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Genome-wide Identification of *HvPP2C* Genes and Expression Profiling in Response to Cold and Heat Stresses in Barley (*Hordeum vulgare L.*)

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Abstract

Background: Protein phosphatases (PP2Cs) are the main classes of protein phosphatases in plants, having pivotal functions in different plant stages and abiotic stresses. The *PP2C* genes are suggested to have critical roles in barley by exposure to heat and cold treatments.

Objectives: We surveyed *HvPP2C* genes from the barley genome in the present study. Phylogenetic analysis, functional annotation, synteny analysis, chromosomal distribution, prediction of cis-elements, and gene expression of *HvPP2Cs* under abiotic stresses were studied.

Methods: In this study, *HvPP2Cs* of barley were surveyed using bioinformatics tools, and *HvPP2C* gene expression profiles under cold and heat stresses in 3 tissues (root, leaf, and stem) were analyzed.

Results: In this study, 61 *HvPP2C* genes were detected from barley, and a phylogenetic tree was divided into 13 subfamilies. The results of chromosomal distribution showed that the *HvPP2C* genes were located on 7 chromosomes. Real-time quantitative reverse transcription PCR (qRT-PCR) analysis of *HvPP2Cs* showed that they were largely expressed in different tissues (root, stem, and leaf) in the Azaran and Jolge barley cultivars. In Azaran, the *HvPP2C* gene expression increased in response to cold stress, whereas, in the Jolge cultivar, the *HvPP2C* gene expression increased in response to heat stress. The analysis of synteny revealed that HvPP2C24b with HvPP2C24a, HvPP2C24 with HvPP2C24a and HvPP2C24b; HvPP2C5 with HvPP2C5a, and HvPP2C41 with HvPP2C41a were paralogous.

Conclusions: Results revealed a broad understanding of the *HvPP2C* gene family in barley, which can be valuable for the functional description of *HvPP2Cs* in plant response to abiotic stresses.

Keywords: Protein Phosphatases, Paralogous, Synteny Analysis, Gene Duplication

1. Background

Phosphorylation and dephosphorylation of proteins are vital post-translational modifications, acting as a reversible change to control different functions of proteins, such as protein-protein interaction and protein localization in response to different stresses such as drought, salt, and cold (1). It is indispensable to probe into the identification and functional description of the *PP2C* gene family, cementing the base for understanding its essential molecular mechanism in stress signaling (2, 3). Protein phosphorylation is one of the important steps that helps regulate some physiological and biochemical reactions. PPs carry out dephosphorylation. Plant protein phosphatase 2Cs (PP2Cs) are important in plant hormone signaling, growth processes, and environmental stress responses (4). The high proportion of *PP2C* genes reveals their evolutionary consequence, prerequisite, and participation in various plant and cellular functions. For instance, it is demonstrated that *TaPP2C* genes are associated with wheat's developmental stages and stress responses (5). The high proportion of *PP2C* genes indicates their evolutionary significance, requirement, and involvement in diverse plant cellular functions (6). The *PP2C* genes have been termed a regulator of water deficit tolerance and act as a negative regulator in the ABA-signaling in plants (7). Transgenic studies confirmed the expression level of the *ZmPP2C10* gene as a negative regulator in drought stress tolerance in maize (5). Evaluating two mutants, abi1-1 and abi2-1, indicated

that two *PP2C* genes are involved in different physiological processes after exposure to abiotic stimuli containing salt, drought, and cold (8).

Gene duplication, critical for gene family development, is considered a type of genome expansion formed through block and tandem duplication (9). Paralogous gene pairs located on the same chromosome are considered as tandem duplication. Gene duplicates are prevalent in plants and, in some cases, contribute to evolutionary novelty. Gene duplication allows the detection of paralogs, where their presence in the genome leads to identifying the gene family.

2. Objectives

This study identified 61 *HvPP2C* genes from the barley genome and grouped them into 12 subfamilies. Comprehensive analyses of functional annotation, gene duplications, chromosomal distribution, and phylogeny of these *HvPP2Cs* were further carried out. To validate in silico analysis, their expression profiles were also investigated by qRT-PCR under cold and heat treatments in 3 different tissues (root, stem, leaf). The results presented here provide a foundation for further functional characterization of *HvPP2C* genes in this model species.

3. Methods

3.1. Physicochemical Characteristics, Functional Annotation, Phylogenetic Analysis, and Chromosomal Distribution of the HvPP2C Genes

As explained earlier, two techniques were utilized to detect putative *HvPP2C* genes in barley. In the first technique, a protein homology search with accessible PP2C proteins from *Arabidopsis* and rice was performed. The second technique included retrieving the PP2C protein sequence using hidden Markov model (HMM) analysis, with Pfam number PF00481 containing the tubule normal domain from the Pfam HMM library. The *Arabidopsis* and rice protein sequences were taken from TAIR and RAP-DB databases. The known *Arabidopsis* PP2C protein sequences were taken from NCBI and utilized as query sequences for the tBLASTn program in barley to search for similar protein sequences. All putative sequences were approved with the SMART database and interproscan. The remaining 10 non-redundant candidates were recognized as HvPP2C proteins. The ExPASy protoparam (www.expasy.org) was used to calculate the theoretical isoelectric point (pI) and molecular weight (Mw) of each HvPP2C protein. The protein sequences of HvPP2Cs were uploaded to the online annotation tool of Mapman (www.plabipd.de/portal/mercator-sequence-annotation) for functional annotation and categorization using default parameters. HvPP2C protein sequences were aligned using the ClustalW function of MEGA 7.0. Then, the phylogenetic tree between barley, rice, field mustard, and *Arabidopsis* was determined using the neighbor joining (NJ) algorithm with 1000 repeated bootstrap test parameters. To locate *HvPP2C* genes on barley chromosomes, *HvPP2C* genes were placed on each chromosome. *HvPP2C* genes were mapped on all chromosomes with MapChart (10).

3.2. Detection of Paralogous and Prediction of TFBS HvPP2C Genes in Barley

Similar gene pairs to HvPP2C proteins were identified by Blastp with more than 85% identity from Ensembl Plants. Similar genes to *HvPP2C* were visualized using the Circos program (http://mkweb.bcgsc.ca/tableviewer/visualize/). After determining the genomic sequence of each gene, 2000 bp upstream of the transcription start region of *HvPP2C* was recovered. Transcription factor binding site (TFBS) prediction was also done using the PlantCARE website.

3.3. Barley Growth Under Heat and Cold Treatments

To study the gene expression of *HvPP2Cs* genes under abiotic stress, seeds of Azaran and Jolge cultivars were grown in peat moss-filled pots and were kept under greenhouse conditions: Day/night temperatures were maintained at 25°C, with a 16/8 h light/dark period. Young root stems and leaves from the 2-week-old seedlings were harvested for tissue-specific expression analysis under cold and heat stresses and treated for four hours at cold (4°C) and heat stress (42°C). Different plant tissues were harvested 4 hours after abiotic stresses and immediately stored at -80°C for further analysis. Barley cultivars that grew normally were used as normal replicates. All experiments were repeated 3 times.

3.4. RNA Extraction and Quantitative Real-time PCR Analysis

Total RNA was extracted from roots, stems, and leaves of cold and heat under stress conditions at the seedling stage four hours after heat and cold treatments using the RNA-Plus kit (Sinaclone, Iran). The purity and concentration of RNA were determined by NanoDrops, and its quality was confirmed using 1% agarose gel analysis. Then, cDNA synthesis was performed according to the instructions of the Easy cDNA Synthesis Kit, Iran. Three replicates were performed to analyze each gene. Barley *actin* gene was used as a reference gene. Gene expression of five *HvPP2C* were analyzed under cold and heat stresses. All

primers used in gene expression analysis are listed in Table 1. Primers were designed based on *HvPP2C* genes using the Oligo program. Real-time PCR (qPCR) was performed on an ABI 7500 using SYBR Green Supermix as described in the manufacturer's instructions. Relative expression was determined through the delta-delta $2^{-\Delta\Delta Ct}$ technique. RT-qPCR was performed to determine the expression profile of five *HvPP2C* genes using different tissues under heat and cold stresses.

Table 1. Primers and Their Sequences Were Used for This Study's Gene Expression Analysis of *HvPP2C*

Primer Name	Sequence (5' - 3')	Annealing Temperature
Hvpp2c16		58
F	CAGGGACGGGGATCAAGTTGT	
R	GCCAGAGTCACTTGCACGA	
Hvpp2c37		60
F	TGCCGTGCTCCTACCATGA	
R	CATCCCTCTCTGTCCTCTGTC	
Hvpp2c70		59
F	TGTGGCAAATGCCGGGGATTC	
R	CCACCAGCGTTCTGAATCCTCTC	
Hvpp2c41		59
F	CACACTGCTGAGCTAGTTGTCC	
R	CTTCACGAGTTACCTGCAAC	
HvPP2C42		60
F	TGTGCGTAGAACCACCGCA	
R	CGACTGCATGGCTCATAGGATTC	
HvActin		58
F	GGTCCATCCTAGCCTCACTC	
R	GATAACAGCAGTGGAGCGCT	

4. Results and Discussion

Based on the bioinformatics analysis, the 61 *HvPP2C* genes were detected. These proteins' detailed information (biochemical properties) are listed in Table 2. Sequence analysis showed that the deduced HvPP2C proteins' length varied from 51 amino acids (HvPP2C6a) to 1266 amino acids (HvPPC61). The predicted MW and pI ranged from 4.56 kDa (HvPP2C36) to 141.01 kDa (HvPP2C61) and from 4.6 (HvPP2C6) to 9.26 (HvPP2C56), respectively (Table 2). When the domain and motifs of HVPP2C proteins are similar, they will be located in the same subfamily, suggesting functional similarities for the genes in the same subfamily (9).

4.1. Phylogenetic Analysis, Chromosomal Distribution, and Duplication of HvPP2C Genes

To assess the phylogenetic relationships of the 61 HvPP2C proteins in barley, we performed a phylogenetic analysis using MEGA7 software (Figure 1). The phylogenetic tree showed that the *PP2C* genes can be divided into 12 subgroups. Previous studies showed that *MtPP2C* genes in *Arabidopsis* and rice were divided into 13 subfamilies (9, 11, 12). Some researchers have revealed that *HvPP2C* genes from monocots can be grouped into 13 subfamilies based on their domains (9, 13). The results show that the exact function of the genes of the subfamilies is due to the conserved motifs.

Most HvPP2C proteins were classified in the same subfamily (Figure 1B). Most proteins from different species were placed in the same cluster, had similar conserved motifs and functional activity, and were paralogous (14). Motifs 1, 2, and 3 were available in most subfamilies, and motif 1 was present in 61 HvPP2C proteins except for HvPP2C58 and HvPP2C60 (Figure 1B). These outcomes propose that the precise functions of various subfamily genes may result from conserved motifs.

Afterward, the 61 *HvPP2C* genes were drawn using the MapChart software, and sixty-one *HvPP2C* genes were located across all seven chromosomes, ranging from 3 to 12 per chromosome (Figure 2). The number of *HvPP2Cs* on each chromosome varied, and chromosome 7 contained the largest number of *HvPP2C* members with 12 genes. However, the least number of genes was detected on chromosome 6, containing only three *HvPP2C* genes. In addition, seven *HvPP2C* were located on chromosomes 1 and 4. The 6 and 10 *HvPP2C* genes were located on chromosomes 2 and 3, respectively. Finally, nine *HvPP2C* were distributed on chromosome 5. Based on the research on rice, *Arabidopsis*, and *B. distachyon*, it has been shown that *PP2C* gene families mainly expanded through whole-genome and chromosomal segment duplications (9, 15). As shown in Fig 2, these 19 pairs of duplicated *HvPP2C* genes are distributed on chromosomes 1, 2, 3, 5, 6, and 7 but not on chromosome 4 (Figure 2).

4.2. Paralogous Genes Study and Gene Duplication in HvPP2C

This study used synteny analysis to detect paralogous *HvPP2C* genes in the barley genome (Figure 3). Based on the results, five barley genes showed high similarity (identity 80%) and were implicated in paralogous. The analysis of synteny revealed that HvPP2C24b with HvPP2C24a, HvPP2C24 with HvPP2C24a and HvPP2C24b, HvPP2C5 with HvPP2C5a, and HvPP2C41 with HvPP2C41a were paralogous. It was observed that tandem duplicates occurred within *HvPP2C24b*, *HvPP2C24*, *HvPP2C24a*, *HvPP2C37*, *HvPP2C6b*,

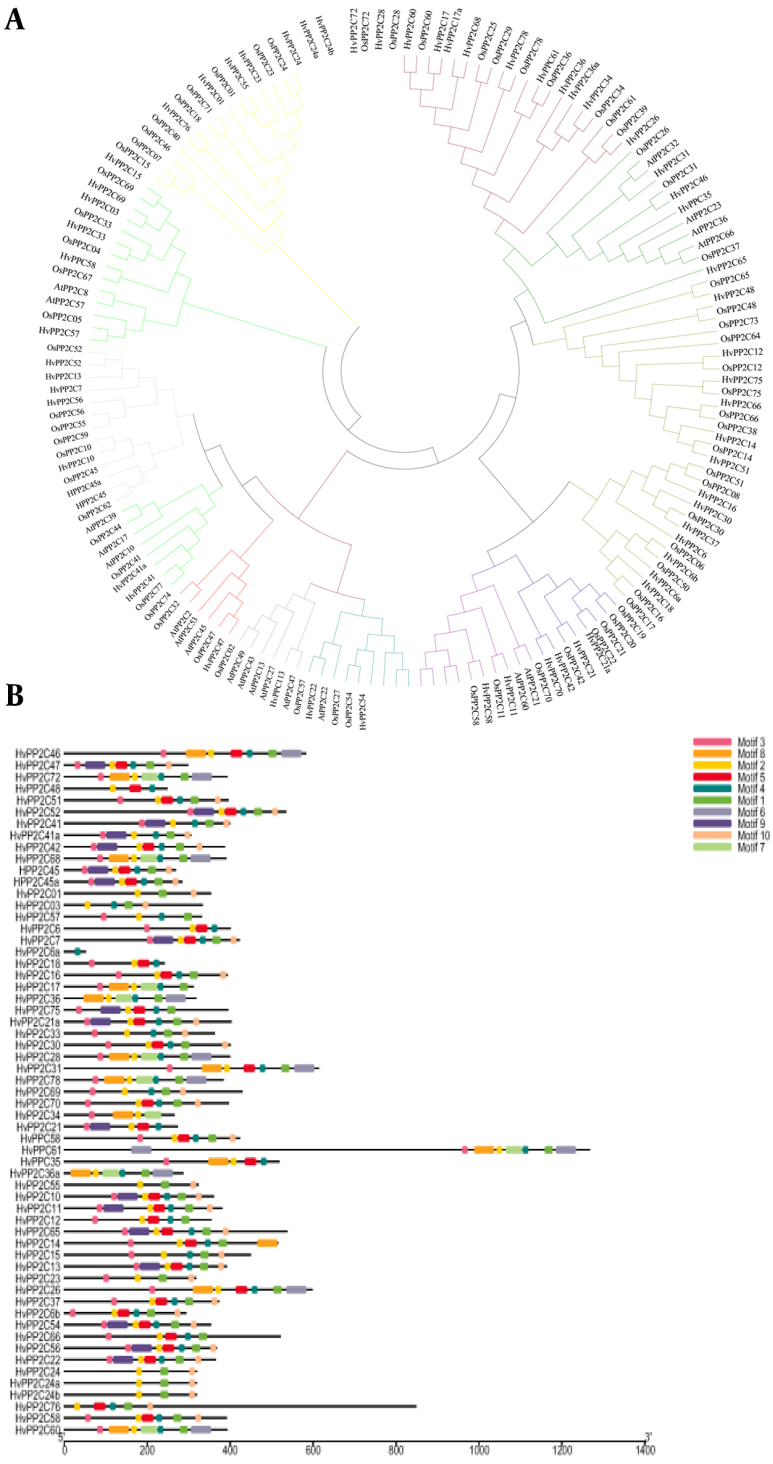

Figure 1. Phylogenetic tree of *HvPP2C* genes constructed by the neighbor-joining (NJ) method (A) in MEGA7.0 and conserved motifs using TBtools (B).

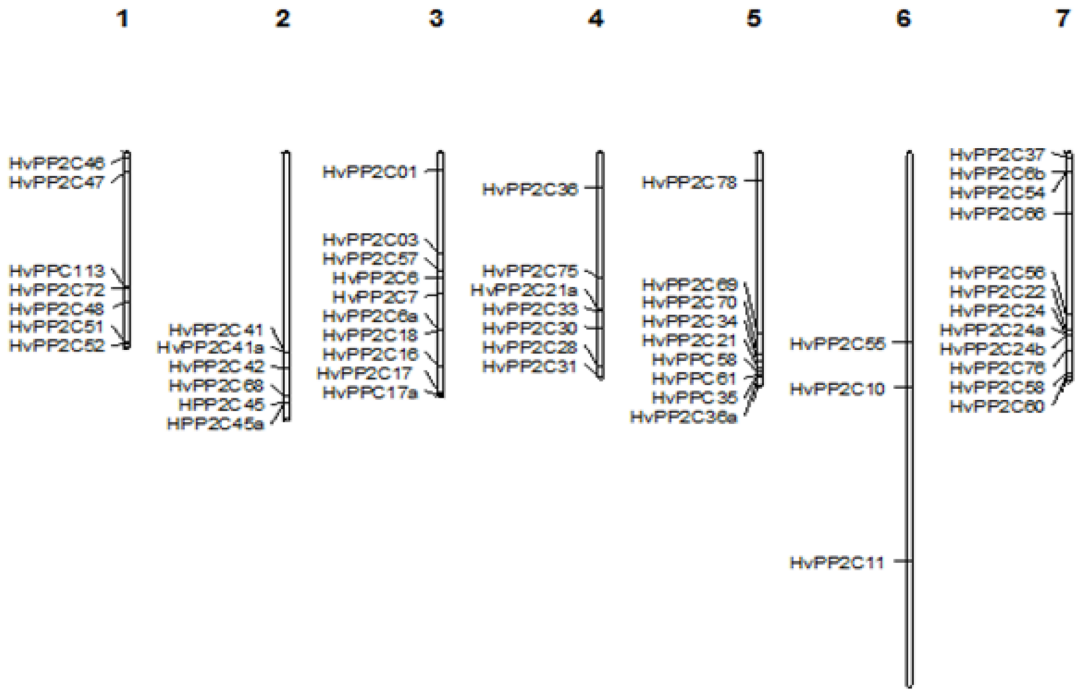

Figure 2. Chromosomal Location and Expansion Patterns of *HvPP2C* Genes Identified in Barley.

HvPP2C12, *HvPP2C61*, *HvPP2C65*, *HvPP2C46*, *HvPP2C45*, *HvPP2C45a*, *HvPP2C48* and *HvPP2C54* genes. Also, block duplication occurred within *HvPP2C11*, *HvPP2C16*, *HvPP2C42*, and *HvPP2C60* genes. Analysis of *HvPP2C* indicated that genome duplication and tandem duplication play key roles in barley genome enlargement (16).

4.3. Prediction of TFBS in the HvPP2C Genes

In the promoter regions of *HvPP2C* genes in barley, TFBs, TCA, and ARE-responsive elements were investigated. In this study, TFBS included ARE (anaerobic responsive elements), TCA elements, TATA-box, and CAAT-box in promoter regions (Appendix 1). *HvPP2C17a* and *HvPP2C37a* genes had the highest TFB in their promoter regions (Appendix 1). Previous studies have shown that *HvPP2C* responds to various stresses (which stress) and stimuli in *Arabidopsis*, tomato, and soybean (9, 17). ARE regulatory elements are important for the induction of anaerobic respiration (11). Enrichment of the TCA element in the promoter regions of most *HvPP2C* genes suggests comprehensive transcriptional regulation by *HvPP2C* itself, indicating a complex regulatory network among them. According to a previous report, TCA elements (salicylic acid elements) have also been associated with abiotic stresses (18). This may be due to the presence of cis-elements in the promoter region of these genes (19). Our findings demonstrated that *HvPP2C*, *HvPP2C37*, and *HvPP2C37* genes have multiple binding sites in the promoter regions and play key roles in response to various environmental stresses. Results also showed that *HvPP2C* genes regulate many important developmental processes in plants, such as seedling and reproductive stages, and are involved in abiotic resistance, such as heat and cold stresses.

4.4. Functional Annotation

In barley, the largest percentage of detected proteins was implicated in protein metabolism (80%), and the second largest class was involved in hormone metabolism (8.57 %) (Figure 4). The percentage of mitochondrial 2-oxoglutarate/malate carrier protein (misc) was 7.14%. Most of the *HvPP2C* genes in the barley were involved in protein metabolism (protein degradation, post-translational modification, and protein synthesis) and hormone metabolism. Different kinds of HvPP2Cs were involved in protein degradation-related proteins, implying that the PP2Cs might play major roles in protein degradation during the barley seedling stage. Our findings showed that *HvPP2C* genes play a key role in abiotic stresses like cold and heat stresses. The HvPP2C

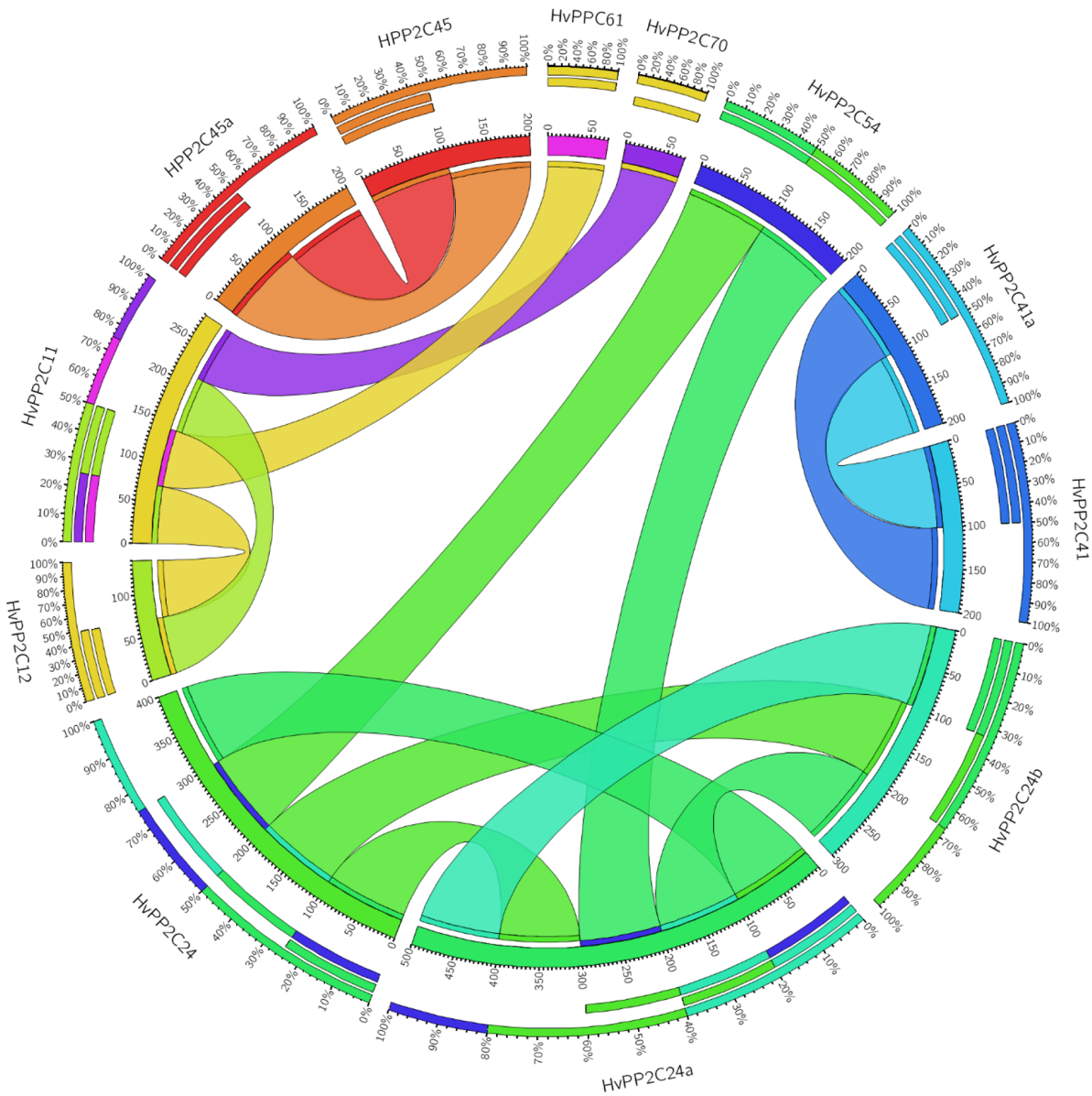

Figure 3. Paralogous relationships of *HvPP2C* genes visualized by Circos database using 61 *HvPP2C* genes.

had TFBs responsive to hormonal stress, indicating its role in response to abiotic stresses.

4.5. Expression Profiles of HvPP2C Genes 3 Tissues Under Cold and Heat Stress Conditions

In Azaran and Jolge cultivars, the expression levels of five *HvPP2C* were determined under heat and cold stress conditions, most of which show a wide range of expression. To evaluate the expression profile of 5 *PP2C* genes and to evaluate the expression profile under heat and cold stress conditions, we utilized qRT-PCR analysis in different tissues: root, stem, and leaf. In the Azaran cultivar, *HvPP2C37* and *HvPP2C70* genes showed low expression in the root under cold stress conditions (Figure 5A), whereas *HvPP2C16* and *HvPP2C42* genes showed increased expression in the stem tissue (Figure 5A). In the leaf tissue, an increased expression of *HvPP2C37*, *HvPP2C41*, *HvPP2C42*, and *HvPP2C70* genes was also shown (Figure 5A). Similarly, the expression levels of *MtPP2C* genes, such as MtPP2C37, were altered significantly during cold stress

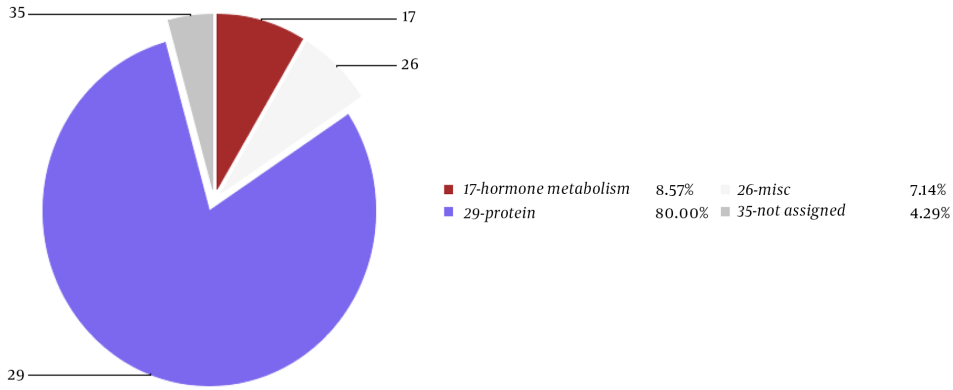

Figure 4. Functional annotation significantly enriched pathways involving the *HvPP2C* proteins in barley. Four BIN codes (17, 29, 26, and 35) were assigned to the functional annotation of *HvPP2Cs*.

(9). In the Azaran cultivar, the *HvPP2C* gene showed decreased expression, except for the gene *HvPP2C70*, which showed increased expression in the stem tissue under cold stress (Figure 5A). Also, *HvPP2C37* and *HvPP2C41* genes were up-regulated in stem tissue under heat stress (Figure 5B). All *HvPP2C* genes showed reduced expression in leaves under heat stress (Figure 5B). Maximum expression was observed for the *HvPP2C16* gene in the root compared with the other genes. It has been reported that among the BdPP2Cs studied, *BdPP2C37* was increased under heat and cold stresses. Previous study has shown that *BdPP2C70* strongly enhanced expression levels in response to abiotic stress, indicating that they may contribute in response to ABA (10). These results showed that the *HvPP2C16* gene can be used as a molecular marker in barley improvement.

The expression profiles of *HvPP2C* genes under heat and cold stresses revealed differential and overlapping expression patterns. In the Jolge cultivar, only the *HvPP2C42* gene showed increased expression in response to cold stress in the root (Figure 5C), whereas the *HvPP2C70* gene showed increased expression in response to heat stress in the shoot tissue (Figure 5D). Further, *HvPP2C16*, *HvPP2C41*, and *HvPP2C42* genes were expressed in the leaves (Figure 5D). On the other hand, *HvPP2C16*, *HvPP2C41*, and *HvPP2C42* genes were increased in the root in response to heat stress (Figure 5D). In the leaf tissue of the Jolge cultivar, *HvPP2C16*, *HvPP2C41*, *HvPP2C42*, and *HvPP2C70* genes showed increased expression under heat stress (Figure 5D). Various expression patterns of *HvPP2C* genes may suggest their various roles in response to heat and cold stress conditions. In contrast to our results, the *MtPP2C41* gene was decreased in response to the cold stress in *Medicago truncatula* (9).

5. Conclusions

In this study, 61 *HvPP2C* genes were detected in barley, and we surveyed their phylogenetic relationships, chromosomal locations, gene duplications, functional annotation, and prediction of TFBS. Also, bioinformatics analysis of these *HvPP2C* genes and their gene expression profiles were performed. Our results showed that the genes and groups with similar protein motifs had similar origins and possibly similar functions. These results provided insights into the evolutionary relationships of HvPP2C in the barley. *HvPPT2C* genes had different functions, indicating the presence of conserved domains in these genes. Based on the *HvPP2C* gene expression pattern, the *HvPP2Cs* gene showed different patterns in response to abiotic stresses. These results showed that the *HvPP2C16* gene can be used as a molecular marker in barley improvement. However, the function of other subfamily PP2C in plant resistance to abiotic stress is poorly understood and needs further investigation. The results of our study establish a foundation for future studies on the functions of *HvPP2C* genes in plant's response to abiotic stresses and provide a basic understanding that may allow us to elucidate the potential functions of *HvPP2C* genes under cold and heat stress conditions in barley.

Footnotes

Authors' Contribution: A.S. carried out a literature search designed and edited the manuscript. Z.H. wrote and drew all the figures of the manuscript AS, and ZH authors contributed to the final manuscript. All authors have read and approved the manuscript.

Clinical Trial Registration Code: NCT00000161.

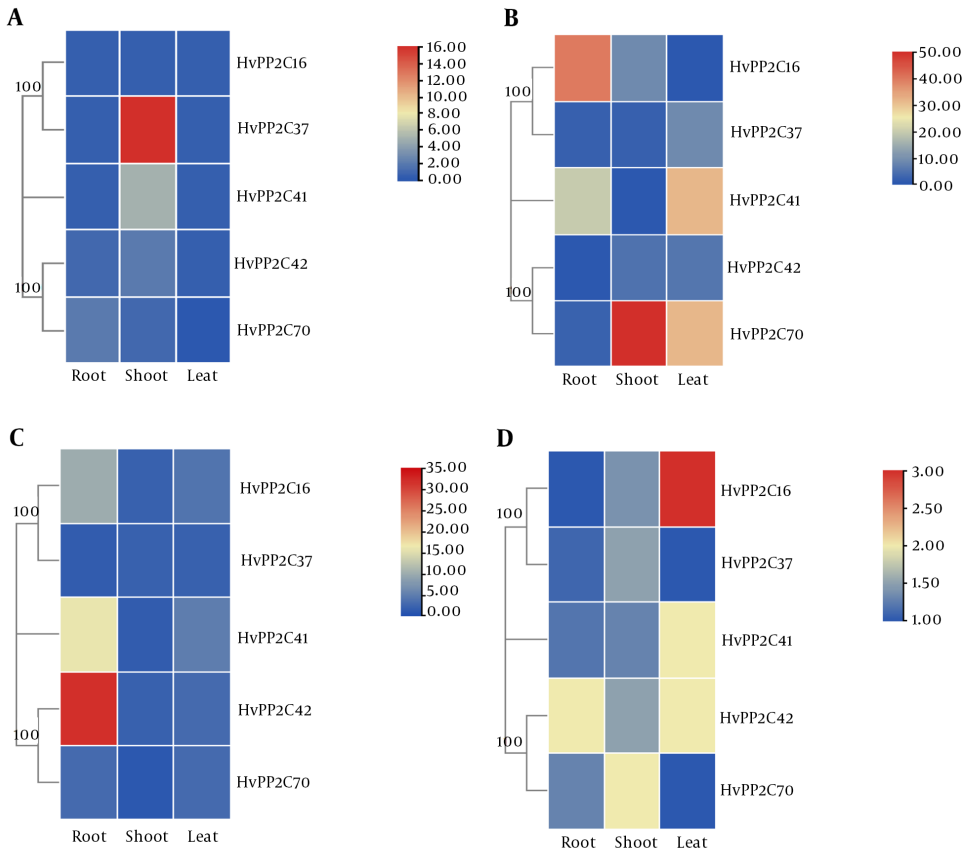

Figure 5. Differential gene expressions under cold (A) and heat (B) stress conditions in Azaran cultivar. The Jolge cultivar shows gene expression under cold (C) and heat (D) stress conditions. Blue and red indicate up and down-regulated genes, respectively. The yellow color indicates no significant expression under stress conditions.

Conflict of Interests: The authors declare that they have no competing interests.

Ethical Approval: This study was approved by the AJUMS Animal Ethics Committee (IR.AJUMS.ABHC.REC.1398.219).

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Table 2. Characteristics of HvPP2C Proteins Found in the Barley Genome

Uniprot accession	Protein Name	Number of Amino Acids	Molecular Weight (MW: kDa)	Theoretical pI	Genome Location	Duplication
M0USW5	HvPP2C46	582	63.33	6.36	chr1H:11138897-11142264	Tandem duplicate
F2EHQ5	HvPP2C47	298	32.76	4.71	chr1H:54286009-54288770	No duplication
A0A287FKC7	HvPPC13	399	43.09	6.67	chr1H:386658800-386662971	No duplication
A0A287FL77	HvPP2C72	392	43.32	8.23	chr1H:390690911-390698376	No duplication
A0A287FT97	HvPP2C48	248	27.25	9.51	chr1H:431534223-431538146	Tandem duplicate
F2DJX7	HvPP2C51	395	41.58	6.32	chr1H:545790189-545793081	No duplication
A0A287GS68	HvPP2C52	534	57.95	4.89	chr1H:552521506-552527955	No duplication
A0A287IKQ2	HvPP2C41	399	43.19	8.68	chr2H:573578815-573582822	Tandem duplicate
AK357653	HvPP2C41a	305	33.17	6.34	chr2H:573626499-573629830	Tandem duplicate
AK357622	HvPP2C42	386	41.88	7.1	chr2H:622771403-622774870	Block duplicate
A0A287JAG6	HvPP2C68	390	42.8	6.9	chr2H:704204949-704207601	No duplication
M0Y7N6	HPP2C45	268	29.30	4.93	chr2H:722484027-722488008	Tandem duplicate
M0Y7N6	HPP2C45a	284	30.91	4.93	chr2H:722563005-722566934	Tandem duplicate
A0A287KAQ4	HvPP2C01	353	37.42	5.68	chr3H:46958934-46961758	No duplication
A0A287KXD0	HvPP2C03	333	35.67	7.99	chr3H:291032153-291037688	No duplication
A0A287KZD6	HvPP2C57	331	35.72	6.67	chr3H:340435807-340459284	No duplication
A0A287L0C7	HvPP2C6	400	41.41	4.6	chr3H:360092700-360098013	No duplication
A0A287L3R5	HvPP2C7	390	45.93	6.7	chr3H:407373335-407383431	No duplication
A0A287LGB2	HvPP2C6a	51	5.51	9.81	chr3H:511604930-511605643	No duplication
A0A287LGC6	HvPP2C18	241	25.79	5.35	chr3H:511676340-511679818	No duplication
M0ZC70	HvPP2C16	393	41.86	5.78	chr3H:614881000-614883250	Block duplicate
A0A287MS52	HvPP2C17	311	34.37	6.42	chr3H:690155525-690157207	No duplication
A0A287MUL9	HvPPC17a	385	42.74	8.97	chr3H:699024910-699050008	No duplication
A0A287NGV0	HvPP2C36	317	4.56	7.59	chr4H:98736380-98739103	No duplication
A0A287NWL4	HvPP2C75	394	43.47	6.07	chr4H:358054764-358061365	No duplication
A0A287P371	HvPP2C21a	402	44.04	5.26	chr4H:449217161-449223939	No duplication
M0UT12	HvPP2C33	362	38.59	5.61	chr4H:456797020-456800448	No duplication
A0A287P934	HvPP2C30	400	43.06	5.68	chr4H:507379979-507384625	No duplication
M0YIW1	HvPP2C28	399	43.78	9.3	chr4H:616241933-616249757	No duplication
M0VSN9	HvPP2C31	613	67.35	6.25	chr4H:645763892-645766806	No duplication
A0A287QIS3	HvPP2C78	383	41.99	9.06	chr5H:79975025-79980048	No duplication
A0A287RMK2	HvPP2C69	429	46.86	5.78	chr5H:519091103-519096484	No duplication
A0A287S6R9	HvPP2C70	396	42.70	4.96	chr5H:582294665-582301638	Block duplicate
A0A287SF58	HvPP2C34	265	28.55	8.85	chr5H:603081538-603088903	No duplication
A0A287SKH9	HvPP2C21	273	30.22	5.43	chr5H:620396504-620402041	No duplication
A0A287SPR2	HvPP2C58	423	46.87	6.1	chr5H:632185424-632196887	No duplication
BAJ98729.1	HvPP2C61	1266	141.015	7.54	chr5H:640812970-640820023	Tandem duplicate
A0A287SS11	HvPPC35	518	54.96	5.3	chr5H:641283216-641287419	No duplication
A0A287SV91	HvPP2C36a	286	31.55	5.36	chr5H:648725477-648728034	No duplication
A0A287TI85	HvPP2C55	323	33.94	4.72	chr6H:54801305-54811859	No duplication
A0A287TKK2	HvPP2C10	360	38.32	4.98	chr6H:67703547-67706602	No duplication
F2E5E2	HvPP2C11	380	41.89	5.58	chr6H:117906254-117913932	Block duplicate
M0ZD44	HvPP2C12	354	37.86	5.5	chr6H:210762786-210767184	Tandem duplicate
A0A287U0S8	HvPP2C65	537	59.84	5.35	chr6H:219263779-219288759	Tandem duplicate

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Table 2. Characteristics of HvPP2C Proteins Found in the Barley Genome (Continued)

A0A287U288	HvPP2C14	515	55.400	6.53	chr6H:246534205-246538678	No duplication
A0A287U425	HvPP2C15	449	47.87	6.07	chr6H:290392640-290399019	No duplication
A0A287U5N1	HvPP2C13	391	41.87	5.25	chr6H:307794396-307808554	No duplication
A0A287UBR2	HvPP2C23	317	34.33	5.48	chr6H:393388996-393389949	No duplication
F2EHY7	HvPP2C26	597	65.12	5.34	chr6H:463066303-463070536	No duplication
A0A287VGJ4	HvPP2C37	372	38.86	4.85	chr7H:11079479-11102169	Tandem duplicate
A0A287VW12	HvPP2C6b	293	32.33	6.72	chr7H:54028669-54033782	Tandem duplicate
A0A287VVL1	HvPP2C54	353	38.47	5.48	chr7H:55007477-55011109	Tandem duplicate
M0VNX5	HvPP2C66	521	56.67	5.21	chr7H:172453523-172458271	No duplication
A0A287X173	HvPP2C56	367	39.46	9.26	chr7H:467679707-467683931	No duplication
A0A287X5E1	HvPP2C22	365	39.19	4.93	chr7H:512082503-512087461	No duplication
A0A287X703	HvPP2C24	319	33.92	5.44	chr7H:524549530-524550486	Tandem duplicate
A0A287X703	HvPP2C24a	320	33.93	5.45	chr7H:524639090-524640046	Tandem duplicate
A0A287X703	HvPP2C24b	330	33.92	5.65	chr7H:524814869-524815825	Tandem duplicate
A0A287XCZ5	HvPP2C76	848	96.84	5.74	chr7H:570046163-570059714	No duplication
F2D7E1	HvPP2C58	391	42.55	4.97	chr7H:636403133-636408251	No duplication
F2E1Q6	HvPP2C60	392	43.53	8.52	chr7H:646432525-646437065	No duplication