American Journal of Plant Sciences, 2022, 13, 277-293
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

In silico Analysis of the Entire *P. glaucum* Genome Identifies Regulatory Genes of the bZIP Family Modulated in Response Pathways to Water Stress

Laura Beatriz Garay Farías[1], Sergio Litwiniuk[2], Cristian Antonio Rojas[1]

[1]Federal University of Latin American Integration (UNILA), Foz do Iguaçu, Brazil
[2]National Council for Scientific and Technical Research of Argentina, Posadas, Argentina
Email: lau.genetica@gmail.com, lbg.farias.2019@aluno.unila.edu.br, sergiolitwiniuk@gmail.com, crojasmail@gmail.com, cristian.rojas@unila.edu.br

How to cite this paper: Farías, L.B.G., Litwiniuk, S. and Rojas, C.A. (2022) *In silico* Analysis of the Entire *P. glaucum* Genome Identifies Regulatory Genes of the bZIP Family Modulated in Response Pathways to Water Stress. *American Journal of Plant Sciences*, **13**, 277-293.
https://doi.org/10.4236/ajps.2022.132017

Received: December 29, 2021
Accepted: February 22, 2022
Published: February 25, 2022

Copyright © 2022 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Abstract

The literature reviewed places *P. glaucum* as a cereal characterized by its nutritional quality and high tolerance to drought stress. However, very little is known about the fine mechanism it uses in response to water stress. To try to clarify this point, we carried out an analysis of the modulation of the expression of regulatory genes of the FT bZIP family. A full genome screening of *P. glaucum* identified 52 putative FT bZIPs, identifying 9 FT PgbZIP differentially expressed under water stress conditions filtered from RNA-seq data from a Transcriptome deposited at the NCBI. The promoter regions of these genes presented multiple elements or *cis* ABREs and DRE motifs, thus suggesting their double modulated participation in the slow or adaptive response and in the rapid response of this cereal to water stress. The findings of this study provide complementary data for the understanding of the mechanism behind the adaptation of *P. glaucum* under water stress, and may be relevant for molecular applications of potential crops.

Keywords

P. glaucum, Water Stress, RNA-seq, *cis* Elements, FT PgBZIP

1. Introduction

The imminent climate change and the consequent difficulties of global food production are a reality [1]. This leads the scientific community to innovate and understand the genetic mechanisms of suggestive crops that serve as tools to deal

with abiotic stress, citing one of the "water stress" that worries and leaves global food insecurity.

Pennisetum glaucum, *synonym Cenchrus americanus* commonly known as millet or pearl millet, is a cereal grown in marginal lands of the arid and semi-arid tropical regions of Africa and India; distinguished by its high tolerance to abiotic stress, such as high temperature, drought and high soil pH; appreciable for the bio-fortification it provides, and the low glycemic index it presents [2], all characteristics that make it attractive to study.

The response mechanism to abiotic stress that begins with an environmental stimulus; leads to a change in cell turgor pressure, triggering a complex signaling pathway, which may or may not be regulated by the presence of a plant hormone called abscisic acid (ABA). Thus, a transduction chain is activated that amplifies the initial signal in which secondary messengers participate, and when this signal reaches the nucleus, the activation of specific genes that code for functional or structural proteins and genes that code for regulatory proteins occurs [3].

Regarding regulatory genes, the bZIP transcription factors in *Arabidopsis*, tomato (*Solanum lycopersicum*), soybean (*Glycine max*) and rice (*Oryza sativa*) have shown greater expression in the face of drought stress, but few bZIP transcription factors have been explored as potential candidates for genetic improvement applications for drought tolerance in crops [4].

The bZIP proteins are families of transcription factors related to abiotic stress tolerance, characterized by presenting a basic motif (made up of around 20 amino acids with six arginine's and four lysine residues) responsible for specific binding to DNA; which many times contains the RKQS sequence, important for signal transduction; and a dimerization motif (made up of about 41 amino acids with leucine residues in each seventh position, which can be homo or heterodimeric), called a leucine zipper given its structural conformation [5].

The proteins bZIP, as well as other transcription factors, bind to DNA at specific recognition sites. These motifs consist of characteristic *cis* elements of promoter boxes that are recognized and paired by the transcription factor. Each bZIP subfamily recognizes specific promoter boxes, the most preferred are box A (TACGTA), box C (GACGTC), box G (CACGTG), box H (CCTACC), TGACG motif, ABREs motifs (abscisic acid response elements).

The regulation of the expression of these regulatory genes activated in response to stress occurs mostly just before the synthesis of the transcript when the pre-initiation protein complex is formed on the promoter region of the gene; whose *cis* regulatory elements could intervene in the dialogue between these signaling routes [1].

A complementary strategy to the use of expensive conventional molecular techniques is *in silico* analysis; a bioinformatics tool that allows us to track and analyze genes expressed as a stress tolerance mechanism. Identified and characterized lectin gene families (an important protein in plant defense and cell signaling) from the analysis of the promoter region, finding that most of the families

responded to light conditions and presented a specific expression according to the organ or tissue of the plant. Furthermore, the presence of hormone-associated *cis* elements, such as jasmonic acid, in the promoter regions of all lectin genes indicated their importance in the response to signaling and culture stress [6].

In this study, the *in silico* analysis of the promoter region seems to indicate a comparative tolerant differential role with other model plant organisms, due to the presence of multiple *cis* elements that modulate the expression of FT bZIP as a regulatory gene *in P. glaucum* involved in pathways of response to water stress.

2. Materials and Methods

2.1. *In silico* Screening of Probable FT bZIP Regulatory Genes throughout the *P. glaucum* Genome

The FT bZIP protein sequences involved in the water stress of model organisms were used as queries for the identification of bZIP proteins in *P. glaucum*. A total of 101 reference sequences (Supplementary **Table S1**) from *Oryza sativa* (18 bZIP), *Arabidopsis thaliana* (25 bZIP) and *Setaria italica* (58 bZIP) were downloaded from uniprot (https://www.uniprot.org/). Once the referential bZIP sequences were downloaded, they were aligned with the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/); obtaining the conserved sequence with the Jalview program (https://www.jalview.org) based on the alignment of multiple sequences and used to generate the HMM profile [7] with the HMMER tool (https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan). The conserved sequence generated was subjected to tBlastn against the *P. glaucum* (*Cenchrus americanus*) Genome database using the NCBI tool, considering an ID ≥ 45% and an E-value of 0.02 at values ≤ 1.

The HMMER bioinformatics tool is used in conjunction with a pre-prepared database of HMM profiles to analyze biological sequences. HMM, from the acronym in English Hidden Markov Model or Hidden Markov Model allows determining the probability with which each sequence of a database has been generated from it. In this way, members of a protein family, or sequences that contain certain functional domains or motifs, can be detected, as well as the prediction of genes, among others. Some of the most important family and domain databases, such as Pfam or Interpro, use HMMER as the basis for building their own HMM profiles [8].

The gene ontology terms (GO) for the putative bZIP proteins or FT recognized were identified using predetermined parameters with the Blast2GO v5 tool, predicting the biological and molecular processes in which they participate.

Blast2GO is a bioinformatics tool that allows the *in silico* functional characterization of a sequence through data mining techniques. Use Blast against the non-redundant DB (Database) of the NCBI (nr) to find the homologues, map to retrieve GO terms and annotate to select reliable functions [9].

The data set of a Transcriptome published in the NCBI as BioProject ID: PRJNA419859, resulting from the experimentation in which millet seedlings of

two drought-tolerant inbred lines ICMB843 and ICMB863 with 21 days after sowing were subjected to water stress with cessation of irrigation for 5 days and root tissue samples taken with their respective controls at 26 days after sowing were used for the analysis of differential gene expression [10] using bioinformatics tools from the Galaxy [11].

The quality of the readings deposited was verified using the online tool FASTQC (Galaxy Version 0.72 + galaxy1), to later filter low-quality readings with the use of Fastp [12]. The scored reads were assigned to the reference genome of *P. glaucum* (*C. americanus*) with the use of the HISAT2 mapper [13]. StringTie allowed the assemblies generated to be merged with the reference transcriptome annotation [14]. The FeatureCounts tool [15] generated a count table for each gene, and for each control condition and water stress of each inbred line, the normalized differential gene expression was analyzed through the use of DESeq2 [16].

Once the presence of the putative bZIP genes identified in the published transcriptome was confirmed, the differential expression of putative bZIP in response to water stress in which it had the highest expression was taken as a selection criterion.

2.2. Tracking the Promoting Region of Selected Putative bZIP FTs

Access was made to the putative bZIP sequences selected according to their induced differential expression from the NCBI Data Bank (https://www.ncbi.nlm.nih.gov/) and were screened using the NCBI Genome Data Viewer, in order to trace ~ 1700 bp upstream of the regulatory gene.

The *cis* elements involved in the water stress of the promoter region of the selected bZIP regulatory genes were identified with the use of plantCARE, to later be compared with the orthologous bZIP promoter regions found in *A. thaliana* and *O. sativa*.

The probable differentially expressed PgbZIPs and with *cis* elements involved in water stress that were suggestive were visualized through IGV Sashimi Plot [17] in order to see if they had isoforms or not, where the alignments in exons are represented as read densities and splice junction reads are drawn as arcs connecting a pair of exons. The isoforms found were confirmed in the presence and expression in the analyzed transcriptome.

3. Results

In silico Identification of Probable bZIP FT of *P. glaucum*

The construction of the HMM Profile based on the alignment of multiple sequences generated a conserved sequence for the plant model organisms used *Arabidopsis*, *Oryza* and *Setaria* (Supplementary **Figures S1-S3**) attributed to the conserved bZIP domain using PFAM (PF00170) [18].

Once traced throughout the *P. glaucum* genome, 52 putatives FT bZIPs (Supplementary **Table S2**) were identified, whose genetic ontology annotation (GO)

functionally categorized them, predicting their participation in the biological regulation of DNA transcription (Supplementary **Figure S4**).

The RNA-seq reading data used were subjected to the FastQC quality analysis (Supplementary **Figure S5**), generating more than 260 million readings, who's *in silico* analysis can be summarized in the **Table S1**, where the inbred line ICMB863 subjected to stress hydric obtained 72% of mapping, and its control ICMB863 without hydric stress 76.68%; while ICMB843 subjected to stress 80% and its control or control 77% of mapped readings. The percentage of mapped readings assigned was lower in the ICMB 843 samples stressed (13.2%) by drought compared to the other samples; coinciding with what was stated in the work carried out by [10] arguing that this could be due to the fact that drought stress would increase the variation in transcripts in ICMB 843 since this inbred line under greenhouse conditions is shown more drought tolerant than ICMB863.

The presence of putative FT PgbZIPs was confirmed, selecting 9 (**Table S2**) of these for having presented the highest differential gene expression (DEG) among the 52 probable PgbZIPs.

Of the 9 FT bZIPs selected *from P. glaucum* with bZIP from plant models indicate Pg5 (bZIP61) and Pg27 (HY5) with an identity of 58% and 66% respectively with *A. thaliana*; while the 9 PgbZIPs selected in millet showed an identity of 59% to 92% with bZIP from *O. sativa*, which is consistent in view of the fact that both cultures are monocots.

In **Table S3** the changes of Log_2 (Fold change) show that in ICM863 4 PgBZIP were negatively regulated (Supplementary **Table S3** and **Table S4**) and 5 were positively regulated while in ICMB843, 6 were negatively regulated and 3 positively under drought stress conditions (Supplementary **Table S5** and **Table S6**). The transcriptomic analysis carried out by [10] highlighted a higher number of negatively regulated genes in ICMB843 under drought conditions compared to ICMB863.

Table S4 shows the *cis* regulatory elements involved in the response pathways to water stress of the selected PgbZIPs (Supplementary **Figure S6**), showing the presence of multiple ABREs, MYC, MYB-MBS and DRE elements.

The Pg47 differentially expressed and positively regulated (log_2 1.44) in ICMB843, visualized four isoforms (**Figure S1**) through the IGV Sashimi Plot bioinformatics tool [17].

When analyzing the differential expression values in ICMB843 (**Table S5**), isoform 3 was the most expressed, even superior to isoform 1; however, its fold change was −0.99, negatively regulated and with a value above −1 and below 1. Comparatively, inbred lines have a difference in expression in isoform 4.

4. Discussion

Despite the fact that *P. glaucum* is an interesting crop due to its rusticity and its available genome, few studies of regulatory genes have been registered in this crop. In order to reveal the early dynamic molecular regulation of *P. glaucum*

subjected to drought stress and to elucidate the key genes that are responsible for tolerance to this type of stress analyzed RNA-Seq data in order to detect the gene expression pattern in the roots in the seedling stage in early phases during three-time points (1 h, 3 h and 7 h) of drought stress. The number of DEG decreased from 1 h to 7 h, suggesting that after exposure to drought stress *P. glaucum* immediately showed responses due to its high drought tolerance capacity, and then the cells returned to a relatively balanced level. This could be a signal to the plants that they were already in normal conditions, so that in a short time it was not essential to express many genes, but simply grew normally. In the research conducted, 12 genes exhibited up-regulation at all three-time points, which could illustrate that they play some important roles in response to drought stress. For these positively regulated genes, some were associated with CCCH-type zinc finger proteins, zinc metalloprotease FtsH proteins and alcohol dehydrogenase (ADH1 gene) and for four of the genes their functions were not clear, but their expression under drought treatment was 20 times higher than those under normal conditions [19].

Here in this work, considering the vital transcription factors in the regulation of genes under stress conditions, we identified probable FT bZIP through the *in silico* analysis of a transcriptome published in millet, which provided probable interesting data that could help to understand the tolerance mechanism of *P. glaucum* to water stress.

The *cis* regulatory elements involved in response pathways to water stress of the PgbZIP selected for their higher DEG show *in silico* the presence of multiple ABREs, MYC, MYB-MBS, and DRE elements, also the function of a promoter is normally determined by the combinatorial action of multiple regulatory elements, which modulate the spatio-temporal expression pattern of genes [20].

At the molecular level, it has been documented that dehydration stress triggers the biosynthesis of ABA, which is an essential tolerance response to this type of stress. The ABREs (Abscisic Acid Response Elements) motifs are important for promoter activity in response to water stress; while the DRE (Dehydration Response Elements) motif participates in independent ABA regulation. Multiple copies of ABREs confer ABA inducibility to a heterologous promoter, whereas a single copy may not be sufficient for expression [21]. The elements MYC and MYB (MBS) are also involved in the response to dehydration. The MYB system may function in a slow and adaptive stress response process [22].

Of the 9 FT bZIP differentially express; Pg1, Pg4, Pg5, Pg21, Pg46, Pg47 and Pg52 present in their promoter regions elements or cis ABREs and DRE motifs; thus suggesting double its modulated participation in the slow or adaptive response and in the rapid response of millet to water stress, agreeing with who highlight that the function of a promoter is normally determined by the combinatorial action of multiple regulatory elements [20].

The comparative results *in silico* found in the present work between the promoters of the orthologues show highly conserved regions, considering them

highly conserved, since they are sequences that would share a similar regulatory network, in relation to ABREs, MYB (MBS) and MYC, coordinated in the response pathways to ABA.

It is known that a large part of the genes that encode the Transcription Factors present isoforms [23]; however, given that the isoforms of a gene share many of their exons, their quantification is one of the most complex processes [24]. When the proportions of the isoforms change in one condition with respect to another, as a consequence of an alteration in the alternative splicing mechanism, it could be relevant in biological terms [25]. Indicate that alternative splicing (AS) plays an important role in stress responses; pointing out that there are studies that show that the post-transcriptional control of RNA metabolism plays an important role [24].

In our *in silico* analyzes, the differentially expressed and negatively regulated PgbZIP selected Pg52 and Pg5 presented a single isoform, while the differentially expressed and positively regulated Pg47 visualized four isoforms under water stress conditions and control without stress. Beyond the little that is known about the participation of bZIP transcription factors in response to water stress, there are some reports that show that different abiotic stresses such as salinity, drought and exogenous ABA caused differential regulation of alternative splicing forms in rice [26]. Likewise, in a transcriptomic analysis of corn carried out by found differentially expressed genes in response to water stress with values of Fold change $\log_2 > 1$, of which approximately 60% were positively regulated and 40% negatively, in which most of these genes had a single differentially expressed isoform, but six upregulated genes and one negatively regulated gene had two differentially expressed isoforms [27].

Isoform 2 of Pg47 showed identity with the chloroplastic DLC protein of *Oryza sativa*. In an attempt to establish the function of DCL, it was found that the dysregulation of the expression of the DCL gene in *Arabidopsis* caused multiple phenotypes such as chlorosis, sterile flowers and abnormal development of cotyledons, suggesting that this gene is necessary in different organs; in addition to being involved in the maturation of plastid rRNA [28]. While both isoform 3 and isoform 4 were present in crops such as *Zea mayz*, *Oryza sativa* and *Setaria italica* but with unknown function.

Due to the results achieved here, PgbZIP47 becomes interesting; showed no identity in *Arabidopsis* but 73% with *Oryza* bZIP23. OsbZIP23 performs a functional activity in response to saline and water stress [29]. When analyzing the promoter region of Pg47, it shows multiple ABREs and DRE elements, which indicates their double participation in response pathways to water stress.

The accumulation of data increases thanks to the development of bioinformatics tools, allowing to give an idea of the composition and functionality of the regulatory elements of the promoters, for which it is proposed to continue with the characterization of the motifs, giving continuity to the processing and *in silico* analysis of RNA-seq repositories in suggestive cultures generated from stress

experiments deposited in the Sequence Readings File (SRA).

Our findings provide a basis for a greater functional characterization and identification of the regulatory mechanism in *P. glaucum* in responses to water stress, and may be a potential candidate to use for crop improvement, molecular breeding techniques and genome editing tools to improve agricultural production and ensure food security in the future.

5. Conclusion

The presence of multiple *cis* elements that modulate the expression of FT bZIP as a regulatory gene in *P. glaucum* indicates its probable participation in response pathways to water stress, both dependent and independent of ABA. The participation of the bZIP Transcription Factors in response to water stress can be considered as a key element for understanding the molecular bases that modulate the biochemical and physiological responses of plants. In our *in silico* analyses, 9 FT PgbZIP differentially expressed under water stress conditions could be identified, suggesting that this family may be one of the key factors for water stress tolerance in this plant species.

Consent to Publish

This article does not involve any clinical studies that need consent of participants to publish.

The following work is part of the dissertation of Laura G to access the title of Master of UNILA. Laura G together with Cristian designed the experimental methodology. Sergio L helped with the bioinformatics analyzes. Laura G wrote the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Atkinson, N. and Urwin, P. (2012) The Interaction of Plant Biotic and Abiotic Stresses: From Genes to the Field. *Journal of Experimental Botany*, **63**, 3523-3543. https://doi.org/10.1093/jxb/ers100

[2] Singh, P., Boote, K., Kadiyala, M., Nedumaran, S., Gupta, S., Srinivas, K. and Bantilan, M. (2017) An Assessment of Yield Gains under Climate Change Due to Genetic Modification of Pearl Millet. *Science of the Total Environment*, **601-602**, 1226-1237. https://doi.org/10.1016/j.scitotenv.2017.06.002

[3] Lindemose, S., Shea, C., Jensen, M. and Skriver, K. (2013) Structure, Function and Networks of Transcription Factors Involved in Abiotic Stress Responses. *International Journal of Molecular Sciences*, **14**, Article No. 5842. https://doi.org/10.3390/ijms14035842

[4] Kuromori, T., MizoI, J., Umezawa, T., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2014) Drought Stress Signaling Network. In: Howell, S.H., Ed., *Molecular Biology*, Springer, Berlin, 383-409. https://doi.org/10.1007/978-1-4614-7570-5_7

[5] Gatz, C. (2011) From Pioneers to Team Players: TGA Transcription Factors Provide a Molecular Lin between Different Stress Pathways. *Molecular Plant-Microbe Interactions*, **26**, 151-159. https://doi.org/10.1094/MPMI-04-12-0078-IA

[6] Ha, C. and Wyckhuys, K. (2017) *In Silico* Identification and Characterlization of the Lectin Gene Families in Cassava (*Manihot esculenta* Crantz). *Academia Journal of Biology*, **39**, 320-332. https://doi.org/10.15625/0866-7160/v39n3.9185

[7] Finn, R., Clements, J. and Eddy, S. (2011) Hmmer Web Server: Interactive Sequence Similarity Searching. *Nucleic Acids Research*, **39**, W29-W37. https://doi.org/10.1093/nar/gkr367

[8] Eddy, S. (2003) The HMMER User's Guide (Biological Sequence Analysis Using Profile Hidden Markov Models). Howard Hughes Medical Institute and Dept. of Genetics Washington University School of Medicine, Saint Louis.

[9] Conesa, A., Gotz, S., Garcia-Gomez, J., Terol, J., Talon, M. and Robles, M. (2005) Blast2GO: A Universal Tool for Annotation, Visualization and Analysis in Functional Genomics Research. *Bioinformatics*, **21**, 3674-3676. https://doi.org/10.1093/bioinformatics/bti610

[10] Dudhate, A., Shinde, H., Tsugama, D., Liu, S. and Takano, T. (2018) Transcriptomic Analysis Reveals the Differentially Expressed Genes and Pathways Involved in Drought Tolerance in Pearl Millet [*Pennisetum glaucum* (L.) R. Br]. *PLoS ONE*, **13**, e0195908. https://doi.org/10.1371/journal.pone.0195908

[11] Afgan, E., Baker, D., Batut, B., Van Den Beek, M., Bouvier, D., Cech, M., *et al.* (2018) The Galaxy Platform for Accessible, Reproducible and Collaborative Biomedical Analyzes. *Nucleic Acids Research*, **46**, 537-544. https://doi.org/10.1093/nar/gky379

[12] Chen, S., Zhou, Y., Chen, Y. and Gu, J. (2018) FASTP an Ultra-Fast All-in-One FASTQ Preprocessor. https://doi.org/10.1101/274100

[13] Langmead, K.D., Salzberg, B. and Hisat, S.L. (2015) A Fast Spliced Aligner with Low Memory Requirements. *Nature Methods*, **12**, 357-360. https://doi.org/10.1038/nmeth.3317

[14] Kovaka, T., Mizoi, J., Umezawa, T., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2014) Drought Stress Signaling Network. In: Howell, S.H., Ed., *Molecular Biology*, Springer, Berlin, 383-409. https://doi.org/10.1007/978-1-4614-7570-5_7

[15] Liao, Y., Smyth, G.S. and Shi, W. (2013) FeatureCounts: An Efficient General Purpose Program for Assigning Sequence Reads to Genomic Features. *Bioinformatics*, **30**, 923-930. https://doi.org/10.1093/bioinformatics/btt656

[16] Love, M., Huber, W. and Anders, S. (2014) Moderated Estimation of Fold Change and Dispersion for RNA-seq Data with DESeq2. *Genome Biology*, **15**, Article No. 550. https://doi.org/10.1186/s13059-014-0550-8

[17] Katz, Y., Wang, E., Silterra, J., Schwartz, S., Wong, B., Mesirov, J., Airoldi, E. and Burge, C. (2015) Sashimi Plots: Quantitative Visualization of RNA Sequencing Read Alignments. *Bioinformatics*, **31**, 2400-2402. https://doi.org/10.1093/bioinformatics/btv034

[18] Sonnhammer, E., Eddy, S., Birney, E., Bateman, A. and Durbin, R. (1998) Pfam: Multiple Sequence Alignments and HMM-Profiles of Protein Domains. *Nucleic Acids Research*, **26**, 320-322. https://doi.org/10.1093/nar/26.1.320

[19] Zhang, A., Ji, Y., Sun, M., Lin, C., Zhou, P., Ren, J., Luo, D., Wang, X., Ma, C., Zhang, X., Feng, G., Nie, G. and Huang, L. (2021) Research on the Drought Tolerance Mechanism of *Pennisetum glaucum* (L.) in the Root during the Seedling Stage. *BMC Genomics*, **22**, Article No. 568. https://doi.org/10.1186/s12864-021-07888-5

[20] Zhang, S., Lian, Y., Liu, Y., Wang, X., Liu, Y. and Wang, G. (2013) Characterization of Maize Wip1 Promoter in Transgenic Plants. *International Journal of Molecular Sciences*, **14**, 23872-23892. https://doi.org/10.3390/ijms141223872

[21] Roychoudhury, A., Paul, S. and Basu, S. (2013) Cross-Talk between Abscisic Acid-Dependent and Abscisic Acid-Independent Pathways during Abiotic Stress. *Plant Cell*, **32**, 985-1006. https://doi.org/10.1007/s00299-013-1414-5

[22] Pandey, S., Reddy, C., Yaqoo, U., Kumar, Y., Arora, S. and Kaul, T. (2015) *In Silico* Analysis of Cis Acting Regulatory Elements CAREs in Upstream Regions of Ascorbate Glutathione Pathway Genes from *Oryza sativa*. *Biochemistry and Physiology*, **4**, 1-7. https://doi.org/10.4172/2168-9652.1000159

[23] Severing, E., Van Dijk, A., Morabito, G., Busscher-Lange, J., Immink, R.G. and Van Ham, R.C. (2012) Predicting the Impact of Alternative Splicing on Plant MADS Domain Protein Function. *PLoS ONE*, **7**, e30524. https://doi.org/10.1371/journal.pone.0030524

[24] Garber, M., Guttman, M. and Trapnell, C. (2011) Computational Methods for Transcriptome Annotation and Quantification Using RNA-seq. *Nature Methods*, **8**, 469-477. https://doi.org/10.1038/nmeth.1613

[25] Soneson, C., Matthes, K., Nowicka, W., Law, C. and Robinson, M. (2016) Isoform Prefiltering Improves Performance of Count-Based Methods for Analysis of Differential Transcript Usage. *Genome Biology*, **17**, Article No. 12. https://doi.org/10.1186/s13059-015-0862-3

[26] Gupta, P., Nutan, K., Singla-Pareek, S. and Pareek, A. (2017) Abiotic Stresses Cause Differential Regulation of Alternative Splice Forms of GATA Transcription Factor in Rice. *Frontiers in Plant Science*, **8**, Article No. 1944. https://doi.org/10.3389/fpls.2017.01944

[27] Song, L., Ma, Q., Zou, Z., Sun, K., Yao, Y., Tao, J., Kaleri, N. and Li, X. (2017) Molecular Link between Leaf Coloration and Gene Expression of Flavonoid and Carotenoid Biosynthesis in *Camellia sinensis* Cultivar "Huangjinya". *Frontiers in Plant Science*, **8**, Article No. 803. https://doi.org/10.3389/fpls.2017.00803

[28] Bellaoui, M. and Gruissem, W. (2004) Altered Expression of the *Arabidopsis ortholog* of DCL Affects Normal Plant Development. *Plant*, **219**, 819-826. https://doi.org/10.1007/s00425-004-1295-5

[29] Zhu, J., *et al.* (2018) Characterization and Alternative Splicing Profiles of the Lipoxygenase Gene Family in Tea Plant (*Camellia sinensis*). *Plant and Cell Physiology*, **59**, 1765-1781. https://doi.org/10.1093/pcp/pcy091

Supplementary Tables and Figures

Table S1. Reference sequences of *S. itálica*, *O. sativa* and *A. thaliana* for the identification of FT bZIP in *P. glaucum*.

https://drive.google.com/drive/folders/1x9cpln70JXbfToV8NlPORtzsRkxe_TiX?usp=sharing.

Table S2. 52 FT bZIP putative.

https://drive.google.com/drive/folders/1x9cpln70JXbfToV8NlPORtzsRkxe_TiX?usp=sharing.

Table S3. Counts Normalized of ICMB 863 (*P. glaucum*).

https://drive.google.com/drive/folders/1x9cpln70JXbfToV8NlPORtzsRkxe_TiX?usp=sharing.

Table S4. DESEq2 of ICMB863 (*P. glaucum*).

https://drive.google.com/drive/folders/1x9cpln70JXbfToV8NlPORtzsRkxe_TiX?usp=sharing.

Table S5. DESEq2 of ICMB843 (*P. glaucum*).

https://drive.google.com/drive/folders/1x9cpln70JXbfToV8NlPORtzsRkxe_TiX?usp=sharing.

Table S6. Counts Normalized of ICMB 843 (*P. glaucum*).

https://drive.google.com/drive/folders/1x9cpln70JXbfToV8NlPORtzsRkxe_TiX?usp=sharing.

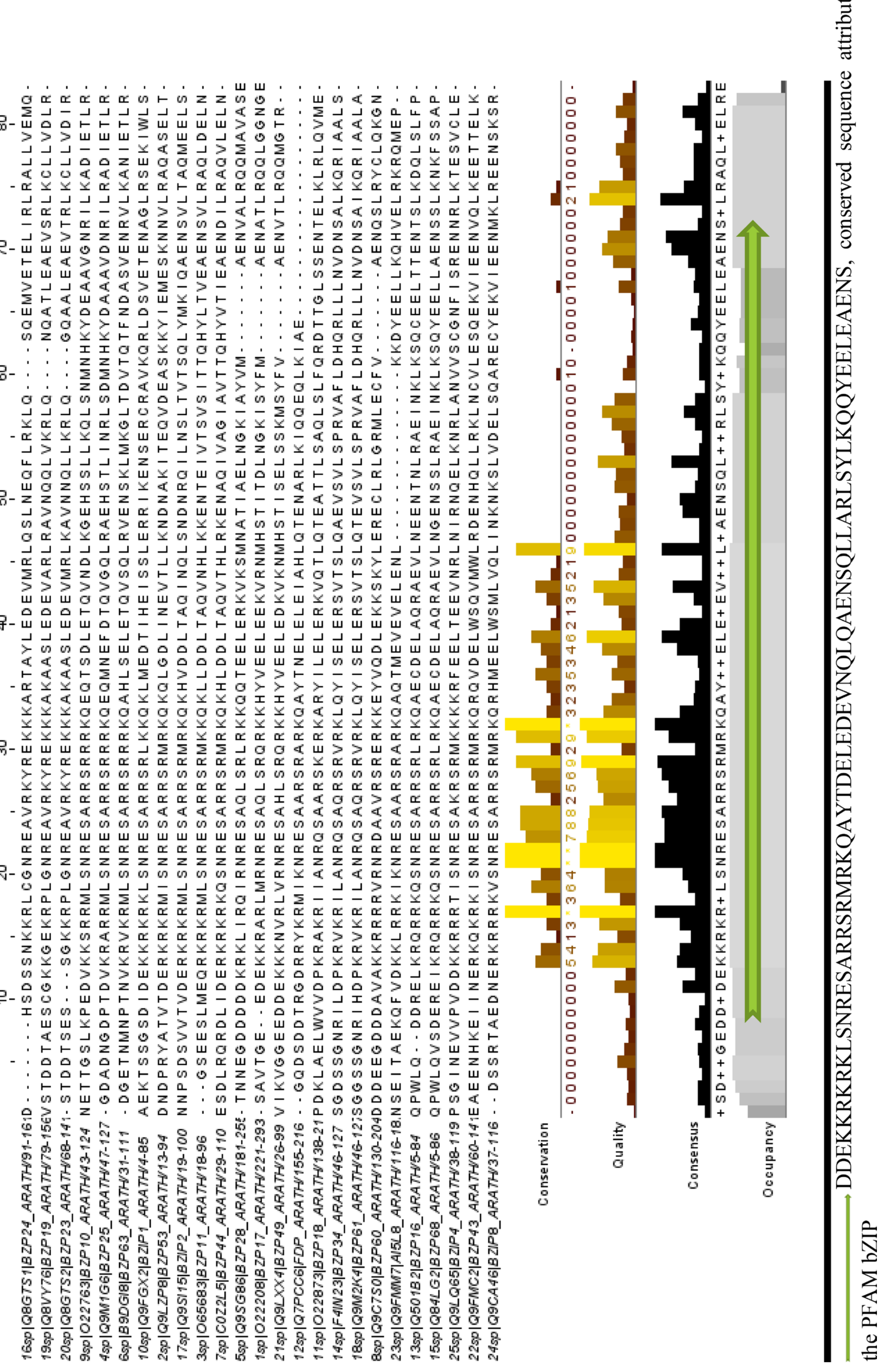


Figure S1. Construction of the HMM Profile Based on the Degree of Conservation of Multiple Sequence Alignment in *A. thaliana.*

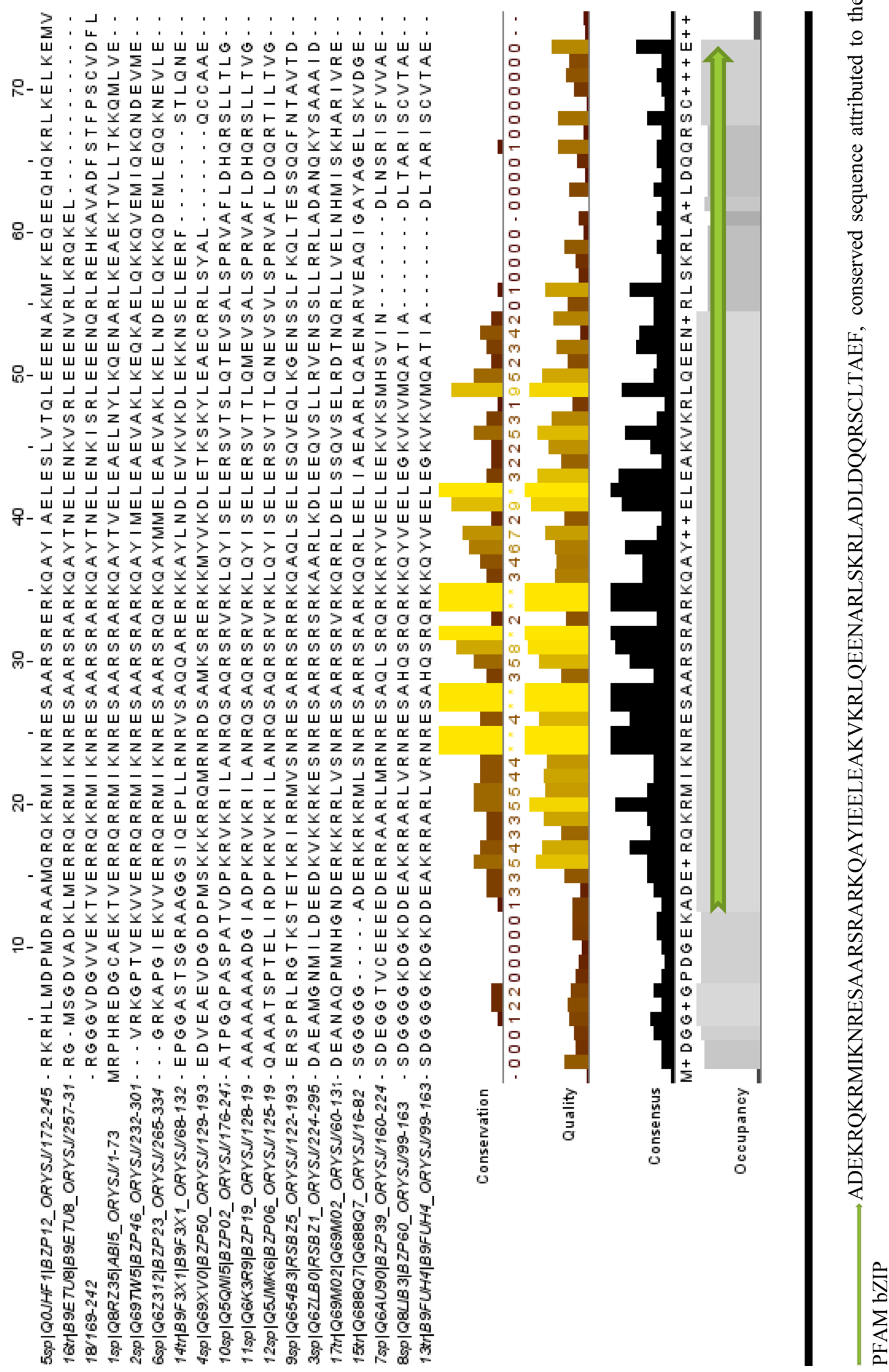


Figure S2. Construction of the HMM Profile Based on the Degree of Conservation of Multiple Sequence Alignment in *O. sativa.*

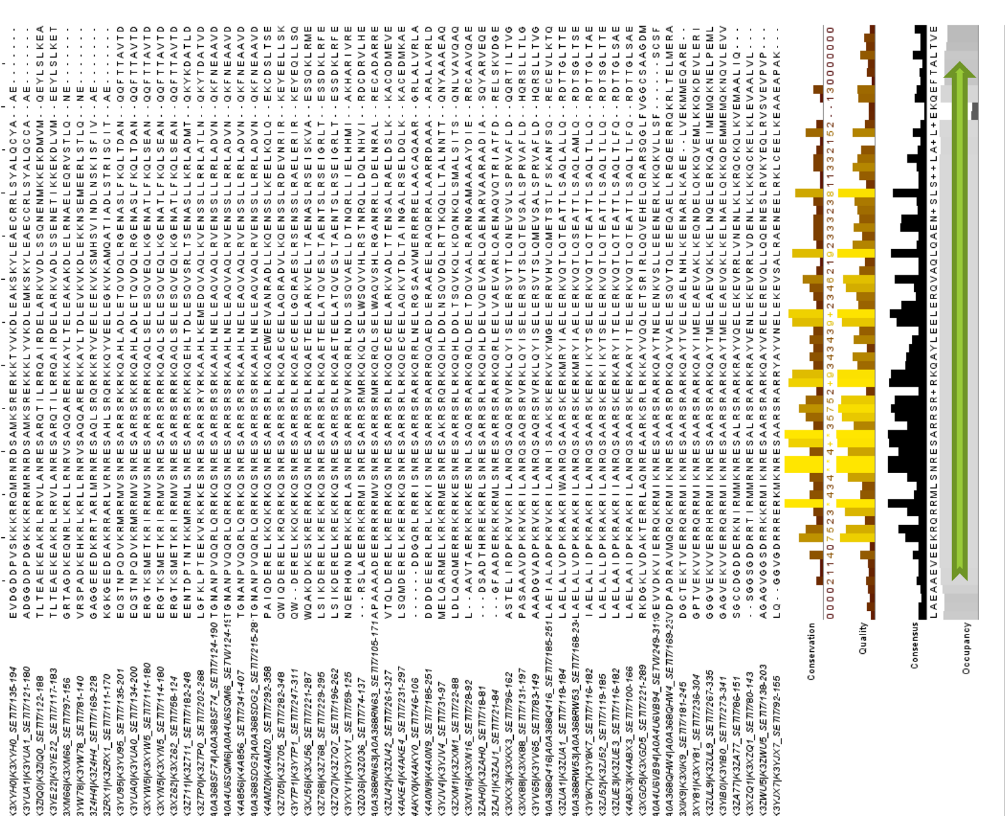
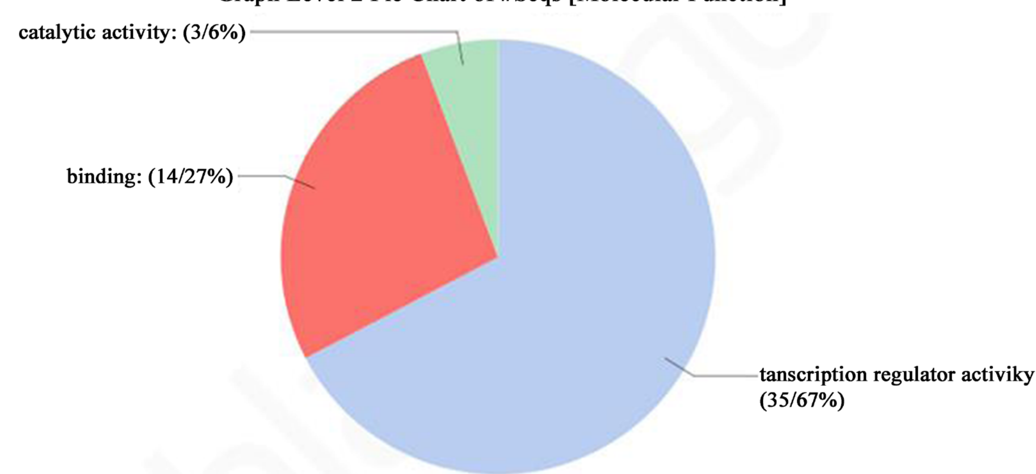


Figure S3. Construction of the HMM Profile Based on the Degree of Conservation of Multiple Sequence Alignment in *S. italic.*

Graph Level 2 Pie Chart of #Seqs [Molecular Function]



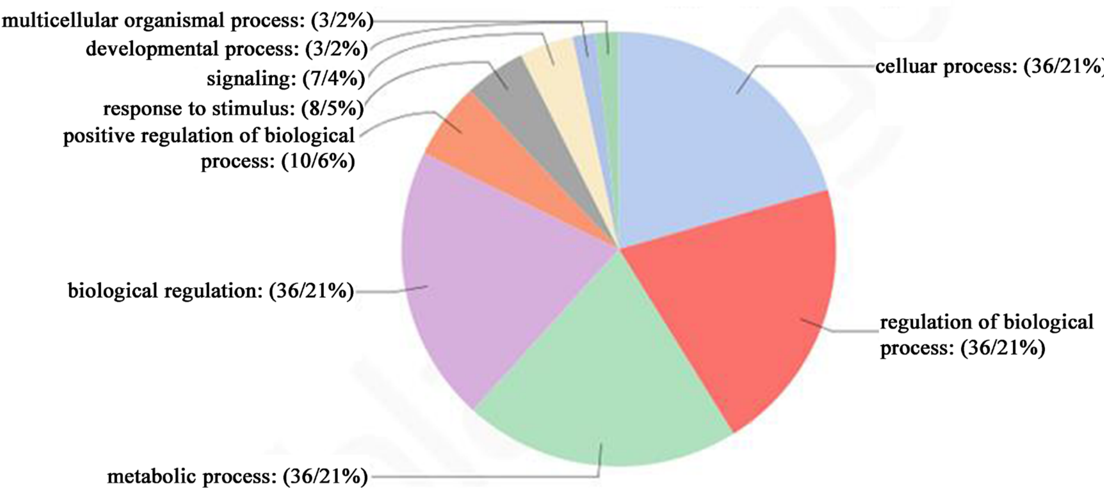


Figure S4. Ontology genetics of 52 putatives bZIP.

Phred quality values for A- ICMB863 Control (3 replicates) B- ICMB863 Stressed (3 replicates) C- ICMB843 Control (3 replicates) D- ICMB843 Stressed (3 replicates).

Figure S5. Reads quality of RNA-seq.

bZIP	Selected upstream sequence
Pg1	Cr2:3978000-3981000
Pg4	Cr2:222689500-22692000
Pg5	Cr2:193196802-93198357
Pg21	Cr7:3989000-3991500
Pg27	Cr6:228475698-28477448
Pg37	Cr1:176831019-76832601
Pg46	Cr3:249786695-49789500
Pg47	Cr3:11481000-11483000
Pg52	Cr5:84373500-84376000
ATbZIP61	Cr3:c21524787-21522243
ATHY5	Cr5:3596000-3593500
ATAREB1	Cr1:17169500-17167000
OsbZIP19	Cr2:8322488-8324100
OsbZIP23	Cr2:c32283000-32281000
OsbZIP39	Cr5:20118098-20116200
OsRISBZ4	Cr2:4108000-4110743
OsRISBZ2	Cr3:c33193500-33191000
OsbZIP60	Cr7:26807783-26811000
OsHY5	Cr1:c3813362-3811900
OsRF2a	Cr9:c20099800-20096675
OsABI	Cr6:30562500-30565000

Figure S6. Location of sequence Upstream selected of putatives bZIPs and orthologous bZIPs.