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ORIGINAL ARTICLE

Molecular cloning, characterization and expression analysis of a heat shock protein 10 (Hsp10) from *Pennisetum glaucum* (L.), a C_4 cereal plant from the semi-arid tropics

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Abstract Heat shock proteins (Hsp10) belong to the ubiquitous family of heat-shock molecular chaperones found in the organelles of both prokaryotes and eukaryotes. Chaperonins assist the folding of nascent and stress-destabilized proteins. A cDNA clone encoding a 10 kDa Hsp was isolated from pearl millet, *Pennisetum glaucum* (L.) by screening a heat stress cDNA library. The fulllength *PgHsp10* cDNA consisted of 297 bp open reading frame (ORF) encoding a 98 amino acid polypeptide with a predicted molecular mass of 10.61 kDa and an estimated isoelectric point (pI) of 7.95. *PgHsp10* shares 70–98 % sequence identity with other plant homologs. Phylogenetic analysis revealed that *PgHsp10* is evolutionarily close to the maize Hsp10 homolog. The predicted 3D model confirmed a conserved eight-stranded ß-barrel with active site between the ß-barrel comprising of eight-strands, with conserved domain VLLPEYGG sandwiched between two ß-sheets. The gene consisted of 3 exons and 2 introns, while the position and phasing of these introns were conserved similar to other plant *Hsp10* family genes. In silico analysis of the promoter region of *PgHsp10* presented several distinct set of *cis*-elements and transcription factor binding sites. Quantitative RT-PCR analysis showed that *PgHsp10* gene was differentially expressed in response to abiotic stresses with the highest level of expression under heat stress conditions. Results of this study provide useful information regarding the role of chaperonins in stress regulation and generated leads for further elucidation of their function in plant stress tolerance.

Keywords ABA · Heat stress cDNA library · Chaperonin · *Pennisetum glaucum*

Abbreviations

Pg	*Pennisetum glaucum*
PgHsp10	*Pennisetum glaucum* heat shock protein 10
ABA	Abscisic acid
SA	Salicylic acid
Cpn	Chaperonin

Introduction

Molecular chaperons are a part of cellular machinery that assists folding of newly synthesized proteins to their native state. Chaperonins are unique, high molecular weight cylindrical complexes which aid protein folding that is unmanageable by simpler chaperon systems [1]. The term chaperonin was first suggested [2] to describe proteins homologous to *Escherichia coli* GroEL, which belongs to a class of molecular chaperones found in prokaryotes and in the mitochondria and plastids of eukaryotes [3]. Most important examples of chaperonins are the prokaryotic GroEL and the corresponding eukaryotic Hsp60. Chaperonins are classified into two subfamilies, the GroE chaperonins (Group I) found in bacteria, mitochondria and chloroplasts (e.g. GroE and chCpn60) and the CCT chaperonins (Group II), found in Archaea and in the cytosol of eukaryotes (e.g. trigger factor 55, thermosomes and the

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TCP-1 ring complex) [4]. Group I Cpn60 (also known as Hsp60) acts in the company of a co-chaperonin Cpn10 (Hsp10) in an ATP-dependent manner. While in bacteria, the Cpn10 is encoded by a single gene *groES*, in algae and plants, the plastid Hsp10 is encoded by multiple genes [5]. In plants, Hsp10 or Cpn10 can be classified as a member of the chaperonin family [6]. PgHsp10, homologous to Hsp10 genes found in other plant species such as *Arabidopsis thaliana* (Gene ID: 838063) and *Zea mays* (Gene ID: 100193174) can thus be categorized as a co-chaperonin. Additionally, Hsp10 as co-chaperonins have been identified from plants such as *Oryza sativa* [7], *Hordeum vulgare* [8], *Triticum aestivum, Phaseolus vulgaris* and *Pisum sativum* [9].

Although the bacterial Hsp10 is a ~10 kDa polypeptide, a ~20 kDa homologue comprising of two subunits is found in plastids. The two subunits are joined by a TDDVKD-linker sequence in head to tail fashion [10]. Hsp10 functions with Hsp60 as double-ring assemblies composed of back-to-back stacked rings of closely related rotationally symmetrical subunits [11], assisting in folding, assembly and sorting of proteins. The functions of Hsps in response to abiotic stress have been unraveled in organisms such as bacteria, yeast, insects, animals, plants and cell cultures [12]. Recent studies shed light on the role of chaperonins with regards to abiotic stress tolerance in plants [13]. Function of heat shock proteins such as Hsp70, Hsp60, Hsp90, Hsp100 and small Hsps belonging to various plants such as *O. sativa* and *Z. mays* have been evaluated with respect to abiotic stress tolerance [14, 15]. The chaperone (Hsp60) and the co-chaperone (Hsp10) enhance the osmotic and salt stress tolerance in transformed *E. coli* and yeasts [13]. Furthermore, plants such as *Nicotiana* spp. and *Arabidopsis* when transformed with Hsps demonstrate tolerance to heat, drought, salinity and cold stress. Along with stress tolerance, certain Hsps such as the Hsp100 also contribute to stress recovery [13].

Pennisetum glaucum (L.) is a resilient plant, mostly cultivated in regions with adverse agroclimatic conditions. Many genotypes of this plant exhibit tolerance to abiotic stresses such as heat, drought and salinity [16]. Stress induced expression of Hsp genes has been reported earlier in a few plants [14, 15, 17]. Similarly, expression of various Hsp genes from *P. glaucum* such as Hsp70, Hsp90 and small Hsps, have also been studied with respect to abiotic stress alleviation [18–21]. The present study investigates the role of co-chaperonin *P. glaucum Hsp10* (*PgHsp10*) in relation to abiotic stress tolerance.

In this study, we report the cloning and characterization of a *PgHsp10* cDNA from *P. glaucum,* a plant well adapted to abiotic stress. To better understand the possible role of *PgHsp10* gene in abiotic stress tolerance, the expression profile of *PgHsp10* gene in response to abiotic stress treatments (salt, high temperature, low temperature, drought), stress hormones (abscisic acid (ABA) salicylic acid (SA) and also in different tissues (seedling, leaf, root, panicle, mature seed) of *P. glaucum* were monitored by qRT-PCR. To the best of our knowledge, this is the first report on the isolation and analysis of *PgHsp10* from pearl millet, an important subsistence crop of the semi-arid tropics.

Materials and methods

Plant material and stress treatments

Pennisetum glaucum (L.) seeds, line 863B (a drought tolerant parental line) and ICMR 1122, ICMR 1086, ICMR 1152 and ICMR 1078 (high resolution cross genotypes) were procured from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The seeds were surface-sterilized and grown in 8" plastic pots filled with 5.5 kg of alfisol mixed with sand and compost mixture under 14/10 h light/dark cycle in a glasshouse. Two sets of plants were grown, wherein one set was treated as control (plants were allowed to grow under normal conditions without subjecting to stress conditions) and the other set was used for various types of stress treatments. For library construction, 14-day-old plants were exposed to heat treatment at 45 °C for different intervals of time. For transcript analysis, 14-day-old seedlings were subjected to different stress treatments for variable time periods. Dehydration stress was administered by withholding irrigation. For cold stress and heat stress, plants were incubated at 4 and 45 °C, respectively. Salt stress was administered via a hydroponic system wherein seedlings were dipped in a tray containing 250 mM salt (NaCl) solution. To induce ABA and SA stress, the seedlings were sprayed with 100 µM ABA/SA solutions. For vapour pressure deficit (VPD) stress treatment at 4.2 kPa, the plants were exposed to high temperature (40 °C) and humidity (38 %). VPD sensitive and insensitive genotypes were selected, based upon our previous studies, wherein, the transpiration rate (Tr, in g cm^{-2} min^{-1}) was assessed on two pairs of pearl millet genotypes which were initially identified as contrasting for the transpiration response to increasing VPD. These lines belonged to a high resolution cross (HRC), which was developed by crossing one of the most promising near isogenic lines (NILs) (ICMR 01029) [22, 23] to drought sensitive H77/833-2. HRC contrasting genotypes were therefore VPD insensitive (ICMR 1122

and ICMR 1086) and VPD sensitive (ICMR 1152 and ICMR 1078). Thus, these were fine mapping recombinant of a population of pearl millet that segregates for the capacity to restrict transpiration under high VPD conditions [24]. Tissue samples from seedlings, leaf, panicle, mature seeds and roots were also collected at different growth stages of the *P. glaucum* under glasshouse conditions. Seedlings and root samples were collected at 6 and 20 DAS (days after sowing) and flowers and mature seeds were collected at 40 and 90 DAS, respectively. After treatment, tissue samples were harvested by flash freezing in liquid nitrogen from both stressed and control plants and stored at −80 °C for RNA isolation.

Cloning of the PgHsp10 cDNA and genomic clone

An EST clone exhibiting maximum homology with the *Hsp10* chaperonin gene (GenBank Acc: CD725310) was identified from the stress responsive EST database [25]. This clone was used as a probe for screening the *Pennisetum* heat stress cDNA library using the plaque hybridization method, as described earlier [25]. Plaques showing positive signals were purified to homogeneity following two rounds of screening. The positive recombinant cDNA inserts were prepared for sequencing [26]. Genomic DNA was isolated by using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The *PgHsp10* genomic fragment was PCR amplified using 150 ng of forward (5′-ATGGCGAAGAGGCTGCTCCCGTC-3′) and reverse (5′-TCAGTCCACAAGTGTACCCAAGAT-3′) primers along with 200 µM of each dNTPs, 2.5 units of Taq DNA polymerase (Invitrogen) and 150 ng of genomic DNA as template in a 50 µl reaction volume. The PCR cycling conditions were: 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min for 30 cycles. A product of 1654 bp was cloned into the topo-TA 4.0 vector (Invitrogen) according to the manufacturer's protocol and sequenced completely (GenBank Acc: KU532916). Sequence comparisons were performed with the MacVector software (Version 14.5.0).

Cloning of PgHsp10 promoter sequence by PCR based directional genome walking

PCR-based directional genome walking method [27] was used for cloning of the upstream region of the *PgHsp10* gene using a specific antisense primer (5′-GCGGTC TTCTTGGGCTGCAC-3′) synthesized in antisense orientation and the T7 primer. PCR was carried out using 150 ng each of the gene specific and T7 primers along with 200 mM of each dNTPs and 2.5 units of Taq DNA polymerase with 150 ng of *P. glaucum* genomic DNA as template in a 50 µl reaction. PCR conditions were 94 °C, 1 min; 55 °C, 1 min and 72 °C, 2 min for 30 cycles. Genomic fragment was cloned into Topo-TA vector 4.0 (Invitrogen) and completely sequenced. The promoter sequences were screened for putative *cis*-acting elements using the PLACE [28] and PlantCARE [29] databases as well as motifs taken from the literature.

Sequence analysis of PgHsp10 gene

To identify genes encoding Hsp10 family members in other plant species, sequence similarity searches were performed using the Blast N, Blast X and Blast P programmes of National Centre for Biotechnology Information (NCBI). Multiple sequence alignments were performed by using clustalW (MacVector programme). ORF length and intron numbers of the *PgHsp10* gene were analyzed and confirmed by comparing the sequences of cDNA and respective genomic clones using the EMBL sequence alignment and MacVector ClustalW programmes. Translated cDNA sequences of different monocot and dicot plant species were used to construct a phylogenetic tree. Theoretical amino acid composition, molecular weight, pI, hydropathy analysis, grand average of hydropathicity, aliphatic index and estimated half-life of the *PgHsp10* protein were determined using the Expert protein analysis system (EXPASY) tools (http://www.ebi.ac.uk/Tools).

Structural analysis and homology modeling of PgHsp10 protein

PgHsp10 molecular model was generated using the homology modeling server SWISS-MODEL [30] using the crystal structure of 10 kDa chaperonin 4pj.1.1.Y as template. The predicted 3-D structure was visualized and compared with *Z. mays* Hsp10 (*ZmHsp10*) by using PyMol. The secondary structure of *PgHsp10* was predicted by using PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) [31].

RNA isolation, cDNA synthesis and qRT-PCR analysis

Total RNA was isolated from *P. glaucum* seedlings exposed to different abiotic stress conditions and their corresponding controls using the TRIzol reagent (Invitrogen GmbH, Karlsruhe, Germany). First-strand cDNA was synthesized separately from these RNA samples using first strand cDNA synthesis kit (Invitrogen GmbH, Karlsruhe, Germany) and used for quantitative PCR amplification using specific oligonucleotide primers (5′-GACTT GGGACATCGAC TACGA-3′ and 5′-GTGGTACTGCTACCGAACAGC-3′ for *PgHsp10* and 5′-TGTGAGCCATACCGTGCCAA-3′ and 5′-GGCAGTGGTGGTGAAGGAGT-3′ for *Pg-ß-Actin* and 5′-AGAAGGCGCTTGCTTACTCAT-3′ and 5′-CAGTTCTGG GTGAGGGAATCT-3′ for *PgMDH* as a housekeeping

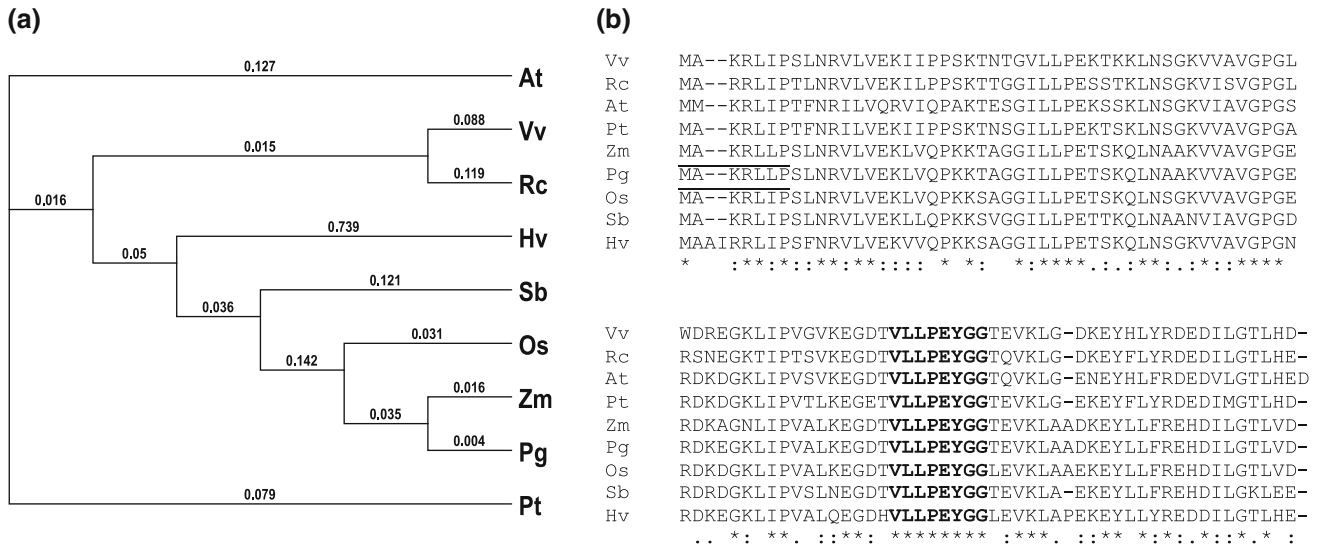

Fig. 1 Phylogenetic analysis of *Hsp10* genes belonging to various plant species. **a** This phylogenetic tree was drawn using the deduced amino acid sequences of different plant species including *Pg Pennisetum glaucum*, *At Arabidopsis thaliana*, *Hv Hordeum vulgare*, *Zm Zea mays*, *Ta Triticum aestivum*, *Pt Populus trichocarpa*, *Sb Sorghum bicolor*, *Os Oryza sativa, Rc Ricinus communis* and *Vv Vitis vinifera*, **b** Multiple sequence alignment highlighting characteristic domains and conserved residues (*Underlined*—MAKRLLP domain, *Bold*—VLLPEYGG domain)

reference genes). Quantitative real-time PCR reactions were performed in optical 96-well plated with an iCycler (BioRad, USA) using SYBR® Green. The reaction conditions were programmed to 2 min at 95 °C (polymerase activation), 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Amplicon dissociation curves were recorded after cycle 40 by heating from 60 to 95 °C with a ramp speed of 1.9 °C min^{-1}. Experiments were performed independently three times, and the average data was considered for further analysis. The relative change in expression levels of *PgHsp10* transcripts in different tissues of the plant or in response to abiotic stress conditions was predicted using REST software [32] using *Pg-ß-Actin* and *PgMDH* as the reference genes [21]. The normalized expressions of *PgHsp10* gene in different tissues were normalized against *Pg-ß-Actin* and *PgMDH* reference genes (qBase plus software (ver: 2.4; Biogazelle, Belgium) [33].

Results

Cloning and sequence analysis of PgHsp10 gene

The full-length cDNA encoding for *PgHsp10* sequence was isolated by screening a *P. glaucum* cDNA library using *PgHsp10* EST (GenBank Acc: CD725310) as a radioactive probe [20]. The cDNA contained a 297 bp long open reading frame corresponding to the *PgHsp10* gene. The *PgHsp10* protein was found to comprise of 98 amino acids with a theoretical molecular weight corresponding to 10.61 kDa and isoelectric point of 7.95 as predicted using the ProtParam tool from Expasy (http://www.ebi.ac.uk/Tools). The protein was predicted to be localized in the mitochondria using Expasy tools (http://www.ebi.ac.uk/Tools).

Phylogenetic analysis and multiple sequence alignment

The phylogenetic relationship between Hsp10 proteins from *P. glaucum* and other selective monocots and dicots was evaluated by constructing a neighbor-joining tree (Fig. 1a). Evolutionarily, *PgHsp10* was found to be closely related with its homologue from *Z. mays*. Also, a distinct relationship between monocot Hsp10 sequences was evident from the branch distances. Multiple sequence alignment indicated that PgHsp10 exhibited 70–98 % sequence identity with homologous sequences from other monocots such as *Z. mays* (98 %), *O. sativa* (92 %), *Sorghum bicolor* (84 %), *Hordeum vulgarae* (84 %), *Vitis vinifera* (74 %) and *Populus trichocarpa* (70 %) (Fig. 1b). VLLPEYGG domain of Hsp10 was found to be conserved throughout all the sequences; moreover, the MAKRLLP domain, a characteristic N-terminus signal peptide was present in Hsp10 sequences of *P. glaucum* and *Z. mays* (Fig. 1b).

Genomic organization of PgHsp10

The structural organization of *Hsp10* genes from various monocot and dicot plants was predicted by comparing the genomic and cDNA sequences (Fig. 2a). The coding region of *PgHsp10* consisted of 3 exons interrupted by 2 introns

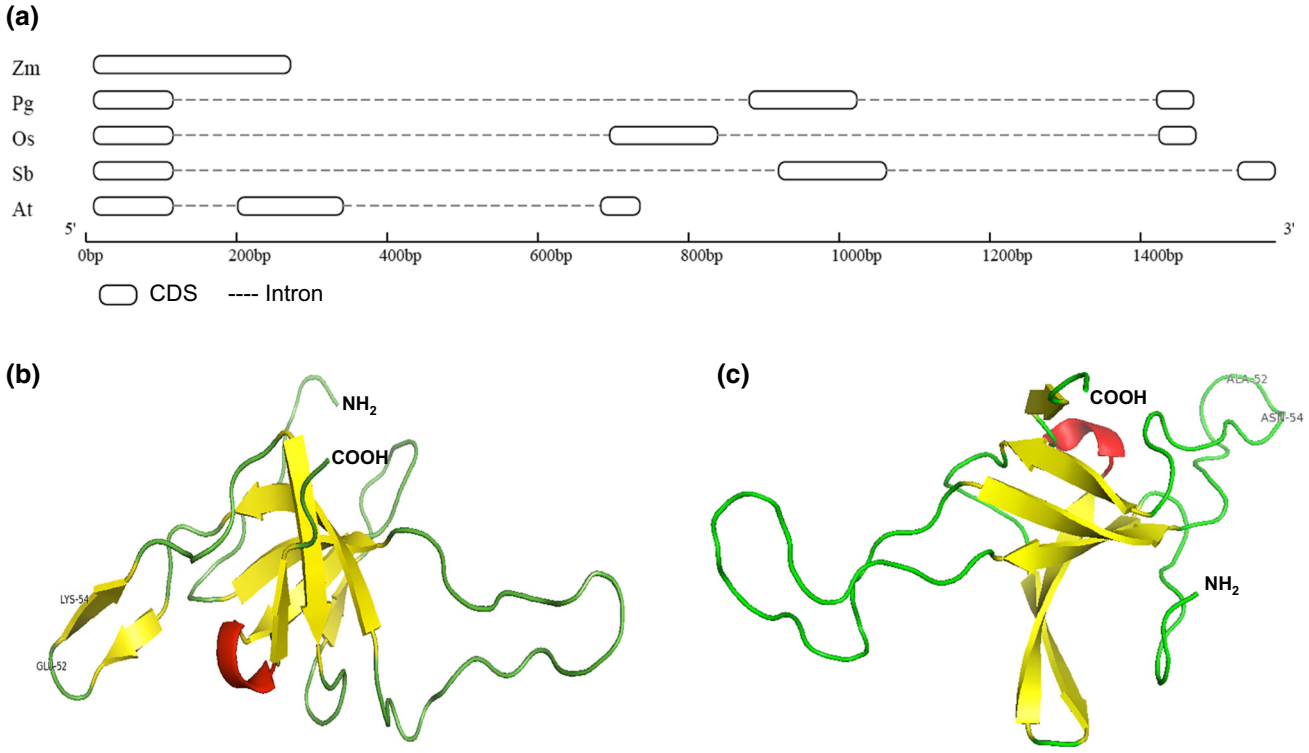

Fig. 2 In silico characterization of *Hsp10* genes and proteins. **a** Genomic organization (*Pg Pennisetum glaucum*, *At Arabidopsis thaliana*, *Zm Zea mays*, *Sb Sorghum bicolor*, *Os Oryza sativa*). Predicted three-dimensional structure of **b** *PgHsp10* and **c** *ZmHsp10*. The difference between *PgHsp10* and *ZmHsp10* at the 52nd and 54th positions is displayed. The conserved domain VLLPEYGG is shown in *red*. (Color figure online)

(GenBank Acc: KU532916). A similar pattern was also observed in the coding regions of *Hsp10* from *S. bicolor*, *O. sativa*, *Populus* sp. and *A. thaliana*. Amongst these genomic regions, the length of exon 1 was found to be 105 nucleotides. Similarly, exon number 3 was predicted to be highly conserved, ranging from 47 to 51 nucleotides. However, the genomic region of *Hsp10* from *Z. mays* (*ZmHsp10*), the nearest neighbor of *PgHsp10*, lacked introns. All exons had similar starting codons; exon 1 starting with adenine (A) and ending with guanidine (G), and exon 2 starting with cytosine (C) and ending with guanidine (G). Similar pattern was also observed with exon 3 that had initial nucleotide as thymidine (T) and last nucleotide as adenine (A). Since the phasing was conserved in *Hsp10* genes from *P. glaucum*, *S. bicolor*, *O. sativa* and *A. thaliana* it suggested that the derivation of these genes was a consequence of duplication of a common ancestral gene.

Molecular structure and homology modeling of the PgHsp10 protein

In silico prediction revealed that *PgHsp10* protein had an pI and molecular weight corresponding to 7.95 and 10.615 kDa, respectively. The aliphatic index of *PgHsp10* was 117.35 which defines relative volume occupied by aliphatic side chains like alanine, valine, isoleucine, and leucine. Estimated value of half-life of *PgHsp10* was 30 h (mammalian reticulocytes, in vitro). A predicted 3-D structural model was constructed on the basis of available information from a closely related heterologous source. Molecular modeling of *PgHsp10* was carried out using template molecule showing highest identity (54.17 %) on target-template alignment and highest resolution (3.15 Å) (Fig. 2b). Predicted 3D structure confirmed a conserved ß-barrel comprising of eight-strands, with conserved domain VLLPEYGG sandwiched between two ß-sheets, as shown in Fig. 2b. The template thus selected was 4PJ1, the crystal structure of the human mitochondrial chaperonin. The same template was used for modeling of *ZmHsp10* (Fig. 2c). The two structures were aligned with each other to exhibit 98 % identity and RMSD value of 0.01. While the structural comparison of *PgHsp10* and *ZmHsp10* revealed very high similarity, the differences were observed only in the presence of two residues (Glu and Lys) in the coil at 52nd and 54th position in the former as compared to Ala and Asn in the later.

Promoter analysis

A PCR-based directional genome walking protocol by using Phi29 DNA polymerase was used to clone the promoter of the *PgHsp10* gene. This method produced overlapping fragments of whole genomic DNA with unique single-stranded walker adapter on their 5′ ends [26]. Subsequently, a specific primer (5′-GCGGTCTTCTTGGG CTGCAC-3′) synthesized in the antisense orientation based on the *PgHsp10* cDNA sequence information was used to amplify a 0.9 kb region of genomic DNA. In silico analysis of this promoter sequence suggested the presence of a number of putative stress cis-regulatory elements, such as the MYB binding site involved in response to drought, Motif IIb for ABA responsive expression, CGTCA and LTR motif for methyl jasmonate responsiveness and GARE-motif gibberellin responsive expression. Other *cis*-regulatory elements such as AE box responsible for light response, and GCN4 motif responsible for endosperm-specific expression were also predicted to be present (Fig. 3).

Expression profile of PgHsp10 gene under different experimental conditions

The expression profiles of *PgHsp10* transcript in response to different abiotic stress conditions such as cold, salt, drought, heat, high VPD and phytohormone treatments were analysed. The relative expression levels of *PgHsp10* gene in tissue subjected to stress was compared with its corresponding control tissue using Relative Expression Software Tool (REST). *PgHsp10* demonstrated varied expression profiles in response to abiotic stress conditions where its expression decreased gradually with time during cold stress and reducing up to 50 % within 24 h (Fig. 4a). As opposed to this, *PgHsp10* transcripts increased in abundance with time when subjected to salt and drought stresses. Although a steady upregulation was observed in response to drought stress, salt stress induced rapid expression after 24 h. An interesting expression pattern was observed when the plants were exposed to high temperatures of 40 and 48 °C, respectively (Fig. 4b). At 40 °C, the gene was upregulated 24-fold in the initial 1 h of heat shock, and down regulated as the plant acclimatized to the temperature. However, at a higher temperature of 48 °C, the transcript abundance of *PgHsp10* slowly increased up to ~6-fold that of control within 4 h. This might point towards the incapability of *PgHsp10* with respect to countering exposure to high temperature. Furthermore, during high VPD treatment, high levels of *PgHsp10* expression were observed in the VPD insensitive genotypes, that are, ICMR 1122 and ICMR 1086, whereas the VPD sensitive genotypes (ICMR 1152 and ICMR 1076) showed down-regulation of the gene. Also, the expression in leaves was higher than that in root tissue in all the tested genotypes except for ICMR 1152 (Fig. 5a).

The expression patterns of *PgHsp10* in response to stress induced by treatment with ABA and SA hormones lacked any specific pattern (Fig. 4c). Plants treated with ABA showed a transient down-regulation at 8 h, while those treated with SA showed transient up-regulation at 4 h that did not deviate significantly from the normalized expression at other time intervals. In addition to these studies, the expression of *Hsp10* in various plant tissues such as seedling, leaf, panicle, mature seed and root were also studied. The normalized expression was found to be approximately 2.5-fold enhanced in the roots followed by 1.6- and 1.3-fold in leaf and panicle, respectively. The seedling and mature seed exhibited slight upregulation in the expression of *Hsp10* (Fig. 5b).

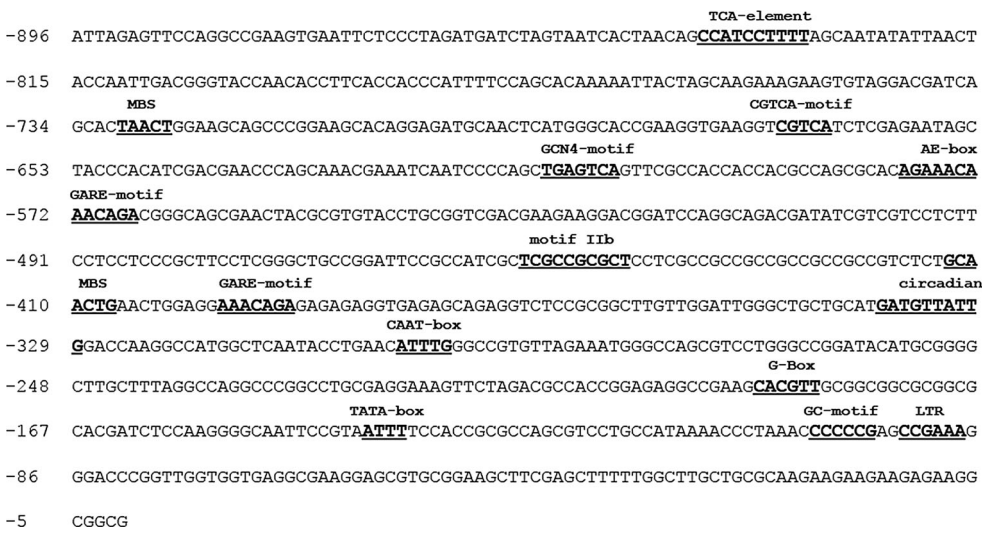

Fig. 3 Promoter sequence of the *PgHsp10* gene(*upstream region* ~0.9 kb). Various putative cis-regulatory elements have been marked in the *upstream region* of the *PgHsp10* gene

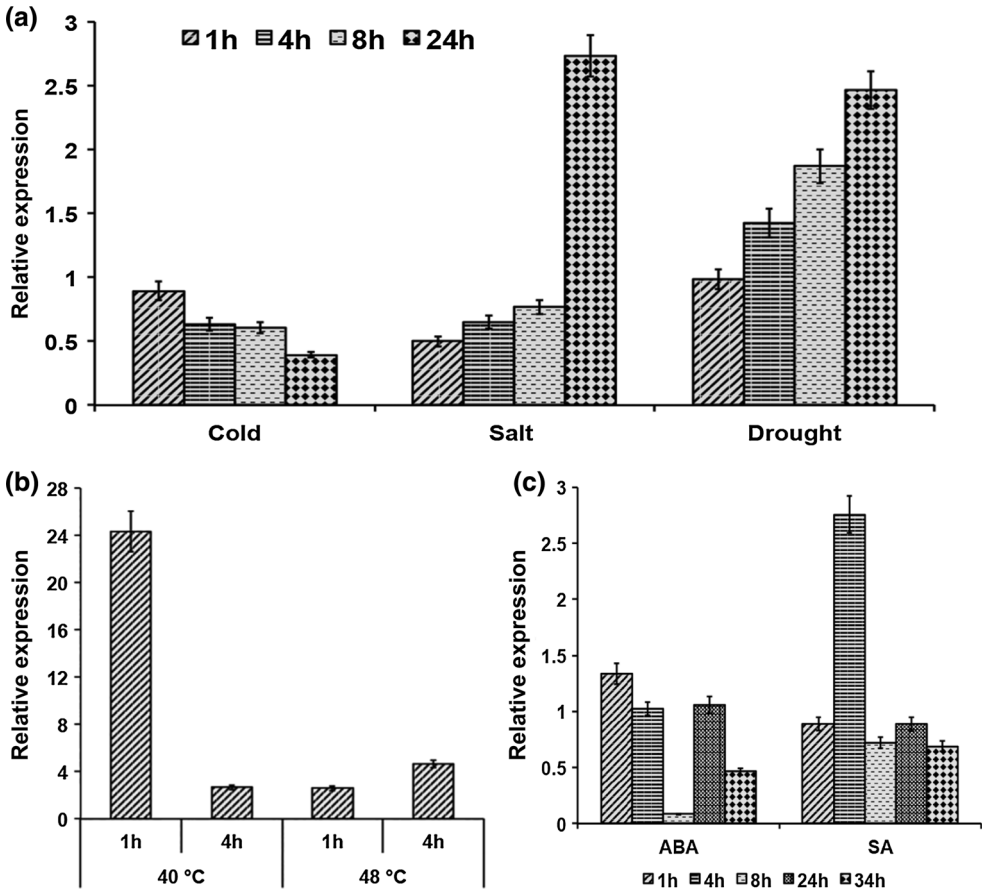

Fig. 4 Relative expression of *PgHsp10* transcript in response to different stress conditions (**a**) cold, salt and drought (**b**) heat (**c**) ABA and SA. The *x-axis* represents the different stress treatments and duration of stress treatment applied to pearl millet plant; the *y-axis* represents the relative fold increase in expression compared to respective controls. Standard *error bars* are shown

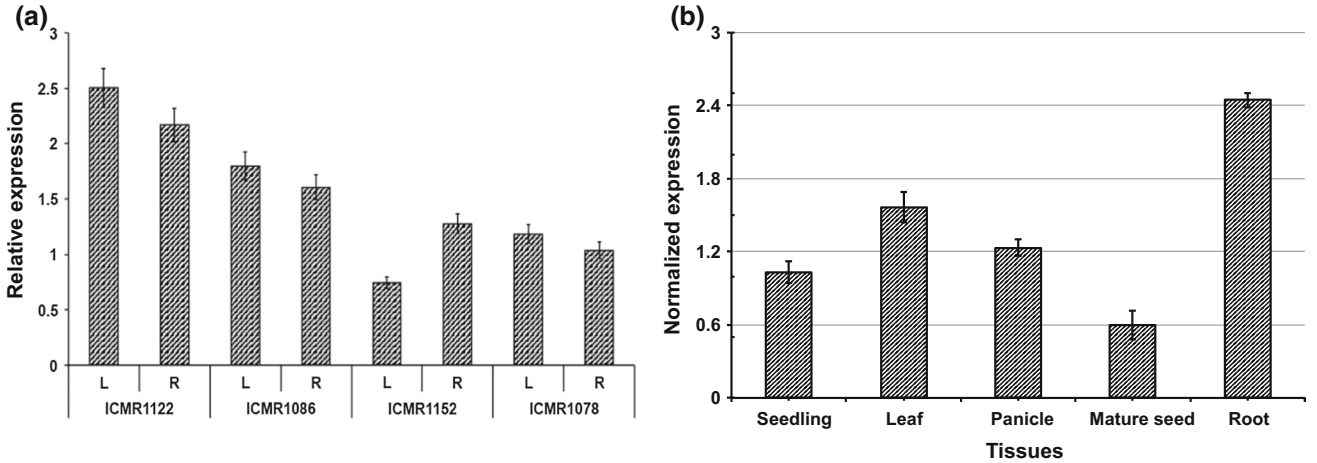

Fig. 5 Relative quantification of *PgHsp10* using qRT-PCR under different experimental conditions. **a** High VPD conditions (High VPD insensitive genotypes: ICMR 1122 and ICMR 1086, VPD sensitive genotypes: ICMR 1152 and ICMR 1076) **b** Normalized expression of *PgHsp10* in different tissues of *P. glaucum*. Standard *error bars* are shown

Discussion

Pennisetum glaucum (Pearl millet) is a robust annual crop belonging to the family *Poaceae*, grown mostly in the semi-arid regions where it is prominently subjected to heat and drought stress conditions. Such abiotic stress conditions result in over 50 % crop yield losses across the world [34]. To counter these constraints, it is important to elucidate the mechanisms of stress tolerance, since many physiological and molecular stress response mechanisms

function synergistically to uphold cellular homeostasis and conclude the plant reproduction cycle [34, 35]. This crop is known to withstand heat stress by achieving rapid growth under high temperature conditions where factors such as root growth, and physiological processes required for growth are stimulated at higher temperatures [36]. As pearl millet exhibits adaptation to heat stress, it is believed to possess a variety of genes necessary to thrive in heat stress. Earlier studies point towards a range of abiotic stress alleviating genes such as chaperonins, heat shock factors and dehydrins, that have been found in the pearl millet genome [19, 20, 37, 38].

Hsps are predominantly involved in signal transduction during high temperature, followed by their role as chaperonins in protein folding and transport. Hsps help the native proteins to retain their functional conformation and avoid the aggregation of non-native proteins, thereby aiding survival under stress conditions. Generally, the expression of Hsps is induced in response to a variety of stress conditions such as extreme temperatures, drought, salinity, wounding and oxidative stress [39]. A large number of ESTs corresponding to stress responsive Hsps have been reported in *P. glaucum* [25]. Particularly, in the present study, the expression profile of *Hsp10* that acts by forming a complex with *Hsp60* has been evaluated in response to abiotic stress conditions. As reported earlier [40], *Hsp60* exhibited enhanced expression under heat stress in *Z. mays*. As *Hsp60* and *Hsp10* function together, the later was believed to demonstrate similar expression profile.

In the present study, cDNA and genomic sequences encoding *Hsp10* chaperonin from *P. glaucum* have been isolated and cloned. Analysis of the nucleotide and deduced amino acid sequences indicated a close relation between *PgHsp10* and *Hsp10* genes from other plants, particularly with *ZmHsp10,* showing a 98 % sequence identity. The genomic organization of the *Hsp10* coding region is highly similar across monocots such as rice and sorghum. The lack of introns in *ZmHsp10* may be attributed to the subcellular localization of this protein, as opposed to the mitochondrial localization of Hsp10 proteins from other plants. The three-dimensional structures of PgHsp10 and ZmHsp10 shed light upon the structural conservation of these two proteins that comprise of 8 antiparallel β-sheets, a structural characteristic displayed by Hsp10 proteins isolated from *Thermus thermophilus* and human mitochondria [41]. The 98 amino acid protein was found to be localized in the mitochondrion that is evident by the presence of an N-terminus signal peptide (MAKRLLP).

In plants, the *cis*-regulatory elements (CREs) are considered to play an important part in regulation of transcription, especially during stress conditions [42]. To evaluate the contribution of CREs in stress induced expression of *PgHsp10* gene, the 5′ flanking region of the gene was analyzed using the PLACE and PlantCare suite. In agreement with earlier studies on PgLEA proteins [20], drought, ABA and methyl jasmonate responsive CREs were found in the sequence upstream of the *PgHsp10* gene. Thus, it can be said that various CREs, combined with their corresponding transcription factors regulate the expression of *PgHsp10* transcript under different abiotic stress conditions.

Hsp10, which belongs to multigenic category, exhibits high sequence similarity amongst its family members. Owing to this, monitoring the expression of individual genes belonging to Hsp10 family by Northern analysis proves to be difficult due to cross hybridization. To date, we lack information on the expression of Hsp10 family genes under wide range of abiotic stress conditions in millets, especially in pearl millet. Under abiotic stress conditions, the typical protein folding and transport patterns in an organism are disturbed. Stress conditions trigger incorrect folding or degradation of proteins, thereby necessitating the presence of molecular chaperons to achieve a functional structure. Thus, during stress conditions, the expression levels of heat shock proteins may be enhanced to compensate for protein degradation. Hsp10 acts as a co-chaperonin in complex with Hsp60. Thus, to enhance the formation of this complex, an acute increase in the expression of Hsp10 is predictable. As widely known, heat shock proteins regulate the folding and transport of proteins under stress conditions. Under abiotic stress conditions, plants incline towards completion of their reproduction cycle at a premature stage [43]. The consequence is very high water uptake and transport through roots, requiring high activity of water transport proteins in the root tissue [44]. This might attribute to high expression of Hsp10 in roots of plants subjected to stress, especially high VPD. Though *PgHsp10* showed high expression in response to heat stress, its up-regulation was not significant at very high temperatures (48 °C). This might point towards the incapability of *PgHsp10* with respect to countering exposure to very high temperature. Also, the panicle being an important organ in reproduction shows significantly high Hsp10 expression.

Conclusion

The cDNA sequences of a gene (*PgHsp10*) encoding for mitochondrial Hsp10 polypeptide from *Pennisetum glaucum* was successfully cloned and characterized. By homology modeling and phylogenetic analysis studies, it was recognized that *PgHsp10* shared 98 % sequence identity with maize Hsp10 homolog and is evolutionarily

close to the *Z. mays* Hsp10. The coding region of *PgHsp10* was governed by three exons and two introns. *PgHsp10* gene was differentially expressed in response to abiotic stresses such as drought, salinity, abscisic acid, salicylic acid, cold and heat with the highest level of expression under heat stress. This expression profile may attribute to counteract the stress-induced protein damage in *P. glaucum*. Plant responses to salinity, dehydration and temperature fluctuations are very complex and facilitated through different protein components involved in stress induced damage repair processes. This study provides useful insights on structural features and response of PgHsp10 when plants are subjected to different abiotic stress conditions in different developmental stages.

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Author contribution Conceived and designed the experiments: PSR, VV and MKR. Performed the experiments: RN, RY, PSR. Analyzed the data: PSR, KKR, VV. Wrote the manuscript: PSR, RN, RY, MKR and KKS.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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