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DROUGHT AFFECTS AQUAPORINS GENE EXPRESSION IN IMPORTANT PULSE LEGUME CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

Aquaporins (AQPs) mediate cross membrane transport of water and other solutes in plants. AQPs also enhance plant performance under environmental stresses including water shortage. Chickpea (*Cicer arietinum* L.) is the most important pulse legume for the people of semi-arid tropics. To understand the evolutionary relationships and role of AQPs in drought tolerance in chickpea, the current study involves an evolutionary analysis coupled with expression analysis and promoter analysis of selected AQPs. A total of 503 non-redundant AQP genes have been studied in 11 plant species including mosses (*Physcomitrella patens)*, monocots (*Oryza sativa* and *Zea mays*) and dicots (*Solanum lycopersicum, Populus trichocarpa, Gossypium hirsutum, Glycine max, Arabidopsis thaliana, Brassica rapa, Solanum tubrosum* and *Cicer arietinum*). Phylogenetic analysis demonstrated a clear divergence of AQP subfamilies as paralogous groups and possible evolutionary direction of AQP subfamilies. Semi-quantitative RT-PCR analysis depicted involvement of PIP2; 2 and NIP6; 3 in increasing plant drought stress tolerance and of SIP1; 1 and PIP2; 3 with a contrary role. *In silico* promoter analysis identified a 49 bp conserved motif among six AQPs and several abiotic stresses related *cis*-elements. The present study is a very first step in deciphering AQPs role in drought stress tolerance with a special perspective from evolutionary relationships and gene expression regulation.

Key words: Chickpea, AQPs, Drought stress, Expression analysis, Promoter analysis.

Introduction

Drought stress along with other environmental constraints limit worldwide plant productivity and yields. An optimum use of water is vital for plant performance especially under stressed environments (Mueller *et al.,* 2012). Cellular membranes-mediated water movement is essential not only for long distance transport of water but also for cell expansion and osmoregulation (Steudle, 1994). Aquaporins (AQPs) are membrane-bound pore-forming proteins that enhance the water permeability of vacuolar and plasma membranes (Maurel, 1997). In plants, AQPs mediate opening and closing of cellular gates (Zhao *et al.,* 2008), which play important roles in water use efficiency and water balance of plants (Moshelion *et al.,* 2015). Moreover, AQPs transport ammonia, glycerol and uncharged small ions, contribute in leaf water homeostasis, promote the expansion of tissue and improve plant abiotic stress tolerance (Ludevid *et al.,* 1992; Javot *et al.,* 2003; Heinen *et al.,* 2009; Péret *et al.,* 2012; Maurel *et al.,* 2015)

AQPs have been identified in wide a range of organisms from *E. coli* to humans (Denker *et al.,* 1988; Fu *et al.,* 2000). Recently, AQPs have been well characterized in many plant species and there exists a significant variation in numbers of AQP members in different organisms e.g. 13 in humans, 31 in *Zea mays,* 33 in *Oryza sativa,* 38 in *Arabidopsis thaliana*, 35 in *Physcomitrella patens,* 47 in *Solanum lycopersicum*, 54 in *Populus trichocarpa*, 66 in *Glycine max* and 71 in *Gossypium hirsutum* (Chaumont *et al.,* 2001; Quigley *et al.,* 2002; Sakurai *et al.,* 2005; Gonen & Walz, 2006; Danielson & Johanson, 2008; Heinen *et al.,* 2009; Park *et al.,* 2010; Reuscher *et al.,* 2013; Zhang *et al.,* 2013).

On the basis of subcellular localization and sequence similarity, there are five major groups of plant's AQPs i.e., NIPs (NOD26-like intrinsic proteins), TIPs (tonoplast intrinsic proteins), PIPs (plasma membrane intrinsic proteins), XIPs (uncharacterized intrinsic proteins) and SIPs (small basic intrinsic proteins) (Quigley *et al.,* 2002; Deshmukh & Bélanger, 2016; Deshmukh *et al.,* 2016). Moreover, lower plants also have two more groups as GIPs (GlpF-like intrinsic proteins) and HIPs (hybrid intrinsic proteins) (Danielson & Johanson, 2008; Deshmukh *et al.,* 2016).

Chickpea (*C. arietinum* L.) is the second most widely grown food legume crop worldwide. The seeds of chickpea are a primary energy source for resource poor farmers. These seeds contain higher amounts of proteins, carbohydrates, lipids, and vitamins (Jukanti *et al.,* 2012). The availability of complete genome sequence of Chickpea (Varshney *et al.,* 2013; Deokar *et al.,* 2015) represents the opportunity for comparative evolutionary studies and characterization of *AQP* gene family (Deokar & Tar'an, 2016). Current study is a very first step for understanding the molecular responses of chickpea AQPs to drought stress. Chickpea is generally grown in water-limited and drought prone environments and hence faces the terminal drought stress resulting significant yield and production losses. In this context, understanding the role of AQP family in chickpea especially under limited water stress conditions would be helpful in devising breeding strategies for developing drought tolerant cultivars.

Materials and Methods

Sequence retrieval of AQPs protein sequences and Phylogenetic analysis: The whole genome nucleic acids and

protein sequences of chickpea are available at NCBI (Varshney *et al.,* 2013). Protein sequences of aquaporins from model plant *Arabidopsis thaliana* (AtAQPs) were used as a query to search AQP gene family in selected plants (Table 1) using NCBI-BLAST (Quigley *et al.,* 2002). To enhance search efficiency, we used Position Specific Iteration BLAST (PSI-BLAST) (Altschul *et al.,* 1997).

For phylogenetic analysis, Molecular Evolutionary Genetics Analysis tool (MEGA v.7) (Kumar *et al.,* 2016) was used. For this purpose, first a multiple sequence alignment was performed and these aligned sequences were used for phylogenetic tree construction by Maximum Likelihood method.

Plant material and growth conditions: Seeds of drought tolerant and susceptible Kabuli cultivars (Susceptible: Punjab-1; Tolerant: K-70005) were obtained from National Institute of Agriculture and Biotechnology (NIAB), Pakistan. Chickpea plants were grown in a growth chamber at 23°C day /21°C night, with a 16 hour light, 8 hour dark photoperiod and approximately 65% humidity. The seedlings were allowed to grow for 30 days in pots. The pots were divided into two groups i.e. plants supplied with ample water and plants subjected to water stress by withholding water for ten days. Each pot contained three plants and at least three replications were performed for each treatment.

RNA isolation and semi-quantitative RT-PCR: The leaf samples from the stressed and control plants from both susceptible and tolerant genotypes were collected for RNA isolation. Trizol reagent was used to extract total RNA from leaves (*Invitrogen*, USA). For cDNA synthesis, 1µg of total RNA was used. Moreover, gene specific primers were used for cDNA synthesis by using Maxima H Minus First Strand cDNA Synthesis Kit, with dsDNase (cat # K1681). The gene specific primers were manually designed (Table 2) 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/).

PCR reactions were performed using The MultiGene OptiMax 96 well Thermal Cycler (Labnet, USA). The amplification products were electrophoresed on 2% agarose gel at 70 V in TAE buffer using 100 bp plus DNA ladder (*Thermo Fisher Scientific*, USA). Gels were stained with ethidium bromide and visualized on *Syngene* gel documentation system (Syngene, England). The reactions were performed in biological triplicates. CaGAPDH was used as internal control gene (Garg *et al.*, 2010).

Promoter Analysis: The promoters of the six AQP genes (*CaPIP1;2, CaPIP2;1, CaPIP2;2, CaPIP2;3, CaNIP6;3, CaNIP7;1, CaTIP2;2, CaTIP1;1 and CaSIP1;1*) (1000 bp upstream from the translation start site) were obtained from NCBI-Gene database (https://www.ncbi.nlm.nih.gov/gene). An online program PlantPan2 multiple promoter analysis tool (http://plantpan2.itps.ncku.edu.tw/promoter_multiple.php) was used to analyze the nature, location and number of *cis*-elements (Higo *et al.*, 1999). Selected *cis-elements* were used as query. For the identification of conserved promoter region, Gapped local alignment of motifs (GLAM2) tool was used from the MEME suite (http://meme-suite.org/tools/glam2).

Table 1. Details of aquaporin genes in different plant species.

Plant Name	Plant type	GIP	HIP	XIP	SIP	PIP	TIP	NIP	Total AQPs	Groups of AQPs	References
Physcomitrella patens	Moss	1 (4.3)	1 (4.3)	2 (8.7)	2 (8.7)	8 (34.8)	4 (17.4)	5 (21.7)	23	7	(Danielson & Johanson, 2008)
Selaginella moellendorffii	Lycophyte	-	2 (10.5)	3 (15.8)	1 (5.3)	3 (15.8)	2 (10.5)	8 (42.1)	19	6	(Anderberg *et al.*, 2012)
Solanum lycopersicum	Dichot	-	-	6 (12.7)	4 (8.5)	14 (29.8.6)	11 (23.4)	12 (25.5)	47	5	(Reuscher *et al.*, 2013)
Populus trichocarpa	Dichot	-	-	6 (10.9)	6 (10.9)	15 (27.3)	17 (30.9)	11 (20.0)	55	5	(Gupta & Sankararamakrishnan, 2009)
Gossypium hirsutum	Dichot	-	-	1 (1.4)	7 (9.9)	28 (39.4)	23 (32.4)	12 (16.9)	71	5	(Park *et al.*, 2010)
Phaseolus vulgaris	Dichot	-	-	2 (4.88)	4 (9.76)	12 (29.27)	13 (31.71)	10 (24.39)	41	5	(Ariani & Gepts, 2015)
Glycine max	Dichot	-	-	2 (3.03)	6 (9.09)	22 (33.33)	23 (34.85)	13 (19.70)	66	5	(Zhang *et al.*, 2013)
Solanum tubrosum	Dichot	-	-	8 (17.02)	3 (6.38)	15 (31.91)	11 (23.40)	10 (21.38)	41	5	(Venkatesh *et al.*, 2013)
Arabidopsis thaliana	Dichot	-	-	-	3 (8.6)	13 (37.1)	10 (28.6)	9 (25.7)	35	4	(Johanson *et al.*, 2001)
Cicer arietinum	Dichot	-	-	-	3 (7.5)	9 (22.5)	12 (30.00)	16 (40.00)	39	4	(Deokar and Tar'an, 2016)
Brassica rapa	Dichot	-	-	-	6 (11.32)	20 (37.74)	14 (26.42)	13 (24.53)	53	4	(Tao *et al.*, 2014)
Brassica oleracea	Dichot	-	-	-	6 (8.96)	25 (37.31)	19 (28.36)	12 (25.37)	67	4	(Bienert *et al.*, 2015)
Zea mays	Monocot	-	-	-	3 (9.68)	13 (41.94)	11 (35.48)	4 (12.90)	31	4	(Chaumont *et al.*, 2001)
Oryza sativa	Monocot	-	-	-	2 (6.1)	11 (33.3)	10 (30.3)	10 (30.3)	33	4	(Sakurai *et al.*, 2005)

Table 2. List of PCR primers used for RT-PCR.

NCBI locus ID	Gene name	Oligo sequence	Tm °C	Amplicon size
LOC101515041	CaNIP6;3F	GGGAGAACTTGCGGGAATTG	59	119
	CaNIP6;3R	TTGTAGCAATTGTTGGGCCC	59	
LOC101510786	CaNIP7;1F	GCCCCAAGTTCAGGAGCTAT	59	116
	CaNIP7;1R	GCACAAAAGGGTATGGGGTG	59	
LOC101495619	CaPIP1;2F	AATCCAGCTCGTAGTCTCGG	59	119
	CaPIP1;2R	TGGTGGTATAGAGCTGCCAG	59	
LOC101513621	CaPIP2;1F	CTGTTTTGGCACCACTACCC	59	103
	CaPIP2;1R	GATCCGAAACTTCTTGCCGG	59	
LOC101513309	CaPIP2;2F	TAGTTGCCGGTCCATTCAGT	59	104
	CaPIP2;2R	GCCAACCCAGTAGATCCAGT	59	
LOC101488859	CaPIP2;3F	CCAGCAGTGACATTTGGGTT	59	103
	CaPIP2;3R	ACCCAACTCCACAAATTGCC	59	
LOC101498244	CaSIP1;1F	GCCATGTCAACCGTCACTTT	59	109
	CaSIP1;1R	ATGTGTTGTGCCGGTTGTTT	59	
LOC101508956	CaTIP1;1F	TCAACTCCTCGGATCCATCG	59	120
	CaTIP1;1R	ACGATCTCCAACACCAAAGC	59	
LOC101505621	CaTIP2;2F	TAGTTGCCGGTCCATTCAGT	59	104
	CaTIP2;2R	GCCAACCCAGTAGATCCAGT	59	

Results and Discussion

Evolutionary analysis: A number of studies have focused on overall evolution of AQPs among different organism (Abascal *et al.,* 2014; Deshmukh & Bélanger, 2016), but literature data lacks details of sub-family divergence among plants. An evolutionary analysis was performed to demonstrate that the AQP gene subfamilies are highly conserved from lower to higher plants. Therefore, a functional conservation could be a strong expectation to be made. A phylogeny-based comparison of number of subfamily members demonstrated that PIP subfamily harbors the largest number of aquaporins genes as compared to others families (Fig. 1, Table 1). Interestingly, the highest number of NIP AQPs were found only in *Selaginella moellendorffii* and *C. arietinum* (Table 1). It could explain a possible functional importance of NIPs for these two plants.

Bacteria and archaea generally possess a single AQP copy, and unicellular eukaryotes and fungi show heterogeneous number of genes, whereas the diversification of AQPs is most outstanding in plants and animals (Abascal *et al.,* 2014). To understand an evolutionary relationship among subgroups of AQPs, we have used sequences mostly from higher plants along with a moss plant *(Physcomitrella patens).* For phylogenetic analysis, 503 aquaporin protein sequences were used from different plants belonging to mosses (*Physcomitrella patens)*, monocots (*Oryza sativa* and *Zea mays*) and dicots (*Solanum lycopersicum, Populus trichocarpa, Gossypium hirsutum, Glycine max, Arabidopsis thaliana, Brassica rapa, Solanum tubrosum* and *Cicer arietinum*). The phylogenetic tree shows a clear divergence of AQPs. It indicates that XIPs and SIPs are originated from same the ancestor and later-on other groups are diverged as NIPs, TIPs and PIPs respectively. Moreover, there are 5 paralog groups in seed plants (Fig. 1, Table 1). It also seems like dicots have more diversity of AQPs as compared to monocots. It is obvious that the paralogous genes of GIPs and HIPs have been emerged in higher plants and based upon sequence homology, they are more similar to SIPs and NIPs. If the subcellular localization of these AQPs is known, a redundant function with the NIPs would explain why GIPs and HIPs are lost in higher plants and would fit in time with the expansion of the NIP subfamily. More importantly, AQP subfamily members of mono and dicots fall in same paralogous groups (Fig. 1). Therefore, it is tempting to speculate that the expansions of AQPs in both the mono and dicots seems to fulfill some common intrinsic requirements of higher plants.

Expression analysis: Drought stress is considered as one of the most damaging factors affecting crop yields by decreasing photosynthesis and lowering stomatal conductance. As a result, the CO_2 fixation is reduced, which limits the production of assimilates for normal plant growth and yields. Certainly, stomatal movements are dependent on water transport. AQPs have been reported to play important role in water transport in different plants (Chaumont *et al.,* 2001; Johanson *et al.,* 2001; Maurel *et al.,* 2015).

Therefore, in an attempt to understand the role of aquaporins in drought stress tolerance in chickpea, the expression pattern of selected chickpea aquaporins genes (Table 2) were analyzed by semi-quantitative RT-PCR. For this purpose, we selected six *CarAQP* genes on the basis of their highest RPKM (Reads Per Kilobase per Million mapped reads; a method of quantifying gene expression from RNA sequencing data) value from Chickpea Transcriptome Database (Verma *et al.,* 2015). Expression analyses of *CaPIP1;2, CaPIP2;1, CaPIP2;2, CaPIP2;3, CaNIP6;3, CaNIP7;1, CaTIP2;2, CaTIP1;1 and CaSIP1;1* genes were performed using semi-quantitative RT-qPCR from two chickpea genotypes (drought tolerant and susceptible) subjected to drought stress. The expression levels of the chickpea aquaporins under drought stress were compared with the expression levels of the control samples.

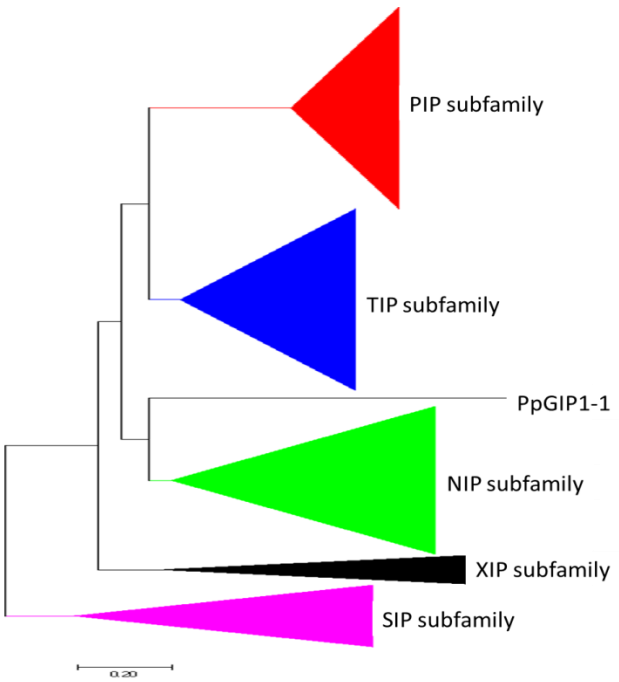

Fig. 1. Molecular phylogenetic analysis by Maximum Likelihood method. The phylogenetic analysis was performed by using by using the Maximum Likelihood method. Tree topology is based on model of the JTT matrix. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. A total of 503 protein sequences were used in this analysis by using MEGA7 (Kumar *et al.,* 2016).

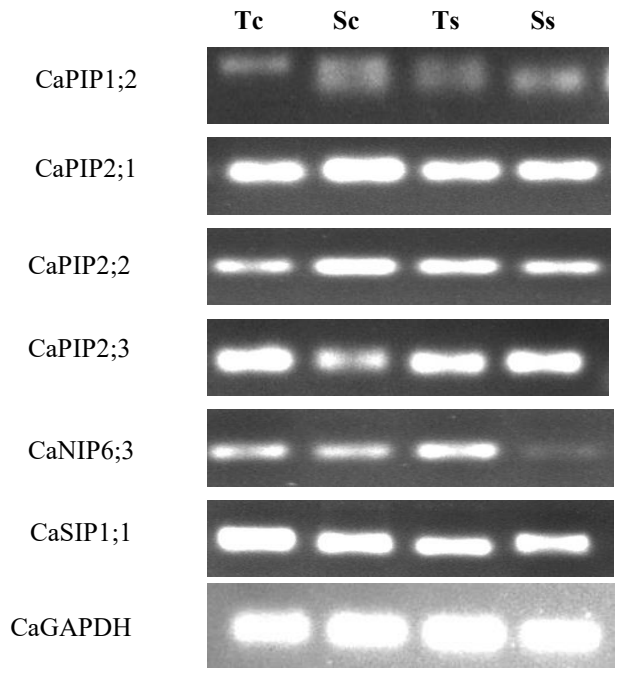

Fig. 2. Semi-quantitative RT-PCR analysis for expression analysis of selected *AQP in Leaves*. Tc, Sc, Ts and Ss represents drought tolerant cultivar under control conditions, drought susceptible cultivar under control conditions, drought tolerant cultivar under drought conditions and drought susceptible cultivar under drought conditions respectively. CaGAPDH is a house keeping gene.

NAME	START	SITES	END	STRAND	MARGINAL SCORE
LOC101515041	756	tataataaaaatgatgtttt.tataataaaaactattggataaaaactt	709	-	28.6
LOC101495619	327	tatatttatttttagaattaaaataatattaatttgatgaaaaaaaata	375	+	32.7
LOC101513621	861	aaaaattataatgataaattatgtgacataatttataataataaaaatt	813	-	40.6
LOC101513309	752	taaaataataataataataataataataataataataataataataata	800	+	44.3
LOC101488859	950	tataataataagaatgaaaagtg.aaaatatatattaacaaaaaaaatt	997	+	33.8
LOC101498244	255	taatttatttagaatattttgtatgaaattatttttta.ataaataatt	208	-	30.0

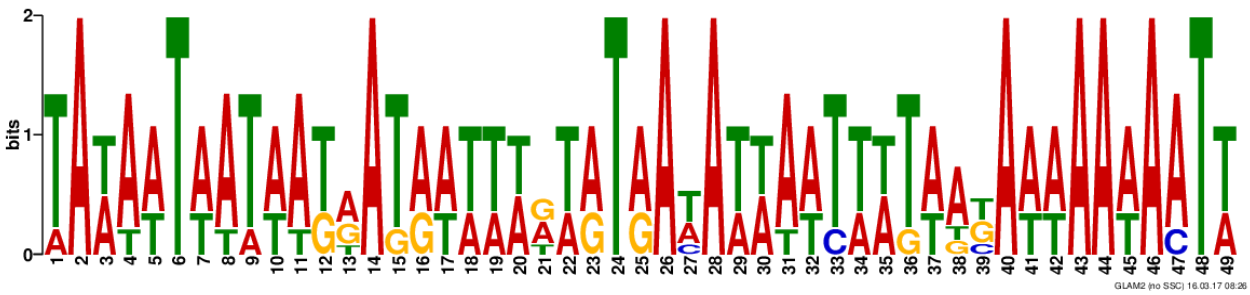

Fig. 3. Conserved motif analysis of selected AQP promoter sequences. A) Local alignment of promoter sequence showing a conserved motif of 49 bp. B) Sequence logo of conserved motif.

Under control conditions, the expression pattern of chickpea aquaporins was found to vary between drought tolerant and susceptible genotypes (Fig. 2). Although the expression levels of *PIP1;2* and *NIP6;3* were almost stable in both genotypes, the expression level of *PIP2;1* and *PIP2;2* was higher in susceptible genotype and the expression of *PIP2;3* and *SIP1;1* was higher in drought tolerant genotype. Under stress conditions in drought tolerant genotype, the expression of *PIP1;2, PIP2;1* and *PIP2;3* remained almost stable. The expression of *PIP2;2* and *NIP6;3* were increased while *SIP1;1* expression was decreased. In drought-susceptible genotype under stress conditions, the expression of *PIP1;2* and *SIP1;1* were almost stable. The expression of PIP2;3 was increased while that of PIP2;1, PIP2;2 and NIP6;3 were decreased. Overall, the expression of PIP1;2 remained almost constant in in both genotypes under control/drought conditions. While the expression of PIP2;2 and NIP6;3 (expression increased in tolerant genotype and decreased in susceptible genotype) seemed to be positively related to plant drought stress tolerance. Contrarily, SIP1;1 (expression decreased in tolerant genotype) and PIP2;3 (expression increased in susceptible genotype) seemed to be negatively related to drought stress.

Table 3. Details of *cis-regulatory* elements in promoter regions of selected AQP genes.

Element	Core sequence	CaPIP1;2	CaPIP2;3	CaSIP1;1	CaPIP2;2	CaPIP2;1	CaNIP6;3	Functions
NAC Core sequence	CACG	1	1	1		1		Response to various stress signal
	CGTR	8	2	4	4		4	
ABRE	ACGTG	2						Response to ABA signal
MYB recognition site	WAACCA	2	5			2	3	Response to drought stress and ABA signal
	YAACKG				4			
MYC recognition site	CANNTG	16	8	12	8	8	8	Response to drought, ABA and cold signal
	TTGAC	3	2	2		2	3	Response to SA, GA and pathogenesis signal
W-box	TGAC	8	8	12	12	14	12	
GT-1 motif	GAAAAA	2	4	9	2	2	10	Response to pathogen and salt signal
EEC	GANTTNC	6	2		2	6		Response to CO2 signal
I box	GATAA	16	8	6	6	2	6	Response to light signal
G-box	CACGTG	2						Response to ABA and water deficit
GARE	TAACAAR		4			6	4	Response to GA signal
TAAAG motif	TAAAG	6	6	4	4	10	2	Response to K^+ influx channel of guard cells
Core motif of DRE/CRT	RCCGAC		1					dehydration-responsive element/C-repeat
CBFHV	RYCGAC		2		2			CBFs are also known as dehydration-responsive element (DRE) binding proteins (DREBs)

PIPs comprise the largest category of AQPs, which mediate water transport by the symplastic and trans-cellular paths. Such transport of water is performed across plasma membranes of cell under normal and drought stressed conditions (Li *et al.,* 2014). Based on sequence homology, there are two subfamilies of PIPs as PIP1 and PIP2. It is reported that PIP2 proteins (compared to PIP1 isoforms) generally perform higher water channel activities in *Xenopus oocytes* (Mahdieh *et al.,* 2008). Likewise, in current study PIP1;2 had almost stable expression while type 2 PIPs showed variable expression. It was tempting to speculate that these PIPs might play similar role in chickpea.

Analysis of putative *cis*-regulatory elements in *AQP* promoters: Based upon expression analysis of AQP transcript abundance, it was anticipated that these genes were regulated at transcriptional level. Abiotic stresses like drought, salinity, high temperature, and low temperature affect plant growth. In plants, a number of genes are up regulated in response to abiotic stresses and the products of these genes encourage plant survival in adverse environmental conditions (Ali *et al.,* 2017; Rasul *et al.,* 2017). These stress inducible genes could promote stress tolerance directly or by controlling other genes (Yasmeen *et al.,* 2016). Transcription factors (TFs) belong to a class of genes that modulate the expression of their target genes through binding with *cis*-regulatory elements present in the promoter region (Nakashima *et al.,* 2009). A number of online databases of genome annotation and cis-element prediction are on our disposal (Priest *et al.,* 2009) to predict the kind of TFs involved in regulation of respective gene. Promoter analysis using these resources shows us the presence of number of cis-regulatory elements modulating gene expression against different stimuli. For *in silico* promoter analysis of selected AQP genes, 1000 bp sequence upstream transcriptional start site was examined using publicly available online tool Plant Pan2 (Chow *et al.,* 2016).

A number of abiotic stress related *cis*-regulatory elements (CREs) were identified in promoter sequences under study (Table 3), which included NAC TF binding sites, MYC recognition site, W-Box and GT1-Box. Moreover, EEC element and TAAAG motif were also present in majority of promoter sequences. These elements are putatively involved in CO_2 based K^+ mediated stomatal movements. In addition, well known drought stress related *cis* elements like ABRE, CBFHV and Core motif of DRE/CRT were also identified in few of these genes (Table 3).

As the pattern of drought regulation of PIPs is generally conserved (Alexandersson *et al.,* 2010) and a common regulatory motif could govern pathway regulation for different genes (Pastori & Foyer, 2002; Zhu *et al.,* 2015). Promoter analysis was performed to identify common regions of promoter sequences. For this purpose, MEME suit GLAM2 was used. As a result, 49 bp long motif was identified which was common among all the AQP promoter sequences under study (Fig. 3). A number of studies have demonstrated the role of plant aquaporins in drought stress tolerance *(Sade et*

al., 2010; Zhou *et al.,* 2012; Pou *et al.,* 2013) but very few studies have focused gene promoter analysis (Ayadi *et al.,* 2014; Chen *et al.,* 2015). Occurrence of several important CREs and common conserved motifs (Wang *et al.,* 2011; Fig. 3), highlight the need to investigate the functional significance of gene promoters through experimental approaches. Since regulatory mutations contributed markedly to plant domestication (Doebley *et al.,* 2006; Swinnen *et al.,* 2016). The combination of in-depth understanding of gene regulatory networks and genome editing to find and alter CREs at the single nucleotide level in plant genomes may provide a promising engineering strategy for future crop improvement (Swinnen *et al.,* 2016).

Conclusion

With the advent of high throughput genome sequencing techniques and extensive transcript abundance studies, the information hidden in plant genomes has been explored to elucidate the mechanisms regulating plant response to environmental factors. Such studies may also facilitate genome and gene evolution studies. In current study, it was attempted to highlight putative role of AQP genes in drought tolerant and susceptible chickpea cultivars. Modulation of the transcript level of CarAQPs in response to drought stress indicates their important role in stress tolerance. Moreover, identification of a conserved region indicates the existence of a possible common regulatory network for these genes.

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