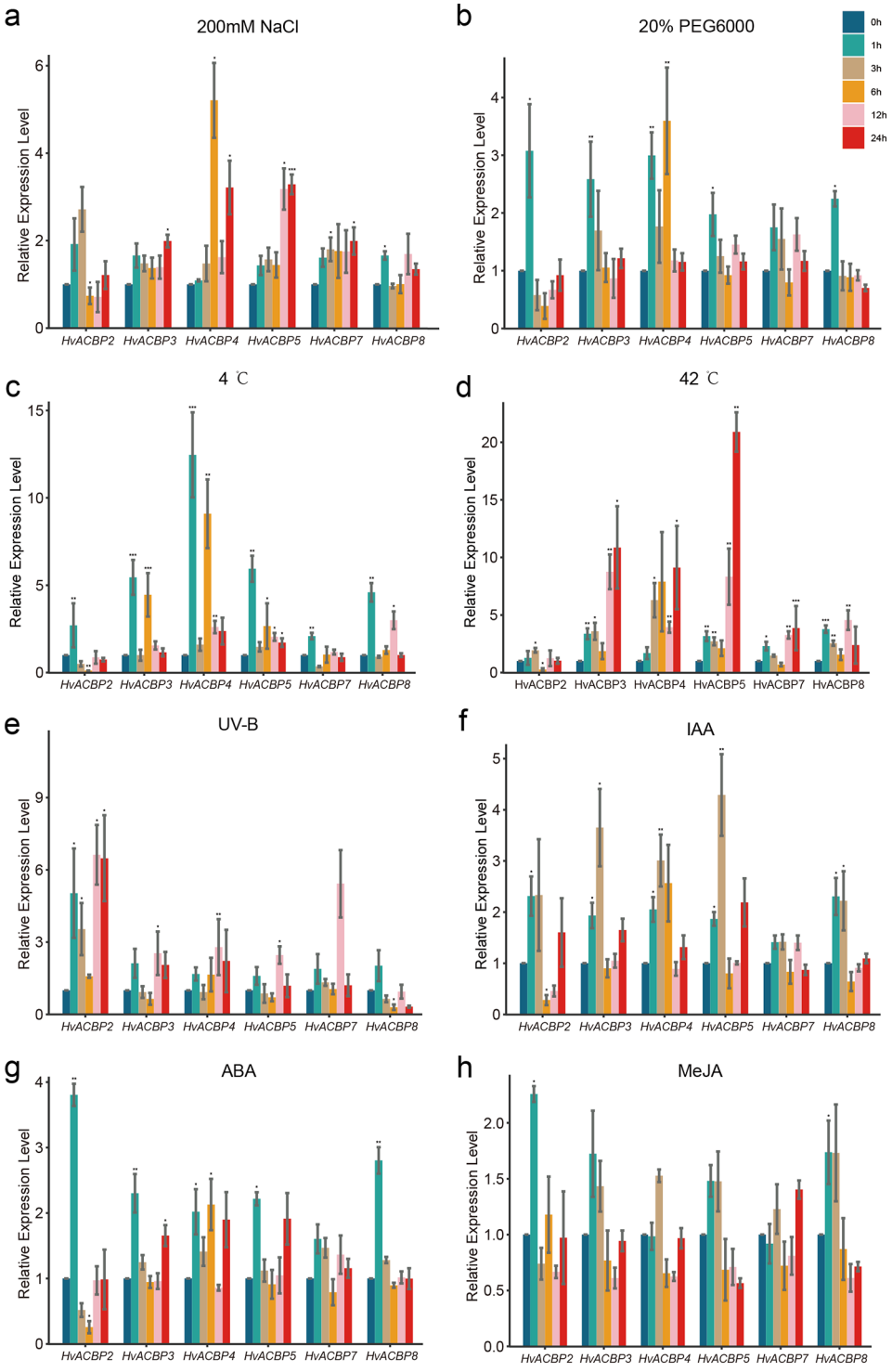

Fig. 6 Acyl-CoA-binding protein gene (*ACBP*) expression in *Hordeum vulgare* L. under hormone treatment and abiotic stress.Stress was induced by hormone treatment with indoel-3-acetic acid (IAA) at 0.15 μmol/L or abscisic acid (ABA) at 100 μmol/L or methyl jasmonate (MeJA) at 100 μmol/L, and *ACBP* expression was measured after 1, 3, 6, 12, and 24 h. Responses to abiotic stressors were measured 1, 3, 6, 12, and 24 h after treatment with 200 mM NaCl, 20% polyethylene glycol (PEG6000), or 30 μW/cm^2 UV radiation (growth lamp was turned off after 12 h of UV treatment, and no UV treatment was provided after 12 h). Cold and heat stress were applied via treatment at 4 °C and 42 °C, respectively. The expression levels of genes in the control were defined as "1". The values are presented as the means of three replicates. "*" as significant at $P \le 0.05$, "**" as significant at $P \le 0.01$, "***" as significant at $P \le 0.001$

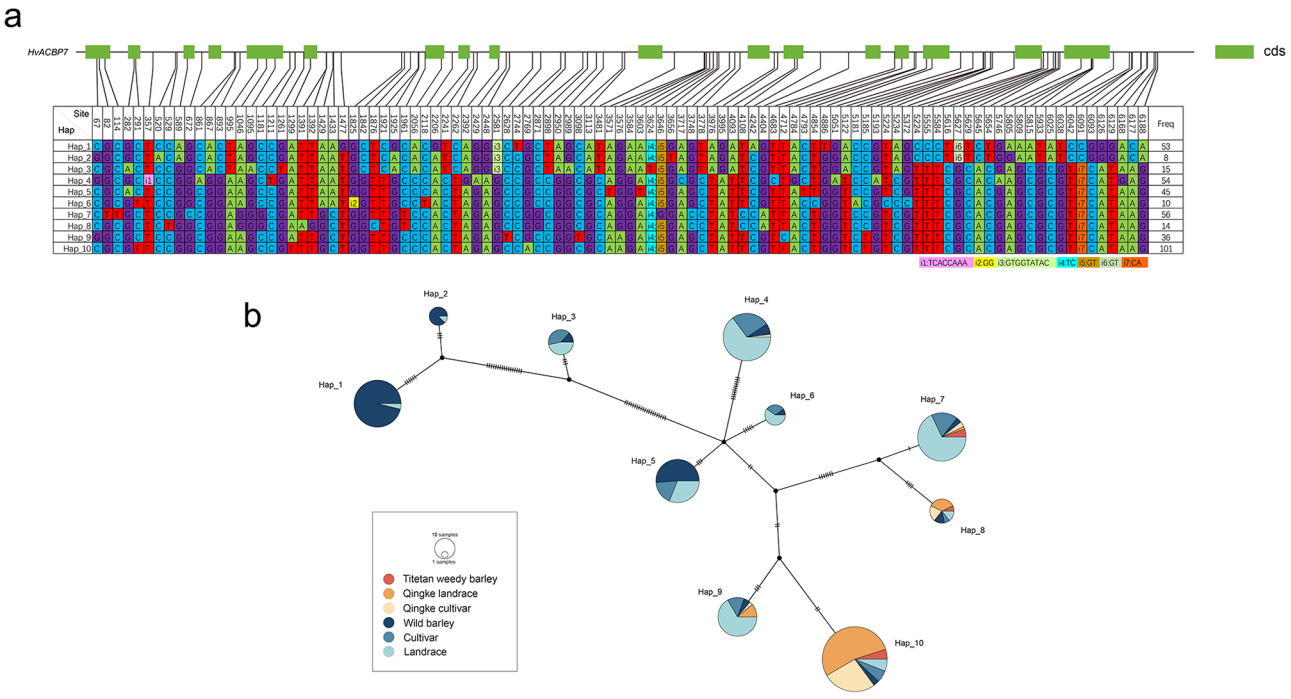

Fig. 7 *HvACBP7* haplotype analysis. (**a**) Single-nucleotide polymorphisms (SNPs) were identified for haplotype analysis. Light-green rectangles: exons; straight lines: introns; SNPs in the promoter and coding sequence are shown in the upper table. Indels are represented by i1–7. (**b**) Proportions of each barley resource having each haplotype, with haplotype network analysis

Table 2 Haploid frequency distribution data

Haplotype	Titetan_weedy_barley	Qingke_landrace_barley	Qingke_cultivar_barley	Wild_barley	Cultivar_barley	Landrace_barley
Hap_1	0	0	0	51	0	2
Hap_2	0	0	0	7	0	1
Hap_3	0	0	0	2	6	7
Hap_4	0	0	1	4	14	35
Hap_5	0	0	0	23	8	14
Hap_6	0	0	0	1	3	6
Hap_7	3	1	2	2	10	38
Hap_8	1	5	3	2	1	2
Hap_9	0	4	1	2	5	24
Hap_10	5	54	27	3	6	6

HvACBP4, which are tandem repeat genes, show similar expression patterns, suggesting their conserved and redundant functions in regulating seed development. These similar expression profiles suggest that *HvACBPs* are involved in barley growth and development. For example, the small ACBP, *AtACBP6*, of *Arabidopsis* is highly expressed in pollen, microspores, and trichome cells [14], whereas *OsACBP1/2* in rice is highly expressed in developing grains [13, 28]. The variation in AtACBP6 affects lipid accumulation in Arabidopsis seeds [30]. The specific high expression of *HvACBP1/8* in grains suggests their function in regulating seed development.

Analysis of cis-elements indicated that *HvACBPs* are involved in the cross-talk of phytohormones, and the effects of hormone treatment also confirmed that *HvACBPs* are involved in the plant hormone response, which is crucial for various aspects of plant growth, development, and stress responses, these results highlighting the potential regulatory functions of *HvACBPs* in barley growth, development, and environmental adaptation.

ACBPs show functional conservation and sub-functionalization in paralogs in responses to abiotic stress

ACBPs are vital in plant responses to various stressors. In *Arabidopsis*, overexpression of *AtACBP1* enhances the sensitivity to salt stress by activating ABA expression via *AtAREB1* [46]. In soybeans, salt tolerance is mediated by *GmACBP3* and *GmACBP4* (Class II) via alternative splicing [39]. The expression level of the *OsACBP4* gene in

rice rapidly increases under high-salt treatment. In barley, *HvACBP4* responds strongly to salt stress, suggesting a role for this gene salt tolerance, whereas *HvACBP3* reacts more slowly, indicating distinct roles for these duplicated genes. In *Arabidopsis*, *AtACBP2* is closely associated with drought response via regulation of ABA-dependent guard-cell closure to improve drought resistance [47–50]. *AtACBP3* and *AtACBP4* are associated with drought responses [51–53]. *OsACBP4* and *OsACBP5* in rice; as well as *GhACBP1*, *GhACBP3*, and *GhACBP6* in cotton, are induced by drought [27, 54, 55]. In barley, the six detected *HvACBPs* upregulated under salt treatment, suggesting that they are related to drought tolerance in barley. *AtACBP6* overexpression enhances stability under cold stress, whereas *AtACBP1* knockout enhances freezing resistance in *Arabidopsis* [29, 56]. Under cold stress, all six *OsACBP*s were downregulated [27]. In cotton, *GhACBP1*, *GhACBP3*, and *GhACBP6* respond to low-temperature stress, whereas *GhACBP6* exhibits significant regulation under heat stress [54]. We found that the *HvACBPs* exhibited varied responses to heat and cold but responded more strongly to heat than to cold. Therefore, *HvACBPs* respond rapidly and in diverse manners to temperature stress, highlighting their importance in temperature adaptation in barley. UV radiation induces membrane damage [57]. Our findings reveal that *ACBPs* participate in repairing UV damage in barley.

The ACBP gene shows sub-functionalization in paralogs. Among Ankry-ACBP subfamily genes, in *Arabidopsis, AtACBP1* is only expressed in the trichomes, whereas *AtACBP2* is only expressed in guard cells, indicating that *AtACBP2* is associated with stomatal opening and the drought response [21, 22, 52, 47]. In barley, *HvACBP3* and *HvACBP4* are tandem repeat genes; *HvACBP3* expression was higher during growth and breeding, whereas *HvACBP4* is more strongly responsive to abiotic stress. *HvACBP2*, a small ACBP in barley, showed greater sensitivity to UV-B than did *HvACBP8*. These results indicate that ACBPs underwent sub-functionalization in paralogs during species evolution.

The Tibetan Plateau, the predominant cultivation region for Tibetan barley, which was domesticated from eastern domesticated barley, is characterized by harsh environmental conditions, including low temperatures, substantial diurnal temperature fluctuations, low latitudes, high elevations, low-density air, intense solar radiation, and high UV-B exposure [48]. ACBPs constitute a well-conserved family of proteins in eukaryotes that are important in stress responses and development [49]. The chromosomal region containing *HvACBP7* was under selective pressure during the domestication of barley in the Tibetan region [50]. Analysis of *HvACBP7* and *HvACBP8* haplotypes showed that during the domestication of barley, *HvACBP7/8* were selected to adapt to the unique environment of the Tibetan Plateau, but their amino acid sequences remained conserved, suggesting the importance and conservation of *ACBPs* in the growth, development, and environmental adaptation of barley; however, the variation of the cis-elements in the promoter requires further analysis.

Conclusions

HvACBPs respond to multiple stressors, underscoring their crucial role in stress resilience in barley, as well as revealing the importance of ACBPs in plant growth, development, and stress adaptation, particularly abiotic stress. Our results provide a foundation for the further functional analysis of *HvACBP* and theoretical basis for improving barley via breeding. We determined mechanisms of plant ecological adaptation in extreme environments.

Materials and methods

Plant material, growth, and abiotic stress conditions

Barley seeds (*H. vulgare* L., Morex) were provided by the Chinese Academy of Agricultural Sciences. The seeds were disinfected with 0.1% NaClO and 75% ethanol, rinsed with distilled water, and placed on moist filter paper into dark for 24 h. The germinated seeds were transferred to hydroponic containers and grown under 24 °C with a 14 h light/10 h dark cycle. The nutrient solution (1/2 Hoagland solution) was replaced every 3 d. Different treatments were applied after 4 weeks.

The 4-week-old seedlings were subjected low-temperature stress (4 °C), high-temperature stress (42 °C), 200 mM NaCl application, 20% polyethylene glycol (PEG 6000) treatment, UV radiation at 30 μW/cm2, 0.15 μmol/L IAA treatment, 100 μmol/L ABA treatment, and 100 μmol/L MeJA treatment. The seedlings were harvested at 0, 1, 3, 6, 12, and 24 h post-treatment, immediately frozen in liquid nitrogen, and stored at −80 °C for subsequent experiments.

Sequence retrieval and identification of *ACBPs*

We performed a BLASTP search using the *ACBP* family from *Arabidopsis* (https://www.arabidopsis.org/) in the barley genome database (http://barlex.barleysequence.org/) with an e-value of 10^{-10}. We also downloaded the ACBP domain file (PF00887) from the Pfam website (http://pfam.xfam.org/) and uploaded it to the HMM website (https://www.ebi.ac.uk/Tools/hmmer/search/hmmsearch) to search for genes in the barley genome potentially containing this conserved domain. Duplicate genes were removed by comparing the results of the two different searches. The remaining genes were validated for the presence of the ACBP-type domain using the CD search (https://www.ncbi.nlm.nih.gov/cdd/) and SMART (http://smart.embl-heidelberg.de/) websites.

HvACBP structure and conserved motif analysis

The structures of the *HvACBPs* were deduced from the coding and genomic sequences using a gene structure displayer (https://gsds.gao-lab.org/). Conserved motifs in each HvACBP were analyzed using MEME Suite (https://meme-suite.org/meme/tools/meme).

Analysis of cis-acting elements in *HvACBP* promoters

A region approximately 2000 bp upstream of the start codon (ATG) was investigated from the reference genome sequence of MorexV3, Cis-acting elements were predicted using the PlantCare website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Expression patterns of *HvACBP* family in different tissues

HvACBPs transcriptome data (fragments per kilobase of transcript per million mapped fragments) were downloaded from the barley genome database (http://barlex.barleysequence.org/). Transcriptome data were normalized by logarithms, and the expression heat maps were drawn using R4.2.1 software.

Phylogenetic analysis of *HvACBPs*

To calculate the correlations between *HvACBPs* and their counterparts in various species, the ACBP protein sequences of Arabidopsis, rice, maize, soybean, sorghum, wheat, and barley were subjected to multiple sequence alignment using the Clustal W tool. A phylogenetic tree was constructed using the maximum likelihood (ML) method via MEGA 7, employing the WAG+G model. The resultant phylogenetic tree was further refined and visualized using iTOL v6 (https://itol.embl.de/).

Protein physical and chemical properties and prediction of secondary and tertiary structures

We used ExPASy (https://web.expasy.org/protparam/) to compute the amino acid content, isoelectric point (pI), molecular weight (average), instability coefficient, aliphatic index, and average hydrophobicity index. The SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and PredictProtein (https://predictprotein.org/) websites were used to predict the secondary structure of the proteins. The tertiary structure of HvACBP proteins was predicted using the SWISS-MODEL Interactive Workshop (https://www.swissmodel.expasy.org/interactive).

RNA isolation, cDNA synthesis, and quantitative real-time PCR analysis

Total RNA extraction was performed using the FastPure Universal Plant Total RNA Isolation Kit (Vazyme, Nanjing, China). RNA was reverse-transcribed into cDNA using the HiScript III RT SuperMix for qPCR (+gDNA wiper) kit (Vazyme, Nanjing, China). Quantitative real-time PCR (qRT-PCR) was conducted using Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). The internal reference gene used for normalization was *HvGAPDH* (*HORVU.MOREX.r3.7HG0703580*). Each sample contained 10 μL of a reaction mixture composed of 5 μL of 2×PCR mix, 1 μL of primer mixture (10 μmol L-1 each upstream and downstream primers), 1 μL of cDNA, and 3 μL of deionized water. The PCR thermal cycling conditions were as follows: initial denaturation at 95 °C for 30 s followed by 35 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s, and extension at 95 °C for 15 s. A final extension step was performed at 60 °C for 60 s, followed by melt curve analysis at 95 °C for 15 s. The experiment was conducted in triplicate. The primers are listed in Supplementary Data.

Haplotype analysis of HvACBP7 and HvACBP8

All single-nucleotide polymorphisms and insertions–deletions used for haplotype analysis were obtained by resequencing of Tibet barley [55]. Haplotype identification was performed using DnaSP_V5 and haplotype network analysis was performed using Arlequin.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-04944-6.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

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Author contributions

HC performed the bioinformatics analysis. HC and MM performed qRT-PCR analyses and drafted the manuscript. MG, SL, and ML planted the crops and collected the materials. GG and GX supervised the research and modified the manuscript. All authors have read and approved the final manuscript.

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Data availability

All datasets supporting the results of this study are included within the article and its supplementary information.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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