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Identification and expression analysis of *HSP100* gene family in *Aeluropus littoralis*

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Abstract: Heat shock proteins (HSPs), molecular chaperones with many activities, are essential to plant growth, development, and stress responses. To make crops more salt- and drought-resistant, plant breeders have considered halophytic plant. *Aeluropus littoralis*, a halophyte monocot grass, is one potential model species to discover new stress-response genes. Here, exon/intron structure, conserved motifs/ domains, and expression patterns of HSP100 gene family were identified in the genome of *A. littoralis*. This study found six unique AlHSP100 non-repetitive genes, revealing remarkable structural and physicochemical variations between the subfamilies. Phylogenetic and motif analyses revealed that proteins from the same subfamily (AlHSP100.1-4) and proteins from other subfamilies (AlHSP100.5-6) have similar types, ordering, and quantities of motifs. Finally, the expression of *AlHSP100.3* gene was analyzed using RT-qPCR under dehydration, salt, cold, and phytohormone abscisic acid stress treatments, revealed that their expression patterns vary in response to abiotic stresses. The presence of stress-dependent regulation of the *HSP100.3* gene, as evidenced by the early response to osmotic stress and the late response to cold stress, is likely associated with the cis-regulatory elements located upstream of this gene. This study provides more valuable information to deepen our understanding of the abiotic stress responses by *HSP100* genes in *A. littoralis*.

Keywords: abiotic stress, gene expression; halophyte; heat shock proteins (HSPs), transcriptome analysis.

Introduction

Due to their unique status, plants exhibit heightened sensitivity to a broad spectrum of environmental stressors, encompassing both biotic and abiotic factors. This susceptibility to environmental constraints is particularly pronounced during the reproductive phase, often resulting in diminished crop productivity (Bita and Gerats, 2013; Esmaeili Tazangi et al., 2022). Understanding the gene expression patterns in response to abiotic stresses, as well as the cross-talk among gene family members and gene networks, is the initial step toward identifying and functionally characterizing stress-responsive genes (Hu et al., 2009a). Heat shock proteins (HSPs) are a protein family found in both prokaryotic and eukaryotic organisms that maintain cellular proteostasis and protect cells against stress (Hu et al., 2022). Protein homeostasis in cells and acquired thermotolerance in plants are both regulated by Hsps, which act as molecular chaperones to prevent misfolding and aggregation of proteins (Gong et al., 2021). There are five groups of Hsps, identified by their sequence homologies and estimated molecular weights: sHsps, Hsp60s, Hsp70s, Hsp90s, and Hsp100s (Chen et al., 2018). A subset of the wider AAA+ family of proteins, HSP100/ClpB are chaperones that use the energy of ATP to restore proteins that have aggregated during times of stress. A subset of the wider AAA+ family of proteins, HSP100/ClpB are chaperones that use the energy of ATP to restore proteins that have aggregated during times of stress (Lee et al., 2007). The HSP 100 proteins consist of five distinct domains: (1) The carboxyl-terminus, (2) the middle domain, (3) the nucleotide-binding domain (NBD) 1, (4) the NBD2, and (5) the amine-terminus (Agarwal et al., 2001). There are two classes in this gene family: ClpATPases class I (ClpA, ClpB, ClpC, ClpD, and ClpE) have two ATP binding domains, while ClpATPases class II (ClpM, ClpN, ClpX, and ClpY) have a single ATP binding domain (Panzade et al., 2021).

It is vital to highlight that the HSP100/ClpB subfamily is present in bacteria, protozoa, yeast, and plants, but not in mammals or humans (Kedzierska-Mieszkowska and Arent, 2020). There are three distinct isoforms of HSP100/ClpB found in plants, each of which is localized to a certain cellular compartment: the chloroplast (ClpB-P), the mitochondria (ClpB-M), and the cytoplasm/nucleus (ClpB-C) (Singh et al., 2010).

Thermostotolerance in plants is facilitated by the Hsp100 family members. Arabidopsis, rice, soybean, wheat, and tobacco are among the plant species whose ClpB-C expression is strongly associated with heat tolerance (Mishra and Grover, 2016). Heat stress isn't the only stress that the ClpB-C family members are involved in. Heavy metals and oxidative stress, for example, lead to the overexpression of ClpB-C genes in rice (Singh et al., 2012). Similarly, oilseed rape mosaic virus (ORMV) infection significantly increases the levels of AtClpB-C transcripts (Carr et al., 2006). In addition to stress regulation, *HSP100/ClpB* genes are also involved in other non-stress conditions such as developmental stages. Several plant species have shown constitutive expression of *ClpB-C* genes throughout reproductive phases. *ClpB-C* were highly expressed during flower and seed development in maize, mustard wheat, rice and soybean (Mishra and Grover, 2016; Razzaq et al., 2023).

Aeluropus littoralis (Gouan) Parl., a member of the *Poaceae* family and a monocot halophyte, can withstand salinities of up to 600 mM and flourishes in areas with intermediate to high salinity (Hashemi et al., 2016). The genomic resources of *A. littoralis* give the possibility to discover the abiotic responsive genes and their cis-regulatory elements (Hashemi-Petroudi et al., 2022). This study provides information on the *A. littoralis* HSP100 gene family, including sequence properties, evolutionary relationships, exon/intron structure, motif organization, and expression pattern analysis utilizing genome and transcriptome data. Furthermore, an investigation was performed to identify the differential expression patterns of the *HSP100.3* gene across root and leaf tissues of the *A. littoralis* plant after treatment with ABA, cold, and drought.

Materials and Methods

Identification of the HSP100 gene family members

The protein sequences of the *HSP100* gene family members from *Arabidopsis thaliana* and *Oryza sativa* were downloaded from the TAIR

(http://www.arabidopsis.org/) and RGAP (http://rice.plantbiology.msu.edu/) databases. *Aeluropus* HSP100 gene family was initially identified via local tBLASTN in BioEdit software (Hall, 1999) by defining *A. littoralis* draft genome (Hashemi-Petroudi et al., 2022) as a subject and protein sequences of *Arabidobsis* and rice as a query sequence (E-value < 1E-10). Finally, the coding sequence (CDS), protein, and genomic sequences of *AlHSP100* family were identified. Three protein database including InterProScan (https://www.ebi.ac.uk/interpro/search/sequence-search) (Jones et al., 2014), Pfam (http://pfam.xfam.org/) (Finn et al., 2016) and SMART (http://smart.embl-heidelberg.de/) (Letunic et al., 2015) were used to confirm the specific domains of the HSP100 proteins.

Physiochemical analysis of HSP100 proteins

The subcellular localization of HSP100 proteins was predicted using the WoLF PSORT algorithm (https://wolfpsort.hgc.jp/) (Horton et al., 2007). The sequences of HSP100 were analyzed with ExPASy ProtParam (http://www.expasy.org/tools/protparam.html) to obtain the number of amino acids, theoretical isoelectric point (pI), and molecular weight, GRAVY, instability index, and aliphatic index.

Exon/intron structures and motif organization

The HSP100 genes' exon/intron structures were visualized via Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn) (Hu et al., 2015). The following parameters were used to find the conserved protein motifs of all HSP100 using the MEME program (http://meme-suite.org/tools/meme/): Twelve is the maximum number of motifs, and the lengths of the motifs range from six to fifty amino acid residues.(Bailey et al., 2009).

Phylogenetic tree construction

HSP100 proteins sequences in soybean, Brachypodium and foxtail millet were obtained from Phytozome database (v12.1) (Goodstein et al., 2012). Multiple sequence alignment of HSP100 proteins in *A. littoralis*, Arabidobsis, rice, soybean, Brachypodium and foxtail millet were performed using MUSCLE and finally the maximum likelihood (ML) phylogenetic tree were constructed using MEGA 11 (Tamura et al., 2013).

Plant material and stress condition

A. littoralis clone samples were used in this investigation. The growth chamber was set up at 25 ± 1 C, with a 16-hour light and 8-hour dark photoperiod and a photon flux density of 100 µmol m-2s-l using cool-white fluorescent light.Transferring the clone samples to a hydroponic culture that contained Hoagland's solution was performed. A stress of 600 mM sodium chloride was applied to plants that were two months old (every three days, 100 mM sodium chloride was received). Polyethylene glycol (PEG) 20% was applied for drought stress. Cold stress was applied at 4 °C. Leaf and root samples were taken at several time-points, including 0 (control), 3, 6, and 48 hours post-stress (hps), as well as one week following treatments. ABA treatment was performed at 100 µM by spraying the hormone solution on the leaves. At 0, 3, 6, 24, and 48 hps, samples of both leaves and roots were taken. The samples that were collected were kept at -80 °C until the next step.

Gene expression analysis of HSP100.3

Based on *A. littoralis* RNA-seq data containing the genes and their expression levels, expression pattern of the *HSP100* gene family was studied in leaves and root tissues, and the results are presented in the Heatmap chart plotted by Clustvis software (https://biit.cs.ut.ee/clustvis/) (Metsalu and Vilo, 2015). In this experiment, *HSP100.3* was selected for RT-qPCR analysis based on down-regulation obtained in RNA-seq data. For the RT-qPCR experiment, the RNA extraction Kit (Threezol, Riragene, Iran) was used to get the total RNA from different samples. The spectrophotometer (Biochrom WPA Biowave II, UK) and 1.2 percent agarose gel electrophoresis were used to check the concentration and integrity of the RNA samples. Subsequently, 1 µg of total DNA-free RNA was reverse transcribed to first-strand cDNA by the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's protocol, and the cDNA was diluted 5-fold for RT-qPCR. To avoid binding to other *AlHSP 100* family members, *HSP100.3* (forward: GCCGATTCCAGCCAGTA; reverse: ATATCTTCACAGCGTCTTCCT) *g*ene-specific primer was designed using AlleleID software

(Premiere Biosoft, CA, USA). For normalization of gene expression, *RPS3* and *UBQ* genes were applied as reference genes according to the previous studies. The minimum information for publishing of quantitative real-time PCR experiments (MIQE) guidelines were followed when conducting RT-qPCR studies (Bustin et al., 2009). Using the CFX96 real-time PCR instrument (Bio-Rad, USA) and Maxima SYBR Green Master Mix (Thermo Scientific), RT-qPCR was carried out. To summarize, the following PCR program was used to conduct each reaction in a total volume of 10 µl: 5 µl Maxima Master Mix, 2 µl diluted cDNA, 0.3 µl per primer, and 3.4 µl RNase-free water. 40 cycles of 15 seconds at 95 °C and 1 minute at 60°C. At the annealing/extension stage, data collection was done. To verify the specificity of the amplification, melting curves were generated by progressively heating the amplicon from 60 to 95 °C.There were non-template controls (NTS) added to every primer master mix. Every qPCR test was conducted with three technical replicates. The 2-ΔΔCt method is applied to measure the relative expression based on the quantification cycle (Cq) values (Livak and Schmittgen, 2001).

Results

AllHSP100 gene family identification

A total of 6 *HSP100* non-repetitive genes coding the specific protein domain of HSP100, including Clp N (PF02861) and ClpB_D2- small (PF10431), were detected in *A. littoralis* plant genome (Table 1). AlHSP100.1 and AlHSP100.2 proteins had two Clp_N domains, one AAA (PF00004) domain, one AAA_lid_9 (PF17871) domain, one AAA_2 (PF07724) and one ClpB_D2-small domain. AlHSP100.3 and AlHSP100.4 proteins concluded one AAA domain, one AAA_lid_9 domain, one AAA_2 and one ClpB_D2-small domain. AlHSP100.5 protein had one AAA_2 domain and one ClpB_D2-small. Also, AlHSP100.6 protein had two Clp_N domains (Figure 1).

Physiochemical properties of AlHSP100

Physiochemical characteristics revealed that AlHSP100 proteins had molecular weights ranging from 30.51 kDa (AlHSP70.16) to 102.38 kDa, and lengths ranging from 278 (AlHSP100.5) to 920 (AlHSP100.2) amino acids (AlHSP100.1). The isoelectric point (pI) values of given proteins tend to be acidic except AlHSP100.4 and AlHSP100.6. The gene length and CDS length changed from 914 bp to 5244 bp and from 837 to 2763, respectively. The instability index was highest for AlHSP100.6, whereas the aliphatic index and GRAVY were both highest for AlHSP100.3. The predicted subcellular localization showed that AlHSP100 proteins activated only in chloroplast and cytoplasm (Table 1).

AlHSP100 exon/intron structure

To further investigate the evolutionary relationships of the *AlHSP100* genes, exon/intron schematics of the *AlHSP100s* were generated using their genome and coding sequences. AlHSP100.5 has the fewest introns of all the *AlHSP100* genes—just one—while *AlHSP100.3* had eleven (the highest) (Figure 1).

Table 1. Physiochemical properties of the *HSP100* genes in *A. littoralis*.

Gene name	Gene ID	AC	PL	MW (kDa)	pI	GRAVY	II	AI	SL
AlHSP100.1	Alg12374	MT647608	912	102.38	6.36	-0.460	38.41	90.14	chlo: 9, mito: 3, nucl: 2
AlHSP100.2	Alg6559	MT647609	920	101.86	6.65	-0.303	46.77	94.32	chlo: 14
AlHSP100.3	Alg8703	MT647610	767	84.06	5.81	-0.179	39.07	98.93	cyto: 8, chlo: 3, nucl: 1, plas: 1, golg: 1
AlHSP100.4	Alg7596	MT647611	642	70.20	7.71	-0.193	33.18	93.91	cyto: 6, mito: 4, chlo: 1, plas: 1, pero: 1, cysk_nucl: 1
AlHSP100.5	Alg13672	MT647612	278	30.51	5.84	-0.403	39.50	87.66	chlo: 7, nucl: 4, cyto: 1, mito: 1, cysk: 1
AlHSP100.6	Alg3898	MT647613	832	89.93	7.74	-0.343	54.68	80.71	chlo: 14

A.C.: Accession number; PL: Protein length (aa); MW: Molecular weight; pI: Isoelectric point; II: Instability index; AI: Aliphatic index; SL: Subcellular localization; Chlo: chloroplast; mito: mitochondria; nucl: nucleus; plas: plasma membrane; golg: golgi apparatus; cyto: cytoplasm; pero: peroxide; cysk nucl: cytoskeleton and nucleus; cysk: cytoskeleton.

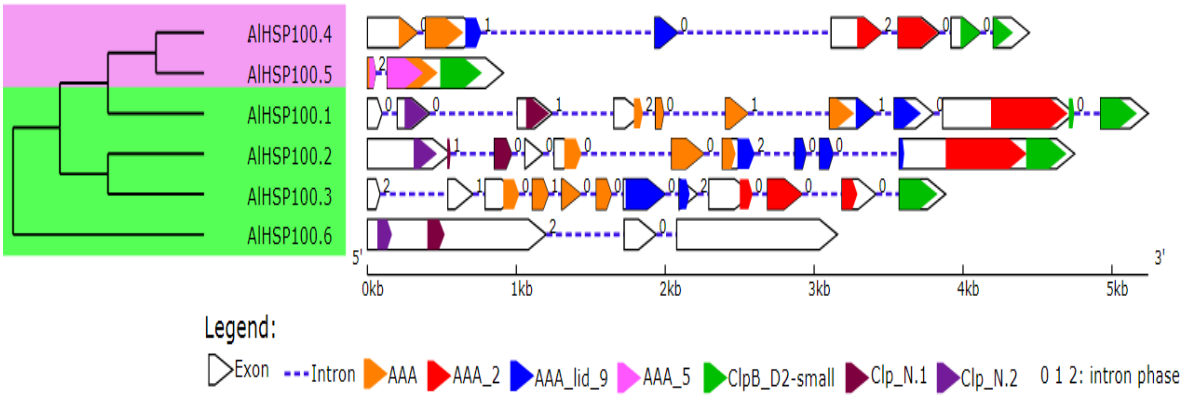

Figure 1. Analysis of *AlHSP100* gene family structures. The orange, red, blue, pink, green, brown, and purple portions denote the AAA, AAA 2, AAA lid 9, AAA 5, ClpB D2-small Clp N.1 and Clp N.2 domains, respectively. The wedges stand for exons, while the blue dashed lines represent introns. The numbers represent the AlHSP100 genes' splicing phases: 0, phase 0; 1, phase 1; and 2, phase 2.

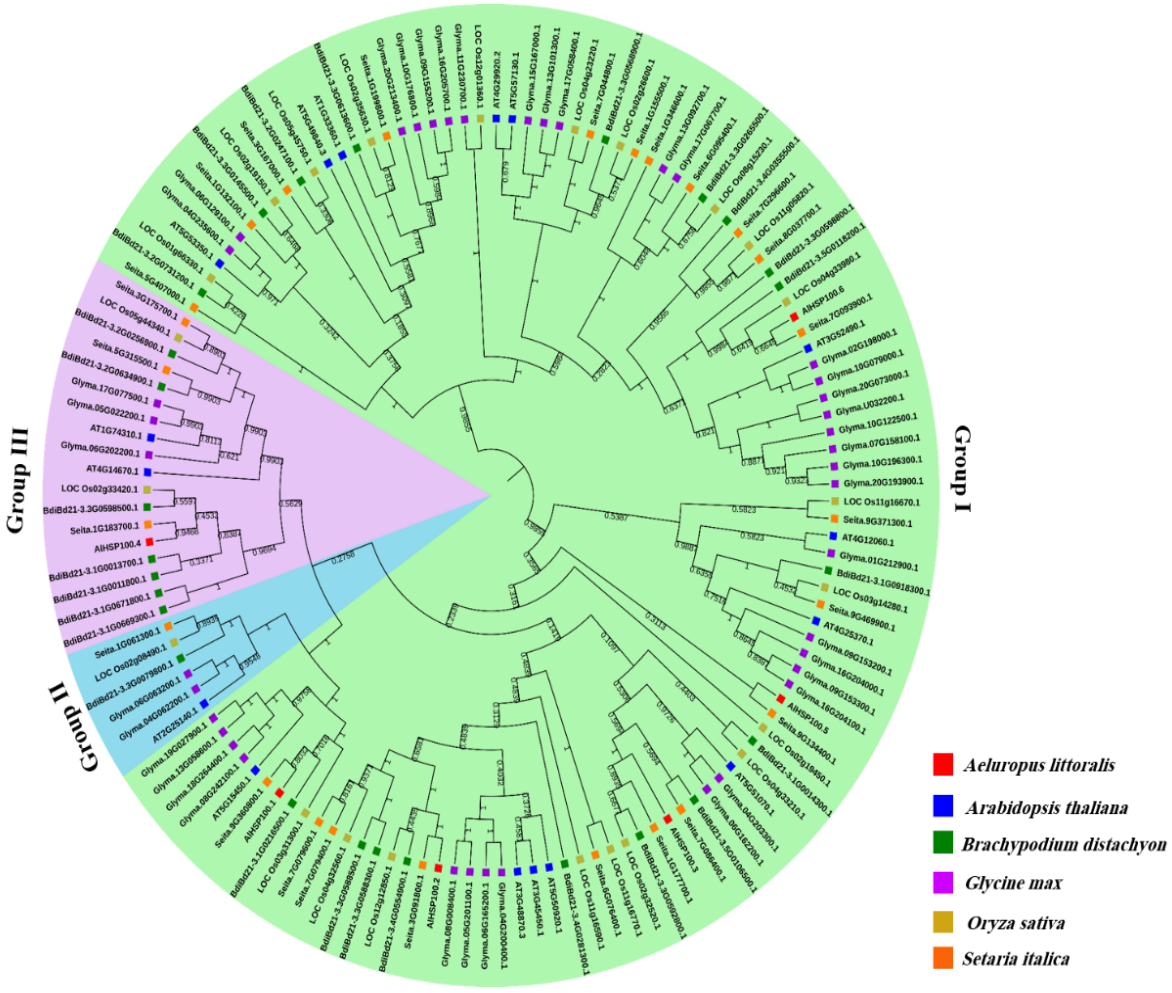

Figure 2. Members of the HSP100 family and their evolutionary relationships across six plant species. MUSCLE method was used to align HSP100 proteins from *A. littoralis*, *A. thaliana*, *Oryza sativa*, *Zea mays*, *Glycine max*, and *B. distachyon*, and MEGA 11 was used to create a phylogenetic tree using the the maximum likelihood.

AlHSP100 motif organization

To study the properties of the AlHSP100 protein sequence, the MEME program predicted 12 motifs independently. All members share a similar motif composition: 1 and 4 (Supplementary Figure 1). Furthermore, protein motifs are highly conserved and dispersed throughout all members, owing to the structural basis for their biological activity. Table 2 lists the sequences and annotations for the motifs.

Phylogenetic Analysis of AlHSP100s

We showed that the 136 HSP100 proteins of the foxtail millet, rice, soybean, *Aeluropus*, *Arabidobsis*, and *Brachypodium* species were divided into three groups by clustering (Figure 2). There were unique patterns and a little difference between *HSP100* distributions in each group. The result showed that each group had similar exon/intron structure and motif composition, while different groups had different exon/intron structure. Moreover, the same pattern was observed inside the species. Subcellular localization analysis indicated that in group I, *HSP100* are active in chloroplast, while in group II, *HSP100* genes were expressed in mitochondria. The third group included activated genes in cytoplasm (Figure 2).

Genes expression analysis

RNA-seq analysis was used to examine the expression patterns of the six *AlHSP100* genes in roots and leaves, providing additional insight into their activities. Figure 3 illustrates that the *AlHSP100.4* gene exhibited no detectable expression. While the other conditions and tissues did not show any noticeable up-regulation, there was a notable increase in expression observed for *AlHSP100.1*. The down-regulation of *AlHSP100.3* transcript level was observed in both stress and recovery conditions.

To gain a clearer understanding of how the *AlHSP100.3* gene responds to different abiotic stimuli and hormone treatments, we opted to investigate its expression in response to cold, salt, PEG, and ABA treatments**.** Although the expression level of AlHSP100.3 reduced modestly at specific time-points in leaf tissue, it remained overexpressed throughout root tissue. The study's findings showed that during salt stress, transcript levels in leaves were noticeably higher than in roots. In leaf, the greatest *AlHSP100.3* transcript level in comparison to the control was detected at the time-point of 6 hours (36.26).

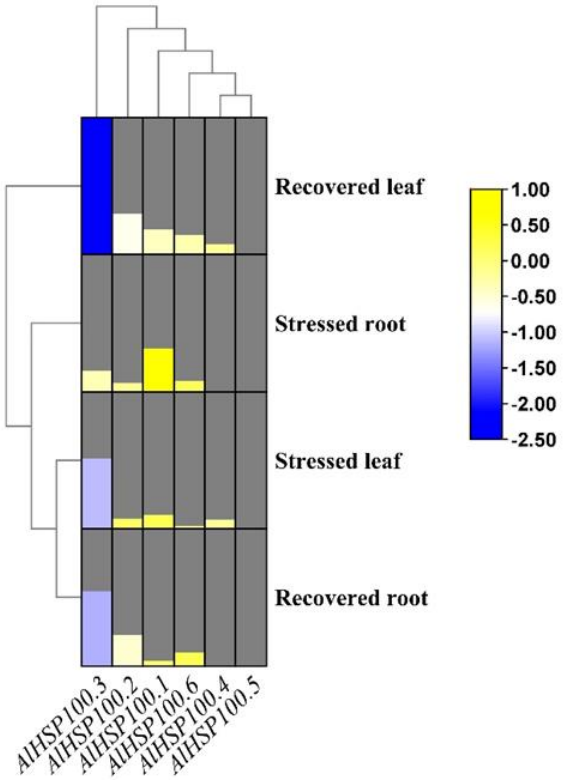

Figure 3. Expression patterns of the *AlHSP100* genes under NaCl treatment and recovery condition. Statistical significance is shown by the color gradient as the log2 fold changes. Significant up-regulation during salt stress is indicated by red, while significant down-regulation is indicated by blue.

The same results were achieved in roots (Figure 4). In drought stress, the lowest *AlHSP100.3* transcript level of leaf was at 1 wps (-3.69) while the highest transcription level was at 48 hps (19.81). The changes of *AlHSP100.3* in roots indicated that the highest transcription level in stressed plant occurred at 48 hps (10.10) compare to the control (Figure 4). The increasing changes of *AlHSP100.3* transcript level was observed in roots in cold stress and the highest amount was at 1 wps (1.60).

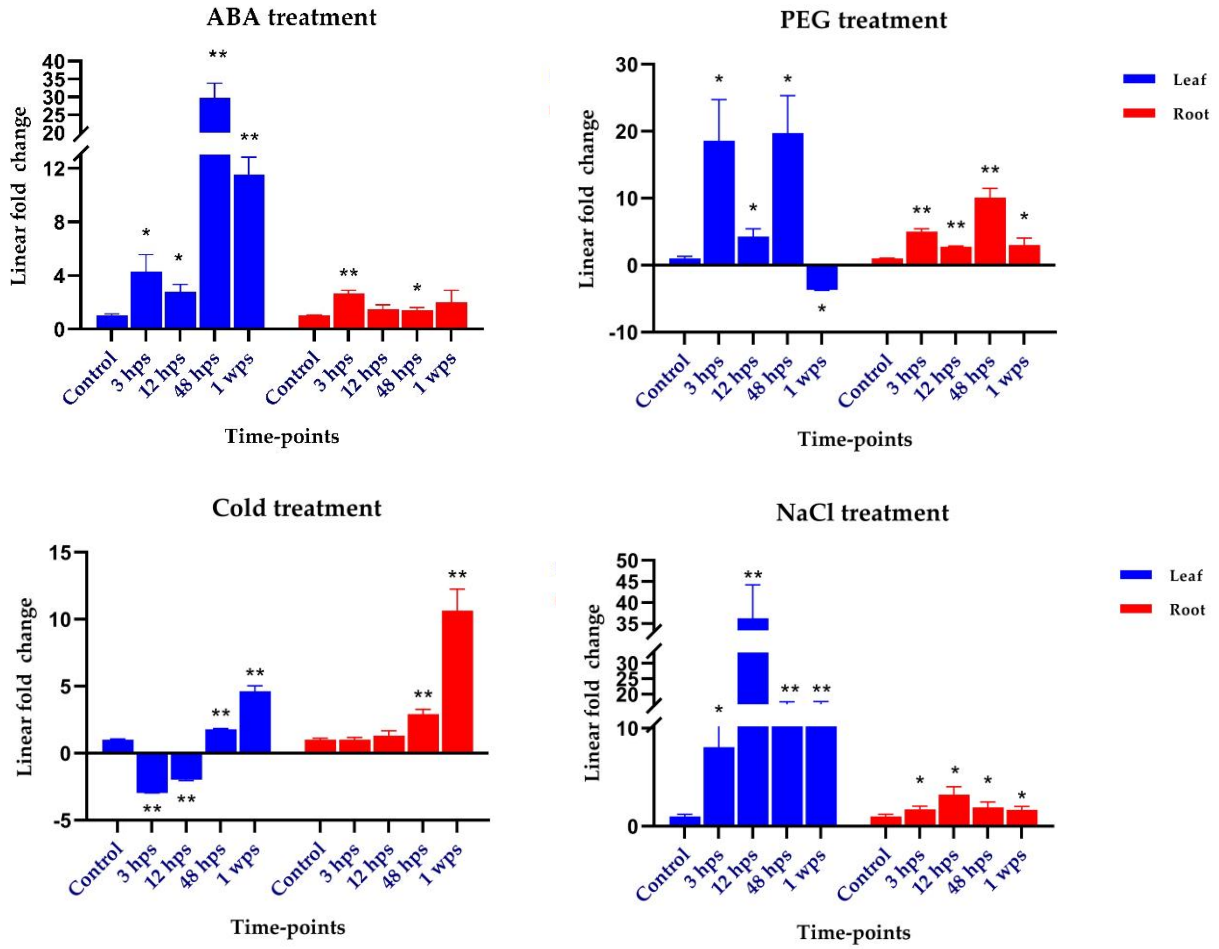

Figure 4. Expression patterns of *AlHSP100.3* gene under abiotic stresses treatments. RT-qPCR was performed to examine the expression profiles of the AlHSP100.3 gene under salt, PEG, ABA, and cold stress. The *RPS3* and *UBQ* genes were applied as reference genes. Error bars were calculated using three technical replicates. Asterisks indicate genes that were significantly up- or down-regulated in response to abiotic stress (* P < 0:05, ** P < 0:01).

At 3 and 6 hps, it was down-regulated in leaf and then increased up to 1 wps (4.62). The transcript level of *AlHSP100.3* in leaf tended to increase from initional point to 24 hps under ABA treatment. The highest and the lowest expression pattern were observed at 3 hps and control, respectively (Figure 4).

Discussion

As molecular chaperones, HSPs play important functions in the growth and development of plants and also provide protection to plant cells when they are under stress (Waters, 2013; Park and Seo, 2015). The HSPs biological function has been studied in wheat (Muthusamy et al., 2017), rice (Ouyang et al., 2009), and Arabidopsis (Lin et al., 2001). This study looked at the *HSP100* gene family in *A. littoralis* on a whole genome level. Six HSP100 genes were identified in the *A. littoralis* plant, which aligns with the findings reported for *Arabidopsis*, which have sixteen HSP100 genes (Goodstein et al., 2012).

The subcellular localizations of HSPs are believed to be tightly linked to their evolutionary relationships.

Isoforms of HSP100 proteins were further classified according to their subcellular localization as chloroplastic, mitochondrial, or cytoplasmic. Subfamilies are commonly designated based on the specific protein localization within each group (Scharf et al., 2001; Sarkar et al., 2009). The same classification was carried out by Singh et al. (Singh et al., 2010). HSP proteins are found in many forms inside plants, each of which is localized in a distinct cellular compartment—the cytoplasm/nucleus, chloroplasts, or mitochondria (Mishra and Grover, 2016). On the other hand, HSP proteins engage in biological processes in numerous subcellular localizations (Razzaq et al., 2023).

Phylogenetic analysis using *Aeluropus* species, *Arabidobsis*, rice, soybean, *Brachypodium* and foxtail millet sequences showed that HSP100 sequences are significantly conserved. The same result of conserved sequences was observed in the reseach of Singh et al (Singh et al., 2010). The tree displayed three main clusters, with each cluster representing a distinct pattern. It is possible that HSP100 proteins play a pivotal role in various cellular contexts due to their distribution to various organelles (Singh et al., 2010).

The importance of genomic organization in the evolutionary interactions of gene families under stress situations has been shown in previous studies (Xu et al., 2012; Chen et al., 2018; Hashemipetroudi et al., 2022). Based on our findings, gene structures shed insight into evolutionary relationships; for example, we found that the structures of the most closely related *HSP100* genes in the same family are quite similar. Additionally, organelle-specific (chloroplast) *HSP100* have more introns, whereas cytoplasmic *HSP100* genes generally have few introns, similar to the *HSP* genes in other plants. The close correlation between intron pattern and protein localization is demonstrated by this finding (Banilas et al., 2012; Guo et al., 2015). In addition, there exist findings that genes, which must be activated in response to stress fast, tend to have decreased intron density during evolution (Guo et al., 2015). The duration of the editing process of primary mRNAs could be affected by intron numbers (Heidari et al., 2023).

Alternative splicing events are possible lead to variations in intron/exon structures within the various AlHSP100 gene families, which may cause an increase in the diversity of gene functions (Panzade et al., 2021).

Within the AlHSP100 family, there was a great deal of variation in the 12 conserved motifs. Proteins belonging to the same subfamily (AlHSP100.1-4) and proteins belonging to distinct subfamilies (AlHSP100.5-6) had similar types, orders, and numbers of motifs. The most conserved protein motifs among HSP100 members are 1 and 4. These motifs may serve as the structural basis for HSP100's biological function (Chen et al., 2018).

We found that the *HSP100.3* genes exhibit distinct responses to different stimuli, indicating a diversity of stress response pathways in *A. littoralis*. Numerous investigations have demonstrated the involvement of the *HSP100/ClpB* genes in the growth, development, and stress response of plants. According to Hu et al. (2009b), heat shock and drought stress stimulate *OsHSP100/ClpB-C* expression in rice, but dehydration and ABA treatment induce *the ClpB-C* ortologous gene expression in wheat (Campbell et al., 2001). In Wu et al. (2009) expriments, the role of HSP100 cis-acting elements to influence desiccation tolerance in rice was disscuased. To detect promoter activity, Singh et al. (Singh et al., 2012) analyzed tissue-specific pattern of rice *ClpB-C* gene by using promoter: GUS reporter system, and shown that *OsClpB-C* gene has higher expression in grain development compare to anther, style and ovary tissues. Despite the complexity and diversity of *HSP100* expression patterns, our data demonstrated that *HSP100.3* was up-regulated in response to salt, drought, cold, and ABA treatment.

Conclusion

Due to climate change and global warming, there is an urgent need to develop transgenic plants with enhanced ability to withstand adverse environmental conditions. In the current study, six members of the *HSP100* gene family were identified in the halophyte Poaceae, *A. littoralis*, using a bioinformatic approach. Subsequently, we investigated the expression of the *AlHSP100.3* gene in response to cold, salt, PEG, and ABA treatments. The induction of the *HSP100.3* gene in response to salt, osmotic, and cold stress suggests its potential contribution to enhanced tolerance to stressful conditions. The presence of stress-dependent

regulation of the *HSP100.3* gene, as evidenced by its early response (3 hps) to PEG treatment and its late response to cold stress, is likely associated with the presence of cis-regulatory elements located upstream of this gene.

Supplementary Materials

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_712459.html.

Supplementary Figure 1. Distribution of conserved motifs of HSP100 in *A. littoralis*.

Supplementary Table 1. The motif sequence, length and annotation in AlHSP100.

Author Contributions

Conceptualization, S.H.H.; software, S.H.H. and S.M.; formal analysis, S.H.H. and S.M.; investigation, F.F. and S.M.; writing—original draft preparation, S.H.H. and F.F.; writing—review and editing, S.H.H.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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مقاله پژوهشی

شناسایی و آنالیز بیان خانواده ژنی *HSP100* در *Aeluropus littoralis*

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چکیده: پروتئین های شوک حرارتی (HSPs)، بعنوان چپرون‌های مولکولی با فعالیت های متنوع، برای رشد و نمو و پاسخ‌های به تنش‌ها ضروری هستند. به‌نژادگران گیاهی برای مقاوم ساختن گیاهان زراعی به شوری و خشک، استفاده از گیاهان هالوفیتی را مد نظر قرار داده‌اند. *Aeluropus littoralis*، بعنوان یک هالوفیت تک‌لپه علفی، یکی از گونه‌های مدل بالقوه برای کشف ژن‌های جدید پاسخ به تنش می‌باشد. در اینجا، ساختار اگزون/اینترون، موتیف‌ها/دامنه‌های حفاظت‌شده و الگوهای بیانی خانواده ژن *HSP100* در ژنوم *A. littoralis* شناسایی شد. در این مطالعه شش ژن منحصر به فرد *AlHSP100* غیر تکراری شناسایی شده که تغییرات ساختاری و فیزیکوشیمیایی قابل توجهی در بین زیرخانواده‌ها مشاهده شد. آنالیزهای فیلوژنتیک و موتیف نشان داد که پروتئین‌های یک زیرخانواده (AlHSP100.1-4) و پروتئین‌های سایر زیرخانواده‌ها (AlHSP100.5-6) دارای انواع، ترتیب و مقادیر مشابهی از موتیف هستند. در نهایت، بیان ژن *AlHSP100.3* با استفاده از RT-qPCR تحت تیمارهای دهیدراتاسیون، نمک، سرما و فیتوهورمون اسید آبسیزیک مورد تجزیه و تحلیل قرار گرفت و نشان داد که الگوهای بیان آنها در پاسخ به تنش‌های غیرزیستی متفاوت است. وجود تنظیم وابسته به تنش ژن *HSP100.3*، همانطور که توسط پاسخ اولیه به تیمار PEG و پاسخ دیرهنگام به تنش سرما مشهود است، احتمالاً با عناصر تنظیم‌کننده سیس واقع در بالادست این ژن مرتبط است. این مطالعه اطلاعات ارزشمندتری در خصوص درک پاسخ‌های تنش غیرزیستی توسط ژن‌های *HSP100* در *A. littoralis* فراهم می‌کند.

کلمات کلیدی: تنش غیر زنده، بیان ژن، هالوفیت، پروتئین‌های شوک حرارتی (HSPs)، تجزیه و تحلیل رونوشت.

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