Indian Res. J. Genet. & Biotech. 9(3) August: 342 – 360 (2017)

1Identification Of Drought-Inducible Promoter Elements In Chickpea *(cicer arietinum* L.)

Prasanth Tej Kumar Jagannadham, Viswanathan Satheesh, Parameswaran Chidambaranathan, Deshika Kohli, Pradeep Kumar Jain, Ramamurthy Srinivasan

National Research Center on Plant Biotechnology Pusa Campus, New Delhi -110012

(Received : June, 2017 : Revised : July, 2017; Accepted : July, 2017)

Abstract

Drought is one of the most common environmental stresses that affect the growth and development of plants. Thus, developing drought-resistance crop-plants with enhanced productivity and improved water-use efficiency is the most promising solution for alleviating future threats to food security. The aim of this study was to identify the promoter elements which were drought responsive as well as up-regulated under drought conditions. In this we have taken SSH libraries data of drought analysis. The ESTs taken downloaded from NCBI were processed through CAP3 assembly leading to the consensus sequences. The duplicates from the consensus were removed using CD-HIT EST. The consensus sequences were used as the query and total proteins of the chickpea genome sequenced by ICRISAT was used as database. The unique sequences were used for the isolation of the promoter sequences. The promoter analysis was done using the genomatix software suite. In genomatix software suite Model Inspector was used to Search for promoter modules. We based our conclusion on the percentage of actual motifs passing through various filtering steps in comparison to random controls and found no significant percentage differences. Furthermore, even for motifs without general orientational preference across all its instances, individual genes and their regulation via promoter elements may very well depend on the correct orientation of such a motif as it may be possible that gene-specific additional factors impose constraints on the orientation of a motif in a particular genomic context that are not evident when probing for genome-wide preferences.

Key words: Drought, Promoter, Motif, Transcription factor Chickpea

Corresponding author's e-mail : shrinivasan53@gmail.com
Published by Indian Society of Genetics, Biotechnology Research and Development,
5, E Biotech Bhawan, Nikhil Estate, Mugalia Road, Shastripuram, Sikandra, Agra 282007
Online management by www.isgbrd.co.in

Introduction

Drought is one of the most common environmental stresses that affect the growth and development of plants (Bray *et al.,* 1997). The global scarcity of water resources has already become a severe environmental problem worldwide. Poor water management, increased competition for limited water resources, and the uncertain threats associated with global warming all highlight the looming water crisis that threatens agricultural productivity worldwide. It has become urgent to elucidate the responses and adaptation of crops to water stress, and improve the drought tolerance of crops (Zhang *et al.,* 1997). Identification of new genes and metabolic pathways involved in plant adaptation to progressive drought stress at the reproductive stage is of great interest for agricultural research (Bray *et al.,* 1997).

Moreover, agro-ecological conditions expected to deteriorate, due to foreseen global climatic changes, towards reduced availability and increased variability of water resources. The ever-increasing human population that is expected to exceed 9 billion people by 2050 (http://www.fao.org/wsfs/world-summit/en) together with the loss of agricultural land, poses serious challenges to agricultural plant research. Thus, developing drought-resistance crop-plants with enhanced productivity and improved water-use efficiency is the most promising solution for alleviating future threats to food security (Peleg *et al.,* 2011, Claeys *et al.,* 2013, Nakashima *et al.,* 2014a).Plants have evolved various adaptive mechanisms to cope with drought stress at multiple levels such as molecular, cellular, tissue, anatomical, morphological and whole-plant physiological level (Abebe *et al.,* 2010, Van *et al.,* 2010, Peleg *et al.,* 2011). Transcriptional profiling analyses, in various species, have been widely used to identify drought-related genes (Ding *et al.,* 2013, Bray *et al.,* 1997).These experiments resulted in condition- and/or genotype-specific genes with little overlaps across studies (Van *et al.,* 2010, Peleg *et al.,* 2011, Ding *et al.,* 2013).

Recently many efforts have been focused on the molecular response of plants to water deficit stress using various model plants (Deyholos *et al.,* 2010, Ingram *et al.,* 1996).Many genes respond to drought at the transcriptional level, and their products are thought to function in drought tolerance and response (Shinozaki *et al.,* 1997, Bohnert *et al.,* 1995, Bray *et al.,* 1997).

Some stress-inducible genes have been used to improve the stress tolerance of plants by gene transfer. Although hundreds of genes have been found to be involved in abiotic stress responses and a number of them have been well characterized the functions of the majority of the genes remain unknown and there are probably more genes yet to be discovered (Shinozaki *et al.,* 1999, Umezawa *et al.,* 2006, Shinozaki *et al.,* 2000).

Plant response to drought stress is a complex course, and several mechanisms known as drought escape, drought avoidance and drought tolerance are involved in adapting the environment of water deficit (Zhang *et al.,* 1997). A great number of dynamic responses to water deficit at physiological, biochemical, and molecular levels are presented in plant, thus enabling them to survive under drought environmental conditions (Franca *et al.,* 2012, Yamaguchi-Shinozaki *et al.,* 2006). Recently, expanding transcriptome data sets have uncovered a global picture of stress responsive genes in Arabidopsis(Urano *et al.,* 2009)' rice (Rabban *et al.,* 2003), maize (Zheng *et al.,* 2010) wheat (Aprile *et al.,* 2009)and other plants. These transcriptome data revealed that drought stress induced genes not only function to protect cells from drought stress through the production of important enzymes and metabolic proteins (functional proteins), but they also regulate signal transduction and gene expression in the stress response (regulatory proteins). The functional proteins include late embryogenesis abundant (LEA) proteins, a variety of transporters, enzymes involved in osmoprotectant synthesis, fatty acid metabolism, cellular metabolism, carbohydrate metabolism and secondary metabolism. Regulatory proteins that are activated in response to water stress, including transcription factors (TFs) such as DREBs (dehydration-responsive element-binding proteins), AREBs (ABA-responsive element-binding proteins) and NAC proteins, have been identified in plant (Yamaguchi-Shinozaki *et al.,* 2006, Nakashima *et al.,* 2009, Fujita *et al.,* 2011). Besides, many genes involved in growth and development, such as chloroplast, cell wall and plasma membrane proteins encoded gene, were down-regulated in response to drought stress (Fujita *et al.,* 2011).The main aim of our study was to identify the promoter elements which are drought responsive as well as up regulated under drought conditions

Materials And Methods

Identification of drought-inducible candidate genes

In this we have taken SSH libraries data of Deokar (Deokar *et al.,* 2011)drought analysis. The sequences were collected from the NCBI using the key words “drought stress and (library name)”as shown in Table 1.The ESTs taken downloaded from NCBI were processed through CAP3 assembly leading to the consensus sequences (http://biosrv.cab.unina.it/webcap3/). The duplicates from the consensus were removed using CD-HIT EST (http://weizhong-lab.ucsd.edu/cdhit_suite/cgi-bin/index.cgi).The consensus sequences were used as the query and total proteins of the chickpea genome sequenced by ICRISAT was used as database. Among the blast hits first three hits were taken up for further analysis. Among these hits after removing the duplicates, the unique sequences were used for the isolation of the promoter sequences. The Promoter sequences were taken up to 2000bp from the transcription start site (TSS) using python script in linux operating software.

Promoter Analysis

Genomatix software Suite (http://www.genomatix.de/?s=f20e11dd1e6f63a71b7591e2c41f991f) was used to analyze the selected promoters. In Genomatix Software suite, MatInspector tool was used to search putative transcription factor binding sites among the promoters drought inducible gene. Quality thresholds are the number of binding sequences (at least 4) and the number of matrix matches expected in a random sequence of 1000 bp (<5). Plants Matrix library was used to identify the transcription factor binding sites.

Motif Identification

An automatic motif finding and score matrix generating program, MEME (http://www.sdsc.edu/MEME/meme/website/meme.html) (Bailey & Elkan, 1994), was used to identify the motif elements common to the drought-inducible promoters. MEME analysis in linux environment was used (http://meme-suite.org/doc/meme.html?man_type=command). Two Types of motifs were analyzed i.e i) Revcomp and ii) Pal.

Results

Drought-inducible candidate genes

In our study we have considered only the SSH libraries of Deokar (Deokar *et al.,* 2011), for two reasons. The SSH libraries developed were taken from the samples those were experienced the drought under the field conditions. The samples also include RIL population developed from the cross between ICC 4958 and ICC1882. The data was compared with the present data available with NCBI database, since the data gets gleaned over the years. So, before for any analysis we have thoroughly checked the available ESTs. Once the ESTs were finalized we used the same set as a query against the chickpea genome database. Even though, chickpea genome was sequenced by two independent groups (Varshney *et al.,* 2013**,** Parween *et al.,* 2015), we have used only one database i.e ICRISAT chickpea Genome available at the NCBI. With Blasting with the genome we are able to identify the genes which were upregulating and downregulating with respect to drought. In this analysis we have identified a total of 3141 upregulating genes and 2741 downregulating genes. Further these genes were screened for the presence of the 2000bp upstream promoter

Promoter ananlysis

In genomatix software suite Model Inspector was used to Search for promoter modules. A total of 8484 (promoter modules) matches were found in 2096 sequences. Sequences searched: 2166 (4334166 bp upregulated genes).A total of 10178 (promoter modules) matches were found in 2554 sequences. Sequences searched: 2638 (5278638 bp). (downregulated genes).As there was a difference in number of sequences used for search of the promoter modules, we calculated the percentage of the promoter modules present based on the number of the sequences. The Fig 1 is representing the percentage of the promoter modules in the their respective conditions (up or down regulated).In both the cases there were 70 promoter modules identified and the surprisingly fact is both upregulated and downregulated genes promoter have same type of modules in total (Fig 1). So we further proceeded to identify the motifs using these promoter sequences

Drought Responsive Motif elements

Two type of motifs were analyzed i.e Revcomp and Pal. In revcomp, the algorithm considers both the given strand and the reverse complement strand when searching for motifs in a complementable alphabet (i.e, DNA) (Fig 2

and Fig 3) whereas in pal method it only look for palindromes in complementable datasets. MEME averages the letter frequencies in complementary columns of the motif (PSPM) together. For instance, if the width of the motif is 10, columns 1 and 10, 2 and 9, 3 and 8, etc., are averaged together. For DNA the averaging would combines the frequency of A in one column with T in the other, and the frequency of C in one column with G in the other. (Fig 4 and Fig 5). Five motifs identified in revcomp method in both upregulating and dwonregulating promoters. Two motifs were identified in Pal Method method in both upregulating and dwonregulating promoters.

Discussion

Drought is a major environmental stress factor that affects the growth and development of plants. Drought or soil water deficit can be chronic in climatic regions with low water availability or random and unpredictable due to changes in weather conditions during the period of plant growth. (Rosegrant and Cline, 2003).Thus, an understanding of drought stress and water use in relation to plant growth is of importance for sustainable agriculture.

To large degree, the expression of genes is regulated at the level of transcription initiation mediated by the specific binding of protein transcription factors (TFs) to short DNA sequence motifs located in gene promoter regions, the DNA-sequence region upstream of genes. Employing both experimental as well as bioinformatic (Bailey *et al.,* 2006, Tompa *et al.,* 2005, Luehr *et al.,* 2012, Agostini *et al.,* 2014) methods, hundreds of cis-regulatory motif sequences, partly also along with the identification of the associated transcription factors binding to them, have been determined across all model organisms and associated database resources have been created Intensive research activities have been devoted towards understanding the principles governing the specific recognition of DNA-motifs by protein transcription factors, their positional preferences relative to transcription start sites, their mode of action - whether to act as single entities or in combinations of different TFs and associated motifs , as well as their evolution . In turn, the principles gleaned from these studies have been applied to identify additional motifs.

With regard to study design, we were able to demonstrate that motif presence irrespective of orientation leads to significant statistical effects documenting motif activity with regard to gene expression regulation and thereby serving as a

positive control (Table 2). Notable progress has been made toward this end by utilizing modern genetics and functional genomics approaches such as transcriptomics, proteomics and metabolomics and consequently, various drought stress responsive genes have been identified and characterized in crops. These key genes mainly code for proteins that have either metabolic or regulatory roles, such as those involved in detoxification, osmolyte biosynthesis, proteolysis of cellular substrates, water channel, ion transporter, heat shock protein (HSP), and late embryogenesis abundant (LEA) protein. On the other hand, the regulatory class primarily comprises of tfs (AREB, AP2/ERF, NAC, bzip, MYC, and MYB), signaling protein kinases [mitogen activated protein kinases (MAPK), calcium-dependent protein kinases (CDPK), receptor protein kinases, ribosomal protein kinases, and transcription regulation protein kinases] and protein phosphatases (phosphoesterases and phospholipase), which synchronize signal transduction and expression of genes during stress responses (Wani *et al.,* 2013). Several of these regulatory genes including tfs were found to play essential roles in multiple abiotic stress responses via regulating downstream stress-responsive genes. Tfs regulate gene expression through binding to *cis*-regulatory elements in the promoter region of different stress-related genes (Nuruzzaman *et al.,* 2013, Franco-Zorrilla *et al.,* 2014).

Tfs Can Induce a Range of Stress Responsive Genes in Plants

In recent years, a wide range of TF families holding relevance with drought stress response have been identified (Anbazhagan *et al.,* 2015). During the signal transduction, tfs directly regulate the expression of the associated genes via serving as molecular switches. These tfs interact specifically with *cis*-elements located in the promoter region of the genes they regulate. In plants, a large proportion of genes in the genome (up to 10%) potentially encode tfs (Franco-Zorrilla *et al.,* 2014), which are categorized into different gene families such as *AREB, DREB, MYB, WRKY, NAC*, and *bzip* based on the distinct structure of their DNA-binding domain (Golldack *et al.,* 2011, Jin *et al.,* 2014).

AREB/ABF tfs

Several research groups have elucidated the molecular mechanism related to drought stress transcriptional networks in plants. Under osmotic

stress conditions, detailed molecular analyses have found abscisic acid-responsive element binding protein (*AREB)/abfs* (ABRE binding factor) as a major transcriptional activator modulating the expression of genes during ABA signaling (Maruyama *et al.,* 2012). ABA-responsive gene expression is controlled by a conserved ABRE (PyACGTGG/TC) *cis*-element in its promoter region.

AP2/ERF tfs

APETALA2/Ethylene Response Element binding Factors (*AP2/ERF*) family covers a large group of plant-specific tfs and is characterized by the presence of a much-conserved AP2/ERF DNA-binding domain (Song *et al.,* 2011). This domain binds with the GCC box, which is a DNA sequence having role in the ethylene-responsive transcription (Rashid *et al.,* 2012).*AP2/ERFBP*. Based on the number and similarity of AP2/ERF domains, the family is further categorized into four major subfamilies: *AP2* (Apetala 2), *RAV* (related to ABI3/VP1), *DREB* (dehydration-responsive element-binding protein), and *ERF (*Rashid *et al.,* 2012, Sharoni *et al.,* 2011).Apropos of the plant abiotic and biotic stress response, *ERF* and *DREB* subfamilies have been extensively studied. Induced, respectively, by cold and dehydration, *DREB1/CBF* (with 11 genes in rice) and *DREB2* (six genes in rice) function in ABA-independent manner (Srivastav *et al.,* 2010, Nakashima *et al.,* 2014a).

Interactions among Multiple tfs

Drought stress is an unpredictable event and it varies in severity and duration. This results in both general and specific effects on plant growth and development. Thus, plant response toward drought stress is dynamic, which involves multiple stress perception and signal transduction pathways, which may crosstalk at various steps in the pathways (Lenka *et al.,* 2011).For this reason, plants have evolved complex regulatory mehanisms, including metabolic adjustment and gene expression toward physiological and morphological adaptation (Baldoni *et al.,* 2015).

Drought responsive gene promoters contain a DRE/CRT motif where ABA-independent *DREB/CBF* TF binds and act as a coupling element for ABRE in ABA-dependent gene expression (Singh *et al.,* 2015).It was already shown that *DREB1A/CBF3, DREB2A*, and *DREB2C* proteins interact with *AREB/ABF (*Li *et*

al., 2015).Thus, there exists a crosstalk between ABA-dependent and independent signaling and regulatory pathways. Under osmotic stress conditions, *AREB/ABF* tfs and *snrk2*s regulate the transcriptional activation of *DREB2A* gene, suggesting a complex interaction between *DREB* and *AREB* regulatory regions at the transcript and protein level (Kim *et al.,* 2011). Similar interactions were also reported in between *AREB/abfs* and nacs. In addition, regulation of ABA-dependent gene expression of ABRE regulons by *SNAC* tfs was confirmed when Jensen (Jensen *et al.,* 2013) reported that *Arabidopsis SNAC* transcription factor *ATAF1* directly modulates ABA biosynthetic gene *NCED3*. By contrast, *SNAC* gene promoter contains ABRE region (Nakashima *et al.,* 2014b) Under dehydration and osmotic stress, *ANAC096* was also found to interact with *ABF2/AREB1* and *ABF4/AREB2 (*Xu *et al.,* 2013).

Motif pairs

Cis-regulatory motifs were reported to frequently operate in combination. Hence, we investigated whether orientation effects become apparent when considering motif pairs. For this analysis, we further reduced the motif set to only those 70 motifs (not considering the core-promoter motifs) that are truly not contained in any other (longer) motif even when considering all possible sequence variants associated with ambiguous bases as part of the motif definitions (see Methods). Otherwise, two motifs would be found unduly coupled (found in the same promoter) as the same mapping positions are (possibly) identified. Furthermore, deciding which of the two respective transcription factors binds to this region may be ambiguous. At the same time, this lessened the penalty associated with the multiple testing corrections as the number of possible pair's scales quadratically with the number of motifs. We first probed all detected motif combinations found in the upstream regions of the same gene for statistical enrichment (gene set overlap) and then examined all eight possible relative orientations of two motifs with respect to their sequence order (position in the upstream region) and orientation (forward or reverse-complement).

Conclusion

Plant response to drought is a complex process comprising many changes from morphological to

molecular level. Under drought stress, many transcription factors operate both exclusively and cooperatively forming a web of interactions. In this article we have identified some of the promoter elements which acts as a binding sites for major transcription factors that play a pivotal role in drought stress response and tolerance.

Tables

Table 1 Comparison of published data and current available data on NCBI

Upregulated ESTs			**Downregulated ESTs**		
Library name	Publication	NCBI	Library name	Publication	NCBI
AS1-1	807	1424	AS2-1	877	940
AR1-1	1424	793	AR2-1	940	845
AB1-1	576	503	AB2-1	576	529
BULK1-1	423	421	BULK2-1	429	427
TOTAL	3230	**3141**	TOTAL	2822	**2741**

Table 2 Summary of Consensus sequences from drought SSH library

	Upregulated ESTs	Downregulated ESTs
Contigs	286	253
Singlets	1414	1802
Total	1700	2055
Consensus	**1650**	**2012**

Table 3 Summary of Promoter sequences selected for analysis

	Upregulated ESTs	Downregulated ESTs
Query	1650	2012
Hits	3336	4300
Unique	2171	2649
insufficient upstream data	5	11
promoter (2kb upstream region)	2166	2638

Figures

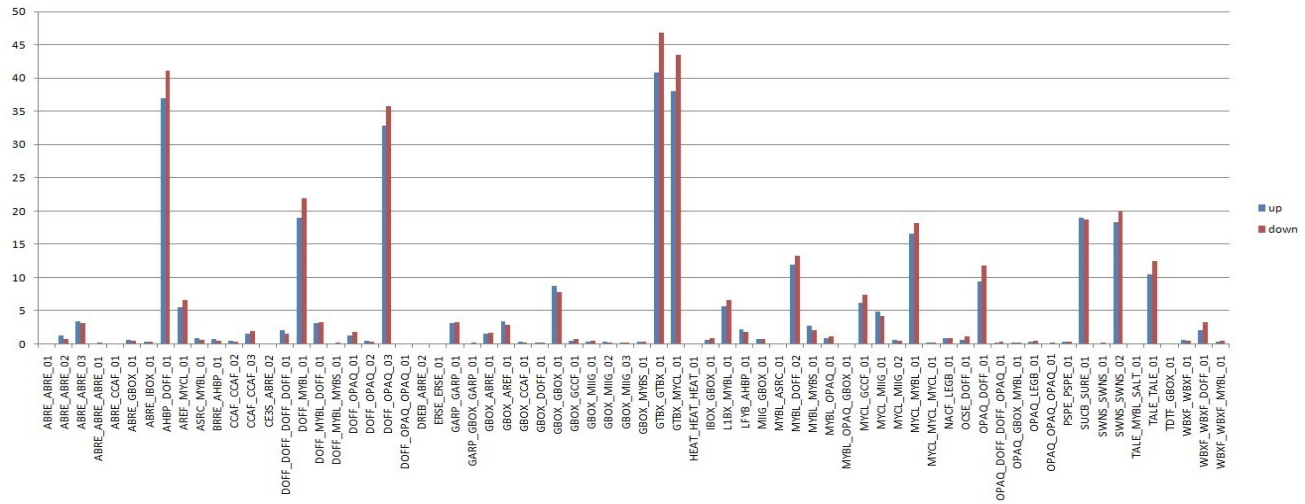

Fig 1 Promoter elements identified in drought responsive genes (both up-regulating and down-regulating) in chickpea

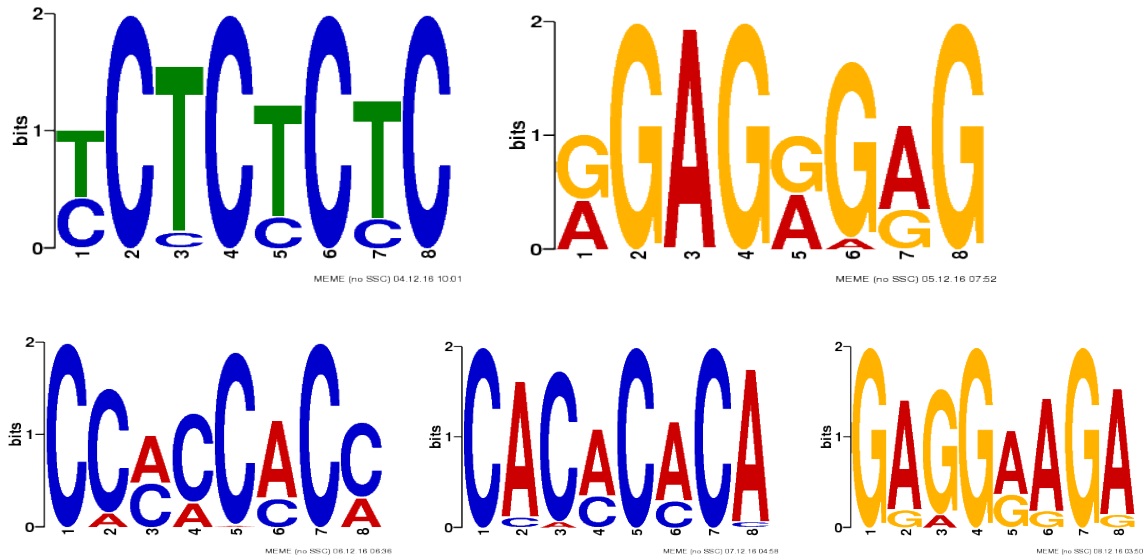

Fig 2 Motifs identified in drought responsive genes which are upregulating under drought in chikpea (revcomp method)

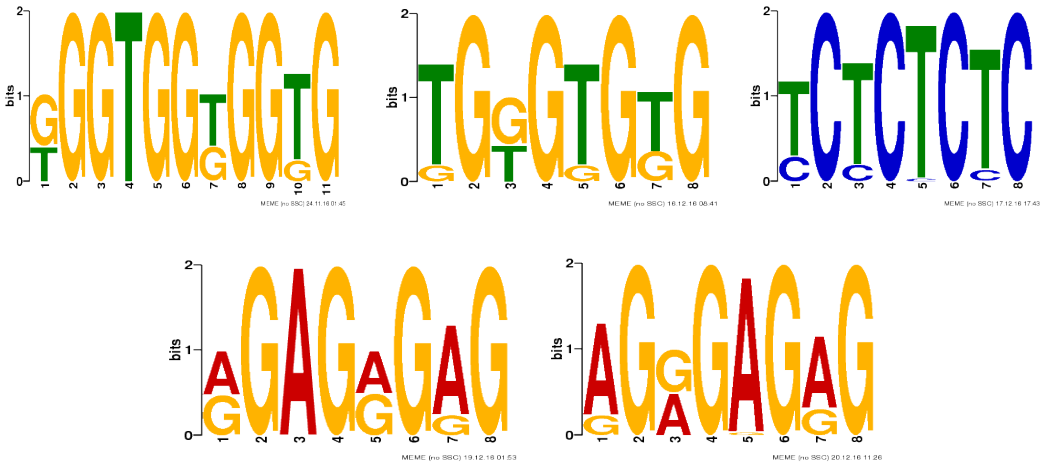

Fig 3 Motifs identified in drought responsive genes which are downregulating under drought in chikpea (revcomp method)

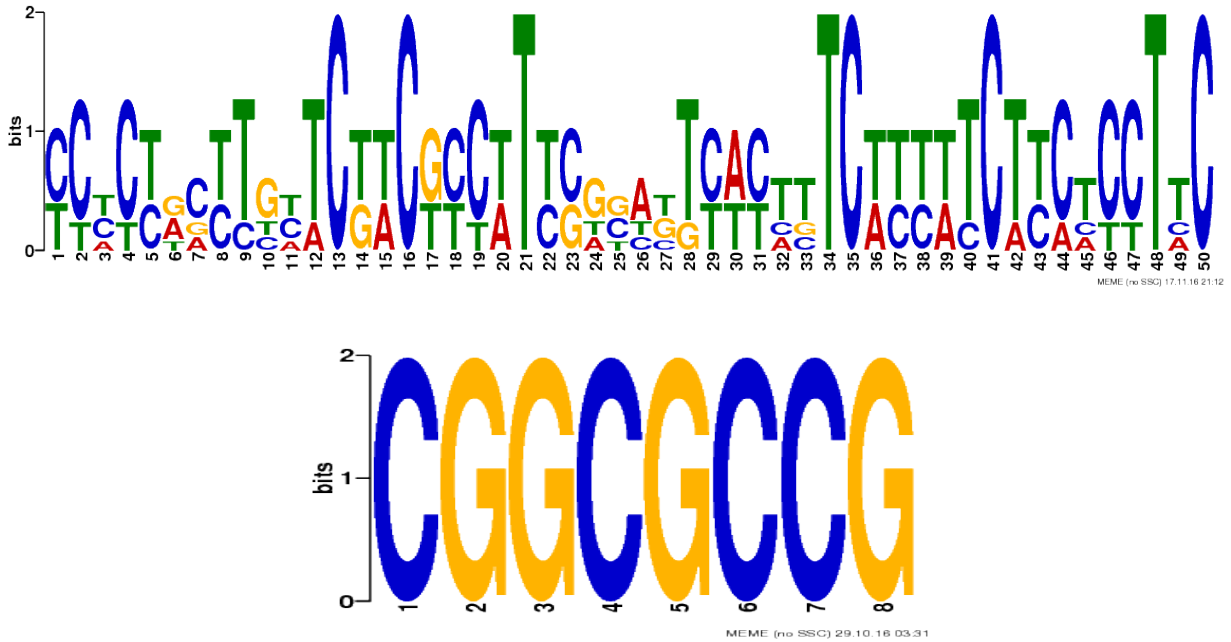

Fig 4 Motifs identified in drought responsive genes which are upregulating under drought in chikpea (pal method)

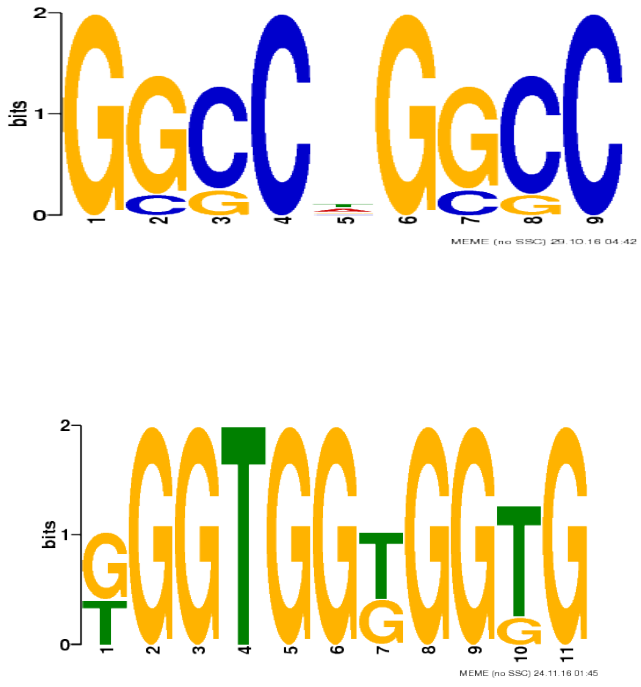

Fig 5 Motifs identified in drought responsive genes which are down regulating under drought in Chickpea (Pal method)

Acknowledment

We thank the Indian Council of Agricultural Research (ICAR), New Delhi, India for funding this work through the Network Project on Transgenics in Crops (NPTC). VS and PTKJ thank the Indian Agricultural Research Institute (IARI) for the Senior Research Fellowship. PC gratefully acknowledges the Senior Research Fellowship awarded by the Council of Scientific and Industrial Research (CSIR).

References

1. **Abebe T, Melmaiee K, Berg V, Wise RP**. 2010. Drought response in the spikes of barley: gene expression in the lemma, palea, awn, and seed. *Funct. Integr. Genomics.,* **10** : 191–205.

2. **Agostini F, Cirillo D, Ponti RD, Tartaglia GG.** 2014. SeAMotE: a method for high-throughput motif discovery in nucleic acid sequences. *BMC Genomics*., **15**:925.

3. **Anbazhagan K., Bhatnagar-Mathur P., Vadez V., Dumbala S. R., Kishor P. B., Sharma K. K.** 2015. DREB1A overexpression in transgenic chickpea alters key traits influencing plant water budget across water regimes. *Plant Cell Rep.,* **34**: 199–210.

4. **Aprile A, Mastrangelo A, Leonardis A, Galiba G, Roncaglia E, Ferrari F, Bellis L, Turchi L, Giuliano G, Cattivelli L.** 2009. Transcriptional profiling in response to terminal drought stress reveals differential responses along the wheat genome. *BMC Genomics*., **10**:279.

5. **Bailey TL, Williams N, Misleh C, Li WW**. 2006. MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res*., **34**: W369 - W373.

6. **Bailey TL, Elkan CP, Altman R, Brutlag D, Karp P, Lathrop R, Searls D**. 1994. Fitting a mixture model by expectation-maximization to discover motifs in biopolymers, *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology.* , Menlo Park : 28-36

7. **Baldoni E., Genga A., Cominelli E.** 2015. Plant MYB transcription factors: their role in drought response mechanisms. *Int. J. Mol. Sci.,* **16**:15811–15851.

8. **Bohnert HJ, Nelson DE, Jensen RG**. 1995. Adaptations to environmental stresses. *Plant Cell.,* **7**: 1099 - 1111.

9. **Bray E**. 1997. Plant responses to water deficit. *Trends Plant Sci.*, **2**: 48-54.

10. Bulyk ML. 2003. Computational prediction of transcription-factor binding site locations. *Genome Biol* 5: 201.

11. Claeys H, Inze D. 2013.The agony of choice: how plants balance growth and survival under water-limiting conditions. *Plant Physiol*. **162** : 1768–1779

12. Deokar AA, Kondawar V, Jain PK, Karuppayil SM, Raju NL, Vadez V, Varshney RK, Srinivasan R. 2011 Comparative analysis of expressed sequence tags (ESTs) between drought-tolerant and susceptible genotypes of chickpea under terminal drought stress. *BMC Plant Biol* **11**:70

13. Deyholos MK. 2010. Making the most of drought and salinity transcriptomics. *Plant, Cell and Environ*., **33**:648–654.

14. Ding XP, Li XK, Xiong LZ. 2013. Insight into differential responses of upland and paddy rice to drought stress by comparative expression profiling analysis. *Int J Mol Sci*., **14**: 5214-5238

15. Franca M, Prados L, Lemos-Filho J, Ranieri B, Vale F. 2012. Morphophysiological differences in leaves of Lavoisiera campos-portoana (Melastomataceae) enhance higher drought tolerance in water shortage events. *J Plant Res*., **125**: 85-92.

16. Franco-Zorrilla J. M., López-Vidriero I., Carrasco J. L., Godoy M., Vera P., Solano R. 2014. DNA-binding specificities of plant transcription factors and their potential to define target genes. *Proc. Natl. Acad. Sci. U.S.A.,* **111** :2367–2372

17. Fujita Y, Fujita M, Shinozaki K, Yamaguchi-Shinozaki K. 2011. ABA-mediated transcriptional regulation in response to osmotic stress in plants. *J Plant Res*., **124**:509–525.

18. Golldack D., Lüking I., Yang O. 2011. Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. *Plant Cell Rep.,* **30** : 1383–1391.

19. Ingram J, Bartels D. 1996. The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* **47**:377–403.

20. Jensen M. K., Lindemose S., de Masi F., Reimer J. J., Nielsen M., Perera V., et al. 2013. ATAF1 transcription factor directly regulates abscisic acid biosynthetic gene

NCED3 in *Arabidopsis thaliana*. *FEBS Open Bio.,* **3**: 321–327.

21. Jin J., Zhang H., Kong L., Gao G., Luo J. 2014. PlantTFDB 3.0*:* a portal for the functional and evolutionary study of plant transcription factors. *Nucleic Acids Res.,* **42** : D1182–D1187.

22. Kim J. S., Mizoi J., Yoshida T., Fujita Y., Nakajima J., Ohori T., et al. 2011. An ABRE promoter sequence is involved in osmotic stress-responsive expression of the DREB2A gene, which encodes a transcription factor regulating drought-inducible genes in *Arabidopsis*. *Plant Cell Physiol.* **52**: 2136–2146.

23. Lenka S. K., Katiyar A., Chinnusamy V., Bansal K. C. 2011. Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. *Plant Biotechnol. J.,* 9:315–327.

24. Li T., Angeles O., Radanielson A., Marcaidalll M., Manalo E. 2015. Drought stress impacts of climate change on rainfed rice in South Asia. *Climatic Change.,* 133: 709–720.

25. Luehr S, Hartmann H, Soding J. 2012. The XXmotif web server for eXhaustive, weight matriX-based motif discovery in nucleotide sequence.*Nucleic Acids Res.,* 40:W104 - W109.

26. Parween S., Nawaz K., Roy R., Pole A. K., Venkata Suresh B., Misra G., et al. 2015. An advanced draft genome assembly of a desi type chickpea (*Cicer arietinum* L.). *Sci. Rep.* **5**:12806

27. Maruyama K., Todaka D., Mizoi J., Yoshida T., Kidokoro S., Matsukura S., *et al.* 2012. Identification of cis-acting promoter elements in cold-and dehydration-induced transcriptional pathways in *Arabidopsis*, rice, and soybean. *DNA Res.* **19**: 37–49

28. Nakashima K, Ito Y, Yamaguchi-Shinozaki K. 2009. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiol.,* **149**:88–95

29. Nakashima K., Jan A., Todaka D., Maruyama K., Goto S., Shinozaki K., et al. 2014a. Comparative functional analysis of six drought-responsive promoters in transgenic rice. *Planta*., 239: 47–60.

30. Nakashima K., Yamaguchi-Shinozaki K., Shinozaki K. 2014b. The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses

including drought, cold, and heat. *Front. Plant Sci.* **5**:170 10.

31. uruzzaman M., Sharoni A. M., Kikuchi S. 2013. Roles of NAC transcription factors in the regulation of biotic and abiotic stress responses in plants. *Front. Microbiol.* **4**:248.

32. Peleg Z, Blumwald E. 2010. Hormone balance and abiotic stress tolerance in crop plants. *Curr. Opin. Plant Biol*., **14**: 290–295.

33. Peleg Z, Reguera M, Tumimbang E, Walia H, Blumwald E. 2011. Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnol. J.*, **9** : 747–758

34. Rabbani M, Maruyama K, Abe H, Khan M, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA Gel-Blot analyses. *Plant Physiol.,* **133**:1755–1767.

35. Rashid M., Guangyuan H., Guangxiao Y., Hussain J., Xu Y. 2012. AP2/ERF transcription factor in rice: genome-wide canvas and syntenic relationships between monocots and eudicots. *Evol. Bioinform. Online.,* **8**: 321–355.

36. Rosegrant MW, Cline SA. 2003. Global food security: challenges and policies. *Science.,* **302**: 1917–1919.

37. Sharoni A. M., Nuruzzaman M., Satoh K., Shimizu T., Kondoh H., Sasaya T., et al. 2011. Gene structures, classification and expression models of the AP2/EREBP transcription factor family in rice. *Plant Cell Physiol.* **52**: 344–360.

38. Shinozaki K, Yamaguchi-Shinozaki K. 1997. Molecular responses to drought and cold stress. *Curr. Opin. Biotechnol.,* **7**:161–167

39. Shinozaki K, Yamaguchi-Shinozaki K, Liu Q, Kasuaga M, Ichimura K, Mizoguchi T, Urao T, Miyata S, Nakashima K, Shinwari ZK, Abe H, Sakuma Y, Ito T, Seki M. 1999. Molecular responses to drought stress in plants: regulation of gene expression and signal transduction. In: Smallwood MF, Calvert CM, Bowles DJ, editors. Plant responses to environmental stress. Bios Scientific Publishers, Oxford, UK:133

40. Shinozaki K, Yamaguchi-Shinozaki K. 2000. Molecular responses to dehydration

and low temperature: differences and cross-talk between two stress signaling pathways. *Curr Opin Plant Biol*., 3:217–223.

41. Singh D., Laxmi A. 2015. Transcriptional regulation of drought response: a tortuous network of transcriptional factors. *Front. Plant Sci.* **6**:895 10.

42. Song S. Y., Chen Y., Chen J., Dai X. Y., Zhang W. H. 2011. Physiological mechanisms underlying OsNAC5-dependent tolerance of rice plants to abiotic stress. *Planta* **234**: 331–345.

43. Srivastav A., Mehta S., Lindlof A., Bhargava S. 2010. Over-represented promoter motifs in abiotic stress-induced DREB genes of rice and sorghum and their probable role in regulation of gene expression. *Plant Signal. Behav.* **5**:775–784.

44. Tompa M, Li N, Bailey TL, Church GM, De Moor B, Eskin E, *et al*. 2005. Assessing computational tools for the discovery of transcription factor binding sites. *Nat Biotechnol.,* **23**:137–144

45. Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K. 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr. Opin. Biotechnol.,* **17**:113–122

46. Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N, Suzuki H, Saito K, Shibata D, Kobayashi M. *et al*. 2009.Characterization of the ABA regulated global responses to dehydration in Arabidopsis by metabolomics. *Plant J.,* **57**:1065–1078.

47. Van Dijk K, Ding Y, Malkaram S, Riethoven JJM, Liu R, Yang JY, *et al*. 2010. Dynamic changes in genome-wide histone H3 lysine 4 methylation patterns in response to dehydration stress in Arabidopsis thaliana. *BMC Plant Biol.,* **10**: 238.

48. Wani S. H., Singh N. B., Devi T. R., Haribhushan A., Jeberson S. M., Malik C. P., *et al*. 2013. "Engineering abiotic stress tolerance in plants: extricating regulatory gene complex," in *Conventional and Non-Conventional Interventions in Crop Improvement*, eds Malik C. P., Sanghera G. S., Wani S. H., editors. CABI, New Delhi: 1–19.

49. Xu Z. Y., Kim S. Y., Kim D. H., Dong T., Park Y., Jin J. B., et al. 2013. The *Arabidopsis* NAC transcription factor

ANAC096 cooperates with bZIP-type transcription factors in dehydration and osmotic stress responses. *Plant Cell* **25**: 4708–4724.

50. Varshney R. K., Song C., Saxena R. K., Azam S., Yu S., Sharpe A. G., et al. 2013. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat. Biotechnol.,*31: 240–246

51. Yamaguchi-Shinozaki K, Shinozaki K. 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.,* **57**:781–803

52. Zhang Q. 1997. Strategies for developing green super rice. *Proc. Natl. Acad. Sci. USA.,* **104**: 16402-9.

53. Zheng J, Fu J, Gou M, Huai J, Liu Y, Jian M, Huang Q, Guo X, Dong Z, Wang H. et al. 2010. Genome-wide transcriptome analysis of two maize inbred lines under drought stress. *Plant Mol Biol* **72**:407–421.