



Full Length Article

Phylogenetic Analyses of the Trithorax Homologs and Related Proteins in *Cicer arietinum*

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Abstract

Epigenetic regulation is a major player for the determination of cell shape and identity. The information, related to gene expression regulation at epigenetic level, is partially preserved in histone proteins as reversible covalent modifications. An important group of chromatin modifying enzymes is comprised of the histone lysine methyltransferases (HKMTs). The *ATX1* is a member of Class III HKMTs, which is involved in flowering time regulation and drought stress response in *Arabidopsis*. Present research involves *in silico* identification, characterization and expression analysis of *ATX*-like genes in chickpea. We have identified eight putative members of class III HKMTs in chickpea, where *CarATX-like* genes harbor the characteristic protein domains like SET, PHD and TUDOR. Moreover, an analysis of gene promoter predicted an occurrence of potential stress responsive *cis*-elements. Digital expression profiling demonstrated the involvement of *CarATX-like* gene in drought stress response and it was confirmed through real-time RT-qPCR. Current study is the very first step for studying chromatin modifying genes of HKMT group in chickpea. It will be helpful for further characterization of these genes in this crop plant. © 2018 Friends Science Publishers

Keywords: Histone lysine methyltransferases; Trithorax homologs; Chickpea; Drought stress; *Cicer arietinum*

Introduction

Plant growth and development is significantly constrained by severe environmental conditions like heat, salinity and drought stress. Climate change is also becoming a significant threat for the crop productivity and agricultural sustainability (Kole *et al.*, 2015). Land plants have evolved a number of specialized strategies to neutralize harsh climatic conditions, which include but not limited to stress responsive signaling pathways. These pathways mediate plant molecular, metabolic and physiological responses for the improvement of plant resistance and survival (Lata and Prasad, 2011). Such pathways are tightly regulated at genetic level by different groups of genes. Apart from conventional genetics, some epigenetic mechanisms are also drawing attention of modern day research.

Epigenetic regulation is a major player for the determination of cell shape and identity. The information related to epigenetic gene expression regulation is partially preserved in histone proteins as reversible covalent modifications. In fruit fly (*Drosophila melanogaster*), there

are two groups of genes that have successfully been characterized for epigenetic regulation i.e., the *trithorax* group (*trxG*) and the *Polycomb* group (*PcG*). Members of these groups are involved in an antagonistic regulation of development related genes (Simon and Tamkun, 2002). At protein level, *PcG* and *trxG* genes harbor a characteristic protein domain called “Suppressor of variegation 3-9, Enhancer of zeste, TRX” or the SET domain. This domain is required for histone lysine methyltransferase (HKMT) activity for the posttranslational modification of lysines (Lys) on H3 and H4 histones as repressive and activating marks (Lachner *et al.*, 2004). The SET domain containing gene family with conserved HKMT activity has also been identified in plants (Pontvianne *et al.*, 2010).

In plants, there are seven classes of genes with HKMTs activity. The class III HKMTs play important role in regulation of flowering time. In the model plant *Arabidopsis*, a genome wide analysis identified seven members of HKMTs i.e., five homologues of fruit fly Trithorax gene named as *Arabidopsis* Trithorax-like proteins 1-5 (*ATX1-5*) and two *Arabidopsis* Trithorax-like related proteins as

ATXR3/ATXR7 (Avramova, 2009). The proteins of Class III HKMTs are characterized by the presence of particular domains like SET, post-SET, PHD (plant homeodomain), FYRN/FYRC (F/Y-rich N or N-terminus) and the PWWP (proline–tryptophane–tryptophane–proline) domain (Alvarez-Venegas and Avramova, 2001). The PHD domain is thought to interact with trimethylated H3K4 (Peña *et al.*, 2006). Different SET DOMAIN genes have been identified to show TrxG-like H3K4-methyltransferase activity in *Arabidopsis* (Berr *et al.*, 2011). The ATX1 and ATX2 proteins are involved in trimethylation and dimethylation of H3K4, respectively (Pien *et al.*, 2008; Saleh *et al.*, 2008a). At plant level, genetic disruption of *ATX1* gene results into early flowering plants with altered leaf morphogenesis (Alvarez-Venegas *et al.*, 2003; Saleh *et al.*, 2008b). A double mutant plant for *ATX1* and *ATX2* exhibits even more earliness in flowering time than *atx1*, which suggests an overlapping role of *ATX1* and *ATX2* in temporal regulation of flowering (Pien *et al.*, 2008; Saleh *et al.*, 2008b). At the same time, *ATX1* and *ATX2* are potentially involved in the regulation of different groups of genes. It was observed that *atx1* mutation could affect almost 7% of overall gene expression while *atx2* mutation affected 0.7% of all the expressed genes (Alvarez-Venegas *et al.*, 2006). Other ATX like proteins (ATX3, ATX4, ATX5) affect H3K4 di/tri-methylation of genes related to vegetative and reproductive development. Similarly, the ATXR3/SDG2 is also involved in regulating genome-wide H3K4me3 profiles but for distinct loci. It suggests the existence of separate regulatory pathways (Yao *et al.*, 2013; Chen *et al.*, 2017). ATXR3/SDG2 has a more important and strong role in H3K4me3 deposition. The knockdown *atxr3* plants exhibit pleiotropic phenotypes, which include impaired development of male/female gametophyte and dwarfism (Berr *et al.*, 2010; Guo *et al.*, 2010; Pinon *et al.*, 2017). ATXR7/SDG25 play an important role for the regulation of Flowering Locus C (Berr *et al.*, 2009).

Chickpea (*Cicer arietinum*) is the second most cultivated legume pulse crop. The grains of this plant contain higher amounts of proteins, fats and carbohydrates (Rasool *et al.*, 2015). The chickpea crop is generally cultivated on marginal lands under severe conditions (Singh *et al.*, 1998). The recent completion of genomic and transcriptomic sequencing projects of chickpea presents a valuable opportunity for genome wide comparative and evolutionary analysis (Jain *et al.*, 2013; Varshney *et al.*, 2013).

Present research work is comprised of identification and characterization of ATX1 homologue in chickpea by using *in silico* approach. Furthermore, phylogenetic relationships have also been studied among homologues of class III HKMTs in commercially important crops. In the end, expression analysis was performed to get an insight of potential functional properties of *ATX-like* genes in chickpea.

Materials and Methods

Genome Wide Identification, Multiple Sequence Alignment and Phylogenetic Analysis

Protein sequences of Arabidopsis class III HKMTs were used as query to identify homologous genes in Monocots (*Brachypodium distachyon*, *Sorghum bicolor*, *Zea mays* and *Oryza sativa*,) and dicots (*Populus trichocarpa*, *Citrus sinensis*, *Brassica rapa*, *Arabidopsis thaliana* and *Cicer arietinum*) using online NCBI-BLAST program. The multiple sequence alignment was performed using Clustal Omega at EMBL (<http://www.ebi.ac.uk>) by using default parameters. The analysis of phylogenetic relationships was performed using default parameters of Neighbor-Joining method in Mega 7 with 1000 bootstrap iterations (Kumar *et al.*, 2016).

Prediction of Gene Structure, Chromosomal Locations and Conserved Domains/Motifs and Promoter Analysis

Genomic DNA and CDS sequences were analyzed for the identification of coding and non-coding regions, by using an online tool called “Gene Structure Display Server” or “GSDS” (Hu *et al.*, 2015). The MapChart program was used to generate a chromosomal map of putative *ATX-like* loci. Conserved motifs within protein sequences were predicted by using default parameters of MEME server (Bailey *et al.*, 2006). For promoter analysis, genomic sequence 1000 bp upstream ATG was used as query in PlantPan2 (<http://plantpan2.itps.ncku.edu.tw/promoter.php>).

Plant Material and Stress Imposition

The seeds of chickpea (genotype K-70005, Kabuli type) were obtained from National Institute of Agriculture and Biotechnology (NIAB), Faisalabad, Pakistan. Plants were grown under controlled conditions: 22/20°C Day/Night temperature, 16/8-h light/dark photoperiod and 65% humidity. At the age of 15 days after germination, plants were subjected to drought, salinity, heat and osmotic stress. For drought stress, plants were subjected to water shortage for a period of 10 days. Similarly, 100 mM NaCl (72 h) and 100 mM mannitol (72 h) were directly applied in pots for salt and osmotic stress respectively. For heat stress, chickpea seedlings were exposed to 42°C for 24 h. After the treatments, leaf samples were immediately frozen in liquid nitrogen and stored at –80°C until use.

Total RNA Extraction, Digital Expression Analysis and Real-Time RT qPCR

The total RNA was extracted from leaf samples using RNeasy Plant mini kit (QIAGEN; Cat No./ID: 74904) following the manufacturer’s instructions. RNA quantification was performed by using NanoDrop spectrophotometer (Colibri spectrometer, Titertek Berthold,

Germany). One microgram of total RNA was reverse transcribed using Maxima H Minus First Strand cDNA Synthesis Kit, with dsDNase (Cat#K1681). Digital Expression data of the *CarATX-like* genes was downloaded from Garg *et al.* (2015). The real-time RT qPCR was performed using a real-time PCR detection system (CFX96 Touch™ Real-Time PCR Detection System) with the iTaq Universal SYBR Green Super Mix. Gene specific primers were designed by using online tool “Oligo Calculator” (<http://mcb.berkeley.edu/labs/krantz/tools/oligocalc.html>) and primer specificity was verified by NCBI Primer-BLAST program (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Following primer sequences were used for *CrATX1-like* (Loc#: LOC101490018, forward primer, 5'-GCTCGAAGTGAGCCATATG -3'; reverse primer, 5'-CACTTG CAGCTGCATCATC -3') expression analysis. The *C. arietinum CarGAPDH* gene (accession number: XM_004515773.2, forward primer, 5'-GAAGCTTGAGAAGGCCGCTA-3'; reverse primer, 5'-TGCCTTTCAACTTGCCCTCA-3') was used as an internal control for normalization of the expression data. Two independent experiments were performed to confirm expression data.

Results

Identification and Phylogenetic Analysis of ATX Like Genes

To identify the *ATX-like* genes in *Cicer arietinum*, class III HKMTs protein sequences of *Arabidopsis thaliana* were used as query. A total of 8 *ATX-like* genes (with a predicted SET domain) were identified in the chickpea genome (Table 1). The chromosome analysis revealed that chickpea *ATX-like* genes are widely distributed in 5 of the 8 chromosomes (Fig. 1). Chromosomes 2, 6 and 8 do not contain *ATX-like* genes. However, *CarATXR3-like2* (XP_004513544.1) gene model has not yet been assigned to a particular chromosome.

To examine the phylogenetics of *ATX-like* genes in chickpea, a phylogenetic tree was constructed from *ATX-like* protein sequences of nine different plant species (Fig. 2). The protein sequences of *Arabidopsis ATX-like* genes were used to identify homologous genes in *Brachypodium distachyon*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Populus trichocarpa*, *Citrus sinensis*, *Brassica rapa* and *Cicer arietinum*. Furthermore, the *ATXR3-like* and *ATXR7-like* genes were also identified. It is because *ATX1-5*, *ATXR3* and *ATXR7* belong to class III histone lysine methyltransferases (HKMTs) superfamily.

According to the phylogenetic tree (Fig. 2), these genes are grouped into four clusters of orthologous genes (COGs). Group I contain *ATX1/ATX2* like gene, while *ATX3/ATX4/ATX5* like genes are present in Group II. Similarly, *ATXR3* and *ATXR7* like genes are located in Group III and Group IV, respectively. Although eight *ATX-like* genes were predicted in chickpea, phylogenetic tree has shown that there is a single *ATX1-like* (*CarATX1-like*), two

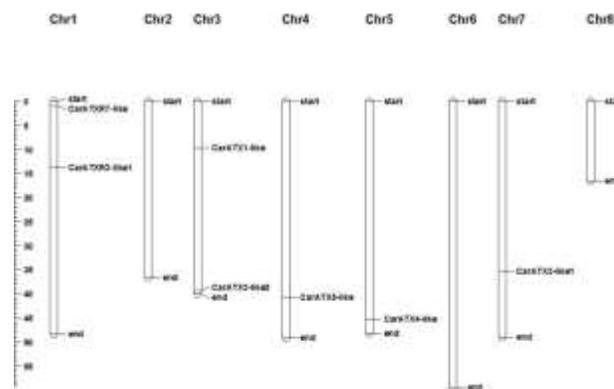


Fig. 1: Chromosomal Map of Class III HKMTs in chickpea

Chromosomal Mapping of Class III HKMTs genes in chickpea was constructed by using the MapChart program and gene positions available in NCBI-GENE database

ATX3-like (*CarATX3-like1* and *CarATX3-like2*), one *ATX4-like* (*CarATX4-like*), one *ATX5-like* (*CarATX5-like*), two *ATXR3-like* (*CarATXR3-like1* and *CarATXR3-like3*) and one *ATXR7-like* (*ATXR7-like*) gene in chickpea. It is worth noting that homologue of *ATX2* is absent in chickpea and monocot plant species under study (Table 1 and Fig. 2).

In each of four groups (Fig. 2), class III HKMTs genes from monocots and dicots are present in distinct subgroups. The *ATX-like* genes from chickpea do not fall in same clade as *Arabidopsis* but the members of *Brassica rapa* are found in the same clade as *Arabidopsis*.

Conserved Domain and Conserved Motifs Analysis

Each protein carries certain conserved domains and motifs, which are required for protein functioning and other processes. The domain architecture of *ATX-like* genes was analyzed for existence of known domains. All members of HKMTs superfamily contain the characteristic SET domain (Table 1). Additionally, Class III HKMTs proteins bear several highly-conserved protein domains including PWWP, FYRC, GYF, TUDOR and PHD domain. Although, a very scanty information is available for biochemical characterization *ATX-like* genes, the presence of various highly-conserved domains within this class of proteins may suggest diverse functions for these proteins (Aquea *et al.*, 2011). In chickpea *ATX-like* proteins, the domain structure is conserved likewise *Arabidopsis* (Table 1). The PWWP, PHD, ePHD and SET domains are commonly present among all members. Additionally, the FYRC and FYRN domains are present in Group I of class III HKMTs (Table 1 and Fig. 2).

The TUDOR domain is the signature domain of *ATX1* protein which is present in *ATX1-like* proteins of *Oryza sativa*, *Brassica rapa* and *Cicer arietinum* only. Group II proteins contain an additional PHD domain. Most of conserved domains are absent in Group III and IV members.

Table1: Details of genomic and proteomic features for ATX homologues in different mono and dicot plant species

Gene	Protein structural domains												
	Chr.#	Exons	Protein Access#	TUDOR	PWWP	FYRC	FYRN	PHD	PHD	ePHD	SET	Post-SET	GYF
<i>Arabidopsis thaliana</i>													
ATX1	2	24	NP_850170.1	215-248	299-389	508-597	448-497		611-557	668-784	898-1017		
ATX2	1	24	NP_172074.6		313-403	524-610	464-513		628-674	685-806	919-1038		
ATX3	3	23	NP_001078326.1		187-290			365-415	548-594	603-714	839-956		
ATX4	4	23	NP_194520.3		205-308			401-451	594-640	649-760	886-1004		
ATX5	5	23	NP_200155.2		221-324			415-466	609-655	664-775	901-1024		
ATXR3	4	21	NP_193253.4								1775-1906		
ATXR7	5	19	NP_001330664.1								1249-1372	1372-1388	
<i>Cicer arietinum</i>													
CarATX1-like	3	26	XP_004492037.1	230-263	315-406	524-593	464-513		623-669	684-798	926-1043		
CarATX3-like1	7	23	XP_004510675.1		220-323			402-452	585-631		876-1013		
CarATX3-like2	3	23	XP_004494815.1		209-312			383-433	566-612		854-972		
CarATX4-like	5	23	XP_004502638.1		245-348			441-491	634-680	689-800	923-1046		
CarATX5-like	4	24	XP_012570669.1		223-326			420-470	613-559	668-779	905-1027		
CarATXR3-like1	1	21	XP_004487363.1								1801-1932		
CarATXR3-like2	NA	20	XP_004513544.1								1865-1996		
CarATXR7-like	1	23	XP_004487923.1								1109-1230	1230-1246	166-216
<i>Brassica rapa</i>													
BrATX1-like	A4	24	XP_009141151.1	179-221	271-359	479-559	419-468		582-628	639-760	874-998		
BrATX2-like	A9	24	XP_009118571.1		259-349	470-556	410-459		574-620	631-752	865-984		
BrATX3-like	A7	23	XP_009104342.1		195-297			368-418	564-610	619-730	857-973		
BrATX4-like	A8	23	XP_018508983.1		153-256			305-356	497-543	552-63	790-912		
BrATX5-like	A10	23	XP_009119888.1		206-309			401-452	596-642	651-762	889-1011		
BrATXR3-like	A5	20	XP_009144717.1								1837-1968		
BrATXR7 like	A9	20	XP_018510432.1										
<i>Citrus sinensis</i>													
CsATX1-like	5	24	XP_006478892.1		334-421	542-613	484-533		650-696	712-828	948-1067		
CsATX3-like	NA	24	XP_006491269.1		232-335			419-469	602-648		894-1016		
CsATX4-like	5	23	XP_006478053.1		259-362			455-505	649-695	704-815	941-1063		
CsATXR3-like	2	20	XP_006469738.1								1886-2017		
CsATXR7-like	NA	15	XP_015381613.1								1156-1279		
<i>Populus trichocarpa</i>													
PtATX1-like1	LGII	25	XP_002301643.2		294-383	486-554	433-477		595-641	652-773	900-1019		
PtATX1-like2	LGXIV	26	XP_002320433.2		300-387	508-585	448-497		615-661	672-793	920-1039		
PtATX3-like1	LGII	23	XP_002302628.2		233-336			434-488	617-663		909-1027		
PtATX3-like2	LGXIV	23	XP_002320864.2		223-323			409-463	592-638		885-1007		
PtATX4-like	LGXV	23	XP_002321418.2		295-398				688-734	743-854	980-1102		
PtATX5-like	LGXII	23	XP_002318412.2		300-404			497-547	690-736	745-856	982-1104		
PtATXR3-like1	LGVII	17	XP_002310475.2								1791-1922		
PtATXR3-like2	LGXVII	17	XP_006372997.1								1917-2048		
PtATXR7-like1	LGV	19	XP_002307834.2								1010-1133	1133-1149	212-267
PtATXR7-like2	LGII	18	XP_002300607.2								1160-1283		246-284
<i>Sorghum bicolor</i>													
SbATX1-like	2		EER96448.2		250-350	400	475			625	850		
SbATX3-like	3	22	XP_002454931		214-316				565-611		852-970		
SbATX4-like	3	23	XP_002456070		243-349			432-485	622-668		910-1028		
SbATXR3-like	7	19	XP_002443954								1602-1743		
SbATXR7-like	8	14	XP_002442769.1								900-1023		
<i>Zea mays</i>													
ZmATX1-like	7	26	XP_008651792.1		267-353	470-532	412-461		627-672	683-803	921-1041		
ZmATX3-like	3	23	XP_008673525.1		186-288			375-424	558-604		845-963		
ZmATX4-like	10	22	XP_008661478.1								1705-1836		
ZmATXR3-like	4	21	XP_008678235.1								1707-1838		
ZmATXR7-like	10	14	XP_008663792								981-1104		
<i>Oryza sativa</i>													
OsATX1-like	9	26	XP_015612383.1	180-219	264-350	467-543	409-458		675-612	623-743	861-984		
OsATX3-like	1	23	XP_015611850.1		190-292			379-428	563-609		849-968		
OsATX4-like	1	23	XP_015621708.1		249-355			439-488	628-874		916-1034		
OsATXR7-like	12	15	BAT18060.1								1037-1155		
<i>Brachypodium distachyon</i>													
BdATX1-like	4	25	XP_010238960.2		272-358	473-549	415-464		573-618	629-749	867-990		
BdATX3-like	2	23	XP_014754682.1		211-313			400-449	585-631		868-990		
BdATX4-like	2	23	XP_003569477.1		229-335			419-468	608-564		896-1014		
BdATXR3-like	3	21	XP_010234335.1								1721-1852		
BdATXR7-like	4	19	XP_010237026.1								1055-1178	1178-1194	

Table 2: Comparison of *cis-elements* in 1Kb promoter regions of *ATX1* and *CarATX1-like* genes

Regulatory Element	Core sequence	<i>ATX1</i>	<i>CarATX1-like</i>	Functions
NAC Core sequence	CACG	2	2	Response to various stress signals
		3	5	
ABRE	ACGTG	1	1	Response to ABA signals
MYB recognition site	WAACCA	2	1	Response to drought stress and ABA signals
MYC recognition site	CANNTG	10	4	Response to drought, ABA and cold signals
W-box	TTGAC	3	2	Response to SA, GA and pathogenesis signals
		6	5	
GT-1 motif	GAAAAA	4	4	Response to pathogen and salt signals
EEC	GANTTNC	4	3	Response to CO ₂ signals
I box	GATAA	4	2	Response to light signals
TAAAG motif	TAAAG	4	1	Response to K ⁺ influx channel of guard cells
CBFHV	RYCGAC	1	1	CBFs are also known as dehydration-responsive element (DRE) binding proteins (DREBs)

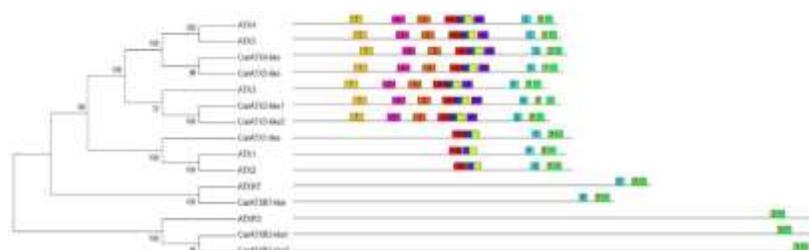


Fig. 3: Comparative Analysis of Conserved Motifs between *Arabidopsis* and Chickpea

A relative comparison of domain positions and conservation in *Arabidopsis* and chickpea was performed by using an online tool MEME. Different colors have been used to represent different motifs. Phylogenetic tree was added for cluster-wise arrangement of genes

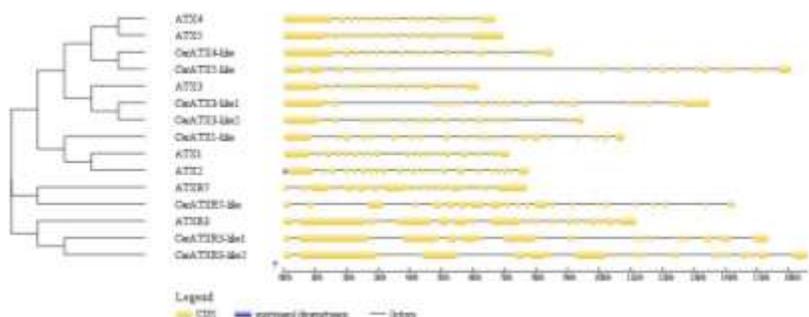


Fig. 4: Comparative Gene Structure Analysis

Comparison of Class III HKMTs gene structure in *Arabidopsis* and chickpea, to determine the conservation of coding and non-coding regions in both species

Discussion

Plant growth and development is severely affected by unfavorable environmental conditions. The major abiotic stresses include drought, osmotic, salinity and heat stress. Plants have adopted a number of sensory and response mechanisms to cope these stresses (Rasul *et al.*, 2017). Over the years, a lot of information have been gathered about genetic mechanisms governing plant responses. Currently, a serious attention has been given to epigenetic mechanisms. It is important for a number of biological processes including transcriptional regulation and formation of heterochromatin (Liu *et al.*, 2010).

Histone methylation is also found to be involved in gametogenesis, embryogenesis, seed development, flowering

time, branching and floral identity (Jarillo *et al.*, 2009; Pontvianne *et al.*, 2010; Chen *et al.*, 2017). Current study involves identification, sequence characterization, phylogenetics and expression analysis of Class III HKMTs in chickpea.

There are seven classes of HKMTs and among them Class III HKMTs contain seven members in model plant *Arabidopsis* (Pontvianne *et al.*, 2010). In current study, 8 genes were predicted as potential members of Class III HKMTs (Table 1) in chickpea. Phylogenetic analysis demonstrated that these genes fall into 4 subgroups, where *Arabidopsis* and *Brassica rapa* fall in the same clade. It is an indication that these genes are highly conserved in family Brassicaceae. It is further strengthened by the existence of *ATX2-like* genes only in *Arabidopsis* and *Brassica rapa*.

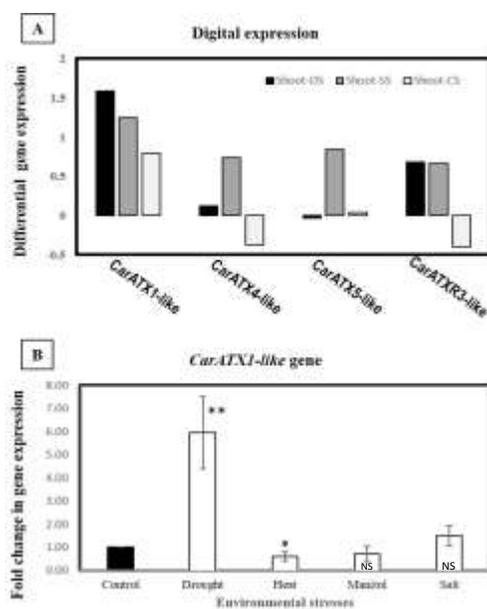


Fig. 5: Expression Analysis of *CarATX-like* Genes in Chickpea Leaves in Response to Abiotic Stresses

Digital expression of Class III HKMTs genes was taken from Garg *et al.* (2015). Shoot-DS, Shoot-SS and Shoot-CS stand for gene expression in aerial plant parts in response to drought stress, salt stress and cold stress, respectively. For drought, salt and cold stress treatments, chickpea seedlings (10 day-old) were kept (for 5 h) on folds of tissue paper, 150 mM NaCl solution and at $4\pm 1^\circ\text{C}$, respectively

Expression of *CarATX-like* gene has been presented for aerial plant parts. Expression was quantified through real-time RT-qPCR in control and stress conditions (Drought, Heat, Mannitol and Salt). Expression has been presented in the form of fold change in expression. Error bars indicate the standard deviation from mean (three replicates, t-test, $P < 0.05$). This experiment was repeated twice to confirm results of expression analysis. For statistical analysis, two sample t-test was performed at 95%

The *ATX2* might have been formed through duplication of *ATX1*. The monocots and dicots appeared in different clusters and among them chickpea appeared as independent clade (Fig. 2). It appears as the Class III HKMTs are following an independent trajectory of evolution in legumes. Therefore, a more detailed analysis is suggested involving HKMTs in chickpea. Gene structure analysis revealed conservation of coding and non-coding regions. But the overall length of non-coding regions was higher in chickpea. The non-coding genic region affects the gene expression (Colinas *et al.*, 2008). The lengthy non-coding genic region could also prevent the re-localization of the gene. Consequently, ensuring the transcribed region attached with the matrix and decreasing the chances of variability of expression. Twelve *cis*-acting regulatory elements (Table 2) were searched in one kb upstream region of *ATX1* transcription initiation codon and its homologue in chickpea. These elements are generally involved in gene regulation in response to stress conditions. Eight of these elements are commonly present in both promoter sequences. The extent of the specificity of gene expression depends on *cis* regulatory elements and their binding and interaction with the transcription factor. It suggests a potentially common

regulatory network for *ATX1* genes and its counterpart in chickpea.

The chromatin modifying elements play with chromatin structure to monitor transcriptional reprogramming during plant development and stress response. The *ATX1* is involved in transcriptional regulation of a number of genes including drought stress related genes. A comprehensive study has identified a group of genes that are simultaneously regulated by *AtMTM* and *ATX1* under drought stress (Ding *et al.*, 2009). We have observed an increase in transcript abundance of *CarATX1-like* gene in response to drought stress. It is tempting to predict that it is involved in similar functions both in chickpea and Arabidopsis. In Arabidopsis, *ATX1* is believed to be involved in discrete dehydration stress response pathways (Ding *et al.*, 2011a) that might be ABA-dependent and/or ABA-independent. The *ATX1* is involved in the regulation of regulatory proteins including transcription factors that participate in multiple signaling cascades (Shafiq *et al.*, 2014; Hou *et al.*, 2016). Another report describes that drought stress increases amount of PtdIns5P (phosphatidylinositol; a cellular lipid signaling molecule) protein, which binds *ATX1*, shift its subcellular localization, represses its activity and subsequently down-regulate *ATX1* target genes (Ding *et al.*, 2011b, a; Hou *et al.*, 2016). There are strong chances that likewise interactions may occur in chickpea and regulate plant development and stress response. A whole genome scanning and comparative analysis would be desirable to detect such counterparts.

Conclusion

It is well established that the alterations in chromatin structure regulate plant response to abiotic stresses and it has offered novel dimensions in plant research. However, the entire correlation network between abiotic stress responses and epigenetic information, is given poor attention in marginal plants like chickpea. The present study concluded that the computational analysis of *ATX-like* genes in Arabidopsis and chickpea provide the fundamental information regarding phylogeny, chromosomal mapping, gene structure, conserved motifs, and promoter analysis. The expression analysis has given important indication of possible functional properties of *ATX1-like* gene in chickpea. Reliable and higher resolution chromatin studies are undoubtedly required to reveal how HKMTs are involved in gene regulation specially under environmental stresses like drought and heat stress. The Class III HKMTs are highly conserved between model plant Arabidopsis and chickpea. Therefore, chickpea could be used as model for other legume plants for the study of HKMTs.

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