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Effect of heat stress on expression of glucose-6-phosphate/phosphate translocators in chickpea leaves

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Abstract

Sugars, besides source of energy, also provide tolerance and acclimation to plants under abiotic stresses. In plants, sugars are transported via specialized protein molecules called as sugar transporters. One of the most ubiquitous soluble sugars in plants, glucose-6-phosphate, is transported from cytosol into the chloroplast by glucose-6-phosphate/phosphate translocators (GPTs). Search of chickpea sequences revealed existence of two phylogentically diverse GPT genes in chickpea named as *GPT1* and *GPT2*. The gene *GPT1* (coding region: 1200 bases, 41.58% GC content) is present on chromosome 5 whereas *GPT2* (coding region: 1164 bases, 38.74% GC content) on chromosome 1. Of these two, *GPT1* was not active in chickpea leaves whereas *GPT2*, under heat stress, over-expressed vis-à-vis control in leaves of heat-tolerant ICC 15614 and down-regulated in heat-susceptible ICC 10685 suggesting that GPT2 is associated with heat tolerance in chickpea. The *GPT2* can be a potential candidate gene for heat-tolerance in chickpea.

Key words: Chickpea, *Cicer arietinum*, Glucose-6-phosphate transporter, Gene expression, High temperature, Heat stress.

Abiotic stresses such as heat, cold, drought and salinity perturb plants' metabolic processes affecting adversely normal growth and yields of agricultural crops. Chickpea (*Cicer arietinum* L., family Fabaceae, subfamily Faboideae, chromosome number 2n = 16, genome size: 740 Mbp; Arumuganathan and Earle 1991), is the third most important food legume in the world (Croser *et al*. 2003). Being a cool season crop, chickpea is susceptible to brief exposures of high temperature (HT, 30-35°C) which results in substantial yield losses (Saxena *et al.* 1988). HT beyond 35°C inhibits pod set in chickpea (Basu *et al.* 2009). At flowering stage, HT disrupts chickpea anther development at pre-anthesis as well as at anthesis stages (Devasirvatham *et al.* 2013). Sugars are ubiquitous molecules in plant systems and play a vital role in growth and development. In plants, sugars are part of various metabolic pathways, are sources of energy and act as signaling molecules. Sugars form carbon skeletons of plants and play a key role in epigenetic modifications by plants to regulate growth and development of vegetative and reproductive tissues. During adverse climatic conditions such as low temperature and HT stress, carbohydrate metabolic pathways or any other cross linked pathways of plants get affected (Kaushal *et al*. 2013; Sharma and Nayyar 2014; Sharma and Nayyar 2016).

Transportation of sugars is a critical component of carbon assimilation within various organs/organelles of plants and transport of various carbon moieties is carried out by molecule specific transporters e.g. glucose-6-phosphate (G6P) is transported by the glucose-6-phosphate/phosphate translocators (G6P/Pi translocators or GPTs) also called as glucose-6-phosphate transporters. There are several GPTs in plants. Disruption of starch metabolism during high CO_2 concentration results in the expression of *GPT2* gene which activates G6P/Pi translocator and exchange the G6P across the membrane of chloroplast (Weise *et al.* 2019). GPTs transport G6P and triose phosphates (3-phosphoglyceraldehyde) but do not transport other hexoses such as glucose-1-phosphate and fructose-6-phosphate (Kammerer *et al.* 1998).

Two G6P/Pi translocator genes – At*GPT1* and At*GPT2* – are present in the genome of *Arabidopsis thaliana* (Niewiadomski *et al.* 2005). At*GPT1* expresses primarily in gametic tissues such as anthers and stamens whereas At*GPT2* expresses in leaf tissues.

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GPT1 imports G6P to non-green plastids of plants and its deficiency affects fatty acid biosynthesis due to defects in the oxidative pentose phosphate pathway (OPPP) (Niewiadomski *et al*. 2005). Increase in *GPT2* expression increases expression of starch synthesis pathway enzymes, transcription factors and anthocyanin biosynthesis enzymes in *A. thaliana* mutant *pho3* indicating that secondary metabolism in plants was regulated by primary metabolism in responses to various stresses (Lloyd and Zakhleniuk 2004). GPT2 is also a key player in sensing the environmental changes, as evidenced in mature leaves of *Arabidopsis thaliana*, where *GPT2* expression modulated early seedling development (Dyson *et al.* 2014). In the present study, we report that two G6P/Pi translocator genes i.e. *GPT1* and *GPT2* are present in the chickpea genome. The effect of HT on modulation of expression of *GPT1* and *GPT2* in leaves of a heat tolerant and in a heat sensitive genotype of chickpea is also described. Besides this, the genes were also characterized.

Materials and Methods

Plant materials and growth conditions

Plants of two genotypes of chickpea i.e. ICC 15614 (heat-tolerant) and ICC 10685 (heat-susceptible) were grown in small pots (10 cm diameter) filled with black vertisol soil, sand and vermicompost (4: 2: 1 by volume). Seedlings were grown under controlled conditions (23±1°C/18±1°C day/night) for 21 days (Fig 1) and thereafter transferred to a growth chamber maintained at high temperature (35°C). Humidity in the chamber was 75-80% and light intensity was ~320 µmol $s^{-1}m^{-2}$ during 12 hours photoperiod. The leaf tissues were harvested at control conditions (0 hour) and after 2 and 72 hours of HT stress. Two hours period allowed us to identify initial changes in gene expression due to heat stress whereas 72 hours period revealed the stabilized expression of the genes.

Gene retrieval, gene characterization and primer synthesis

The cDNA sequences of chickpea genes, *GPT1* and *GPT2* were retrieved from the NCBI (National Centre for Biotechnology Information, NIH, USA) database (https://www.ncbi.nlm.nih.gov/). Open reading frames of the genes (nucleotide and amino acid sequences) were deduced by using the open reading frame finder tool of the NCBI (https:// www.ncbi.nlm.nih.gov/ orffinder/). GC content of the sequences was calculated using the software, https:// www. biologicscorp.com /tools/ GCContent/ index. The cDNA sequences of Arabidopsis genes, *GPT1* and *GPT2* were also retrieved from the NCBI database. Diversity in cDNA sequences of the genes was calculated using clustalW followed by phylogenetic tree construction using neighbor joining method (https:// www.ebi.ac.uk/Tools /services/web/toolresult.ebi– jobId=clustalo-I20200413-095304-0679-93618513-p1m&analysis= phylotree) of EMBL-EBI (European Molecular Biology Laboratory-European Bioinformatics Institute) database.

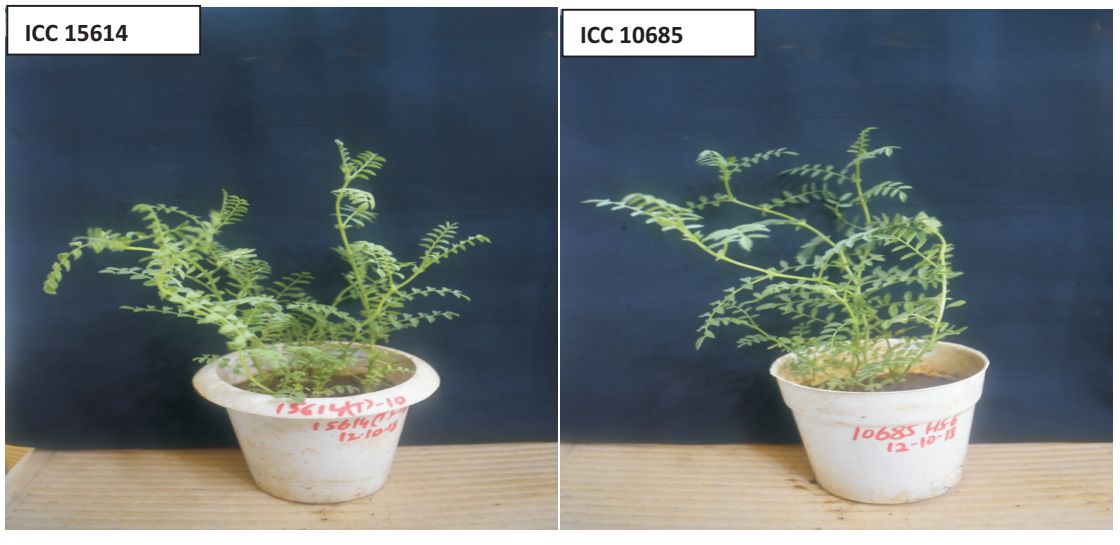

Figure 1. Plants of two genotypes of chickpea, ICC 15614 (heat-tolerant) and ICC 10685 (heat-susceptible), at the time of heat stress

The cDNA sequences of the target genes *GPT1* and *GPT2* were used to design gene-specific primers using primer3 software (http://bioinfo.ut.ee/primer3/). The primers were got custom synthesized from Integrated DNA Technologies (www.idtdna.com) (Table 1). Primer sequences of two chickpea housekeeping genes, *ATP-binding cassette transporter (ABCT*) and *Clathrin adaptor complexes* medium subunit family protein (*CAC*), (Reddy *et al.* 2016) were used to synthesize the primers for these genes.

RNA Extraction and cDNA synthesis

Total RNA from control and treated leaves of ICC 15614 and ICC 10685 was extracted using Trizol method as per manufacturer's instructions (Ambion Life Sciences, Thermo Fisher, USA). RNA pellet was dissolved in nuclease free water and treated with RNase-free DNase I (Thermo Fisher Scientific USA). The quantity of RNA was measured using nanodrop (Thermo scientific) at 260 nm and quality using 1 % denaturing (formaldehyde) agarose gel electrophoresis. The quantity of RNA was equalized in all the samples and cDNA was synthesized from total RNA using oligo (dT) 18 primer (Thermo Scientific) and M-MLV Reverse Transcriptase enzyme (Promega, USA) by incubating the reaction tubes at 42°C for 60 minutes as per manufacturer's instructions.

Quantitative polymerase chain reaction (qPCR)

The transcript abundance of *GPT1* and *GPT2* was analyzed by qPCR in Step One™ Real-Time PCR System (Applied Biosystems, USA) with the SYBR Green PCR Master Mix (TB Green™ Premix Ex Taq™, Takara Clontech, USA) using recipe for qRT PCR (Table 2). The thermal profile for PCR was: 95°C for 10 min; 40 cycles of 95°C for 15 s, and 56°C for 1 min during which fluorescence was measured. This was followed by a melting curve analysis at: 95°C for 15 s, 60°C for 15 s, a ramp from 60°C to 95°C for 20 min period and fluorescence was monitored in this stage, followed by 95°C for 15 s. Transcript amounts were normalized using the *ABCT* and *CAC* housekeeping genes. The relative expression ratios of target genes were calculated using comparative Ct values by using the Livak method (Livak and Schmittgen 2001) and data were further analyzed as per Taylor *et al.* (2019). The experiment had three replicates per sample and the data were expressed as mean values ± standard error (SE) of the Ct values of respective sample.

Table 1. Sequence of gene primers

Gene name	Primer name	Sequence 5'-3' (Forward)	Sequence 5'-3' (Reverse)
ATP-binding cassette transporter	*ABCT*	TCACAGGTTGTGATGGAGTCTG	CCTCAAATCTTGTTGGGGTGTC
Clathrin adaptor complexes	*CAC*	CATGGACTAGACCACCAATTCA	AACAGTGTTGTACCCGCTCTTT
Glucose-6-phosphate/phosphate translocator 1	*GPT1*	ACCCTTACCCTTGGCTCACT	AGGCTCGCCACTCTTGATAA
Glucose-6-phosphate/phosphate translocator 2	*GPT2*	CTTGGTGGGCTTTGAATGTT	TCAACTTTTGGGGAATCAGC

Table 2. Master-mix composition for qPCR

Sybr Green	5.00 µL
Rox	0.20 µL
Forward primer	0.20 µL
Reverse primer	0.20 µL
cDNA	1.00 µL
Nuclease-free water	3.40 µL
Total volume	10.00 µL

Results and Discussion

Gene retrieval and characterization

Search of chickpea genome and other sequences available in NCBI database (https://blast.ncbi.nlm.nih.gov) revealed the presence of two *glucose-6-phosphate transporter* genes in chickpea named as *GPT1* and *GPT2*. NCBI BLAST (BLASTn and BLASTt) further confirmed the identity of the genes. cDNA sequence of *GPT1* gene was 1953 base pairs in length whereas that of *GPT2* was 1621 base pairs long. The 5′ untranslated region (5′ UTR) of the cDNA sequence of *GPT1* gene was 420 bases long whereas 3′ UTR region was 333 bases long (Figs 2 and 3). Similarly, the 5′ UTR region of the cDNA sequence of *GPT2* gene was 136 bases long and 3′ UTR region was 321 bases. Coding region of *GPT1* was 1200 nucleotides in length with 41.58% GC content whereas that of *GPT2* was 1164 nucleotides in length with 38.74% (Figs 2, 3, 4 and 5). Predicted protein of the *GPT1* was comprised of 399 amino acids whereas that of *GPT2* was comprised of 387 amino acids (Figs 6 and 7). The *GPT1* and *GPT2* were not clustered together on the same region of the genome or present on same chromosome but were on different chromosomes – *GPT1 on* chromosome 5 and *GPT2* on chromosome 1.

5' UTR

AACGCCTCCACCACCAAACCCCACAACAACAACAGTATCCCCTAAAACTATTTTATACTAAA
ACTAAACAACCATCAACAAGAAACTACAACCAAATTTGACCATATAAAAATATTCATTGCAT
TTATACTTTTTTATTTTAATTTTAATTCATTCAATACCAGAGATTCCAAGTGTTTTGGTTTCCA
ATAAACCTTTCAAGATCTCAAAGGGGCATGATCAGAACCTAAAAATACATTCTTTTTACATA
TAAACAGTGTGTTCAGGATCGTGAGAGTTGATTTCACGACCCTGACACAATAGGGTTGTCTC
TGATTTTTGTTTTTCTGGTTGGTTTCTTTTGTTTTTTTTTCCTTTTCCTTTGAGTTAAGGTTTTGG
TCGGAAAGTTTTGATTTTTAAGATACCCTTTTCTTAAAAACC

Open reading frame

ATGATTTCATCGTTGAGACAACAACCTAGTATAGCGATCAGTGGTTCTGATGTTGTTTTAAG
GAAAAGACATGGAACCCTAGTTCAACCACAGTCGTTTTTACCTTCACTGGTAAGAGGAAAAT
CACAGAGATCTCTTGTTTCAGTGCAAAAGCCTCTTCACCTTGCATGTGTTGGTGTTGGAAATT
TTGGGTCAGTGAAGAGTTTTGATTCAGTGAAGGGTTTTGGAAGTGATGATTTGGTTAAGTGT
GAAGCCTATGAAGCTGATAGATCGGAGGTTGAAGGTGCAGCAACACCATCAGAAGCTGCAA
AAAAAGTGAAAATTGGGATATATTTTGCAACTTGGTGGGCTTTGAATGTTGTTTTCAATATTT
ATAATAAGAAGGTTTTGAATGCATACCCTTACCCTTGGCTCACTTCAACTCTCTCACTTGCTT
GTGGCTCCCTTATGATGTTGATCTCTTGGGCCACTAGGATAGCTGAAGCACCTAAAACTGAT
TTTGAGTTTTGGAAGACTTTGTTCCCTGTTGCTGTTGCTCACACAATTGGACATGTTGCCGCT
ACGGTCAGTATGTCGAAAGTTGCGGTATCATTTACACATATTATCAAGAGTGGCGAGCCTGC
TTTTAGTGTTCTGGTTTCGAGATTTATTTTGGGTGAGACCTTCCCAGTGCCAGTCTATCTGTCC
TTACTTCCAATCATTGGTGGATGTGCACTTGCTGCTGTGACTGAGCTCAATTTCAATATGATT
GGTTTTATGGGGGCTATGATATCAAATTTGGCATTTGTGTTCCGTAACATCTTTTCGAAAAAG
GGGATGAAGGGAAATTCTGTTAGCGGAATGAACTACTATGCTTGTTTATCTATTTTGTCCCTT
GCAATTCTCACACCCTTTGCAATTGCCGTGGAAGGGCCACAAATGTGGGCTGCTGGATGGCA
AACAGCTCTCTCTGAAATTGGACCTCAATTCATATGGTGGGTAGCAGCTCAGAGTATATTCT
ATCATCTATACAATCAGGTGTCTTACATGTCCTTGGATGAGATCTCTCCCTTGACATTTAGCA
TTGGAAACACCATGAAACGTATATCTGTCATAGTATCTTCAATTATCATCTTCCACACACCAG
TTCAGCCCGTCAATGCTCTTGGAGCTGCAATTGCTGTCTTCGGGACCTTCTTATACTCACAGT
CAAAACAATAG

3' UTR

AGTTGTGGAACATAAATTCCTCAAGAGACTACTACAAGGAAGTTGATTTTGGAACATGTGGC
TGCTGGATTTTTGTAAGACTAGGATGATCCGGCTGTTAGTATCTGTAGATAAACAATAATAA
TAATCAGAAATGATGATGCTGAGAAACATTATCATGAAACAGATGCTTAGAGAGGTTTTTTA
TTTTTCAATTTCTTAAATGCCAATGGATTGAAATAAAAGTTCCTGTTTATATAGTCCGAGAAT
AATAATTTCTCAAAACATTGTTTACTATATAAGGAGTTTTATCTATAATAACATTATGCGTGT
TATCTATTTGACGCTTTTTTT

Figure 2. Predicted cDNA sequence of the *glucose-6-phosphate/phosphate translocator 1* gene of *Cicer arietinum*

5' UTR

ACCAACCCATCAAGGCATAAACTACTAAGTGCAAAACACAATTATACAAACACATCTAT
TTTAACAACCTATTATCGTTTATTCTTTCATTTATTCATTCTGTTACACTACAACACATTCA
TATAAACTAAGAATC

Open reading frame

ATGATCTCTTCAATCAAATGCACAACATCATCACTCACAAAGCTTCCTCTTCAAAGGCCT
CAAATTTCAAATCTACCAACCATTCAAAATGTTGAACAAAACATGTCTCTTTCTCTACTCT
CTTCAAAGAAACCTCTCTACCTATCATCCACTGAGAATTTTGCATTATTGACAAAACCCA
ATAGAAAGAATGTGACTTTGTGTCAAGCCTATGAAGCTGATAGATCAAGGCCACTTGAG
ATTAACATTGAGCTTCCTAACGAAGAAGAAGCTCAAAGACTCAAGATTGGATTGTATTTT
GCTACTTGGTGGGCTTTGAATGTTGTTTTCAACATATACAACAAGAAAGTTTTGAATGTTT
TTCCTTATCCTTGGCTTACTTCCACTCTGTCCTTAGCTGCTGGTTCTCTCATTATGTTAATT
TCATGGGTCACTAGGGTTGCTGATTCCCCAAAAGTTGATTTGGATTTCTGGAAGGCCCTT
TTTCCTGTTGCTATGGCACACACAATTGGGCATGTTGCTGCAACTGTGAGCATGTCAAAA
GTAGCAGTTTCATTCACTCACATCATCAAGAGTGGAGAACCAGCTTTCAGTGTCCTAGTT
TCAAGATTCTTGCTTGGAGAACCATTCCCTATGCCAGTTTACTTCTCATTGTTGCCAATAA
TTGGTGGTTGTGCACTAGCTGCTGTAACTGAGCTCAATTTCAATATGATTGGATTTATGG
GGGCTATGATATCAAATTTGGCATTTGTGTTCAGGAATATATTCTCAAAGAAAGGAATGA
AGGGAAAGNNNNNNNNTGGAATGAACTATTATGCTTGTCTCTCAATGATGTCTCTATTAA
TTCTCACACCTTTTGCCATTGCTGTGGAAGGTCCCAAAGTTTGGGCTGTAGGCTGGCAAA
CTGCAGTGTCTCAAATTGGTCCCAATTTTGTATGGTGGGTGGTTGCTCAGAGTGTGTTCTA
TCATTTGTATAATCAAGTATCATACATGTCTCTTGATCAGATTTCACCCTTAACATTTAGT
ATAGGAAACACAATGAAGAGAATTTCTGTAATAGTCTCTTCAATCCTTATTTTTCACACT
CCACTTCAACCTATCAATGCTCTTGGAGCCGCCATTGCAATTCTTGGCACCTTCATCTATT
CACAGGCTAAACAGTGA

3' UTR

GGTTGTGTTTTTGAAGCTGTTATGGAACACAAAGGAATGATGACTCATGTCATGGCTTGA
GTTCTAAGGGTACTATTATGTTGTTAGACTGTGATTAGTACTATGATGTGAATTAGAGAG
TGTATTATGTTGTCAGTGATTAGGAGTATATGATGTTAATTAGAGATTGCATTGTGTTGTT
AATAGGAATTTTCTCTGAGGTCATTATAACATTGTAATTTCGTTTTTATGTGTGTAAATGA
GTTTTCTGCTGCTTTTCTGTCAAGAGCTTTAGAAGAATAGACTGTATTGAATTATAAGATT
GATGTGTCATAAGAGTTA

Figure 3. Predicted cDNA sequence of the *glucose-6-phosphate/phosphate translocator 2* gene of *Cicer arietinum* L.

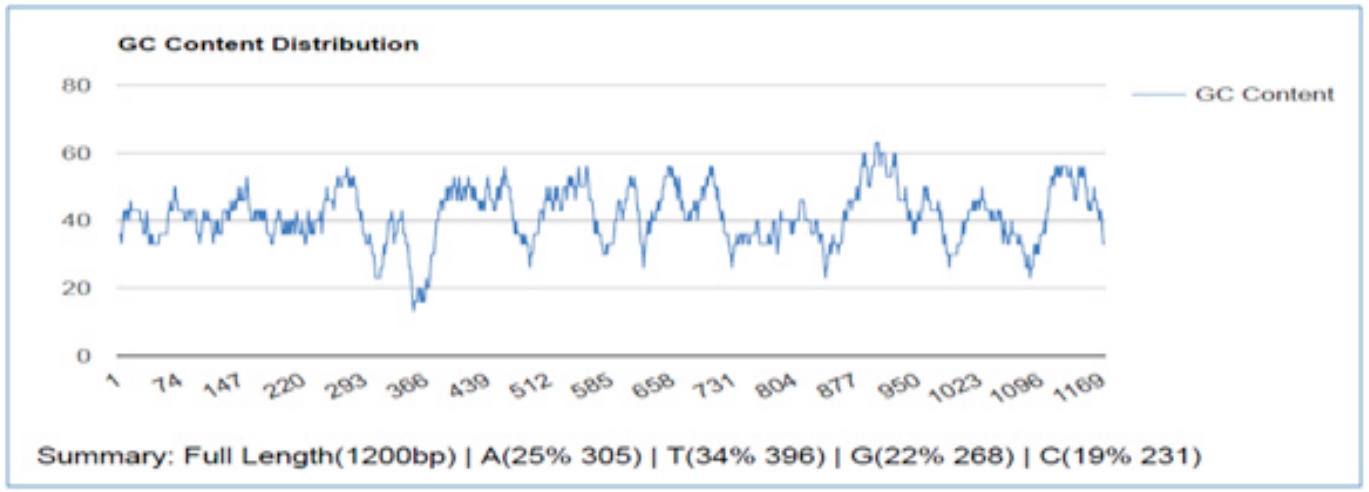

Figure 4. GC content of coding region of predicted cDNA sequence of *glucose-6-phosphate/phosphate translocator 1* gene of *Cicer arietinum*

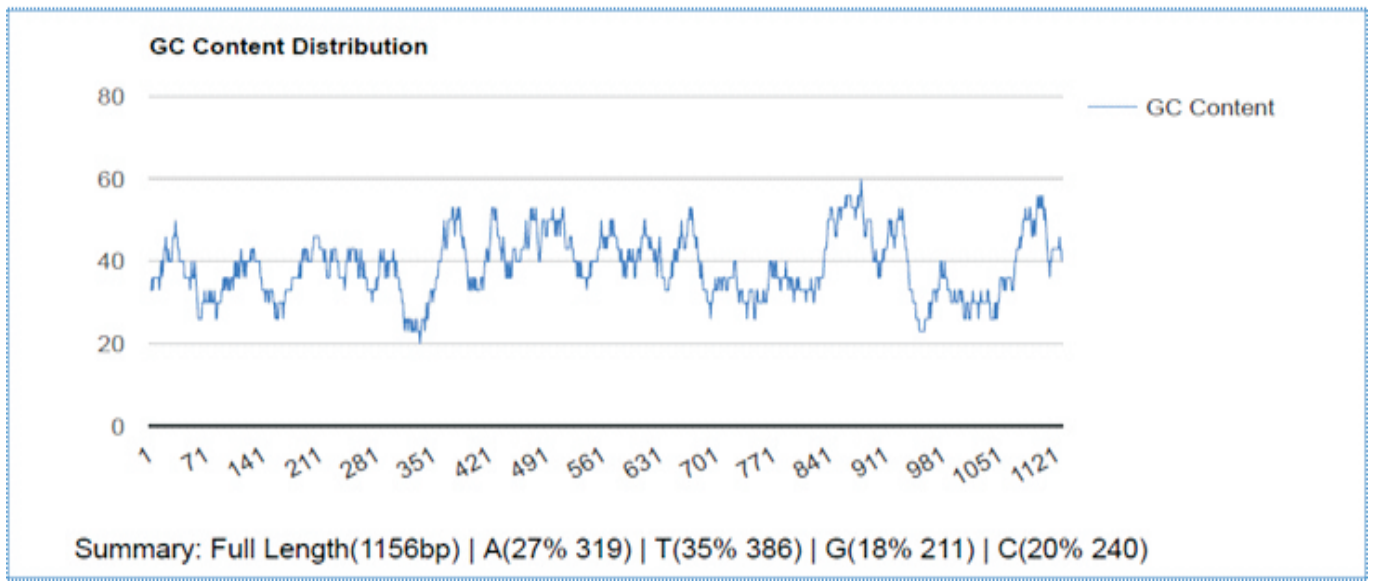

Figure 5. GC content of coding region of predicted cDNA sequence of *glucose-6-phosphate/phosphate translocator 2* gene of *Cicer arietinum*

MISSLRQQPSIAISGSDVVLRKRHGTLVQPQSFLPSLVRGKSQRSLVSVQKPLHLACVGVGNF
GSVKSFDSVKGFGSDDLVKCEAYEADRSEVEGAATPSEAAKKVKIGIYFATWWALNVVFNI
YNKKVLNAYPYPWLTSTLSLACGSLMMLISWATRIAEAPKTDFEFWKTLFPVAVAHTIGHVA
ATVSMSKVAVSFTHIIKSGEPAFSVLVSRFILGETFPVPVYLSLLPIIGGCALAAVTELNFNMIG
FMGAMISNLAFVFRNIFSKKGMKGNSVSGMNYYACLSILSLAILTPFAIAVEGPQMWAAGW
QTALSEIGPQFIWWVAAQSIFYHLYNQVSYMSLDEISPLTFSIGNTMKRISVIVSSIIIFHTPVQP
VNALGAAIAVFGTFLYSQSKQ

Figure 6. Predicted protein sequence of *glucose-6-phosphate/phosphate translocator 1* of *Cicer arietinum*

MISSIKCTTSSLTKLPLQRPQISNLPTIQNVEQNMSLSLLSSKKPLYLSSTENFALLTKPNRKNVTLC
QAYEADRSRPLEINIELPNEEEAQRLKIGLYFATWWALNVVFNIYNKKVLNVFPYPWLTSTLSLA
AGSLIMLISWVTRVADSPKVDLDFWKALFPVAMAHTIGHVAATVSMSKVAVSFTHIIKSGEPAFS
VLVSRFLLGEPFPMPVYFSLLPIIGGCALAAVTELNFNMIGFMGAMISNLAFVFRNIF SKKGMKGK
XXXGMNYYACLSMMSLLILTPFAIAVEGPKVWAVGWQTAVSQIGPNFVWWVVAQSVFYHLYN
QVSYMSLDQISPLTFSIGNTMKRISVIVSSILIFHTPLQPINALGAAIAILGTFIYSQAKQ

Figure 7. Predicted protein sequence of *glucose-6-phosphate/phosphate translocator 2* of *Cicer arietinum*

Phylogenetic similarity between *GPT1* and *GPT2*

The cDNA and predicted protein sequences of *GPT1* and *GPT2* from chickpea and *A. thaliana* were used for phylogenetic analysis. Phylogenetic analysis of cDNA sequences revealed that two genes of chickpea were distinct from each other; however, genes from *A. thaliana* were diverse from both of the chickpea genes as evident from less homology (Fig 8). Protein sequences of *GPT1* and *GPT2* from chickpea were more diverse from each other than cDNAs wherein *GPT1* of chickpea had more similarity with *GPT1* of *A. thaliana* and *GPT2* of chickpea with *GPT2* of *A. thaliana* (Fig 9) suggesting more conservation between chickpea and *A. thaliana* protein sequences unlike gene sequences.

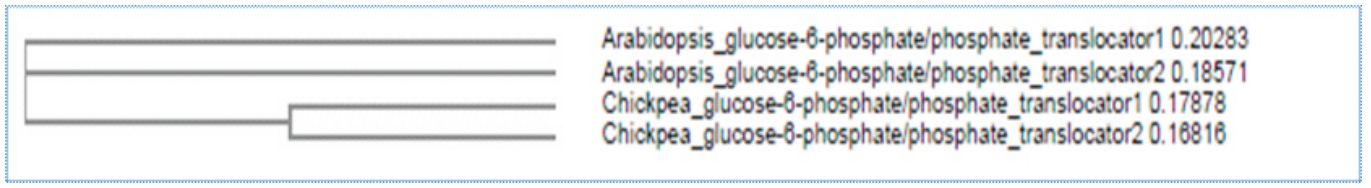

Figure 8. Phylogenetic tree showing homology among the cDNA sequences of the *glucose-6-phosphate /phosphate translocator 1* and *glucose-6-phosphate/phosphate translocator 2* genes of *Cicer arietinum* and *Arabidopsis thaliana*

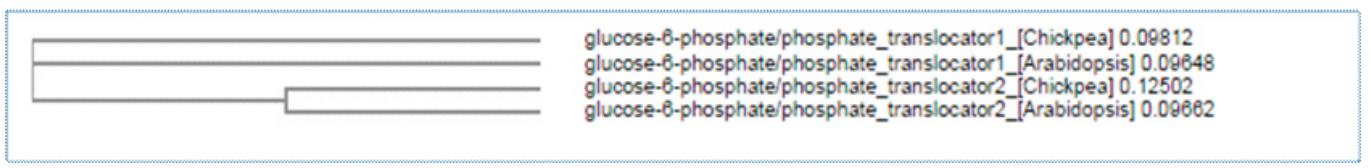

Figure 9. Phylogenetic tree showing homology among protein sequences of *glucose-6-phosphate/ phosphate translocator 1* and *glucose-6-phosphate/phosphate translocator 2 of* *Cicer arietinum* and *Arabidopsis thaliana*

***GPT1* and *GPT2* expression under controlled and heat stress conditions**

The RNA isolated from different samples was of high quality as evident from ratios of absorbance at 260/280 nm which ranged from 1.85 to 2.10 and were close to the value of 2.0 for RNA (Table 3). The quality of RNA was further confirmed by denaturing (formaldehyde) agarose gel electrophoresis wherein intact rRNA bands were detected with no signs of degradation (Fig 10).

Table 3. A260/A280 ratio and quantity of RNA isolated from leaves of control and heat treated plants of two chickpea genotypes after DNase 1 treatment

RNA samples	A260/A280 ratio	Quantity (ng/µL)
ICC 15614 control	2.10	145.00
ICC 15614 (2 hrs; 35°C)	1.90	1360.00
ICC 15614 (72 hrs; 35°C)	1.90	730.00
ICC 10685 control	2.00	245.00
ICC 10685 (2 hrs; 35°C)	1.85	1040.00
ICC 10685 (72 hrs; 35°C)	1.90	1204.00

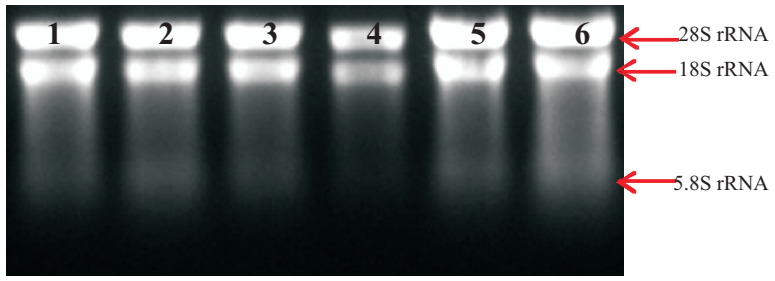

Figure 10. Denaturing (formaldehyde) agarose gel electrophoresis (1%) showing quality of total RNA. Total RNA was isolated from chickpea samples grown at normal temperature (control) and at high temperature stress (35°C). Lanes 1 to 3: ICC 15614, Lanes 4 to 6: ICC 10685, Lanes 1 and 4: control; Lane 2 and 5: 2 hrs at 35°C, Lane 3 and 6: 72 hrs at 35°C

Of the two genes used in the current study, the expression of *GPT1* remained low in control as well as heat treated plants indicating that this gene is neither leaf specific nor a major player in heat tolerance in chickpea leaves (Fig 11A). The *GPT1* expression did not change in ICC 15614 at 2 hours (-0.09 fold) as well as 72 hours (-0.15 fold) of HT, whereas its expression was slightly increased in ICC 10685 with fold change of 0.33 at 2 hours and 0.95 at 72 hours (Fig 11B). Similar to chickpea, *AtGTP1* of *A. thaliana* was also leaf non-specific. *GPT1*, in *A. thaliana*, was reported to play role in gamete formation and embryo development but not in leaves. Loss of the activity of GPT1 disturbed the oxidative pentose phosphate pathway (OPPP) due to deficiency of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and resulted in non-viable embryo formation as well as death of plant embryos during early stages due to accumulation of reactive oxygen species (ROS) in *Arabidopsis* embryos (Andriotis *et al.* 2010). In another study, gametogenesis and viable embryo formation was arrested in mutants of *GPT1* lines in *A. thaliana* (Niewiadomski *et al.* 2005) suggesting the vitality of this transporter in gametogenesis and embryogenesis. Low expression of *GPT1* in leaves of chickpea (present study) confirms the earlier findings in *Arabidopsis thaliana* that *GPT1*

is not leaf specific and plays no or limited role in leaves of plants.

The *GPT2* expressed in leaves of both the genotypes under normal temperature (NT) as well as under heat treatment (Fig 11A and 11B). The expression of *GPT2* under heat treatment increased significantly in heat-tolerant ICC 15614 (relative expression ratio at 2 hours = 87.21, 72 hours = 21.45) and decreased in heat-sensitive ICC 10685 (relative expression ratio at 2 hours = 0.39, 72 hours = 1.68). Between the two genotypes, *GPT2* under heat treatment was highly up-regulated at 2 hours (6.45 fold) as well as at 72 hours (4.42 fold) in the heat-tolerant genotype, whereas in heat sensitive genotype gene expression was down-regulated at 2 hours (-1.35 fold) and slightly upregulated at 72 hours (0.74 fold). Differential regulation of *GPT2* in the heat tolerant and heat sensitive genotypes of chickpea suggested that this gene had a role in heat tolerance by chickpea. *GPT2* in *Arabidopsis thaliana* plays a vital role in health of plants growing in changing environments (e.g. heat, cold, drought, high light irradiances) which directly affects plants' metabolism (Athanasiou *et al.* 2010). Immediately after high light stress, the activity of GPT2 transporter enhanced in leaves of Arabidopsis (Athanasiou *et al.* 2010). Earlier findings in *Arabidopsis* and over expression of *GPT2* immediately (2 hrs) after heat stress in chickpea (present study) suggested that this gene is one of the initial players in imparting heat stress tolerance in chickpea leaves.

Over-expression of *GPT2* under heat stress in heat tolerant genotype pointed towards GPT2 mediated enhanced transport of G6P under heat stress in chickpea resulting in provisions of vital carbohydrates for tolerating heat. The role of GPT2 in sugar responses of plants is documented in model crop *A. thaliana*. In *A. thaliana*, GPT2 is vital for the rapid induction of sugar responses which requires W-box cis-elements in the promoter region of *GPT2* gene (Chen *et al.* 2019). WRKY18 and WRKY53 transcription factors bind directly to the W-Box cis-elements in the promoter regions of sugar response genes such as *GPT2* and

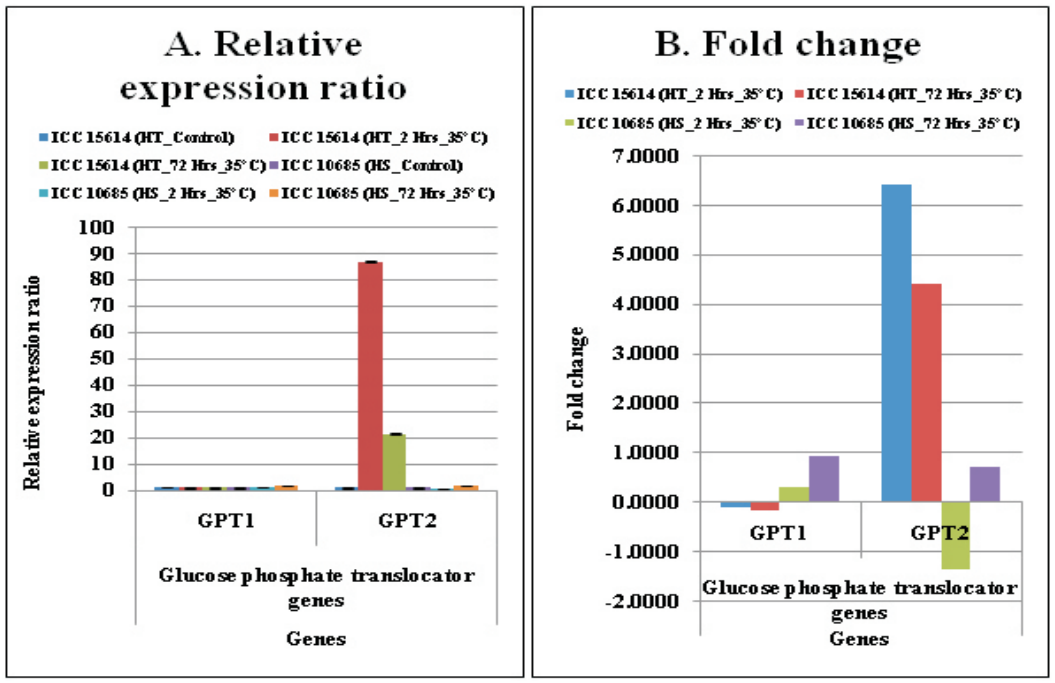

Figure 11 A. Relative expression ratios of two *glucose-6-phosphate/phosphate translocator* genes, *GPT1* and *GPT2*, at 0, 2 and 72 hours after heat stress (35°C) in chickpea genotypes ICC 15614 (heat-tolerant) and ICC 10685 (heat-susceptible). Expression was quantified using quantitative real-time PCR and normalized to *ATP-binding cassette transporter (ABCT*) and *Clathrin adaptor complexes* (*CAC*) housekeeping genes. Quantitative data show the mean (±SE) of three replicates.

B. Fold change in expression of *GPT1* and *GPT2* at 2 hours and 72 hours after heat stress (35°C) in ICC 15614 and ICC 10685.

result in their expression (Chen *et al.* 2019). *GPT2* gene is regulated at transcriptional level and for its expression redox responsive transcription factor 1 (RRTF1) and triose phosphates (such as glyceraldehyde 3-phosphate) export is necessary (Weise *et al.* 2019). Non-reducing sugar, sucrose, induces the expression of *GPT2* by importing the G6P into the chloroplasts resulting in cell proliferation (Dingenen *et al.* 2016).

Not only sucrose, decrease in starch accumulation also induced GPT2 mediated transport in plants as evident from increased activity of GPT translocator in leaves of *A. thaliana* mutants that were impaired in starch metabolism (Kunz *et al.* 2010). Consequent to increased GPT2, it imported larger amounts of G6P into the chloroplast from cytosol, that resulted in starch accumulation and acclimatization of *A. thaliana* plants to high light and maintained the photosynthetic capacities (Dyson *et al.* 2015). Heat stress, in other crops, influences starch synthesis; fatty acid synthesis; nucleotide biosynthesis and GPT2 provides necessary impetus to regulate positively the sugar and starch metabolism (Kunz *et al.* 2010, Dyson *et al.* 2015, Chen *et al.* 2019).

It is concluded that chickpea harbours two G6P transport genes, *GPT1* and *GPT2* wherein *GPT2* expresses in leaves and *GPT1* is non leaf-specific. These genes are unlinked and are located on different chromosomes on the genome of chickpea. The present study and those reported earlier in model crop, *A. thaliana*, show that *GPT2* plays role in heat stress tolerance in leaves of plants and this tolerance is possibly due to *GPT2* induced changes in carbohydrate metabolism in leaves under heat stress.

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