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Genome wide characterization of Hsp 100 family genes from pigeonpea

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Abstract

Pigeon pea [*Cajanus cajan*] is an important grain legume of South-East Asia and East Africa. It is mainly grown as a *kharif* crop and is known to sustain prolonged drought and temperature stress. The Hsp 100 family gene [ClpB] is one of the major and critical genes required for acquired thermo tolerance. The current study was taken up to identify and characterize the Hsp100 family genes in pigeon pea since very little information is available for this thermo tolerant plant. *In silico* genome wide characterization was carried out and 5 Hsp100 like gene sequences were identified. Domains were predicted and three proteins were found to possess Class I type ClpB domains. Expression of these genes was observed at control 2, 6 and 24 h post heat stress conditions indicating their heat inducible nature. Out of the 5 genes predicted, two Clp genes show constant expression under heat stress and could be important components of the heat stress response in pigeon pea as they are targeted to the chloroplast. To identify additional members, candidate gene approach was taken up. *Glycine max* Hsp100 genes were downloaded and their expression studied under heat stress conditions in Asha, Pusa dwarf and *C. platycarpus* genotypes. Out of 23, 20 genes were expressed in pigeon pea indicating the presence of more Hsp100 family genes in the genome that need further investigation. This study would help in understanding the molecular components governing thermo tolerance in pigeon pea.

Key words: Pigeonpea, thermo tolerance, Hsp100 genes, ClpB, *Glycine max*

Introduction

Pigeon pea [*Cajanus cajan* L. Millsp.] is one of the major grain legume apart from greengram, blackgram, chickpea and lentil. It is 5th most important pulse in developing countries. More than 85% of the world pigeon pea is produced and consumed in India. Pigeon pea is an important source of protein and rich in sulfur containing amino acids. Often termed as orphan legume, pigeon pea cultivation requires minimal input including water and generally tolerates high temperatures prevalent during the monsoon season. Pigeon pea also fixes atmospheric Nitrogen and is useful for producing green manure. Like many other crops, pulses are also sensitive to heat stress at bloom stage and only a few days exposure to high temperature can cause heavy yield loss through flower drop or pod abortion [1]. Hsp 100 family gene is one of the major and critical genes required for acquired thermo tolerance. This protein has been reported in Arabidopsis, soybean, tobacco, rice, maize, lima bean and wheat etc. These genes function in protein disaggregation and/or protein degradation. It removes non-functional but potentially harmful polypeptides arising from misfiling, denaturation or aggregation and is important for the maintenance of cellular homeostasis [2].

These are a class of chaperon proteins that are highly inducible by high temperatures. Under heat stress condition, transcription of new proteins stops; many proteins get degraded and lose their 3D structure. This is followed by formation of non-native proteins, leading to tissue damage or plant death [2]. An increase in up-to 5^{o}C temperature induces the expression of many hsps and Hsp100 proteins are foremost in terms of expression [3]. These Hsp proteins carry out the function of protein folding, assembling and assist in refolding of mis-aggregated proteins in proper way thus saving the plants cells in

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severe heat stress condition by re-establishing normal protein conformation and cellular homeostasis [4].

The Hsp 100 family proteins are members of the AAA+ family of proteins. AAA+ where + indicates ATPase associated with diverse cellular activities [5-6]. Hsp 100 proteins contain nucleotide binding domain [NBD], sensor motifs walker A and walker B, and C-terminal domain [7]. The NBD domain is required for ATP binding and hydrolysis of ATP to generate chemical energy required for remodeling of bound substrates. It also leads to oligomerization of AAA+ proteins. By oligomerization of proteins, these form barrel shaped oligomer and central channel [8]. Depending upon number of NBD domains in Hsp proteins, Hsp 100 family proteins are divided in two classes - Class I and Class II. The class I proteins contain two NBD domain, amino terminal, middle spacer and carboxyl terminal regions. This class contains Clp A, B, C, D and E protein. Class II contains only one NBD which resemble the second NBD of Class I domain [7]. The C-terminal D-2 small domain is a mixture of alpha-beta structure. It is essential for oligomerization. C-terminal domain is rich in alpha helical structure and acts as lid for substrate binding domain. The function of lid depends upon Hsp 70 protein assisting in binding with ATP or ADP [2].

Arabidopsis contains eight Hsp 100 related genes [9]. Rice has nine Hsp 100/ClpB family genes, [10], out of which only 3 are regulated by heat stress and rest may be involved in other growth and developmental pathways. Among the major legumes, Soybean contains 23 Hsp100 related genes [Soybase] but is not completely characterized for their expression under heat stress. Therefore, characterization of these Hsps is important to deduce structure and functions in pigeon pea, since hsp100 family genes are the foremost proteins involved in alleviating the damage arising out of heat stress. This preliminary study will give useful insights into the heat stress response pathways in pigeon pea.

Materials and methods

Leaf sample of Asha, Pusa Dwarf and *Cajanus platycarpus*, a wild species of pigeon pea were collected from field.

Heat treatment

For heat treatments plants were raised in pots for 2-3 weeks. Heat treatment was given at 45^0C for a time period ranging from 30 minutes to 24 hours in a Thermo Scientific MaxQ 6000 Incubator. For field grown plants heat treatment was carried out using the detached leaf method. The intact leaf samples were placed on 0.8% agar plates and wrapped with parafilm. Samples were stored immediately in the deep freezer after a quick dip in liquid Nitrogen.

Estimation of membrane stability index

The membrane stability index [11] of leaves from the varieties Asha and Pusa dwarf under control 2,6 and 24hrs of heat stress was estimated using the formula MSI = (1-CI/CF) x 100

In silico analysis

The gene sequences were downloaded from accession AFSP000000. Genes were predicted by FGENESH. Homology searching of predicted genes was done by BLASTP (NCBI). The sequences having high hits, large query coverage and least percent of gaps were downloaded. The predicted protein sequence and its homologous sequences were taken and alignment was done by BLASTP. Domain prediction was done using Pfam database. Multiple alignment of sequences was done using MULTALIN. Phylogenetic analysis was done by using neighbor joining method of MEGA software.

Expression analysis

RNA isolation was carried out by Sigma Spectrum Plant Total RNA kit using standard protocols. cDNA was prepared by using ABI-RT kit as per manufacturer's protocol. RT-PCR was carried out with designated primers (Table 1, Supplementary data) and using standard procedures.

Results and discussion

Thermal stress leads to cellular membrane dysfunction, which is manifested in increased permeability and leakage of ions. This is measured by the efflux of electrolytes. Hence the estimation of membrane dysfunction under stress is measured by cellular electrolyte leakage from affected leaf tissue into an aqueous medium [12]. Physiological studies were conducted on two varieties of pigeon pea *viz*., Asha and Pusa dwarf. In the variety Asha (Fig. 1), membrane stability started decreasing marginally after 2 h of heat stress, and declined by 25% and 56 % after 6 and 24 h respectively. In case of Pusa dwarf membrane stability reduced by 36%, 43% and 76% under 2, 6 and 24 h of heat stress respectively. There is a marginal decrease in membrane stability in Pusa

Table 1. List of primers used for RT-PCR

S.No.	Gene call	Forward Primer sequence	Reverse Primer sequence
1	CcClpB4	AATTTTGGGGCAAGACCAG	TAGAGGAGGACGCTCTTTGC
2	CcClpB3	ATGGTGCTAGGCCAGTCAAG	AAGCTTTTGCTGGGGAAGTT
3	CcClpB4.1	TGCCAAGGGTATTCTTAGAGGA	AAGCCTTCCCAGCTGTCTTT
4	CcClpB	TGTCGATTGAAGTGCAGGAG	CGTCGTCTTCGAGCCTAAAC
5	CcHsp70KD	GGCTTGAGAAGAAGGTGGTG	TGACTTCTGCCCAGTTGTAGC
6	Glyma06g17360	AGGGACAGAGTGGTGGAGGA	AACAGAGTCGCCCTCCTTGA
7	Glyma09g28260	TGGTGCCAGGGGTTTAAGAG	CCAGGGGCAGTTAGTGATCC
8	Glyma05g00540	ATGGGGGCGAGTTGGTCTA	CATCTTCTTGACTGCTTGAACAGC
9	Glyma13g05920	GCTAGGCCAGTCAAGCGAGT	TGTTGGGGAAGTTGACCATT
10	Glyma04g06610	AGCCTCTGGATTCCGAACAG	TGGATCAAAACCCAGTACACCA
11	Glyma04g41400	GGGAGAGGGGCTAAAATCCTT	CATGCTGGCAACAATGGAAG
12	Glyma04g37740	GGAGGACAGCATGGCTGAG	GAAGTGCCTCTGGCAAGGA
13	Glyma06g17020	ATATGGTGCTCGCCCTCTG	TCGTAACAAAGGGGTTTCCA
14	Glyma16g33080	CTCCTGGATGTGGAGGGAAA	GGCTCTTGATGAAAGCTCCAA
15	Glyma05g38510	TTGTCTTCCGGCAACTCACA	TCCTTCGTCAACCACCCTCT
16	Glyma18g36010	GATGAGTTTGGGGGCAGTTATC	GATGAGTTTGGGGGCAGTTATC
17	Glyma04g38050	CCAGATATTGGATGTGCTGCTG	TCTAAGAGGGCGAGCACCAT
18	Glyma19g03440	TTGGAGCGTGTGCAGAAGAG	CACGTTTTGCTGAATCACTCG
19	Glyma20g35600	TGGTGCCAGGGGTTTAAGAG	CCAGGGGCATTTACTGATCC
20	Glyma08g01140	CAAGAGCTTGGTGACTGAGGAA	TGGAACACCTCCTTCAGCATT
21	Glyma10g32040	TGGTGCGAGGGGTTTAAGAG	CCAGGGGCATTTACTGATCC
22	Glyma18g49930	CCCCAACTATGGTGCTAGGC	GCCGTTGGTAAATGCTGTGA
23	Glyma06g06690	ACAAGTCGTTGAGTTGGCTAGG	CCTGTTTTTCACTCGCTCCA
24	Glyma06g13450	TGGGAGAGGGGCTAAAATCC	ACATGCTGGCAACAATGGAA
25	Glyma08g26410	ACAGCGCGTAATGGATGCT	CTCTTTTGCACACGCTCCAG
26	Glyma20g17360.2	TCAAGGATGTGGCTTTTGAAGA	GGCAAGCTACTAGCAATGCAAA

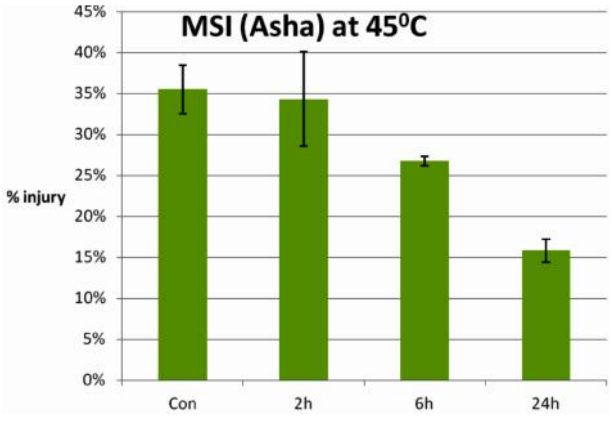

Fig. 1. Changes in the MSI of variety Asha in response to 2h, 6h and 24 h of heat stress at 45°C

dwarf (Fig. 2) as compared to Asha. Membrane stability is a direct measure of the cell's capacity to withstand thermo tolerance. In both the genotypes tested the MSI remained quite stable even after 24 h heat stress, and did not completely disintegrate. This however was not confirmed by microscopic analysis. Both the varieties showed significant ability to withstand heat stress as evident from % injury to the tissues. However the real test of the effect of high temperature can only be measured by evaluating its growth parameters. It is known that such basal levels of tolerance on leaf tissues are not a direct measure of a plant's response to thermo tolerance. The effect of high temperatures may lead to temporary shutdown of photosynthetic and respiration machinery and may not reflect in impact on yield. The true thermo tolerant ability of a plant

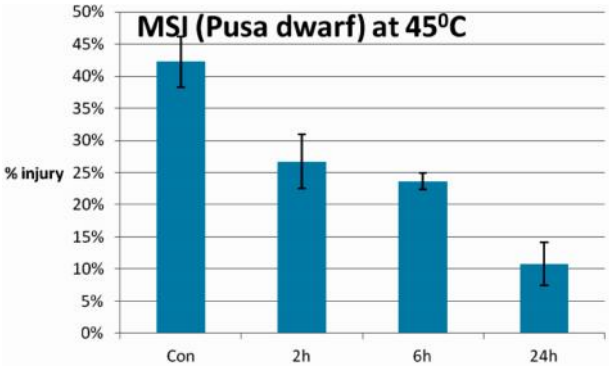

Fig. 2. Changes in the MSI of Pusa Dwarf in response to 2h, 6h and 24 h of heat stress at 45°C

depends upon its reproductive stage capacity to withstand heat beyond its optimal range [15].It is largely accepted that loss of electrolytes in a cell due to damage to the membrane integrity during heat stress leads to cell death eventually. This has a significant impact on yield, but in some cases like wheat and soybean, such yield penalties were not detected. There seems to be another mechanism operating in plants to overcome the effects of high temperature stress [16]. This is a preliminary study and will require detailed phenotyping in field as well as on different stages of growth and development to actually narrow down to parameters that define its maximum threshold as far as thermo tolerance is concerned.

Draft genome sequence of pigeon pea contains 152 contigs related to abiotic stress [13]. Out of these, five contigs were found to be displaying homology to Hsp 100 family genes, three were based on putative castor ClpB genes, one is 105kd protein and one *Arabidopsis* (Table 2). The contigs were assigned names according to its homology with similar protein (labeled as Cc).Gene prediction was done by FGENESH. *Medicago tranculata* and *Arabidopsis thaliana* were used as the reference organisms. These predicted proteins were then used for homology searching by BlastP. All three putative ClpB genes have nearly 928-978 amino acids which is in agreement with those in Arabidopsis [9]. The result of predicted proteins is given below (Table 3). CClpB4, CcClpB3 and CcClpB4.1 displayed strong homology to soybean sequences where B4 was predicted as mitochondrial and B3 and B4.1 as chloroplast targeted in nature. The result of homology search is summarized (Table 4). Similarly, the corresponding homologue from soybean genome was also found out for the CcClpB genes (Table 5). MULTALIN programme was used to

Table 2. Result of genome wide identification of Hsp 100/*ClpB* gene in pigeon pea

Contig No.	Gi No.	Gene homology with
22425	255548768	chaperone Clpb, putative [Castor]
33893	255570232	chaperone Clpb, putative [Castor]
34576	25557023	chaperone Clpb, putative [Castor]
00122	255563893	Hsp 105 kDa, putative [Castor]
10072	15242850	Heat shock protein-related Arabidopsis

Table 3. Result of gene structure prediction of Hsp100/ *ClpB* genes in pigeon pea

Gene Name	No. of Amino acid	No. of exons
Cc22425	977	10
Cc33893	978	11
Cc34576	928	11
Cc00122	777	8
Cc10072	1023	3
Cc 56651	871	6

align protein sequences of pigeon pea Clp proteins with Arabidopsis hsp101 protein as a reference. CcClpB gene did not show any putative domain structure as it had different amino acid sequence. The genes show a high degree of conservation of amino acid residue with AtHsp101. There were slight differences in between all proteins, which might give them specific secondary structure and ultimately specificity in functioning. Alignment results are shown in Fig. 3.

The phylogenetic analysis of the predicted pigeon pea Hsp protein sequences and 23 ClpB protein sequences of *Glycine max* using the software MEGA5 [14] is shown in Fig. 4. The phylogenetic tree reveals that these four genes fall into four different groups and have a high degree of similarity with the soybean genes. As also evident from homology and domain studies, CcClpB falls into a different group and confirms it as a non ClpB protein. The phylogenetic tree also shows clear synteny between pigeon pea and soybean hsp100 genes. The three CcClpb genes B4.1, B4 and B3 displayed a tight similarity with corresponding soybean genes. This is expected as the previous predictions were also based on soybean genes. Such evolutionary evidences indicate the universal nature of these genes. Similar studies among different plant

Table 4. Targeted location of Hsp100/ClpB genes

Gene name	Gene	Location	Query	Identity
CcClpB4	Clp4	Mitochondrial	100%	95%
CcClpB3	ClpB3	Chloroplast	100%	95%
CcClpB4.1	ClpB4	Chloroplast	100%	93%
CcHsp97KD	Hsp97KD	Chloroplast	95%	89%
CcClpB	ClpB	Chloroplast	100%	77%

Table 5. *Cajanus cajan Clp B* genes and corresponding *Glycine max* genes

Cajanus cajan gene	*Glycine max* gene
CcClpB	*Glyma07g33250.1*
CcClpB3	*Glyma18g49930.1, Glyma08g26410.1*
CcClpB4	*Glyma04g.06610*
CcClpB4.1	*Glyma13g05920*

hsp100 genes have also concluded on the high degree of conservation of these genes across species irrespective of the different growing conditions and tissue/stage [25].

The present study was conducted to undertake a genome wide identification and characterization of the pigeon pea hsp100 family genes from the draft genome sequence of pigeon pea. The Hsp100 family of proteins is important for both basal and acquired thermo tolerance. The common feature of these genes is their unique ability of being induced very early post minor temperature changes. The heat shock protein was first discovered and described in yeast as Hsp104 and then in *E. coli* [17-18]. Later on, the first plant hsp100 gene was described in soybean and Arabidopsis where the authors demonstrated the role of Hsp101 in complementing yeast mutants susceptible to high temperatures [19].Since then it has been demonstrated in many crops that Hsp101 protein is a key factor for acquired thermo tolerance [20-22].

The Hsp100 proteins are highly conserved across species signifying a universal role in heat stress pathways. As far as legumes or related species are concerned not much information is available except for lima bean [23]. Pfam prediction resulted in identification of only three genes, *viz*., CcClpB4, CcClpB3 and CcClpB4.1 that were found to contain the Hsp 100 family class I specific domains, especially the 2 AAA domains [24] (Fig. 5). These also contained 2 Clp N amino terminal domains and one C terminal D-2 small domain of Clp B (Table 6). There are only slight differences between B4 and B4.1 as evident from the domain position and size of the AAA sequence.CcHsp97KD gene did not show any homology domains typical of the Hsp 100 family proteins, but was rather a hsp 70 gene. Since Cchsp97 did not show any characteristic feature of the hsp100 gene, it was not taken for further studies.

Semi-quantitative RT PCR amplification of the pigeon pea heat shock proteins in the variety Asha (Fig. 6) showed that Cc ClpB, CcClpB4, CcClpB4.1 and CcHsp 101 (taken as a reference) gene were expressed under all stress conditions. Cc ClpB3 shows reduced expression as compared to above three genes. B4 and B4.1 show a high expression level till 6 hours and decline there- after. It has been reported that the cytoplasmic ClpB genes show a high induction post heat stress in Arabidopsis and rice, whereas the organelle targeted genes show a constitutive expression which get highly induced after heat stress [25]. It has also been shown that hsp100 genes are also involved in other housekeeping activities during embryo development and seed maturation. It has been reported that the organelle targeted ClpB genes not only provide thermo tolerance to the organelles but are also involved in protein transport. In case of durum wheat, four of the reported ClpB genes showed a differential expression under heat stress [26]. In case of Arabidopsis and in Lima bean a pre treatment at 38°C is required for proper induction of the ClpB gene family genes. B3 and B4.1 are chloroplast targeted and show good levels of expression in control. A similar observation was reported for Lima bean where the Cp targeted ClpB genes also behaved in a similar manner [23]. Another study suggests the key role of cp targeted ClpB genes for chloroplast growth and development especially under stress to keep the photosynthetic machinery working. Lee *et al.* 1994 reported the reduction in chlorophyll or abnormal chloroplast development when the cp targeted ClpB gene was knocked out, indicating the role of these genes in other cellular functions [19].

The genome wide analysis did not reveal the expected number of hsp100 gene family. This could be either due to poor gene prediction or largely due to incomplete genome sequence data. Hence a candidate gene approach was used. Synteny studies of pigeon pea have found it to be closest to soybean [13]. A

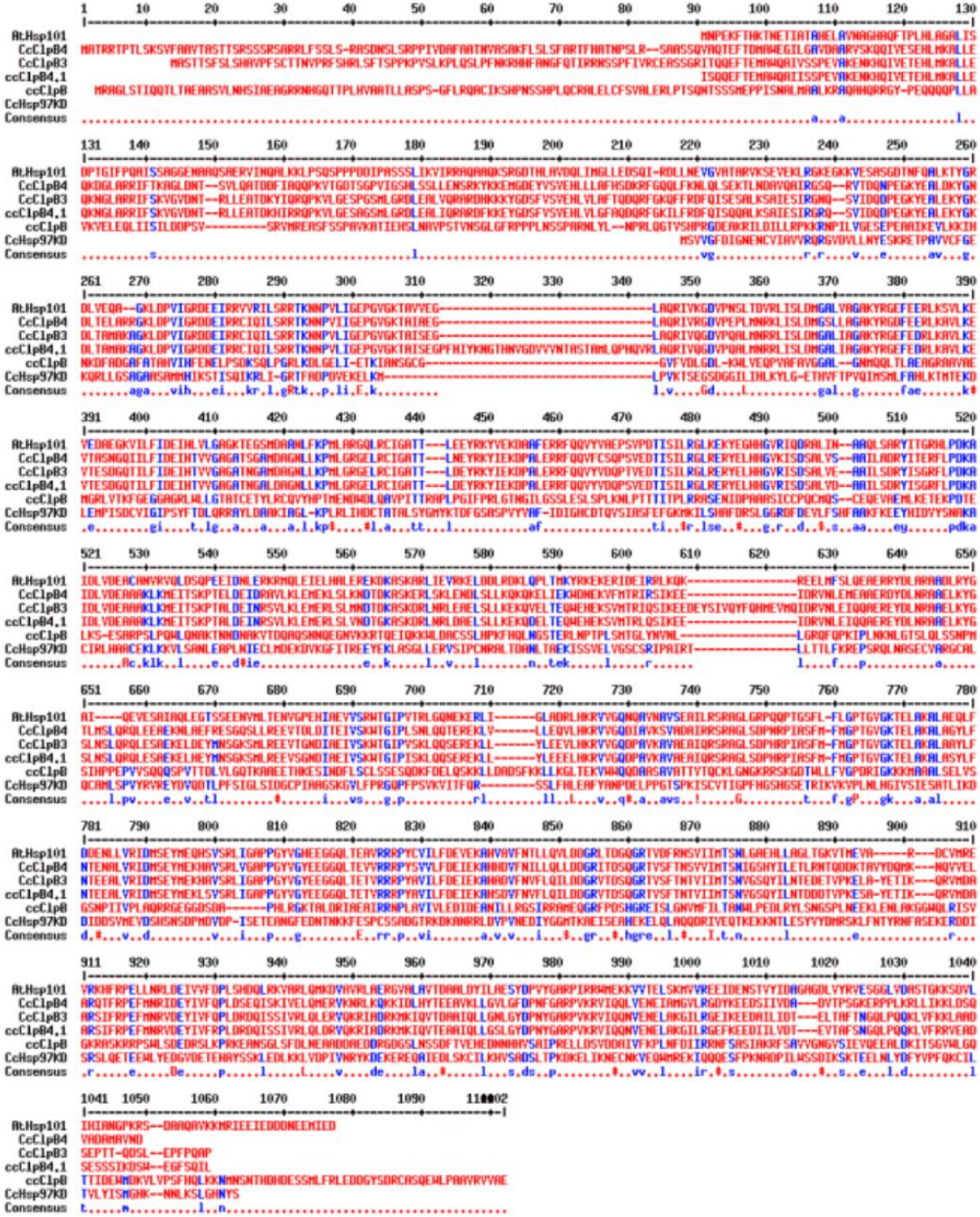

Fig. 3. Sequence alignment of *Cajanus cajan* Hsp 100 family genes and their related homologous genes [Multalin]

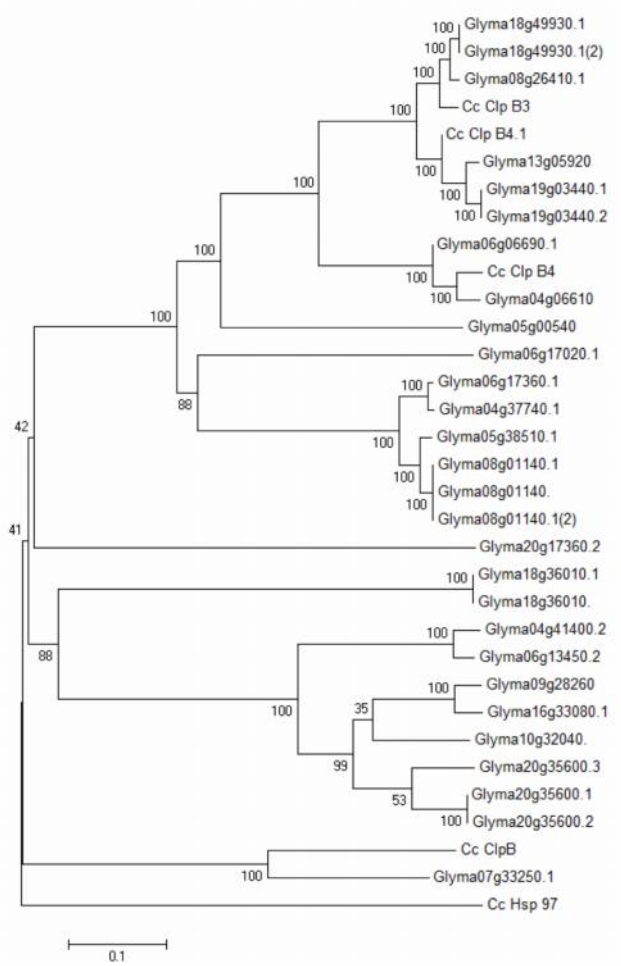

Fig. 4. Phylogenetic analysisof *Glycine max* and *Cajanus cajan* Hsp 100 family genes

Table 6. Domain positions in ClpB proteins of pigeon pea

Domains	Cc*ClpB4*	Cc*ClpB3*	Cc*ClpB4.1*
$ClpN_1$	44-69	34- 86	34-85
$ClpN_2$	121-172	111-162	-
AAA_1	229-367	219-35 6	142-310
AAA_2	627-797	633-802	571-740
$ClpBD_2$	803-883	808- 888	746-825

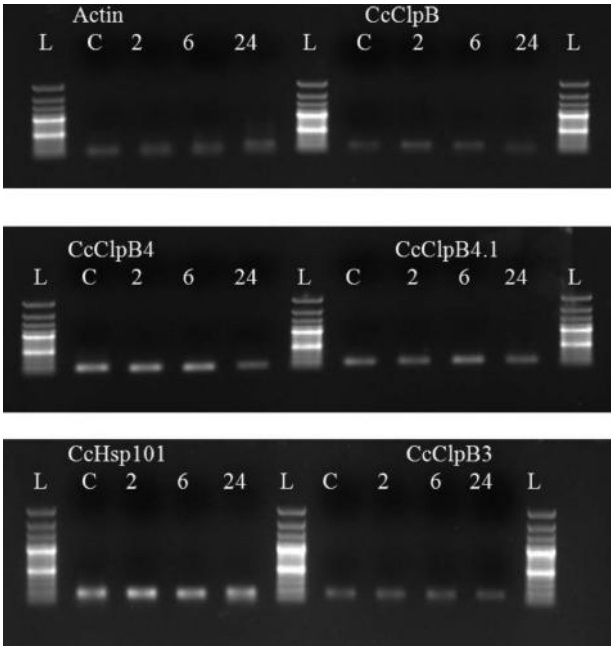

Fig. 6. RT-PCR profile of pigeon pea *ClpB* genes under different heat stress periods [C= control, Stress treatments:2, 6, 24 hrs at 45^0C]

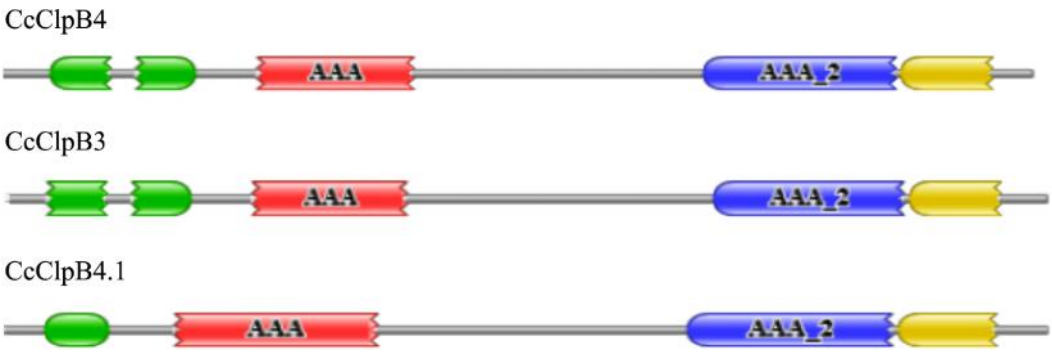

Fig. 5. Pfam analysis for *Cajanus cajan* Hsp 100 family protein domains

total of 23 CLpB genes were downloaded from the soybean genome and used for expression analysis in pigeon pea varieties Asha, Pusa Dwarf and a related wild species *C.platycarpus* at normal and heat stress conditions (Fig. 7). These 23 soybean genes were then analyzed for their expression under different periods of heat stress in pigeonpea. Expression analysis indicated mixed results with no consensus seen among the two pigeon pea genotypes indicating probably some experimental variations (Asha and Pusa Dwarf). At this stage a wild species *C.platycarpus* was also included to investigate the synteny theory and also

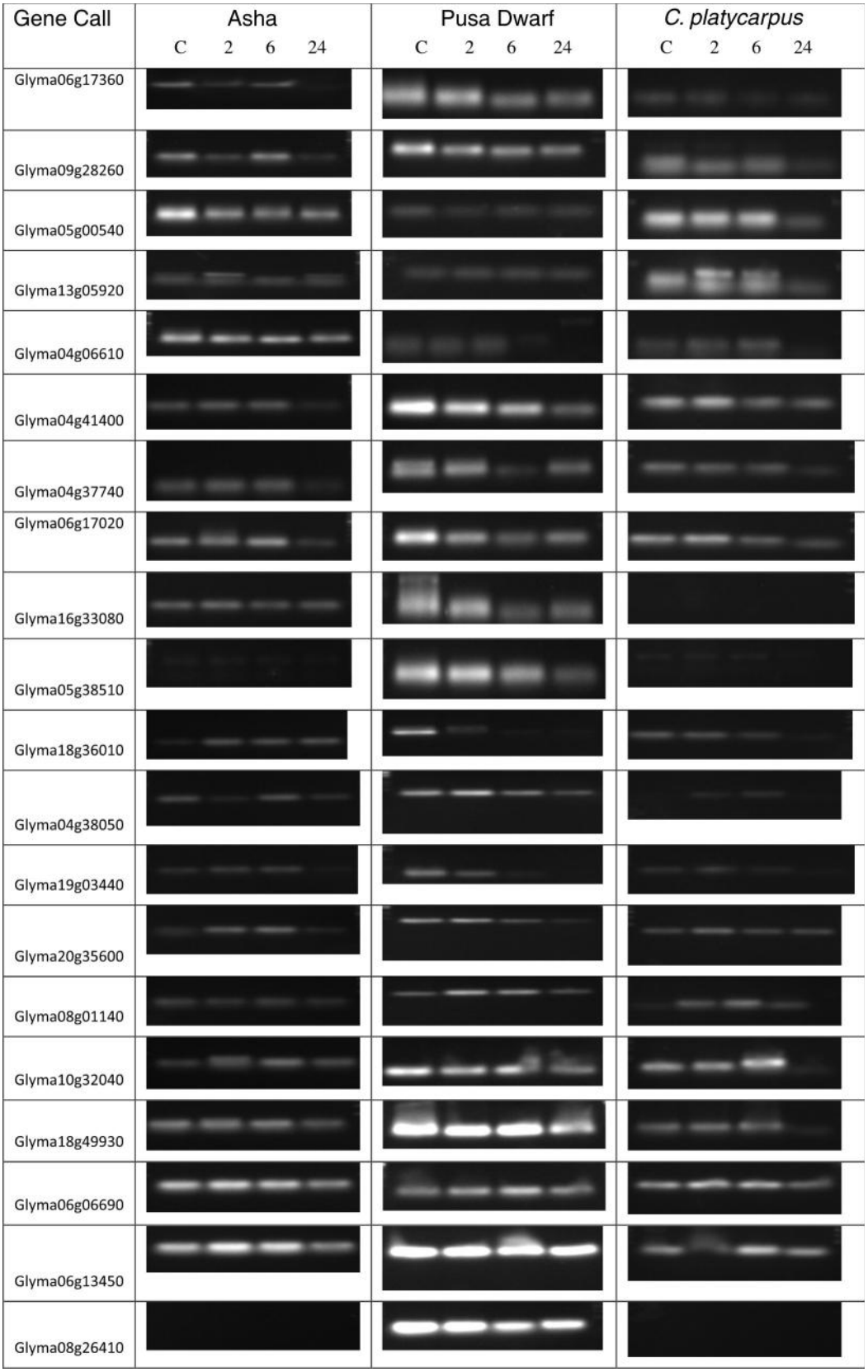

Fig. 7. Expression profile of *G. max ClpB* genes under different heat stress periods in Asha, Pusa dwarf and *C. platycarpus*; C=Central, Heat stress at 45oC:2, 6, 24 hr

add to the validation of the universal conserved nature of these hsp100 proteins.Out of the 23 genes selected, only 20 genes showed expression under one or the other periods under heat stress. The homologoue of pigeon pea hsp100 i.e.Glyma18g49930, 13g05920, and 04g 06610 did come true at the expression level. The other two protein CcClpB and Cchsp97 which failed the pfam domain requirement did not show any expression and the corresponding soybean genes also did not light up under heat stress. Out of these three, at least one i.e. Glyma18g49930 which is a homologue of CcB3 displayed a similar expression profile. In *C.platycarpus* most of the genes displayed expression of the heat induction kind except for Glyma16g33080,05g38510 and 08g26410 which are either not present in *C.platycarpus* or not induced by heat stress. It is also possible that these genes are expressed at higher temperatures or require whole plant based heat exposure for expression studies. In case of tomato and lima bean Hsp 100 proteins do not express at optimal growth temperature but are induced under high temperatures [23-24]. In soybean Hsp 100 proteins were found to be expressed from 28°C onwards, but highest expression was observed at 40-42°C [19]. In Arabidopsis under optimal growth conditions AtHsp101 is almost undetectable in vegetative tissues and accumulates during seed maturation [21]. Thus plant stage, growth and temperature variables may help to define the exact expression profiles for a set of Hsp100/ClpB genes.

It is too early to draw any conclusions on the number of Hsp100/ClpB genes present in the pigeon pea genome, but based on the expression data of soybean hsp100 genes, it can be expected that additional hsp100 genes may exist in pigeon pea other than the three detected in this study. As far as legumes are concerned, not much information is available for these genes. This data should provide some insight into the pigeon pea heat stress response machinery and help in isolation of important factors governing thermo tolerance.

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References

1. **Siddique K. H. M., Loss S. P., Regan K. L. and Jettner R. I.** 1999. Adaptation and seed yield of cool season grain legumes in Mediterranean continents of south-western Australia. Aust. J. Agri. Res., **50**: 375-387.
2. **Schirmer E. C., Glover J. R., Singer M. A. and Lindquist S.** 1996. *HSP100/Clp* proteins: a common mechanism explains diverse functions. Trends Biochemistry Sci., **21**: 289-296.
3. **Buchanan B., Gruissenm W. and Jones N. R. (eds)** 2002. Biochemistry and Molecular Biology of plants. American society of plant physiologists, Wiley and sons, 1367pp.
4. **Wang X., B. Vinocur, O. Shoseyov and Altman A.** 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci., **9**: 244-252.
5. **Burton B. M. and Baker T. A.** 2005. Remodeling protein complexes: insights from the AAA+ unfoldase ClpX and Mu transposase. Protein Sci., **14**: 1945-1954.
6. **Sauer R.T., Bolon D. N., Burton B. M., Burton R. E., Flynn J. M., Grant R. A., Hersch G. L., Joshi S. A., Kenniston J. A., Levchenko I., Neher S. B., Oakes E. S., Siddiqui S. M., Wah D. A. and Baker T. A.** 2004. Sculpting the proteome with AAA[+] proteases and disassembly machines. Cell, **119**: 9-18.
7. **Schirmer E. C. and Lindquist S.** 1997. Interaction of the chaperone Hsp104 with yeast Sup35 and mammalian PrP. Proc. Natl. Acad. Sci., U S A. **94**: 13932-13937.
8. **Mogk A., D. Huber and Bukau B.** 2011. Integrating Protein Homeostasis Strategies in Prokaryotes .Cold Spring Harbour Laboratory Press Cold Spring Herb Perspect Biol., **3**: a004366.
9. **Agarwal M., Katiyar-Agarwal S., Sahi C., Gallie D. R. and Grover A.** 2001. *Arabidopsis thaliana* Hsp100 protein: kith and kin. Cell Stress, Chap. **6**: 219-224.
10. **Singh A., Singh U., Mittal D. and Grover A.** 2010. Genome-wide analysis of rice ClpB/HSP100, ClpC and ClpD genes. BMC Genomics, **11**: 95.
11. **Sairam R. K., Deshmukh P. S. and Shukla D. S.** 1997. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agron. Crop Sci., **178**: 171-177.
12. **Sullivan C. Y. and Ross W. M.** 1979. Selecting for drought and heat resistance in grain sorghum. In: Mussell H. and Staples R.C. (eds), Stress Physiology in Crop Plants. John Wiley and Sons, New York, pp. 263-281.
13. **Singh N. K., Gupta D. K., Jayaswal P. K., Mahato M. K., Dutta S., Singh S., Bhutani S., Dogra V., Singh B. P., Kumawat G., Pal J. K., Pit A., Singh A., Rawal H., Kumar A., Prashant G. R., Khare A., Yadav R., Raje R. S., Singh M. N., Datta S., Fakrudin B., Wanjari K. B., Kansal R., Dash P. K., Jain P. K., Bhattacharya R., Gaikwad K., Mohapatra T., Srinivasan R. and Sharma T. R.** (2011).The first draft of the Pigeonpea

genome sequence. J Plant Biochem. Biotechnol., **21**: 98-112.

14. **Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S.** 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol., **28**: 2731-2739.
15. **Weaver M. L. and Timm H.** 1988. Influence of temperature and plant water status on pollen viability in beans. J. Am. Soc. Hortic. Sci., **113**: 31-35.
16. **Wahid A., Gelani S., Ashraf M. and Foolad M. R.** 2007. Heat tolerance in plants: An overview. Environ. Exp. Botany, **61**: 199-223.
17. **Sanchez Y. and Lindquist S. L.** 1990. Hsp 104 required for induced thermotolerance. Science, **248:** 1112-1115.
18. **Squires C. L., Pedersen S., Ross B. M. and Squires C.** 1991. ClpB is the *Escheria coli* heat shock protein F84.1. J Bacteriol., **173**: 4254-62.
19. **Lee Y. R., Nagao R. T. and Key J. L.** 1994.A soybean 101-kD heat shock protein complements a yeast HSP104 deletion mutant in acquiring thermo-tolerance. The Plant Cell, **6**: 1889-1897.
20. **Queitsch C., Hong S. W., Vierling E. and Lindquist S.** 2000. Hsp101 plays a crucial role in thermo tolerance in Arabidopsis. The Plant Cell, **12**: 479-49.
21. **Hong S. W. and Vierling E.** 2000. Mutant of Arabidopsis thaliana defective in the acquisition of tolerance to high temperature stress. PNAS, **97**: 4392-4397.
22. **Nieto-Sotelo J., Martínez L. M., Ponce G., Cassab G. I., Alagón A., Meeley R. B., Ribaut J. M. and Yang R.** 2002. Maize HSP101 plays important roles in both induced and basal thermotolerance and primary root growth. Plant Cell, **14**: 1621-33.
23. **Keeler S. J., Boettger C. M., Haynes J. G., Kuches K. A., Johnson M. M., Thureen D. L., Keeler C. L.Jr. and Kitto S. L.** 2000. Acquired Thermo-tolerance and Expression of the HSP100/ClpB Genes of Lima Bean. Plant Physiol.,**123:** 1121-1132.
24. **Yang J. Y., Sun Y., Sun A. Q., Yi S. Y., Qin J., Li M. H. and Liu J.** 2006. The involvement of chloroplast HSP100/ClpB in the acquired thermotolerance in tomato. Plant Mol. Biol., **62**: 385-395.
25. **Singh A. and Grover A.** 2010. Plant Hsp100/ClpB-like proteins: poorly-analyzed cousins of yeast ClpB machine. Plant Mol. Biol., **74**: 395-404.
26. **Gullì M., Corradi M., Rampino P. , Marmiroli N. and Perrotta C.** 2007. Four members of the HSP101 gene family are differently regulated in *Triticum durum* Desf. FEBS Lett., **581**: 4841-4849 .