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Expression analysis of six chromatin and remodeling complex genes (SWR1) in chickpea in different tissues and during heat stress

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Abstract

Nucleosome responds to heat stress through histone H2AZ variants. Based on homology searches, we have identified seven genes from the chickpea genome which are homologous to chromatin remodeling complexes (SWR1) in *Arabidopsis*. Chickpea homologs of *PIE* (Photoperiod independent early flowering 1), *ARP6* (Actin related protein), two *SEF* (Serrated leaf and early flowering) and three *H2AZs* (histone 2A variant-Z, a thermosensor in plants) genes were analyzed for expression in chickpea under heat stress and in five different tissues. Expression analysis of these genes during heat stress at pod development stage showed only *PIE1* gene was upregulated and heat stress at seedling stage resulted in significant down regulation. Tissue specific expression analysis showed that the expression of *PIE1* and *SEF* was relatively higher in root, flower, pod wall, and grain tissues as compared to that in shoots. Thus, *CarPIE1* gene might play an important role in chromatin remodeling complexes during heat stress in chickpea and all the three histone *CarH2AZ* variants can be good candidate genes for the characterization of basic processes of gene responsiveness in chickpea.

Key words: SWR1 complex, heat stress response, phylogenetic analysis, pod development stage and gene expression

Introduction

Plants being sessile have to respond immediately upon stress perception to regulate the expression of stress-responsive genes. Chromatin remodeling has become one of the prime pathways for induction of stress-responsive genes (March Diaz and Reyes 2009). Chromatins, which are made of nucleosomes and additional proteins, are required for regulation of transcription. A nucleosome is DNA wrapped in protein complexes and histones are major proteins in this complex required for basal compaction of DNA strands inside the nucleus (Weber and Heinkoff 2014). Histones are highly conserved group of proteins in eukaryotes. There are five major classes of core histone proteins namely, H2A, H2B, H3, H4 and H1 proteins. The former four classes of histones as a pair form an octamer complex among them and wrap DNA around it, and later histone protein H1 links the wrapped DNA: histone octamer complex for higher level of compaction. In addition to these core histone proteins, there are also histone variants found in eukaryotic cells such as H2AZ, H2AX functioning in cellular processes providing responsiveness to external or internal stimuli (March Diaz and Reyes 2009). For example, histone variant *H2A.Z* is responsible for the ambient temperature responsiveness in Arabidopsis and an important thermo sensor for plants. H2A.Z is selectively enriched in nucleosome at +1 position of gene which assist in tight binding of nucleosome at this region in non-stress condition which prevents transcription. In addition H2AZ enriched nucleosome is present only in stress-responsive genes. Upon changes in ambient temperature of even one degree celsius, physical processes enable eviction of *H2A.Z* from the nucleosome and the gene becomes amenable for transcription. Interestingly, this process is reported to be independent of transcription (Kumar and Wigge 2010). Thus, apart from genome organization in nucleus, histone proteins are reported to be involved in important roles such as regulation of gene expression in response to different stresses, DNA repair, recombination and epigenetic modification among other processes.

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SWR1 (sick with RSC/Rat1 complex) is a complex of proteins involved in chromatin remodeling function through deposition of H2AZ dimers in the nucleosome on +1 position of stress-responsive genes (Weber and Heinkoff 2014). The proteins in this complex belong to SNF2 (Sucrose Non-Fermentable) protein family. This family protein performs helicase like function utilizing ATP as energy for chromatin remodeling. There are at least 13 proteins identified in Arabidopsis to be involved in this complex. These protein complexes are well characterized in yeast and humans. (March Diaz and Reyes 2009). In plants, three genes from Arabidopsis were characterized to be homologs of SWR complex found in yeast and humans, they are, mutants of *PIE1* gene (Photoperiod independent early flowering 1) which showed early flowering phenotype with pleiotropic effects and failed to deposit H2AZ variant in responsive genes (Noh and Amasino 2003). Another gene mutant namely, *ARP6* (Actin related protein 6) in Arabidopsis showed constitutive warm transcriptome response and also failed in deposition of variant histone H2A.Z (Kumar and Wigge 2010). In addition, SEF protein in Arabidopsis was reported to physically bind to these two proteins and all these three proteins forms a chromatin remodeling complexes in *Arabidopsis* for the deposition of histone variants (March Diaz et al. 2007). In addition, *Arabidopsis* mutants of *H2AZ* also showed similar phenotypes to that of *pie1*, *arp6* and *sef* (Der and Gilberman 2012). These genes along with histones H2AZs were characterized to be involved in many cellular processes like flowering time regulation (Choi et al. 2007), abiotic stress response, inhibition of salicyclic acid responsive genes (March Diaz et al. 2008), epigenetic pathway (Weber and Heinkoff 2014), DNA repair, meiosis, recombination (Rosa et al. 2013), phosphate starvation response (Smith et al. 2010), metabolic response (Nutzman and Osborn 2014), and genome stability (Clemens Bonisch and Sandra B. H. 2012) in *Arabidopsis*. But, to the best of our knowledge no analysis of all these genes has been reported in other plants.

Chickpea is an annual *rabi* crop grown predominantly in residual soil moisture. Reproductive phase of the crop growth is most sensitive to drought, cold and heat stress (Devasirvatham et al. 2013). Heat stress especially affects the pod development stage of the crop (Thudi et al. 2013). There are several reports of drought, cold stress responsive ESTs and transcriptome available in chickpea (Jha et al. 2014). We constructed a SSH library of chickpea seedlings subjected to heat stress (unpublished). In the initial analysis of stress library, one of the contigs was found to be homologous to Arabidopsis PIE1 protein. Thus, based on the designed model for the deposition of histone variants during temperature responsiveness (Fig. 1), we designed an experiment to identify the

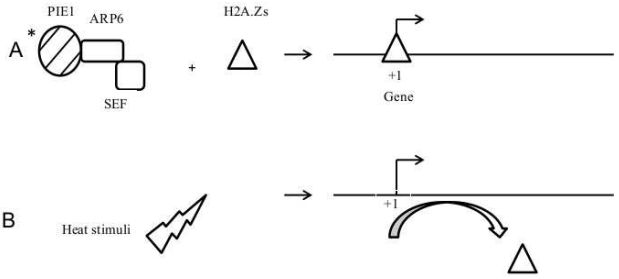

Fig. 1. Schematic model for the activity of selected SWR1 complex genes. A) In non heat stress condition, PIE1, ARP6 and SEF genes interact and deposit the histone variant H2AZ into nucleosome on the +1 position of gene. It makes gene amenable for transcription, B) under heat stress, physical eviction of H2AZ proteins occurs and inducible expression of genes takes place

homologs of Arabidopsis *PIE1, ARP6, SEF*, and *H2A.Zs* in chickpea and performed an expression analysis in different tissues and during heat stress in seedling and pod development stages of the crop.

Materials and methods

Plant materials and stress treatments

Chickpea variety ICC4958 was used for the study and the experiment was performed in National Phytotron Facility, New Delhi. Heat stress was given to the plants during the pod development stage. Surface sterilized chickpea seeds were sown in 6" pots and grown in control condition (24±2^{o}C) in a glass house with 14/10 hours light and dark condition respectively. At the pod development stage, 8 plants were shifted to another glass house and stress treatment (37±2^{o}C) was given for the duration of three hours. After the stress treatment, the pots were again shifted to control glass house for recovery. Similar heat stress schedule was followed for five days and after the fifth day stress treatment, shoot samples were harvested, immediately frozen in liquid nitrogen and stored in –80^{o}C until RNA isolation. Tissue specific expression analysis was done for field-grown plants. ICC4958 seeds were line

sown during last week of October, 2013 in the field. Samples of shoot, root, flower, pods were collected from the plants during the pod development stage on first week of February, 2014. Samples were immediately frozen in liquid nitrogen, and stored in –80^{o}C until RNA isolation.

For stress treatment at seedling stage of 15-day-old seedlings, chickpea ICC4958 seeds were sown in 4" pots and kept in control glass house (24±2^{o}C). Fifteen days after germination, seedlings were subject to heat stress treatment at 37±2^{o}C in the incubator. Stress treatment for several time points (24 h., 6 h., and 1 h., 30 and 15 min.) was given in similar order and both control and treated seedlings were collected at the same time. Samples were immediately frozen in liquid nitrogen, and stored in –80^{o}C until RNA isolation.

Total RNA isolation, cDNA synthesis and qPCR

Trizol reagent, Invitrogen was used for the isolation of total RNA from the tissues using the product protocol. Quality of the total rna was checked in 1% agarose gel and nano-drop spectrophotometer. 10 µg of total rna was used for the DNase treatment using NEB enzymes. cDNA was synthesized from 1 µg of DNase treated RNA using thermo scientific maxima cDNA synthesis kit. 1µL of cDNA was used for qPCR using the step one plus, ABI biosystems. For each sample, three biological and three technical replicates were used. *GAPDH2* was used as internal reference gene. Melt curve analysis was done for confirming the specificity of the amplification. Fold change was determined using ΔΔct method (Livak, K. J and Schmittgen 2001). The expression in control sample was kept as one and fold change was calculated for stress samples. For tissue specific expression analysis, expression in shoots was kept as one and compared with root, flower, pod wall and grain samples. The sequences of the primers used in the study are given in Table 1.

Table 1. Sequence details of primers used in the study

S.No.	Locus id	Sequence
1	LOC101497893-FP	GGAGTATCTGACTGC
	LOC101497893-RP	TGAAGTATT
		CTCCAGCAATTGTT
		CCCTTTATC
2	LOC101507294-FP	GCAACGGCTGCAG
	LOC101507294-RP	TTTATTC
		GTTCTTCATCTCCACGG
		ATAGC
3	LOC101489506-FP	GGTGGTGTCATCCCTCA
	LOC101489506-RP	TATTC
		CTGTGATCACCATTTCAA
		GTCATC
4	LOC101490716-FP	TCCAGGATACAATCTT
	LOC101490716-RP	CTGGAAAG
		TCGGCATTGGCT
		GGAATAA
5	LOC101507453-FP	ATGCTGGCAGACT
	LOC101507453-RP	GGTATTT
		ATCCGTCGAATACCACA
		TTAGTT
6	LOC101490497-FP	CTTGAGCTCTTACATGA
	LOC101490497-RP	TGCAAAT
		CACGTGTAATTGGCA
		GAGAAAC
7	LOC101491360-FP	CCTCCACATGTTCCC
	LOC101491360-RP	TCATATT
		GTAATTCGCAGAGAAA
		CCACATAC
8	GADPH-FP	ACCTACGACGAAATC
	GADPH-RP	AAGGCTGCT
		ACAATGAGGTCAACG
		ACACGGGTA

In-silico analysis

Arabidopsis protein sequences (*ATPIE1*- AT3G12810; *ATARP6*-AT3G33520; *ATSEF*- AT37055; *HTA11*-AT3G54560; *ATHTA8*-AT2G38810; *ATHTA9*-AT1G52740) sequences were retrieved from TAIR database. NCBI, BLAST program (BLASTP, TBLASTN) was used for the homologous protein identification in chickpea. BLAST 2 sequence analysis was used for pair-wise alignment. Multiple sequence alignment and phylogenetic tree reconstruction were performed in MEGA 6 program. For phylogenetic tree reconstruction, neighbor joining method with 1000 bootstrap replicates was used. SMART tool is used for domain analysis. Chickpea transcriptome database (CTDB) was used for the retrieval of expression value of the chickpea homologs. Digital expression analysis was done using the ESTs available in NCBI database. GENEVESTIGATOR tool was used for the analysis of expression of Arabidopsis. Promoter sequences of 1 kb from predicted translation start site are retrieved from NCBI chickpea database and analyzed in PLANTCARE database.

Results

Identification of chickpea homologs

BLASTN analysis in NCBI nucleotide collection (nr/nt) database of one of the heat stress SSH contigs

showed homology to helicase-SRCAP (Snf2- related CREBBP Activator Protein) like protein found in humans. The complete mRNA (LOC101490716) and protein sequence were retrieved from chickpea database in NCBI. Further, Arabidopsis homolog was identified through BLAST P by using chickpea protein sequence as query against Arabidopsis non redundant protein database. The best hit showed identity of 63% and e-value of 0.0 and named as photoperiod independent early flowering protein 1 (*PIE1*, AT3G12810). As PIE1 forms chromatin remodeling complexes with ARP6 and SEF on Arabidopsis for the deposition of histone2AZ variants, we also identified these homologs in chickpea through BLAST and phylogenetic analysis (Table 2).

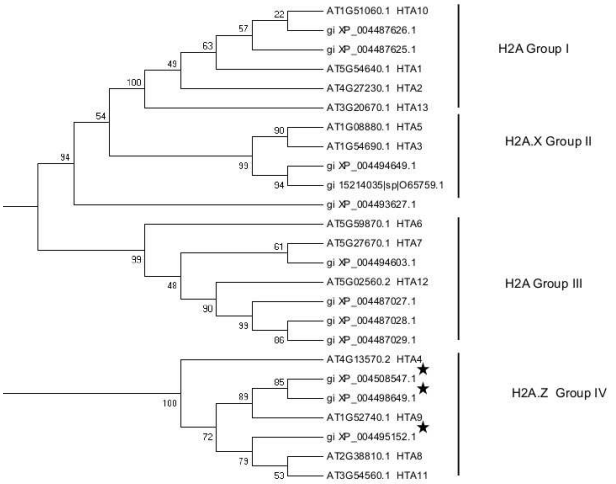

Fig. 2. Neighbor joining phylogenetic tree of histone H2A proteins of Arabidopsis and chickpea. * represents the chickpea H2AZ proteins. AT represents Arabidopsis gene id and XP represents chickpea protein id. Values at the branch points indicates boot strap support for the branching

Table 2. Details of seven chickpea genes of SWR1 complex

Gene id	Protein id	Homologs size (aa)	Protein (Kda)	MW	pI
LOC101490716	XP_004497900	PIE 1	2053	234.7	5.15
LOC101507453	XP_004488416	ARP 6	438	49.3	5.9
LOC101490497	XP_004500321	SEF 1	173	19.23	8.8
LOC101491360	XP_004500324	SEF 2	173	19.17	8.8
LOC101507294	XP_004498649	H2AZ.1	134	14.27	10.39
LOC101489506	XP_004495152	H2AZ. 2	134	14.31	10.39
LOC101497893	XP_004508547	H2AZ. 3	131	14.05	10.28

Chickpea homologs of *Arabidopsis ARP6* protein was identified through BLASTP search. TBALSTN analysis showed at least 9 ARP proteins in chickpea. Both the analysis were used to identify the correct homolog with had e-value of 0 to that of *Arabidopsis* ARP6 protein. There is one homolog of *Arabidopsis ARP6* in chickpea with locus id LOC101507453. There are two *SEF* homologs in chickpea (LOC101491360, LOC101490497) found to be duplicated and present adjacent to another gene LOC10149082. There is 99 and 100% identity and similarity, respectively, between these two *SEF* homologs in chickpea. Chickpea homologs of histone 2A variants were identified by employing phylogenetic analysis. All the 13 *Arabidopsis* histone 2A proteins were retrieved from TAIR database. One of the *Arabidopsis H2A.z* variants (*ATHTA8*-AT2G38810) was queried against chickpea reference genome and non-redundant protein database using TBLASTN and BLASTP searches. Both the results showed chickpea genome consists of 12 histone 2A proteins (Table 3). After multiple sequence alignment in MUSCLE, neighbor joining phylogenetic tree for 13 *Arabidopsis* and 12 chickpea histone 2A proteins were constructed using MEGA 6 program. Among four distinct groups in the tree, fourth group has *Arabidopsis* histone H2A.z variants (Fig. 2). Accordingly, 3 chickpea proteins (LOC101497893, LOC101507294, LOC101489506) present in this group were identified to be homologs of H2A.z proteins and taken for further analysis.

Domain and sequence analysis

The domains identified in chickpea proteins are given in Table 4. Chickpea homolog of *Arabidopsis* PIE1 protein consist of three domains namely HSA (domain in helicases), DEXDc (dead like helicase superfamily) and HELICc (Helicase superfamily C-terminal domain). This protein is one of the largest proteins of size 2053 amino acids in chickpea and only the sequences in these domains are highly conserved between Arabidopsis and chickpea. HSA, DEXDc, HELICc domains has 90, 97, 95% identity between Arabidopsis and chickpea homologs of PIE1 respectively. Chickpea ARP6 homolog consist of single actin domain of 432 amino acid length. There is 81% identity between the actin domain of Arabidopsis and chickpea ARP6 homologs. Zn-HIT (Zinc finger-HIT) domain was identified in two chickpea SEF

Table 3. Groups of histone 2A genes identified from the chickpea genome

Gene id	Protein id	Groups	Protein size (aa)	MW (Kda)	pI
LOC101514392	XP_004487028	III	150	15.95	10.75
LOC101514067	XP_004487027.1	III	149	15.93	10.96
LOC101514719	XP_004487029.1	III	148	15.75	10.73
LOC101492287	XP_004493627.1	II	143	15.08	10.47
LOC101500089	XP_004494603.1	III	146	15.42	10.35
LOC101514555	XP_004494649.1	II	139	14.62	10.36
—-	O65759.1*	II	139	14.6	10.32
LOC101489870	XP_004487625.1	I	135	14.06	10.05
LOC101490207	XP_004487626.1	I	134	14.05	10.05
LOC101507294	XP_004498649	IV	134	14.27	10.39
LOC101489506	XP_004495152	IV	134	14.31	10.39
LOC101497893	XP_004508547	IV	131	14.05	10.28

proteins, and 85% identity was present between Arabidopsis and chickpea proteins. All the three histone 2A.Z proteins have highly conserved histone domain. There is only a difference of 9 amino acids between the chickpea histone H2AZ proteins.

EST analysis

BLASTN search of the chickpea genes (*CarPIE1, CarARP6, CarSEF1* and *2, CarH2AZ.1, 2* and *3*) mRNA sequences against chickpea ESTs database showed only two of the histone variants (LOC101497893, LOC101507294) had an ESTs present in the database. The first histone variant was found to be present in salinity EST and the latter was found to be present in ESTs of drought and immune response.

Tissue-specific expression analysis

Normalized values retrieved from chickpea transcriptome database for various tissues were used to develop heat map for five genes (Fig. 3). Chickpea *PIE1* (LOC101490716), *ARP6* (LOC101507453), and one of the histone *H2AZ* (LOC101497893) showed uniform expression in all the tissues. In comparison, other two histones (LOC101507294 and LOC101489506) showed higher level of expression than *PIE1*, *ARP6* and one of the H2AZ but uniform expression level in all the tissues. One of the histone H2AZ (LOC101489506) expressions was found to be less in leaf tissues compared to other tissues. Similar analysis was also performed for various flower stages, germinating seeds, young flowers and shoot apical meristem. In comparison between the genes, there is no variation in the expression pattern in these tissues. All these genes showed uniform expression in all the stages analyzed.

Genevestigator analysis

To compare the expression pattern of *Arabidopsis* SWR1 complex homologs, we analyzed the *Arabidopsis* expression pattern for these genes in various tissues in genevestigator (Fig. 4). For example, *PIE1* was found to be expressed higher only during the bolting stage, whereas *ARP6* and *HTA9* were found to be expressed throughout the developmental stages except in mature flowers. Also, there is no expression of *HTA8* and *HTA11* in developing seeds. SEF gene in *Arabidopsis* was found to be expressed only in germinating seed and senescing leaves.

Quantitative real time – PCR analysis of chickpea genes

The expression of seven chickpea genes in different tissues collected from field grown chickpea plants is given in (Fig. 5). The fold change expression in shoots is kept as one and compared with expression in other tissues. Compared to shoots, root tissues of chickpea PIE1 (LOC101490716) gene showed highest fold change expression of 18 fold followed by flower and pod walls of 16 and 10 fold, respectively. The expression of this gene in grain tissue is more or less equal to that of shoot tissues. Chickpea *ARP6* gene (LOC101507453) showed similar expression level in shoot and flower tissues and compared to shoots there was slightly reduced expression level in roots, pod wall and grains tissues. The fold change expression of one of the *SEF.1* gene (LOC101490497) showed 2 fold change in flower tissue compared to shoots whereas roots, pod wall and grain tissues showed lower level of expression than shoots. Interestingly, another *SEF.2* gene (LOC101491360) showed higher expression of 40, 37 and 27 fold change in grain, flower, and pod wall tissue, respectively, compared to shoot tissues. All the three histone variant genes showed lower level of expression in roots and grains compared to shoot tissues. In addition, the expression in flower and pod wall were also lower than shoot tissues.

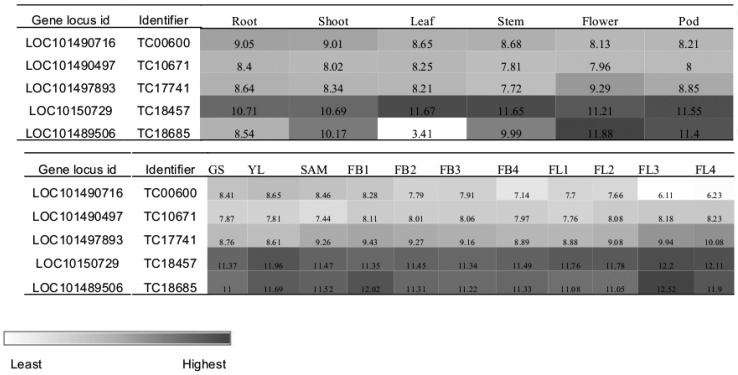

Fig. 3. Heat map expression values of five chickpea SWR1 complex genes. TC represents tentative contigs in chickpea transcriptome database. GS- Germinating seed; YL-Young leaf; SAM-Shoot apical meristem; FB1-FB4-Flower bud stages; FL1-FL4-Flower stages. Values in each cell represents normalized reads per million

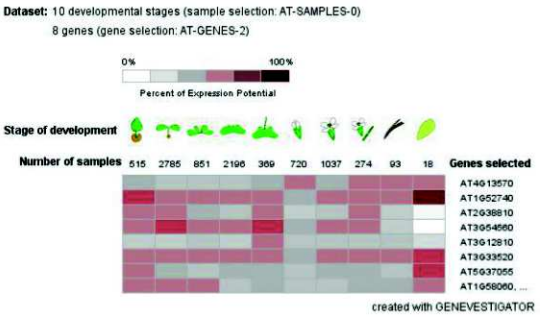

Fig. 4. Expression analysis of *Arabidopsis* SWR1 complex genes using GENEVESTIGATOR in different development stages. ATPIE1-AT3G12810; ATARP6-AT3G33520; ATSEF- AT37055; HTA11-AT3G54560; ATHTA8-AT2G38810; HTA9-AT1G52740

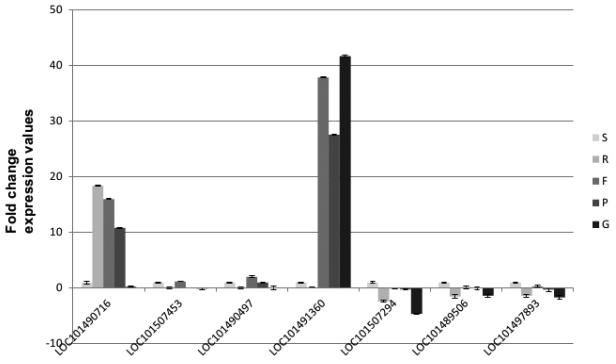

Fig. 5. qPCR expression analysis of seven chickpea genes in different tissues collected from the field. S-Shoot; R-root; F-Flower bud; P-Pod wall; G- developing grains. Expression in shoots was kept as one. CarPIE1-LOC101490716; CarARP6-LOC101507453; CarSEF1-LOC101490497; CarSEF2-LOC101491360; CarH2AZ1-LOC101507294; CarH2AZ2-LOC101489506; CarH2AZ3- LOC101497893

The expression of these genes at pod development stage during heat stress showed that only the chickpea *PIE1* gene (LOC101490716) is upregulated up to 2.4 fold during heat stress (Fig. 6a). Chickpea ARP6 gene showed down regulation of 0.87 fold in heat stress, both the chickpea SEF genes showed slight down regulation of up to 0.3 fold in heat stress. In comparison, all the three chickpea H2AZ gene showed down regulation. LOC101489506 and LOC101497893 showed downregulation of up to three fold in comparison with control condition.

Table 4. Pfam analysis of seven chickpea proteins

Chickpea gene	Domain name	Region	E value
PIE 1	HAS	23-95	7.19E-29
	DexDc	517-710	5.01E-36
	HELICc	1092-1175	4.87E-25
ARP 6	Actin	5-437	4.34E-25
SEF 1	HIT Zn finger	132-160	2.80E-11
SEF 2	HIT Zn finger	132-160	2.42E-11
H2AZ 1	H2A	11-131	1.64E-72
H2AZ 2	H2A	14-134	4.64E-72
H2AZ 3	H2A	14-134	2.88E-68

HSA = Domain in helicase, DexDc = Dead like helicase super family, HELICc = Helicase super family C = Terminal domain, Actin = Actin domain, HIT Zn finger = Zinc finger domain, H2A = Histone domain

Time point analysis of heat stress samples (Fig. 6b) at seedling stage showed expression of *PIE1* gene was down regulated only after 6 hours of heat stress treatment, whereas in other time points the expression level of the gene was similar to that in control conditions. *CarARP6* gene showed up regulation only at 24 hours of heat stress treatment, but showed relatively similar expression in all other time points with respect to control. Both the *SEF* gene showed downregulation in all the time points compared to control and one of the *SEF* gene (LOC101491360) showed relatively greater down regulation between them. Two histone *H2AZ* genes (LOC101507294, LOC101489506) were down regulated within 15 minutes,

1 hour and 6 hour of heat stress treatment but showed similar expression with respect to control in 24 hour of stress treatment. Another chickpea histone gene (LOC101497893) showed highest down regulation of 10 fold in 15 minutes of heat stress treatment and remained down regulated up to 6 h of heat stress. But it got upregulated in 24 h of heat stress in comparison with control samples.

Discussion

We had identified from our analysis, one homolog of chickpea *PIE1*, *ARP6* and two homologs of *SEF* and three homologs of *H2AZ* genes which are homologues to chromatin remodeling complex SWR1 like in Arabidopsis. Chickpea homolog of PIE1 protein is single copy gene as that in *Arabidopsis* and showed greater conservation only in the domains rather than the full length protein. This is in accordance with human and mice homologs of *PIE1* showing higher conservation only in the domain regions (Diaz and Reyes 2009). Also, similar to Arabidopsis, humans, and yeast, the ATPase domain in chickpea *PIE1* is bipartite as DEXDc and HELICc of ATPase domains in *PIE1* are separated by an insertion of several amino acids. The conservation of domains in chickpea PIE1 probably indicates similarity in molecular function of *CarPIE1* to that of *Arabidopsis* PIE1. In difference, it was mentioned by Riaz and Reyes (2009) that the diversification of C-terminal region of PIE1 orthologs in humans, mice, and *Arabidopsis* might provide organism specific functions. As there is very little conservation in the c-terminal region of PIE1 between *Arabidopsis* and chickpea, chickpea specific function of PIE1 cannot be ruled out. In addition, varied spatio-temporal expression of the gene also is likely to provide diversity in function.

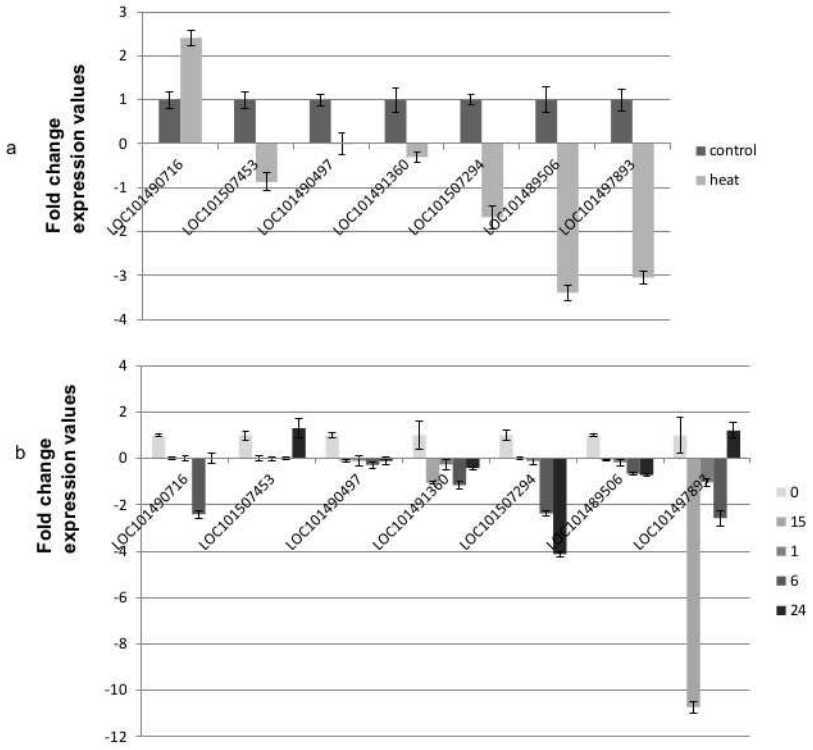

Fig. 6. qPCR expression analysis of seven chickpea genes in heat stress. a) Analysis during heat stress at pod development stage shoots samples. Expression at control was kept as one. b) Analysis in different time points at seedling stage. 0-control; 15-Fifteen minutes; 1- One hour; 6- Six hours; 24- Twenty four hours. CarPIE1-LOC101490716; CarARP6-LOC101507453; CarSEF1-LOC101490497; CarSEF2-LOC101491360; CarH2AZ1-LOC101507294; CarH2AZ2-LOC101489506; CarH2AZ3-LOC101497893

As *PIE1* is a single copy gene in Arabidopsis and chickpea, *ARP* genes occur as gene family. In a comprehensive analysis of actin related proteins in 20 genomes by Muller et al. (2005), among the 11 *ARP* sub-families grouped, *ARP6* and *ARP4*, are found to be nuclear located proteins and also these two sub families of ARPs are conserved in eukaryotic phyla. Thus, due to its higher conservation, through simple protein blast analysis, we could identify the best homolog of ARP6 protein in chickpea among its gene family members indicating its conservation of sequence in also chickpea genome. In addition, 9 *ARP like* gene family proteins identified in chickpea have to be analyzed further. There are two homologs of *SEF* genes in chickpea. It is interesting to note that other than tandem duplication some other mechanism might have operated to position these two *CarSEF* genes adjacent to another gene. Alternatively, one of the *SEF* genes might have lost in *Arabidopsis* during evolution. Thus, a synteny analysis could provide an answer to this puzzle. We employed phylogenetic analysis and identified three histone variant *H2AZ* in chickpea. Chickpea histone *H2A* gene family has all the

four groups as identified in *Arabidopsis* (Yi et al. 2006), rice (Hu and Lai. 2015) and *Brachypodium* (Boden et al. 2013). Similarly, *Arabidopsis*, rice, *Brachypodium*, and chickpea also have three H2AZ proteins in the genome.

Tissue-specific expression analysis of transcriptome data showed similar expression levels for these genes in all the tissues analyzed. This expression pattern is similar to that observed in Arabidopsis. The mutants of *Arabidopsis*, *pie1, arp6, sef1* and *h2az* showed phenotypes such as altered leaf size, early flowering, reduced inflorescence length (Rosa et al. 2013), in addition *h2az* mutants showed reduced siliques size and defects in ovule development (Der and Gilberman 2012). Thus, it can be said that the expression of these genes is required in most of the tissues of plants. In our qCPR analysis of field grown chickpea plants, except for chickpea *PIE1* and *SEF2*, other genes showed more or less similar level of expression in five different tissues. As, there are two *SEF* genes in chickpea, it is possible that one of the SEF homolog in chickpea might have evolved for diversified expression in tissues. Accordingly, the 1 kb promoter sequence of these genes did not show any similarity even though the protein showed 99% similarity in amino acid sequence. But the function of higher level of expression of *SEF* gene has to be analyzed. Similarly, higher expression of chickpea *PIE1* gene in roots, flowers and pod wall have to be analyzed in future.

In *Arabidopsis*, as these six proteins are part of SWR1 complex, similar phenotypes of flowering time deregulation were observed in their mutants of *arp6, pie1*, *h2az*, and *sef*. But not all the phenotypes were similar between them indicating its additional functions. In our expression analysis, only one gene *CarPIE1* showed up regulation during heat stress at pod development stage. Thus, it is possible that in addition to chromatin remodeling, *PIE1* in chickpea might perform different function during heat stress. Also, its down regulation at one of the time points in seedlings stage stress treatment shows the heat acclimation treatment given at pod development stage might elicit different transcriptional regulation compared to acute heat stress given at seedling stage. Accordingly, it was reported that heat acclimation stress treatments showed up-regulation of different set of genes compared to non acclamation stress treatment (Larkindale and Vierling 2008). Alternatively, *CarPIE1* gene might have a specific role in pod development stage of crop growth. However, this aspect needs further studies. In humans, *Pie1* ortholog known as S*RCAP* in humans has been reported to be one of the important modulators of heat stress response (HSR). *SRCAP* gene, along with histone acetyl transferase complex including *EP300* acetylates the HSF1 protein and increases its turnover time inside nucleus, and acetylating HSF1 at specific residues delays the attenuation and prolongs the heat stress response (Raychoudari et al. 2014). In plants, acetylation of *Hsfs* is not reported yet, thus the upregulation of PIE1 in chickpea has to be studied with respect to HSR in chickpea and *Arabidopsis*. *Arabidopsis* mutants of *arp6, h2az* genes showed constitutive heat stress response. Thus, the downregulation of chickpea *ARP6, SEFs, H2AZs* in pod development stage if translated into reduced protein levels could ensure constitutive transcription of heat stress responsive genes during heat stress. Also, acclimation responses to long term heat stress in *Chlamydomonas reinhardtii* showed histones and ribosomal proteins were entirely down regulated and cell division was immediately stopped (Hemme et al. 2014). Thus, the outcome of downregulation of chickpea histone genes upon heat stress not only might arrest cell division but also makes heat stress-responsive genes constitutively active thus providing tolerance to heat stress. There are several reports of constitutive expression of stress-responsive genes providing tolerance to different stresses (Charulata and Manoj Prasad 2011). Thus, through SWR1 complex, plants might evolve a strategy to up regulate stress responsive genes constitutively to provide heat tolerance during heat stress. Since, *H2AZ* ESTs in chickpea are identified in drought and salinity stresses, *H2AZ* gene role in other stresses also need to be studied. It is also reported in rice that rice *H2AZ* genes were downregulated in shoots in salt and drought stress (Hu and Lai 2015).

Expression analysis for the seven chickpea genes during heat stress at seedling stage showed most of the genes were downregulated in heat stress. Specifically, one of the *H2AZ* genes (LOC101497893) showed greater down regulation within 15 minutes of stress treatment. Thus, this highly downregulated histone gene immediately administering heat stress might function as thermo-sensor for heat stress at seedling stage of chickpea. In an interesting comparison, *H2AZ* containing nucleosome responded differentially with respect to tissues and stage of development in *Brachypodium* (Boden et al. 2014). Since all the three histone genes were uniformly

downregulated at pod development stage, it can be postulated that all three *H2AZ* gene might function as thermo-sensor during pod development stage.

Chickpea cultivar improvement strategies emphasize on early flowering trait as one of the mechanism for terminal stress avoidance during reproductive stage of crop growth. As SWR1 complex are involved in flowering time regulation in Arabidopsis, the identification and expression analysis of seven SWR1 genes in chickpea could be useful in genetic dissection of flowering trait in chickpea. Also, upregulation of *PIE1* gene during heat stress at pod development stage might be the initial point in evaluating the role of this gene in heat stress responses. Also, higher level of expression of one of the *SEF* genes in reproductive tissues (LOC101491360) could be taken forward for further analysis. It is reported in *Brachypodium* that thermal stress response in developing grains occurs via *H2AZs* containing nucleosomes. Also, compared to *H2AZs* containing nucleosomes in vegetative stage, reproductive stage *H2AZ* nucleosomes are more sensitive to heat stress (Boden et al. 2013). In chickpea, even in non heat stress condition, later formed pods showed early maturity and reduced seed size. During heat stress, pod abortion and reduction in seed size was observed. Approaches like overexpression of SWR1 complex genes might misregulate transcription of several genes, as *H2AZ* overexpression resulted in cellular proliferation of human cancer cells (Svotelis et al. 2010). Thus, cultivar development in chickpea for heat tolerance can exploit natural polymorphism present in histone expression pattern. Accordingly, screening of chickpea cultivars for heat tolerance could incorporate expression analysis of three histone *H2AZ* genes in developing grains during stress and it can be hypothesized that those cultivars which show least responsiveness in expression of *H2AZ* genes during heat stress in developing grains might show more tolerance to heat stress. Thus, such an analysis will provide additional window of opportunity to increase the grain development period during heat stress in chickpea, which presumably can increase the yield of the crop. In addition, such a strategy might also be used for drought and salinity tolerance screening in chickpea.

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