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Genome-wide analysis of HSP70 gene family in *Beta vulgaris* and in-silico expression under environmental stress

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Abstract

Heat shock proteins, HSP70, are vital for plant stress response mechanisms, particularly under abiotic stresses, such as salinity and drought. However, its role in *Beta vulgaris* is still unknown. We conducted a comprehensive genome-wide analysis of the *BvHSP70* gene family in *B. vulgaris* roots to elucidate their diverse functions, regulatory mechanisms, and roles in abiotic stress adaptation. We identified 22 *BvHSP70* genes, characterized by conserved motifs and *cis* elements associated with stress response in the gene promoters. miRNA interactions suggest regulatory roles, while gene duplication and syntenic analysis were utilized to reveal evolutionary trends with insights into gene expansion and conservation across species. These findings indicate the involvement of *BvHSP70* genes in stress adaptation and broader biological processes. Key regulatory miRNAs were identified in two *BvHSP70* genes. Expression analysis under salt stress indicated significant upregulation of *BvHSP70-2* gene, *BvHSP70-15* gene, and *BvHSP70-17* gene after 1 day, whereas *BvHSP70-18* showed notable upregulation after 7 days. Under drought stress, *BvHSP70-4*, *BvHSP70-13*, and *BvHSP70-14* were significantly downregulated, whereas *BvHSP70-17* and *BvHSP70-20* were significantly upregulated. These findings demonstrate the critical function of the BvHSP70 family in *B. vulgaris* stress adaptation. Understanding the functional and regulatory mechanisms of *BvHSP70* can facilitate the development of strategies to enhance stress tolerance in *B. vulgaris* and other crops, thereby contributing to agricultural sustainability and food security.

Keywords *BvHSP70* family, Genome-wide, Abiotic stress, Gene expression, Salt tolerance

Introduction

The Heat Shock Proteins-70 (HSP70), widely involved in responses to biotic and abiotic stresses within the bacterial, plant, and human kingdom [1]. They are crucial for managing the multiple stresses that plants transition from the endurance of heat, cold, drought, and salt to biotic stresses, pathogens, and nematodes [2–3]. Structurally, HSP70 encompasses three conserved domains: an N-terminal ATPase domain of 40-kDa that is responsible for client protein interactions, an 18-kDa substrate-binding domain that is useful for protein folding because of its capability to recognize hydrophobic regions, and a 10-kDa variable C-terminal that serves as a domain

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[4–5]. Depending on their functions and regulatory patterns, these genes are categorized as constitutively expressed HSPs or inducible heat shock proteins. They enable protein folding, preservation of the proteostatic system, degradation of nonnative proteins, movement of proteins across the membrane, and inhibition of protein aggregation [6]. HSP70 proteins are integral components of plant cell organelles and play a critical role in cellular functions and protein stability [7–8]. HSP70 proteins are localized in various plant cell organelles and contribute to diverse cellular functions and protein homeostasis. Plant HSP70 gene family are classified into cytosolic, endoplasmic reticulum (ER), plastid, and mitochondrial subfamilies based on their subcellular localization [9–10]. Most importantly, specific members of *NtHSP70-1* were identified and assigned in *Nicotiana tabacum* plants. The evolution of HSP70 from lower to higher plants has elucidated how these proteins display mechanisms, such as co-chaperones and cooperative mechanisms, for maintaining protein folding homeostasis [6, 11–12].

Understanding the role of HSP70 members in plant responses is a major focus of plant research [13–14]. For example, *Arabidopsis* mutants lacking *cPHSC70-2* and *cPHSC70-1* exhibit impaired phenotypes under high-temperature conditions during seed germination [13–14]. Additionally, deficiencies in *AtHSP70-15* in Arabidopsis have been linked to increased plant mortality under heat stress, whereas overexpression of *AtHSP70-1* enhances thermotolerance [15]. The *BIP1* gene, which encodes HSP70, plays a crucial role in various contexts: it facilitates rice immunity via *Xa21*, participates in responses to water stress in tobacco plants, and contributes to rice seed development [16]. Recent studies have indicated a connection between HSP70 and abscisic acid (ABA)-induced antioxidant responses in *Zea mays* L. Furthermore, the induction of *NtHSP70-1* by ABA treatment in tobacco plants has enhanced cyto-protection and stress tolerance [17]. Despite this, the specific functions of HSP70 genes, including those in *B. vulgaris*, remain unclear [1].

Sugar beets (*B. vulgaris* L. ssp. *vulgaris*) are important in the food and sugar industries, contributing to approximately 20% of the world's annual sugar production [18]. The resilience to high salt concentrations and drought conditions can be attributed to specific gene families. The resilience of *B. vulgaris* to high salt and drought conditions has been investigated with a focus on specific gene families; however, the role of HSP70 in this context has not been thoroughly examined [18–19]. We performed a genome-wide analysis of the HSP70 gene family in sugar beets in this study, examining their phylogeny, chromosomal distribution, gene structures, conserved motifs, cis-acting regulatory elements, and expression patterns under salt and drought stress. This systematic investigation offers new insights for further functional exploration and utilization of HSP70 genes in crop improvement, particularly in the development of salt-tolerant and drought-resistant sugar beet varieties that benefit sugar crop cultivation [20].

Materials and methods

Sequence retrieval and physicochemical and predicted subcellular localization analysis

The amino acid sequences of *B. vulgaris* were retrieved from the Phytozome v13 database (https://phytozome-next.jgi.doe.gov) using BLAST-P with the PF00012 domain as a query. Sequences were verified against the NCBI Conserved Domain Database (https://www.ncbi.nlm.nih.gov/genome/) [21]. Data on 22 HSP70 proteins, including chromosome location, gene orientation, mRNA length, and peptide size, were predicted, and ProtParam was used to provide information on the theoretical pI, molecular weight, grand average of hydropathy (GRAVY), and stability index. Subcellular localization was predicted using the WoLF PSORT database, and the results were visualized as a heat map using TB-tools [22].

Comparative phylogenetic, evolutionary and gene structure

Motifs in the amino acid sequence were analyzed using the MEME program (http://meme.sdsc.edu/meme/website/intro.html), and the sequences were further examined using the NCBI Conserved Domain Database [23].

HSP70 protein sequences from *B. vulgaris*, *Solanum tuberosum*, *A. thaliana*, and *Solanum lycopersicum* were aligned to construct a comparative phylogeny using the MEGA 11 and NJ methods with 1000 replications. Finally, the phylogenetic tree was visually refined using iTOL (https://itol.embl.de/) [24].

We investigated the HSP70 genes in *B. vulgaris*, focusing on functional annotation, structural analysis, gene duplication, and synteny. Protein sequences were aligned using the MUSCLE program, and Ka/Ks substitution rates were calculated using TBtools with default parameters to assess evolutionary rates. Divergence time was estimated using the formula that $T = Ks/2$ where $\lambda = 6.56 \times 10^{-9}$. Gene copies were detected using MCScanX, and syntenic maps were generated using TBtools [24–26]. Gene structure and intron-exon patterns were investigated using GSDS v2.0. (http://gsds.cbi.pku.edu.cn/) [27].

Cis-regulatory elements and miRNA analysis

Cis-regulatory elements were identified using the PlantCare database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) [22].MiRNA target sites were identified using the PmiREN database (https://www.pmiren.co

m/), and interactions between target genes and predicted miRNAs were visualized using Cytoscape [27].

Gene ontology (GO) and protein–protein interaction analysis

GO enrichment and protein–protein interaction analyses were performed for *B. vulgaris* HSP70 genes. Functional annotation was sourced from UniProt (https://www.uniprot.org/) [28] and analyzed using the ShinyGo v0.761 web tool (https://www.bioinformatics.sdstate.edu/go/) [29]. Protein interactions were examined using the STRING database (https://string-db.org) [27]. revealing a complex network of interactions among HSP70 protein domains [28–29].

Expression analysis under salinity and drought stress

RNA-seq data for *BvHSP70* genes were obtained from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/) to analyze gene expression under salinity and drought stress conditions under accession numbers GSE114968 and GSE205413 treated with mixed neutral-salt solution (300mM, NaCl, KCl, and $CaCl_2$-equimolar) [30] and under drought stress (DR) respectively [31]. All data were statistically analyzed using Statistix 8.1 software.

Results

Identification and molecular characterization of hsp70 gene family in B. Vulgaris

The *B. vulgaris* genome was downloaded from Phytozome v13 to identify *BvHSP70* genes within the *B. vulgaris* genome using the PF00012 domain as a query in a BLAST-P search. Furthermore, 22 BvHSP70 members were detected, and their sequences were further scrutinized for heat shock protein domains using Pfam, InterPro tool, and SMART database. Furthermore, the molecular weights of *BvHSP70* genes ranged from 64,716.60 to 75,749.28 Da. The approximate isoelectric point (pI) varied from 4.98 to 6.51, with an average of 5.5219. The grand average of hydropathicity (GRAVY) ranges from – 0.274 to – 0.474, with an average of – 0.34814. The stability of the proteins was predicted based on the instability index (II), and the proteins having an II value smaller than 40 were classified as stable and those with an II value greater than 40 as unstable. Among the 22 identified proteins, one (*BcHSP70-13*) was predicted to be unstable due to having higher II values of more than 40. Of the 22 *BvHSP70* genes analyzed, 13 were located on chromosome 1, with chromosomes 2 and 6 hosting 2 genes each. Furthermore, there was one gene each on chromosomes 9 and 7. A closer examination of gene orientations revealed that 11 *BvHSP70* genes were in the forward (F) direction, whereas the remaining 11 were oriented in the reverse (R) direction (Table 1).

The analysis of cis-elements

Specific elements such as TGA have also been implicated in auxin sensitivity. Simultaneously, the GARE motif and P-box are associated with the gibberellin response and the ERE for ethylene-responsive expression. In another category, the CGTCA motif is a prevalent cis-element, along with the TGACG motif, which is linked to methyl jasmonic acid responsiveness. Among the phytohormone response-related cis-elements, ABRE (25%), P-box (3%), TGACG motif (31%), TCA element (8%), GARE motif (2%), and CGTCA motif (31%) were associated with salicylic acid (SA), ABA, ethylene, and MeJA responses, respectively (Fig. 1). Furthermore, various stress-related elements were identified, including MBS (8%), LTR (19%), ARE (32%), and W box (28%), which were correlated with light, cold, and drought stress responses, respectively. These insights shed light on the intricate regulatory mechanisms underlying the *BvHSP70* gene family's ability to enhance abiotic stress responses.

The subcellular localization of HSP70 genes

Examination of the subcellular localization of the 22 *BvHSP70* genes revealed a range of distribution patterns. Significantly, 53% of the proteins were detected in the cytoplasm, 18% in the chloroplasts, and 14% in the Cysk_Nucl. Other locations, such as vacuoles, mitochondria, nuclei, and extracellular spaces, were minimally present. These findings underscored the diverse subcellular functions performed by *BvHSP70* proteins within plant cells, as shown in Fig. 2.

Conserved motif analysis and domain prediction

Our analysis of the conserved motifs among the 22 *BvHSP70* genes revealed that motifs 1–7 were consistently conserved across the entire gene set. This suggested that each HSP70 gene has a distinct function. The unique composition of these motifs implied specific regulatory elements or functional attributes that differentiate them from other gene families (Fig. 3). Domain analysis of the 22 *BvHSP70* genes revealed remarkable uniformity, and all genes containing a singular domain were identified as HSP70. This primary domain was divided into subfamilies, including the PTZ00009, PLN03184, dnak, and SQR_QFR_TM superfamilies. Notably, the PTZ00009 superfamily was present in 18 *BvHSP70* genes, PLN03184 in 2 genes (*BvHSP70*-3 and *BvHSP70-2*0), and dnak and SQR_QFR_TM superfamilies were present simultaneously in *BvHSP70-1* and *BvHSP70-1*9. This underscored the significant conservation of the HSP70 domain across all 22 *BvHSP70* genes (Fig. 4). When analyzing the exon–intron structures of the *BvHSP70* gene family, notable patterns emerged, revealing distinct genomic arrangements. *BvHSP70-1*2 and *BvHSP70-1*3 exhibited a major profile characterized by a single exon

Table 1 *BvHSP70* gene family information for 22 non-redundant genes discovered in the *B. vulgaris* genome

Gene ID	Source Accession No.	Chromosome No.	Chromosome Location		Strand	pI	GRAVY	Molecular weight (Mw)	Size (AA)	Instability Index(II)
			Start	End					Peptide	
Bevul.9G216900.1	BvHSP70-1	9	53,396,127	53,400,049	F	5.53	-0.309	72587.20	678	39.42
Bevul.7G208800.1	BvHSP70-2	7	58,530,806	58,533,833	R	5.17	-0.474	71279.66	648	34.74
Bevul.1G202400.1	BvHSP70-3	1	62,338,543	62,342,375	F	5.14	-0.320	75414.26	700	31.74
Bevul.1G090400.1	BvHSP70-4	1	44,354,854	44,358,019	F	6.10	-0.305	69298.01	624	30.87
Bevul.1G090100.1	BvHSP70-5	1	44,281,628	44,284,549	F	6.19	-0.274	66138.39	599	30.20
Bevul.1G091700.1	BvHSP70-6	1	44,631,075	44,636,345	R	5.60	-0.332	71178.97	641	32.92
Bevul.1G091000.1	BvHSP70-7	1	44,509,935	44,512,652	F	6.51	-0.281	69957.06	627	32.78
Bevul.1G091100.1	BvHSP70-8	1	44,529,997	44,532,252	F	5.63	-0.375	69650.06	624	27.49
Bevul.1G090500.1	BvHSP70-9	1	44,382,936	44,385,824	R	5.28	-0.278	71980.90	650	33.59
Bevul.1G090200.1	BvHSP70-10	1	44,297,699	44,301,109	R	5.62	-0.308	71198.36	642	34.73
Bevul.1G092000.1	BvHSP70-11	1	44,676,343	44,678,748	R	5.45	-0.266	64716.60	579	31.10
Bevul.1G209700.1	BvHSP70-12	1	63,045,404	63,047,372	F	5.37	-0.423	71782.73	655	34.66
Bevul.1G090600.1	BvHSP70-13	1	44,406,102	44,409,299	F	5.11	-0.344	69829.37	631	40.77
Bevul.1G091800.1	BvHSP70-14	1	44,656,257	44,658,479	R	6.41	-0.345	71304.33	637	33.78
Bevul.1G090700.1	BvHSP70-15	1	44,455,943	44,458,203	F	5.57	-0.415	70244.67	627	30.07
Bevul.1G091300.1	BvHSP70-16	1	44,558,377	44,563,405	R	5.46	-0.332	70961.17	638	35.22
Bevul.4G205100.1	BvHSP70-17	4	60,350,839	60,354,923	F	5.15	-0.470	73774.78	664	32.99
Bevul.3G034500.1	BvHSP70-18	3	3,983,061	3,986,578	R	4.98	-0.449	73344.03	665	29.91
Bevul.6G225800.1	BvHSP70-19	6	69,082,801	69,086,473	F	5.43	-0.282	72786.39	679	37.60
Bevul.6G153600.1	BvHSP70-20	6	48,801,175	48,805,229	R	5.16	-0.302	75749.28	713	30.56
Bevul.2G099800.1	BvHSP70-21	2	29,549,366	29,552,481	R	5.10	-0.427	71022.52	647	34.28
Bevul.2G099600.1	BvHSP70-22	2	29,478,230	29,481,934	R	5.13	-0.395	71303.83	651	33.49

and absence of introns. Conversely, *BvHSP70-17* and *BvHSP70-20* shared a conserved structure with eight exons and seven introns, which was the highest among the examined genes. These findings underscored the substantial genomic diversity within the *BvHSP70* gene family, showing variations in their exon–intron organization (Fig. 5).

Analysis of the HSP70 gene family's phylogeny, gene duplication and synteny

In the phylogenetic analysis, HSP70 members from four plant species *B. vulgaris (Bv)*, *S. tuberosum (St)*, *S. lycopersicum (Sl)* and *(A) thaliana (At)*, were systematically grouped into four clades, labeled I-IV. We analyzed 93 HSP70 genes, including 22 from *(B) vulgaris*, 18 from *A. thaliana*, 29 from potatoes, and 24 from tomatoes. Each clade was distinguished using a specific color scheme to improve clarity and facilitate a comprehensive understanding of the phylogenetic relationships: Clade I was represented in light yellow, clade II in blue, clade III in green, and clade IV in red (Fig. 6).

The distribution of HSP70 spanned multiple chromosomes, with notable concentrations on chromosomes 1, 2, 3, 4, 6, 7, and 9. Chromosome 1 hosted the majority of HSP70 genes, including HSP70-4 through HSP70-16. Other chromosomes also contained specific HSP70 genes, such as HSP70-21 and HSP70-22 on chromosome 2, HSP70-17 on chromosome 3, and HSP70-3 and HSP70-12 on chromosome 4. Chromosomes 6, 7, and 9 each harbored one HSP70 gene. These findings provided insight into the chromosomal organization of HSP70 genes (Fig. 7).

During the analysis of Ka/Ks ratios, 168 duplicate pairs of HSP70 members were identified in the *B. vulgaris* genome. Ka/Ks analysis showed that *BvHSP70-5_BvHSP70-13 and BvHSP70-11_BvHSP70-13* exhibited the highest Ka/Ks values (2.571 and 2.326, respectively). In contrast, *BvHSP70-2_BvHSP70-21* and *BvHSP70-2_BvHSP70-22* showed lower Ka/Ks ratios (0.0321 and 0.0177, respectively). The divergence time of *BvHSPs* genes was estimated to be one million years ago (MYA). The lowest MYA pair values, such as those of *BvHSP70-4_BvHSP70-5 and BvHSP70-11_BvHSP70-14*, indicated a more recent divergence, and those of *BvHSP70-9_BvHSP70-19*, *BvHSP70-14*, and *BvHSP70-18* suggested ancient divergence from a common ancestor (Fig. 8).

The syntenic relationship among *BvHSP70* members revealed tandem and segmental duplication events in the *B. vulgaris* genome. Chromosomes 1, 2, and 6 showed evidence of segmental duplication, whereas chromosomes 3, 4, and 6 showed tandem duplication (Fig. 9).

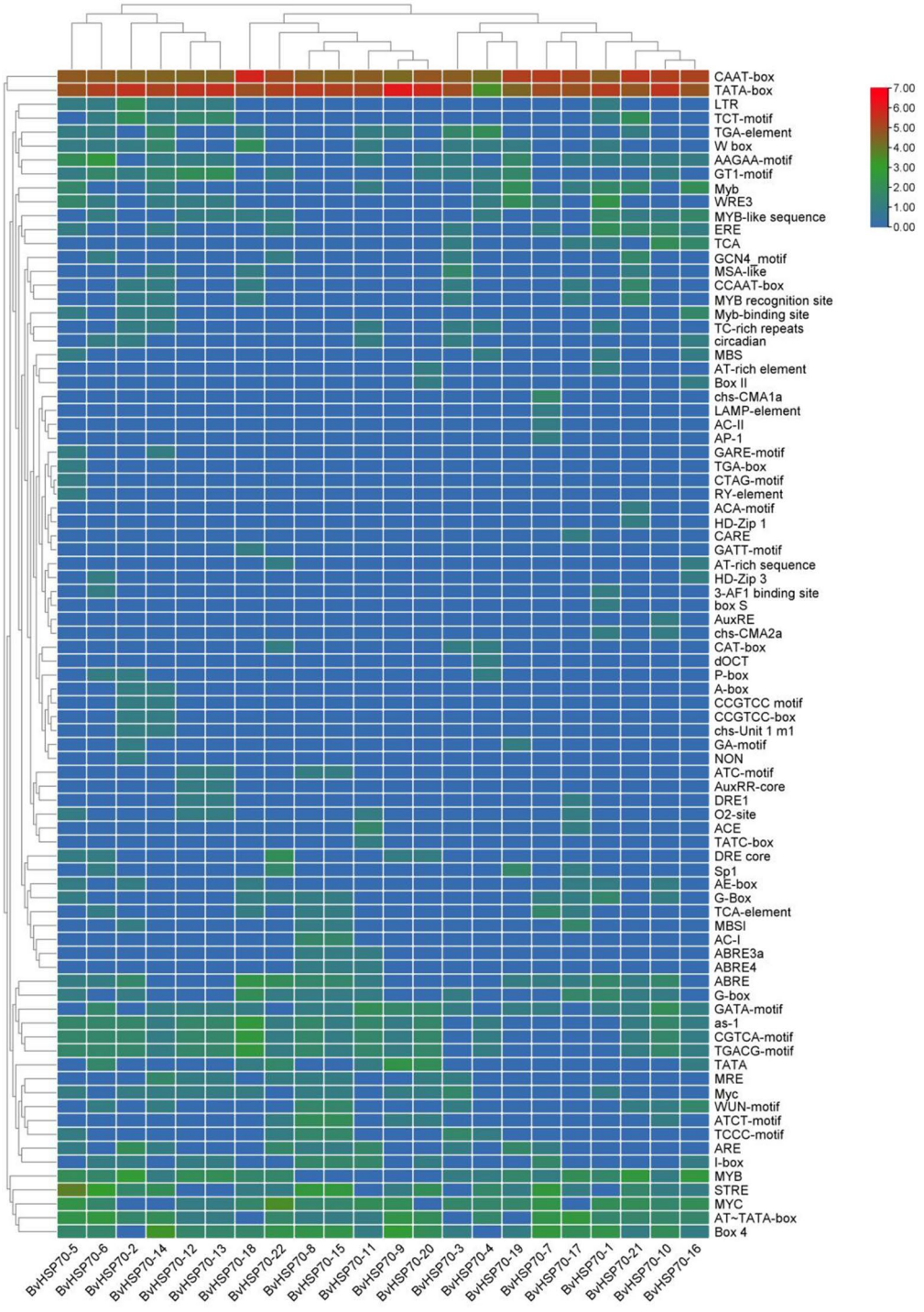

Fig. 1 Linear representation of the BvHSP70 genes, where the length corresponds to the sequence of each gene. Highlight regions with varying densities of cis-elements using a heat map gradient: Red: High density of cis-elements. Green: Low density of cis-elements. cis-elements into Panel B categories based on function or type (e.g., stress-responsive, hormonal regulation, development-related along with their respective percentages

GO analysis and protein–protein interaction

GO enrichment analysis provided significant insights into the functional roles of *BvHSP70* genes. Specifically, these genes were identified as major contributors to the calnexin cycle for the folding of N-glycosylated proteins (GO:0030544), cellular networks of molecular chaperones, and MreB protein formation (Fig. 10). During the protein interaction investigation, 7 nodes and 14 edges were observed. The average node degree was calculated to be 4, with an average local clustering coefficient of

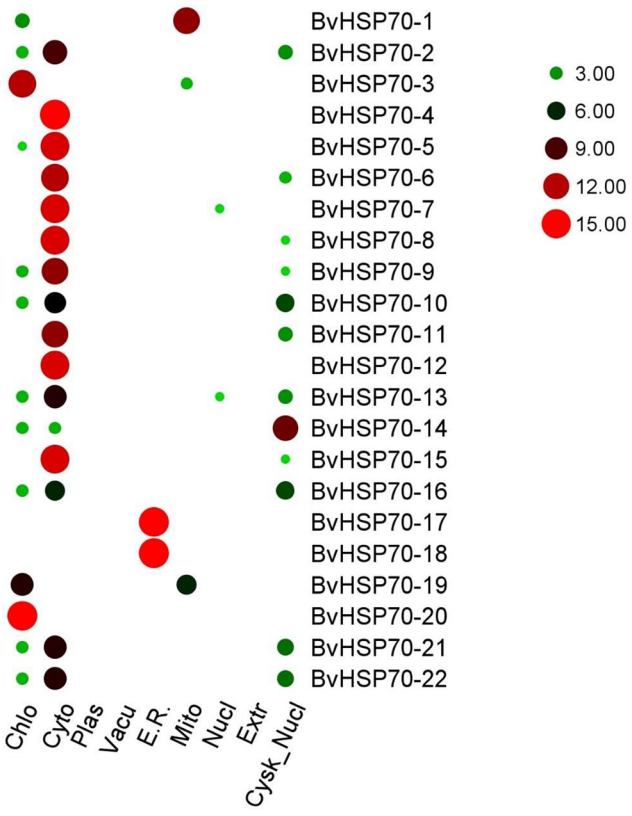

Fig. 2 The heatmap depicting the subcellular localization of BvHSP70 genes identifies 22 members distributed across plant cell compartments, including the nucleus, cytoplasm, chloroplast, Golgi apparatus, mitochondria, plastids, and peroxisomes. White represents minimal presence, while red highlights regions of high functional significance

0.571. Interestingly, the expected number of edges in this scenario was 25, and the p-value for protein–protein interaction enrichment was remarkably low at 0.971, suggesting a vital enrichment interaction. A low-confidence threshold of 0.150 was used to achieve the minimum interaction score. Among the 22 *BvHSP70* proteins, interactions were analyzed for only seven proteins (*BvHSP70-2*, *BvHSP70-16*, *BvHSP70-18*, *BvHSP70-19*, *BvHSP70-20*, *BvHSP70-21*, and *BvHSP70-22*). Notably, *BvHSP70-18* showed the highest number of interactions with the six proteins, whereas the remaining proteins primarily interacted with each other (Fig. 11).

microRNA (miRNA) analysis

During miRNA analysis, 35 miRNAs were identified (Table 1S). miRNA analysis of HSP70 genes revealed various interactions between distinct miRNAs, each differing in length. The shortest miRNA, miR5676, spanned positions 1–22, whereas the longest, miR9483a, extended from position 1 to 24. Among these miRNAs, 15 were implicated in inhibiting translation, whereas the remaining 20 were implicated in inhibiting cleavage. Notably, miR7487 and miR5676 targeting *BvHSP70-3* and *BvHSP70-11* exhibited the minimum cleavage inhibition expectation value of 3.5 and a UPE of -1. Conversely, a maximum cleavage inhibition expectation value of 5 was observed for multiple miRNAs targeting *BvHSP70-3*, *BvHSP70-4*, *BvHSP70-9*, *BvHSP70-11*, *BvHSP70-13*, *BvHSP70-14*, *BvHSP70-6*, *BvHSP70-15*, *BvHSP70-16*, and *BvHSP70-8*. These miRNAs target miR7523, miR8722, miR6198, and miR8722, which are mediated by *BvHSP70-6*, *BvHSP70-15*, *BvHSP70-16*, and *BvHSP70-8*, respectively, and are involved in translation inhibition.

Expression analysis of BvHSP70 gene family

Salt-responsive mRNAs expressed in B. vulgaris roots were systematically identified and characterized in the present study. Statistical analysis revealed that 13 of 22 BvHSP70 genes were expressed. During the first treatment (1 day), only three genes (BvHSP70-2, BvHSP70-15, and BvHSP70-17) showed significant upregulation in treated plants compared with the control plants. Similarly, during the second treatment (7 d), only one gene (BvHSP70-18) was significantly upregulated in the treated plants Fig. 12. RNA-Seq data were retrieved from the NCBI for Biotechnology Information GEO database to clarify the mechanism of drought stress in B. vulgaris with respect to the BvHSP70 gene family. Statistical analysis revealed that 20 of 22 BvHSP70 genes were expressed. Three genes (BvHSP70-4, BvHSP70-13, and BvHSP70-14) were significantly downregulated under drought stress, whereas only two genes (BvHSP70-17 and BvHSP70-20) were significantly upregulated plants Fig. 13.

Discussion

Heat shock proteins (HSPs) are fundamental components of plant stress responses, particularly under abiotic stresses such as heat, drought, salinity, and cold [32]. These proteins are essential for maintaining cellular homeostasis by assisting in protein folding, stabilizing denatured proteins, and preventing aggregation under stress conditions [33]. In addition, HSPs are essential for assisting plants to cope with these stresses by promoting protein folding, stabilizing proteins, and preventing aggregate formation [34]. In addition, ribosomes are involved in protein transport, assembly, and breakdown, ensuring cellular homeostasis, even under stressful conditions. The levels of HSPs were found to increase under these stress conditions to adapt to the genome-wide identification of HSP70. The enhanced amounts of HSPs help safeguard key proteins against damage due to stress, play a role in the repair of injured proteins, and participate in establishing new cellular homeostasis. In addition, HSPs are involved in signal

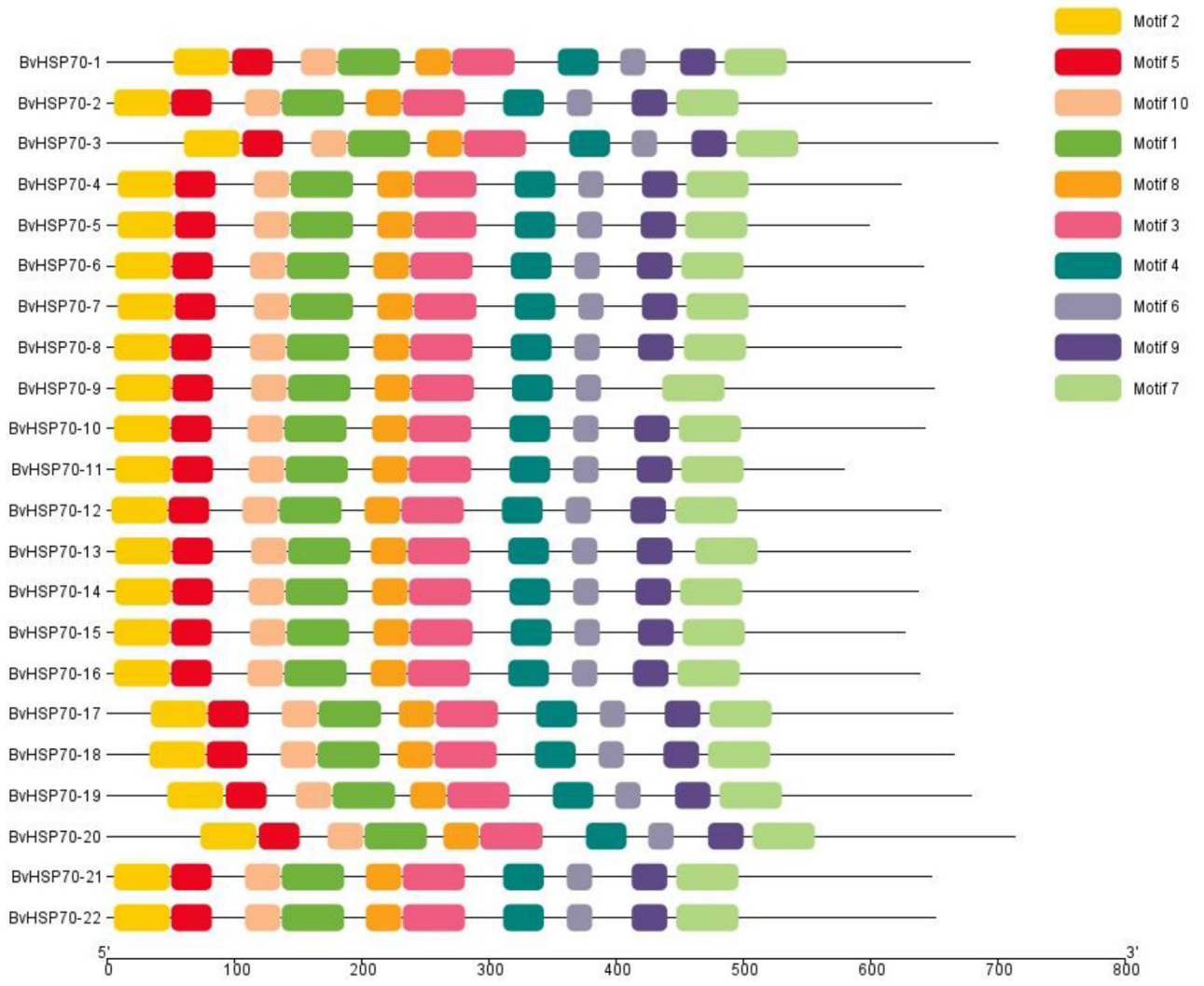

Fig. 3 The distribution of ten motifs along the 22 *BvHSP70* protein family members. The unique composition of suggested specific regulatory attributes that differentiate them from other gene families. Domain analysis of the 22 BvHSP70 genes revealed remarkable uniformity, with all genes containing a single domain identified as HSP70

transduction pathways that mediate stress responses, such as the expression of stress-responsive genes and hormones that regulate stress. HSPs are vital links in the plant defense system for plants to cope with stress caused by extreme environmental conditions [35, 36].

A genome-wide examination was performed to identify and describe *BvHSP70* genes in *B. vulgaris*. The physicochemical properties of these 22 *BvHSP70* proteins were intensively investigated to analyze the divergence between members of the specified protein family. This study revealed that all *BvHSP70* proteins have hydrophilic features, as reflected by their negative GRAVY values (aqueous interaction tendency and dependence on pH) and electric charges (positively or negatively charged, 36). Furthermore, analysis using the instability index revealed that 21 proteins exhibited features indicative of stability, whereas only one protein exhibited traits suggestive of instability Table 1. Subcellular localization analysis revealed the presence of *BvHSP70* proteins in diverse organelles spanning the chloroplast, mitochondria, cytoplasm, cytosol, ER, nucleus, and plasma membrane. Notably, 53% of the proteins were found in the cytoplasm, whereas 18% were localized in the chloroplasts, indicating their predominant presence in these two compartments. This suggested that *BvHSP70* proteins may play crucial roles within the cytoplasm and chloroplasts, such as in chaperone formation during abiotic stress, signaling, and protection against damage, thereby contributing to plant resilience [37] (Fig. 2).

The conserved motifs present in the BvHSP70 genes provide further insights into their regulatory functions. The analysis revealed that motifs 1 to 7 were universally present across all 22 genes, emphasizing the essential role

Fig. 4 The figure illustrates the domains present in each identified HSP70 gene in *Beta vulgaris*. Domain analysis of the 22 BvHSP70 genes revealed remarkable uniformity, with all genes containing a singular domain identified as HSP70

of these motifs in stress-induced regulatory mechanisms. The conserved nature of these motifs supports the idea that BvHSP70 genes share a common regulatory framework that enables them to respond effectively to environmental stressors (Fig. 3) [38]. This finding aligns with previous research, which has highlighted the functional significance of conserved motifs in mediating stress tolerance mechanisms in plants. The conservation of the HSP70 domain across all 22 *BvHSP70* genes in a genome-wide study implied its fundamental role in abiotic stress responses. This uniformity underscored HSP70's evolutionary significance as a key player in adaptation to stress under various environmental conditions. Additionally, categorizing the HSP70 domain into subfamilies such as PTZ00009, PLN03184, dnak, and SQR_QFR_TM highlighted the functional diversity within the HSP70 gene family. Specific subfamily prevalence among genes suggested potential functional specialization or regulatory functions in stress responses [39]. This analysis emphasized the critical role of the HSP70 domain in enhancing plant resilience to abiotic stress and provides insights into the underlying molecular mechanisms (Fig. 4). *BvHSP70* genes are equipped with a wide range of cis-regulators known to regulate plant stress responses, growth, and signaling mechanisms [37]. Thus, the diverse cis-acting elements in all 22 *BvHSP70* genes suggested their versatile roles in plant stress responses and development [40]. These elements from the promoter regions are involved in phytohormone responses, stress responses, and growth pathways. Therefore, particular motifs, namely CAT-box, MRE, and Box-4, played pivotal roles in meristem expression, zein metabolic control, and light-induced growth. In addition to TGA, GARE, and P-box, the ethylene response occurred mainly through elements, including the ERE. In addition, the existence of stress-related components was a sign of the key role that *BvHSP70* genes play in improving plant tolerance to harsh abiotic stresses such as light, temperature, salinity, and drought. These findings shed light on the *BvHSP70* gene family's adaptivity in controlling plant stress responses and development [41] (Fig. 1).

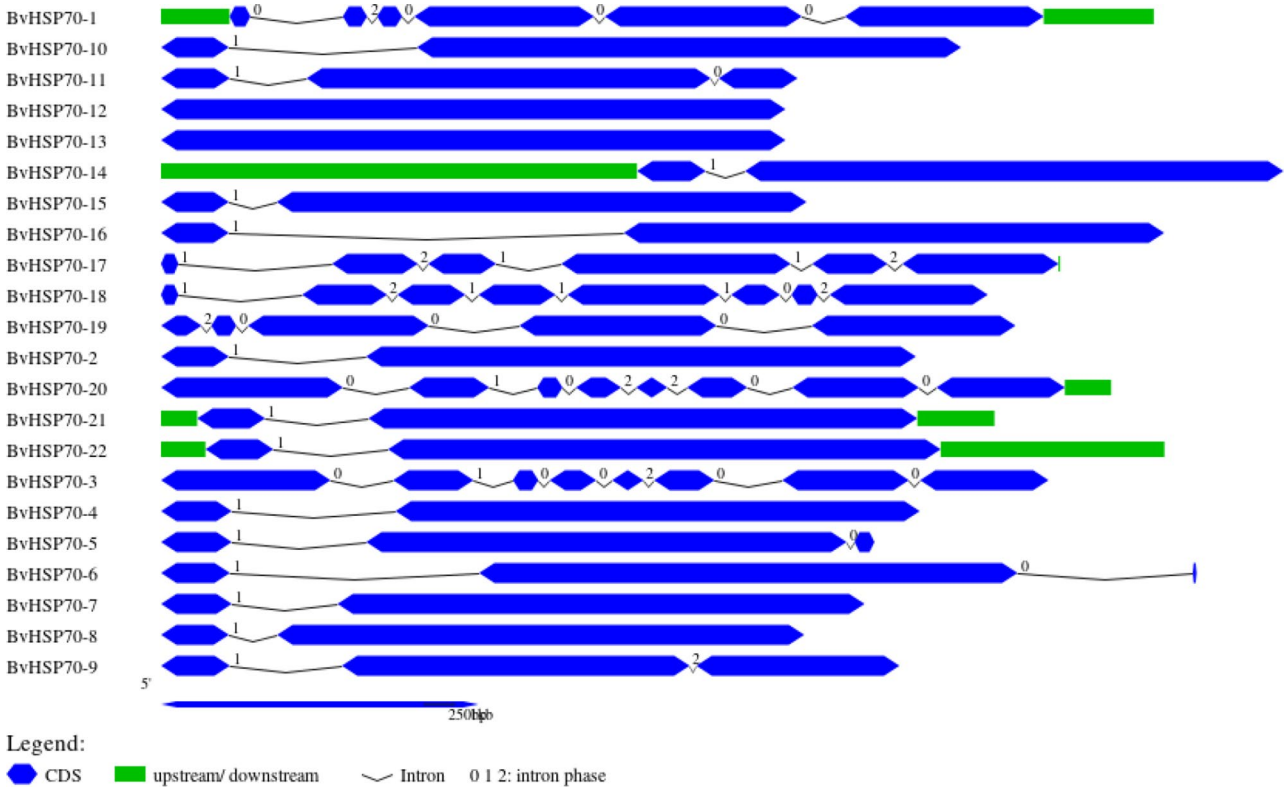

Fig. 5 The intron and exon structure analysis are phylogenetically represented; the blue color represents exons, and the black line shows introns. Furthermore, analysis of exon–intron structures revealed notable patterns in their genomic arrangements

A comparison of exon–intron structures in 22 *BvHSP70* genes revealed a considerable distance. Some genes had a single exon without introns, like *BvHSP70-12* and *BvHSP70-13*, but others, such as *BvHSP70-17* and *BvHSP70-20*, had exons with introns, indicating that they are more complex. This genetic variation among *BvHSP70* genes implied that the gene family can execute various functions across different biological processes [42–43] (Fig. 5). The phylogenetic relationships between *BvHSP70*, *StHSP70*, *AtHSP70*, and *SlHSP70* were analyzed through phylogenetic analysis. The genes were divided into four groups (I, II, III, and IV) that reflected their evolutionary relationships. Overall, 93 genes were studied. Historical and functional similarities were analyzed in the ancient *BvHSP70* gene family, especially stress responses in various plant species [28, 29, 38] (Fig. 6). A duplication study of the HSP70 gene family was conducted using different analyses, which helped to illuminate its evolutionary history and duplication events. The representation of HSP70 genes on various chromosomes, namely chromosomes 1, 2, 3, 4, 6, 7, and 9, implied that they were widely dispersed, with chromosome 1 containing most of the genes. This distribution indicates that the evolutionary process may have a very complex genetic arrangement and may be responsible for both the duplication and divergence of genes [44]. Analysis of the Ka/Ks ratios revealed duplicate molecular pairs with degrees of divergence from the basic sequence, varying from *BvHSP70-5_BvHSP70-13* to *BvHSP70-11_BvHSP70-13*, demonstrating higher Ka/Ks values, implying rapid divergence. In accordance with this finding, the *BvHSP70-2-BvHSP70-21* and *BvHSP70-2-BvHSP70-22* pairs showed lower Ka/Ks values, indicating conservation. Divergence time estimation confirmed these findings, showing that multiple gene pairs underwent recent and ancient divergence [45]. In addition, genome syntenic analysis indicated segmental and tandem duplications, which also suggested the highly complex evolutionary nature of the HSP70 gene family in the *B. vulgaris* genome [46]. These findings revealed the ancestral history, functional similarity, and evolutionary mechanisms of gene duplications within the *BvHSP70* gene family. Gene duplication within HSP70 gene family members, including the *BvHSP70* family, is important in strengthening plant tolerance to abiotic stress. These duplications cover all aspects, from redundancy to functional divergence and may result in the evolution of new stress response mechanisms. The duplication of genes in this study indicates that plants can be more resilient to environmental stresses through genetic toolkit diversification (Figs. 7, 8 and 9).

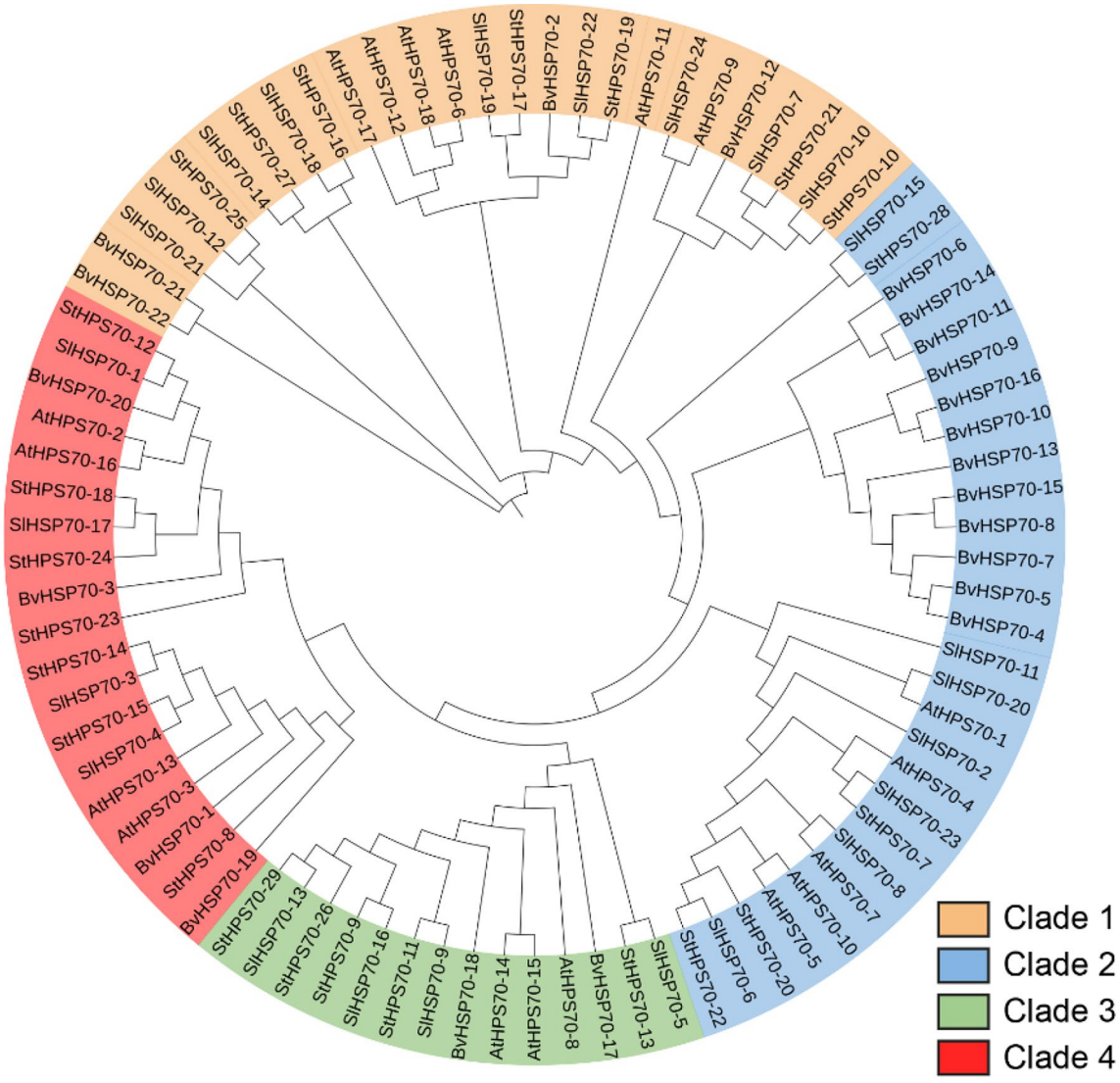

Fig. 6 Phylogenetic analysis of HSP70 genes from four plant species 22 from Beta vulgaris, 18 from Arabidopsis thaliana, 29 from potatoes, and 24 from tomatoes revealed that they were systematically grouped into four distinct clades (I–IV). Clade I was represented in light yellow, clade II in blue, clade III in green, and clade IV in red. A total of 93 HSP70 genes were included. Each clade was distinguished by a specific color scheme to enhance clarity and facilitate a comprehensive understanding of the phylogenetic relationships

The *BvHSP70* gene family was annotated as a key player in abiotic stress responses using GO analysis. Specifically, they are involved in the folding of N-glycosylated proteins, part of the calnexin cycle (GO: 0030544), which signifies their contribution to protein homeostasis. They are part of the cellular network of other molecular chaperones and play a central role in the quality control and stability of proteins under stress conditions [47]. They also participate in synthesizing the MreB protein and have significance in cell shape characterization and enhanced stress tolerance. This analysis highlighted *BvHSP70* genes that play the role of middle-catalysts in stress responses and adaptation to stressful environments (Fig. 10). This study showed that high protein–protein interactions among HSP70 family members, particularly under stress conditions, underscored its vital function during stress response processes. The most meaningful enhancement of connections, with a p-value < 0.05, indicated the importance of this subset of connections in stress responsiveness. The most prominent proteins, *BvHSP70-18*, *BvHSP70-19*, and *BvHSP70-20*, with high interaction levels, perform major functions in the stress response process. This meshwork allows various family members to advance cooperative actions, direct protein folding,

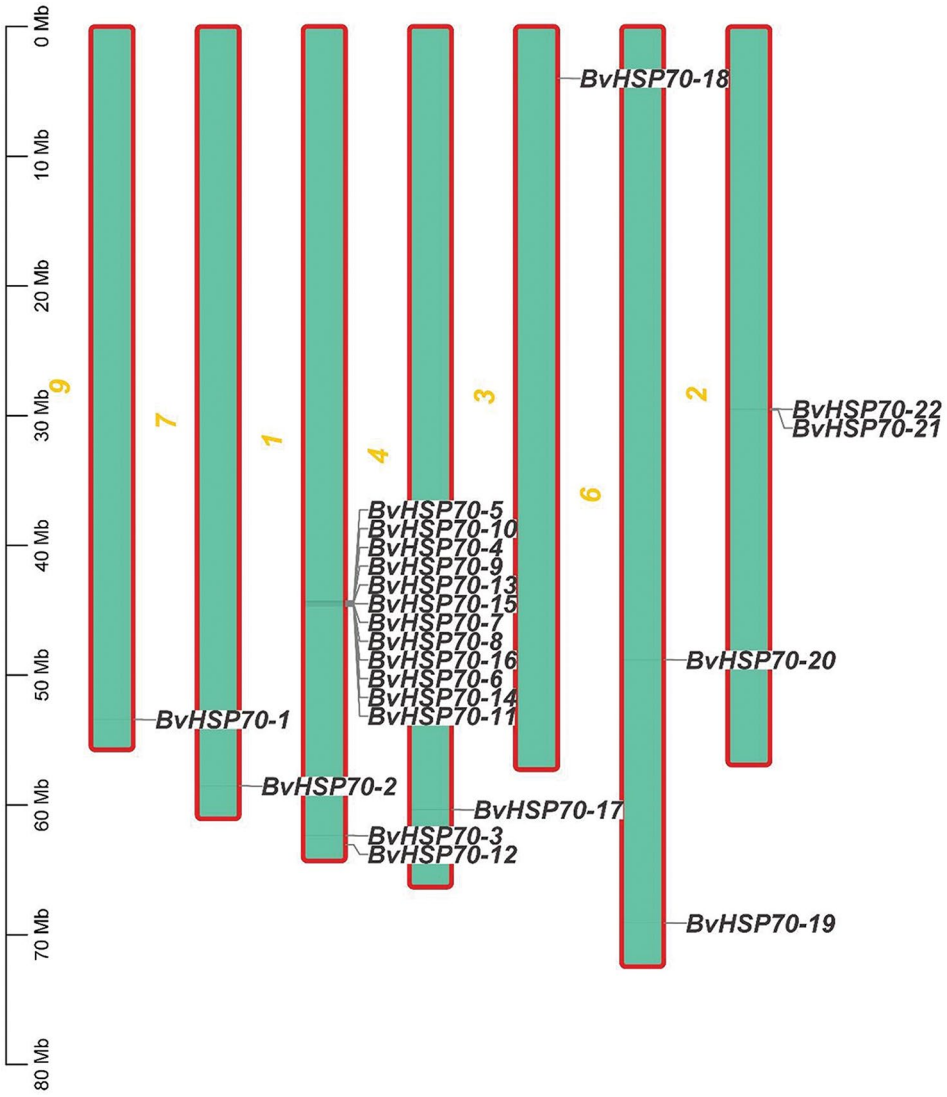

Fig. 7 The chromosomal distribution of HSP70 genes reveals their widespread dispersion across various chromosomes. Notably, chromosomes 1, 2, 3, 4, 6, 7, and 9 each harbor specific HSP70 genes, with chromosome 1 containing the largest cluster, including HSP70-4 through HSP70-16. Chromosome 2 contains HSP70-21 and HSP70-22, chromosome 3 holds HSP70-17, and chromosomes 4, 6, 7, and 9 feature HSP70-3, HSP70-12, and one HSP70 gene, respectively

and stability [48]. The primary purpose of this study was to clarify the pivotal role of the *BvHSP70* family of genes in the molecular chaperone stage and as a stress response regulator to maintain overall cellular homeostasis and survival of plants under stress [49] (Fig. 11).

The number of miRNAs targeting specific *BvHSP70* genes in the *B. vulgaris* genome indicated complicated regulatory pathways in response to abiotic stress. These miRNA-HSP70 connections, which can be explained using miRNA biology, are the most important for regulating gene expression under stress. Unlike simple translation inhibitors or cleavage agents, miRNAs control HSP70 expression through multiple mechanisms, indicating their intricate regulatory roles. These deviations in the targets of miRNAs and their cleavage inhibition values shed light on the sophisticated regulatory mechanism of HSP70 family miRNAs under stress, which is an essential process for adaptation [50].

HSP70 genes, specifically in *B. vulgaris* roots under salinity stress, were upregulated in this study, implying

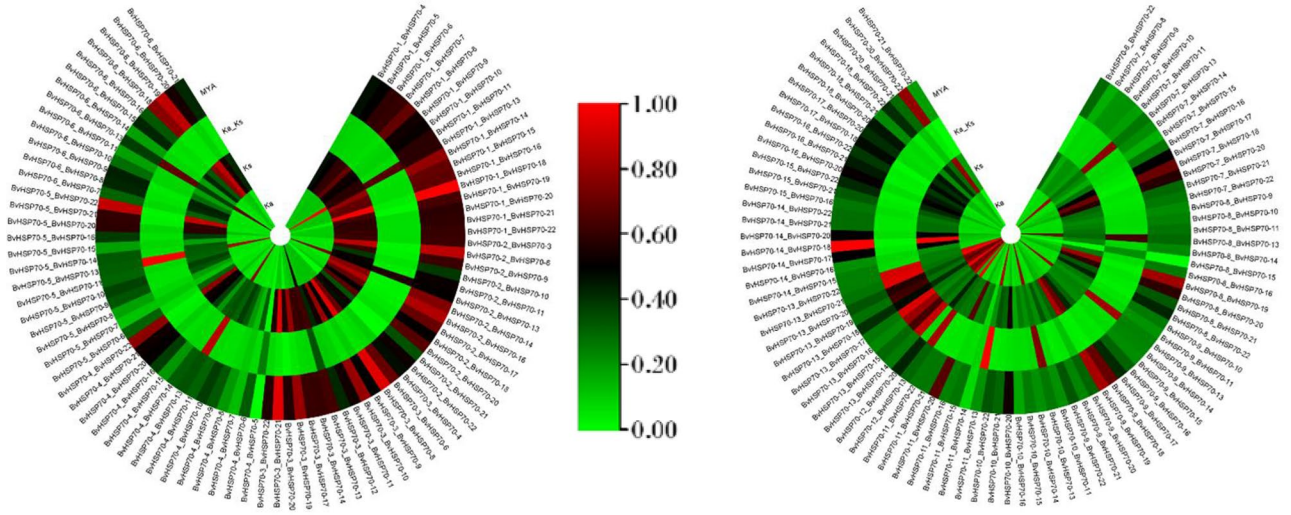

Fig. 8 The Ka/Ks represent the ratio of mutations involving synonymous substitutions (Ks) to mutations involving non-synonymous substitutions (Ka). During the analysis of Ka/Ks ratios, 168 duplicate pairs of HSP70 members were identified in the B. vulgaris genome. Ka/Ks analysis showed that BvHSP70-5_BvHSP70-13 and BvHSP70-11_BvHSP70-13 exhibited based on duplication events and the selection pressure on paralogous pairings of B. vulgaris HSP70s genes assessed based on the calculated Ks and Ka values

their key role in salt tolerance mechanisms. *BvHSP70-2*, *BvHSP70-15*, and *BvHSP70-17* were markedly overexpressed on day 1 of treatment. By day 7, *BvHSP70-18* levels increased tremendously. This suggested their implications in stress response pathways and protein-folding processes. They also provide resistance to stress-induced damage. This information may lead to improved salt tolerance through hybrid mating and genetic engineering. The differential expression of HSP70 genes under drought conditions provides informative and valuable evidence for their activity in drought tolerance pathways. *BvHSP70-4*, *BvHSP70-13*, and *BvHSP70-14* suggested that these genes were downregulated, possibly suppressing stress-responsive pathways and consequently reducing heat stress tolerance. *BvHSP70-17* and *BvHSP70-20* were upregulated, indicating that unraveling the molecular responses of HSP70 genes to drought stress could provide the basis for strategies that improve drought tolerance in crops by targeting downregulated genes for expression manipulation and leveraging the upregulated HSP70s, such as *BvHSP70-17* and *BvHSP70-20*, through genetic engineering or breeding approaches. These differential expression patterns point to the potential of targeting specific genes for crop improvement through genetic engineering or breeding strategies aimed at enhancing stress tolerance.

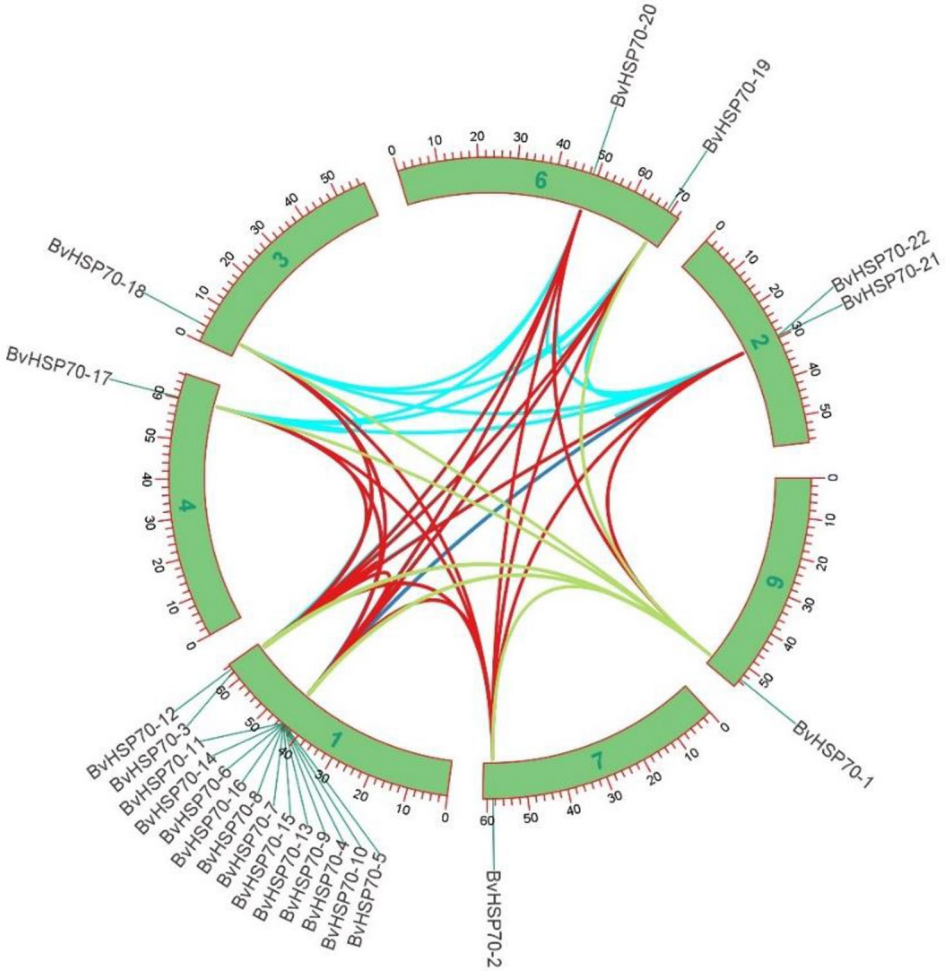

Fig. 9 Syntenic association of *Bv*HSP70 genes, structurally similar sequences of plant species *B. vulgaris (Bv)*, *S. tuberosum (St)*, *S. lycopersicum (Sl)* and *A. thaliana (At)* share conserved areas. The technique visually represents the degree of similarity across genomic regions using green and pink colors

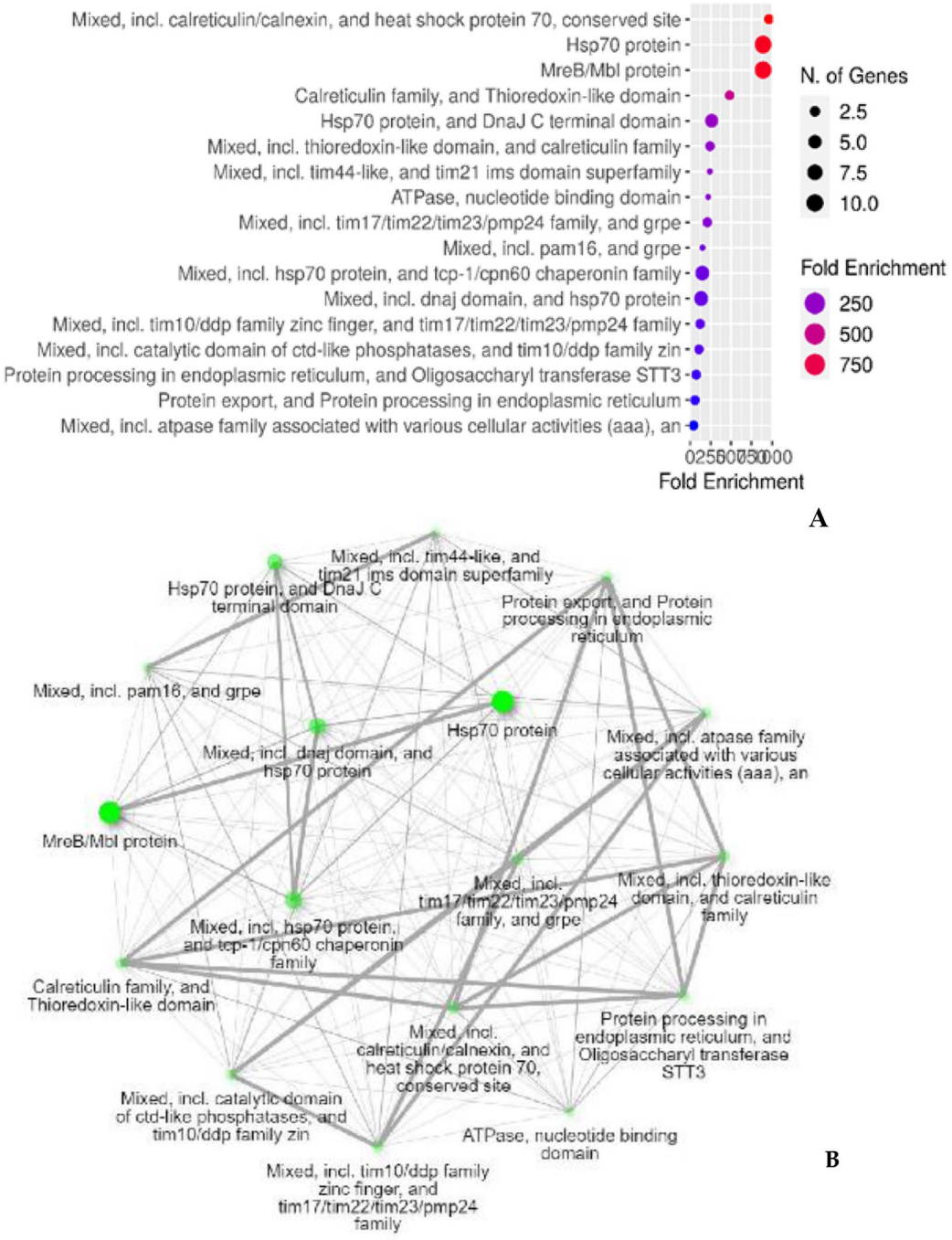

Fig. 10 **A**. Functional annotation of *Bv*HSP70 genes predicted HSP70 genes. The GO enrichment analysis in this study offered valuable insights into the functional roles of HSP70 genes, with red indicating high gene function values and blue representing low gene function values. **B**. The GO enrichment analysis in this study, the network showed offered valuable insights into their functional roles

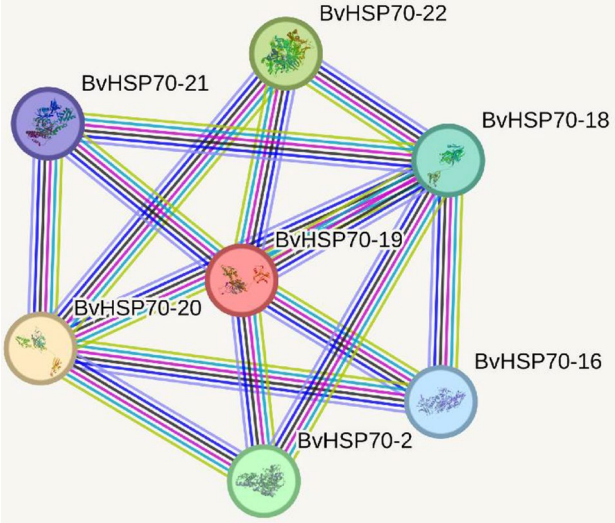

Fig. 11 Protein-protein interactions were analyzed for seven of the 22 BvHSP70 proteins: BvHSP70-2, BvHSP70-16, BvHSP70-18, BvHSP70-19, BvHSP70-20, BvHSP70-21, and BvHSP70-22. Among these, BvHSP70-18 demonstrated the highest number of interactions, engaging with all six other proteins, while the others primarily interacted with each other

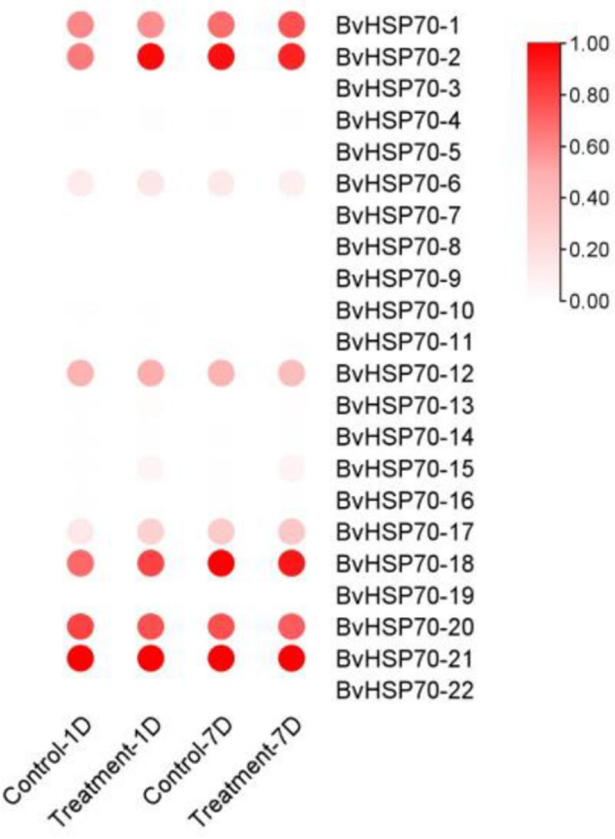

Fig. 12 Genome-wide analysis identified and characterized salt-responsive mRNAs expressed in B. vulgaris roots. High expression is indicated in red, while low expression is indicated in white

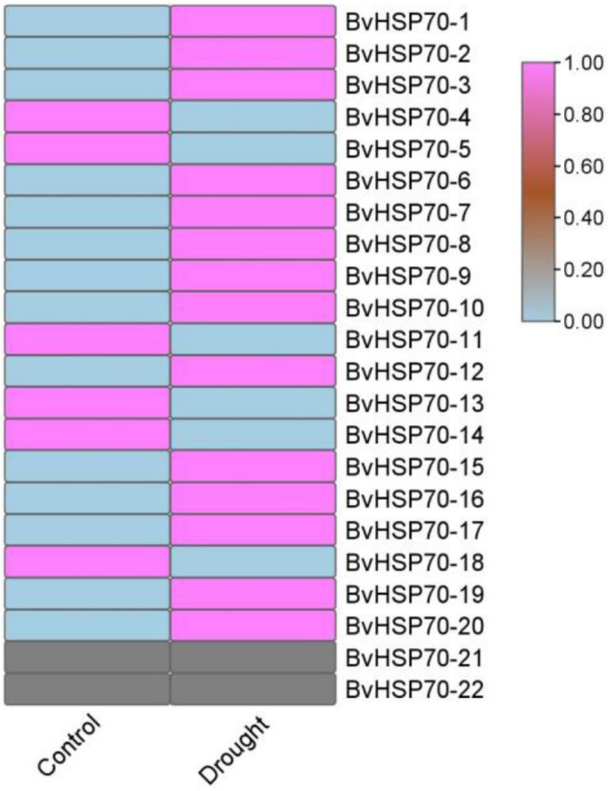

Fig. 13 RNA-seq data from NCBI GEO were used to explore the role of the BvHSP70 gene family in *B. vulgaris* drought stress response. High expression is depicted in red, and low expression in black

Conclusions

In this study, we conducted a genome-wide analysis of the HSP70 gene family in *B. vulgaris* and identified 22 *HSP70* genes in *B. vulgaris* and elucidated their conserved sequences, motifs, and regulatory elements. Comprehensive in silico analyses were performed, including gene structure, chromosomal distribution, phylogenetic relationships, and syntenic studies, providing insights into the evolutionary characteristics of the HSP70 gene family in *B. vulgaris*. Promoter analysis underscored their roles in abiotic stress responses, hormone signaling, and developmental processes. miRNAs are crucial regulators of apoptosis. RNA-seq data revealed distinct expression patterns under salinity and drought conditions. During salt stress, *BvHSP70-2*, *BvHSP70-15*, and *BvHSP70-17* were significantly upregulated after 1 day of treatment, while *BvHSP70-18* showed significant upregulation after 7 days. Under drought stress conditions, *BvHSP70-4*, *BvHSP70-13*, and *BvHSP70-14* were downregulated, whereas *BvHSP70-17* and *BvHSP70-20* were upregulated. This dynamic gene expression revealed the crucial role of the *BvHSP70* family in stress adaptation mechanisms. The findings revealed that *B. vulgaris* HSP70 genes are regulated by 35 distinct microRNA families, are highly responsive to light, temperature, and phytohormones,

and play crucial roles in the biological, cellular, and molecular functions of *B. vulgaris*. Furthermore, three-dimensional protein structural insights offer potential for enhancing crop traits, such as stress resistance and biomass yield. Collectively, these analyses establish a foundation for future research into the molecular and physiological roles of HSP70 genes in *B. vulgaris*. Such an understanding can enlighten targeted approaches for enhancing crop stress tolerance, ultimately contributing to agricultural sustainability and food security.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06214-5.

Supplementary Material 1

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Author contributions
PA supervised, conceived and designed the experiments; IAS and MAM contributed reagents, materials, and analytical tools; and IAS and TA provided guidance on the entire manuscript. All the authors have read and approved the final version of the manuscript.

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Data availability
The amino acid sequences of ***B. vulgaris*** were retrieved from the Phytozome v13 database (https://phytozome-next.jgi.doe.gov). RNA-seq data for BvHSP70 genes were obtained from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/) to analyze gene expression under salinity and drought stress conditions under accession numbers GSE114968 and GSE205413, respectively.

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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