Characterization of cis-elements in hormonal stress-responsive genes in *Oryza sativa*

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Abstract. Phytohormones play a key role in plant growth and development. The process of plant's perception and response to abiotic and biotic stresses is controlled mainly by the phytohormones which act as an endogenous messenger in the regulation of the plant's status. They can be activated by different signaling pathways in response to environmental stresses. Plants respond to environmental stress through interaction of transcription factors with a handful of *cis*-regulatory elements (CREs). Some examples of cis elements include abscisic acid-responsive element (ABRE), G-box (CACGTG) element, and W-box. In order to investigate the effects of different hormonal stresses which have a key role in response to biotic and abiotic stresses in rice, microarray data was used. Of the available data, 931 genes revealed significant differences in response to different hormonal stresses such as auxin, cytokinin, abscisic acid, ethylene, salicylic acid, and jasmonic acid. The present results showed that 388 genes were up-regulated, and 543 genes were down-regulated. Most of the genes were up-regulated in response to Indole-3-acetic acid (IAA) hormone. Genes Ontology analysis revealed that they respond to various hormones involved in auxin- responsive genes, auxin-activated signaling pathway and cellular responses to environmental stimuli. G-box had the highest number of cis elements involved in hormonal stress and was regulated by auxin signaling and various stresses. *Dehydrin* was the only gene up-regulated in response to the six hormones. This gene can be activated in response to abiotic and biotic stresses. As such, *dehydrin* gene can be used in crop breeding programs to increase tolerance to different environmental stresses in various plant species.

Keywords: Cis elements prediction, Microarray data, Phytohormones, Regulatory elements.

INTRODUCTION

Plants are exposed to many abiotic and biotic stresses during the course of life (Jung *et al.*, 2008). Plants use phytohormones which are important regulatory elements involved in response to different conditions such as stress, defense, and adaptation (Nemhauser *et al.*, 2006).

When plants are exposed to external stimuli such as pathogen and insect attack, drought, and salt stress, hormonal signal transduction pathways are induced which in turn cause changes

in the hormone metabolism and its distribution within plant (Cheong *et al.*, 2002). It has been reported that phytohormones such as auxin, cytokinin, gibberellin, abscisic acid, ethylene, jasmonic acid and salicylic acid can play a role as important regulators in response to different stresses. Therefore, it is important to increase our understanding about role of phytohormones in molecular and cellular mechanisms in various pathways (Li *et al.*, 2016). Rice is an ideal model to

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study the regulation of gene expression in response to different stresses (Rabbani *et al.*, 2003; Yazaki *et al.*, 2003). Based on previous research performed, auxin and abscisic acid are the most important phytohormones in response to different hormonal stresses (Uddin and Singh, 2017). ABA has an important factor in the response of plants to abiotic stresses. Thousands of phytohormones-regulated genes are activated in response to hormonal, abiotic and biotic stresses (Depuydt and Hardtke, 2011). Numerous number of hormone-responsive genes with differential expression have been detected, offering a cross-talk between hormone and abiotic stress signaling (Garg *et al.*, 2012).

Among up-regulated genes, genes involved in flavonoid biosynthesis are key roles in response to stresses. Auxins influence flavonoid biosynthesis and the reduction of flavonoid levels leads to IAA transport in plant. Flavonoid biosynthesis is related to the biosynthesis of aromatic compounds such as tryptophan, which is a precursor for auxin (Li and Zachgo, 2013). Analysis of gene expression profiling increase understanding to the biological pathways that one might wish to derive (Garber *et al.*, 2001). There is a requirement for more insights of transcriptional responses of plant hormone under stresses, that extensive crosstalk and signal integration has been uncovered (Sharoni *et al.*, 2012). High-throughput technique such as microarray has been extensively used for evaluation of plant expression profile under stress conditions (Lenka *et al.*, 2011). Microarray data has been used to survey gene expression patterns providing new dimensions in gene expression related to growth and development (Cramer *et al.*, 2011).

In the current study, microarray data was used to survey gene expression patterns on six hormones in rice. Interaction between cis regulatory elements and transcription factors forms transcriptional regulation network. This network can regulate gene-controlling morphogenesis, development of anatomy, and other aspects of developmental biology and hormone responses (Sato *et al.*, 2012; Smita *et al.*, 2011; Wilmoth *et al.*, 2005). Gene network can detect relationships between genes and transcription factors, also co-expression genes (Wilmoth *et al.*, 2005).

Different reporters have shown that co-

expressed genes are involved in a common biological pathway and cellular processes, plant growth, and reproduction (Michalak *et al.*, 2008; Koç *et al.*, 2018). Integration of data with promoter structure in plants shows that promoters of co-expressed genes induced a common cis-regulatory elements (CAREs) (Reiss *et al.* 2006; Werner 2001). CAREs functions were predominantly associated with light response, hormonal regulation and stress response and were detected in the upstream of DGEs under drought and salinity stress conditions in rice (Shariatipour *et al.*, 2018). Most of cis elements expressed in response to abiotic, biotic, and hormonal stresses can be suggested to be the ABA responsive elements (ABREs), also termed as G-box, with promoters of the hormonal responsive genes (Ross and Shen 2006). The hormonal regulatory elements such as Methyl jasmonate (MeJA), Ethylene (ET) and abscisic acid (ABA) were responsible for activation of ABRE, P-box, and TGACG-motif elements. These motifs are present in majority of DGEs under drought stress conditions (Shariatipour *et al.*, 2018). This work aimed to characterize cis elements related to responsive genes in *Oryza sativa* under hormonal stress.

MATERIALS AND METHODS

Microarray data related to the expression patterns of hormonal stresses response of rice (*Oryza sativa* subspecies indica variety IR64) (GSE37557) was retrieved from NCBI GEO database (www.ncbi.nlm.nih.gov/geo/). In this study, microarray data required for control and stress conditions were collected from 16 samples in water (control) and in a 50 µM solution of IAA and BAP and 100 µM solution of ABA, ACC, SA and JA for 3 h (treatment) with 2 biological replications from gene bank (<http://www.ncbi.nlm.nih.gov/gds>). Analysis of data was performed using fold change which is based on log2ratio, followed by normalizing the data (the ratio expression of the genes examined under hormonal stress to control) and selected by P-value <0.05 as the statistical significance (Mehdizadeh *et al.*, 2013). T-test was used to calculate the p-value of the expression change of each probe, and differentially expressed genes (DEGs) were detected using the Affy

package (Gautier *et al.*, 2004). DEGs with p-values <0.05 and log fold-change values ≥ 2 and ≤ -2 were selected for genes. The filtered data with DEGs >2 was considered as up-regulated genes and DEGs <-2 were determined to be down-regulated. Up-regulated and down-regulated genes in response to hormonal stress are shown in table 2. Probe sets were mapped to MSU Rice Genome Annotation Project gene set (release 6.1). Heatmap of DEGs was performed by package graphics to represent differential expression. To convert probe set to ID genes, DAVID sites (<https://david.ncifcrf.gov>) was used. One-way ANOVA showed significantly different genes among the studied genotypes at a five percent significance level. In addition, the corrected P-value of Benjamin-Hookberg allowed a more stringent selection of up-regulated genes showing less than five percent significance level. To obtain the possibility functions of hormone-responsive genes, their annotation was taken from the rice genome database (<http://rice.plantbiology.msu.edu/>). Gene ontology was performed on biological pathways of genes using Gene Ontology Enrichment Analysis Software Toolkit (Yazaki *et al.*, 2003). STRING 10.0 (<http://string-db.org/>) was done for identifying co-expression genes and drawing the genes network (Szklarczyk *et al.*, 2017). In order to predict the *cis* regulatory elements, genes promoter analysis for each gene was obtained from PlantCARE; database (Lescot *et al.*, 2002) (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

RESULTS AND DISCUSSION

Differentially-expressed gene in response to hormonal stresses. According to microarray data in different hormonal stress conditions, the genes are up-regulated and down-regulated in response to IAA and ethylene, respectively (Fig. 1). The data were based on p-value > 0.05 and the threshold of 2 by which a total of 931 genes were induced. A set of 388 up-regulated and 543 down-regulated genes were detected in response to different hormonal stresses. Results of clustering of expressed genes showed that almost all genes were up-regulated in response to IAA (Table 1).

Therefore, it can be suggested that auxin may be involved with other hormones and can be a main component in regulation of reactions to environmental stresses. Similar to previous studies, several auxin-responsive genes are regulated by biotic and abiotic stresses (Ghanashyam *et al.*, 2009). It was shown that IAA levels significantly increased in response to pathogen infection (O'Brien and Benková, 2013). Among six hormones evaluated, analysis of phytohormones transcriptome showed that auxin and ABA were the most important hormones induced under abiotic and biotic stresses (Ohme-Takagi and Shinshi, 1995).

Figure 1. Results of clustering of expressed genes in microarray test. Microarray data was normalized based on Log 2. Up-regulated genes are represented by red color, genes showing decreased expressions are indicated by blue color, and no significant change in gene expression is shown by white colour. The colour scale representing log signal values is shown at the right of the heatmap.

Table 1. Up and down-regulated genes in response to different hormonal stress.

	IAA	ABA	BAP	SA	JA	ACC	Total
Up-regulated	171	50	39	60	38	30	388
Down-regulated	0	98	100	86	123	136	543

Detection of responsive gene-network under hormonal stresses. Figure 2 shows gene network analysis of genes involved in response to different hormonal stresses and their relationships. The responsive genes to hormonal stress include CYPs (*CYP450*, *CYP72A* and *CYP734A*), GSTs, the gene family of auxins (*OsLAA*s), and transcription factors of auxin (*ARF*s). CYPs are vital for signaling molecules and environmental responses (Sato *et al.*, 2002). Cytochrome P450 genes are engaged in biosynthesis of brassinosteroids and their metabolism have been shown to be up-regulated by auxin (Fujioka and Yokota, 2003). *CYP72A1* gene is involved in brassinosteroids inactivation and regulation of BRs homeostasis (Fujioka and Yokota, 2003). Findings revealed that cytochrome P450 is the main component in the synthesis and degradation of external steroid hormones as a monooxygenase (Chapple, 1998). Recently, *ARF* genes have extensively been investigated using molecular and bioinformatics analysis (Spartz *et al.*, 2014). *ARF19* gene acts redundantly in reining leaf expansion and lateral root growth (Wang *et al.*, 2007). *OsLAA*s gene family is induced in response to different hormonal stresses. Many auxin-responsive genes are responsive to at least two or more hormones. Similar findings indicated that *OsLAA* genes play a key role in particular growth stages or are involved in specific stress or hormones (Hagen and Guilfoyle, 2002; Jain *et al.*, 2007; Greenham and McClung, 2015). Similar results revealed that the four *OsLAA1* genes were increased by >2.5-fold in response to auxin, suggesting that many of the *OsLAA* genes are auxin activated (Greenham and McClung, 2015; Hagen and Guilfoyle, 2002). According to a previous study, *OsLAA* genes were also expressed in response to six different hormonal treatments and other stresses (Hagen and Guilfoyle, 2002). Furthermore, several auxin-response gene families (*Aux/LAA* and *ARF*) showed differentially-expressed genes under various hormonal stress situations (Jain *et al.*, 2007). *LAA* genes code for short-lived proteins that are main components in cell growth (Song *et al.*, 2009). The

glutathione S-transferase (GSTs) and cytochrome P450 genes exhibited, in most cases, increased expression in response to different environmental stresses (Fig. 2). *OsGSTU6* gene was expressed by different environmental stresses and *OsGSTU35* gene was induced by biotic stresses (Jain *et al.*, 2007).

Figure 2. Predicted gene network for responsive genes to various hormonal stresses is drawn up in string site where the lines drawn between genes represent the relationship between genes.

Gene ontology enrichment analysis. Gene ontology at three levels of cellular components, molecular function, and biological processes is presented in Table 2. The nucleus, membrane-bound organelles and intracellular organelle were related to cellular components and that the molecular function was associated with catalytic activity, glutathione transferase activity and binding. Gene ontology of biological processes includes response to hormone, response to auxin and auxin-activated signaling pathway in rice (Table 2). Similar researches have shown that most of genes are involved in response to environmental stresses and are related to auxin signaling (Jain *et al.*, 2006).

Table 2. Analysis of gene ontology of molecular function, biological processes and cellular components, respectively in response to different hormonal stresses in rice plant.

GO ID	Ontology	Term	P-value
GO:0043226	Cellular_component	Organelle	0.025
GO:0043229	Cellular_component	Intracellular organelle	0.025
GO:0005634	Cellular_component	Nucleus	0.025
GO:0043227	Cellular_component	Membrane-bounded organelle	0.02
GO:0010181	Molecular_function	Binding	4.87e-05
GO:0003824	Molecular_function	Catalytic activity	7.50e-08
GO:0004364	Molecular_function	Glutathione transferase activity	0.005
GO:0009725	Biological_process	Response to hormone	7.50e-08
GO:0009733	Biological_process	Response to auxin	1.54e-08
GO:0009734	Biological_process	Auxin-activated signaling pathway	1.25e-06
GO:0009755	Biological_process	Hormone-mediated signaling pathway	4.57e-06
GO:0032870	Biological_process	Cellular response to hormone stimulus	4.57e-06
GO:0071365	Biological_process	Cellular response to auxin stimulus	1.25e-06

Up-regulated gene in response to different hormonal stress. Analysis of microarray data showed that only *dehydrin* gene expression increased in response to the six hormones used. It has been reported that dehydrin expression may be corresponding with hormonal regulation under cold acclimation (Zhang *et al.*, 2017). As a result, *dehydrin* gene may be produced in response to other stresses, such as salinity, cold and drought. A report showed that expression of dehydrins in seeds undergoing a maturation drying has to be considered as a direct response to drought stress. *OsDHN1* gene belongs to acidic dehydrin family (Kleinwächter *et al.*, 2014). *Dehydrin* genes are the main component in membrane hardening and osmotic regulation. Moreover, dehydrin is the key element in drought tolerance of seeds (Hanana *et al.*, 2014). Previous report has shown that *OsDHN1* gene is overexpressed in rice, hence confirmed its high tolerance to abiotic stresses (Kumar *et al.*, 2014). In wheat, this gene is expressed in response to ABA (Koike *et al.*, 1997). *Dehydrin* genes were up-regulated by many phytohormones, indicating the involvement in ABA dependent pathways (Allagulova *et al.*, 2003).

Cis-regulatory elements analysis. Cis regulatory elements detected in the 1000-bp upper region are presented in Figure 3. Cis-regulatory

components in the promoter sequences provided a good understanding of plant responses under hormonal stress conditions. Gene promoter analysis revealed that TATA-box and CAAT-box were present in majority of promoter regions of genes. In the present study, many light-responsive components were revealed in the promoter sequence of all genes from rice, including ACE, Box-W1, CATT motif, G box, GC motif, Skn-1 motif, and TCT motif.

The presence of circadian in many of studied genes causes plant to respond appropriately to cellular processes such as physiological processes, plant growth and reproduction. Additionally, cis regulatory elements (G-box, Circadian, and TGACG-motif) expressed in response to hormonal stress. Other cis-elements (box E and Box-W1) are induced in response to pathogens such as fungi (Jain *et al.*, 2007). Skn-1 motifs regulate specific expression pattern of endosperm and CAT-box, specifically expressed in the meristem region.

The G-box provides binding sites for specific bZIP proteins and this cis-element are regulated to environmental stresses such as UV light, ABA, red light and injuries (Ciarmiello *et al.*, 2011; Saidi and Hajibarat, 2018). It has been reported that G-box motif related to photosynthesis, hormone (ethylene and ABA) metabolism, and stress

responses was common in grapevine and *Arabidopsis* (Ma *et al.*, 2013).

Our results showed that various regulatory elements are involved in salicylic acid (W boxes) which are expressed in response to hormonal stresses. W-boxes are the main components of binding site for WRKY transcription factors and play a key role in activating transcription of auxins, salicylic acid, and light. This finding is also reported by other studies (Liu *et al.*, 2016; Park *et al.*, 2004).

P-box is responsive to gibberellin, TCA-element to salicylic acid, and TGACG-motif are involved in methyl jasmonate which agrees with the results obtained by other researchers as reported by Kaur *et al.* (2017). TGACG motif is involved in methyl jasmonate response affecting biological processes like response to abiotic and biotic stresses. MBS is activated in response to low temperature, drought and salinity stress (Jaspers *et al.*, 2009).

Figure 3. Cis regulatory elements detected in 1000-bp upper region. Frequency of cis elements detected in upstream of responsive genes to different hormonal stresses.

CONCLUSION

Phytohormones are key elements in growth processes and development, as well as mediators in response to different environmental stresses. Our findings showed that the responsive genes were expressed in response to six hormones at seedling stage in rice, but the most detected genes up-regulated are in response to auxin hormone. The present study can provide a comprehensive understanding of hormonal cross-talk in plant development and stress responses. G-box was the most abundant among the cis-regulatory elements and was regulated in response to auxin and different stresses. This cis-element, a sequence-specific protein-binding site, has an

interaction with transcription factors such as bZIP and *dehydrin* gene network. The interaction between genes and transcription factors lead to the formation of gene network. Overall, insight of relationships among these components can help researchers to engineer plants resistant to stress conditions.

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REFERENCES

- Allagulova, C. R., Gimalov, F. R., Shakirova, F. M., & Vakhitov, V. A. 2003. The plant dehydrins: structure and putative functions. *Biochemistry (Moscow)* 68(9): 945-951.
- Chapple, C. 1998. Molecular-genetic analysis of plant cytochrome P450-dependent monooxygenases. *Annual Review of Plant Biology* 49(1): 311-343.
- Cheong, Y. H., Chang, H. S., Gupta, R., Wang, X., Zhu, T., & Luan, S. 2002. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. *Plant Physiology* 129(2): 661-677.
- Ciarmiello, L. F., Woodrow, P., Fuggi, A., Pontecorvo, G., & Carillo, P. 2011. Plant genes for abiotic stress. In *Abiotic Stress in Plants: Mechanisms and Adaptations* InTech.
- Cramer, Grant R., Kaoru Urano, Serge Delrot, Mario Pezzotti, & Kazuo Shinozaki. 2011. Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biology* 11(1): 163.
- Depuydt, S. & Hardtke, C. S. 2011. Hormone signaling crosstalk in plant growth regulation. *Current Biology* 21 (9): R365-73.
- Fujioka, S., and Yokota, T. 2003. Biosynthesis and metabolism of brassinosteroids. *Annual Review of Plant Biology* 54(1): 137-64.
- Garber, M. E., Troyanskaya, O. G., Schluens, K., Petersen, S., Thaessler, Z., Pacyna-Gengelbach, M., ... & Altman, R. B. 2001. Diversity of gene expression in adenocarcinoma of the lung. *Proceedings of the National Academy of Sciences* 99(2): 13784-13789.
- Garg, R., Tyagi, A. K., & Jain, M. 2012. Microarray analysis reveals overlapping and specific transcriptional responses to different plant hormones in rice. *Plant Signaling and Behavior* 7(8): 951-956.
- Gautier, L., Cope, L., & Bolstad, B. M. 2004. Irizarry RA affy—analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics* 20(3): 307-15.
- Ghanashyam, C. & Jain, M. 2009. Role of auxin-responsive genes in biotic stress responses. *Plant Signaling and Behavior* 4(9): 846-8.
- Greenham, K. & McClung, C. R. 2015. Integrating circadian dynamics with physiological processes in plants. *Nature Reviews Genetics* 16: 598.
- Hanana, M., Daldoul, S., Fouquet, R., Deluc, L., Leon, C., Hoefler, M., & Ghorbel, A. 2014. Identification and characterization of a seed-specific grapevine dehydrin involved in abiotic stress response within tolerant varieties. *Turkish Journal of Botany* 38(6): 1157-1168.
- Hagen, G. & Guilfoyle, T. 2002. Auxin-responsive gene expression: genes, promoters and regulatory factors. *Plant Molecular Biology*. 49(3-4):373-85.
- Hirose, N., Makita, N., Kojima, M., Kamada-Nobusada, T., & Sakakibara, H. 2007. Overexpression of a type-A response regulator alters rice morphology and cytokinin metabolism. *Plant and Cell Physiology* 48(3):523-39.
- Jain, M., Nijhawan, A., Arora, R., Agarwal, P., Ray, S., Sharma, P., Kapoor, S., Tyagi, A. K., & Khurana, J. P. 2007. F-box proteins in rice. Genome-wide analysis, classification, temporal and spatial gene expression during panicle and seed development, and regulation by light and abiotic stress. *Plant Physiology* 143(4): 1467-83.
- Jain, M., Kaur, N., Garg, R., Thakur, J. K., Tyagi, A. K., & Khurana, J. P. 2006. Structure and expression analysis of early auxin-responsive Aux/IAA gene family in rice (*Oryza sativa*). *Functional And Integrative Genomics* 6(1): 47-59.
- Jain, M., Kaur, N., Tyagi, A. K., & Khurana, J. P. 2006. The auxin-responsive GH3 gene family in rice (*Oryza sativa*). *Functional and Integrative Genomics* 6(1): 36.
- Jaspers, P., Blomster, T., Brosché, M., Salojärvi, J., Ahlfors, R., Vainonen, J. P., & Overmyer, K. 2009. Unequally redundant RCD1 and SRO1 mediate stress and developmental responses and interact with transcription factors. *The Plant Journal* 60(2): 268-279.
- Jung, K. H., An, G., & Ronald, P. C. 2008. Towards a better bowl of rice: assigning function to tens of thousands of rice genes. *Nature Reviews Genetics* 9(2): 91.
- Kaur, A., Pati, P. K., Pati, A. M., & Nagpal, A. K. 2017. In-silico analysis of cis-acting regulatory elements of pathogenesis-related proteins of *Arabidopsis thaliana* and *Oryza sativa*. *PLoS One* 12(9): e0184523.
- Kleinwächter, M., Radwan, A., Hara, M., & Selmar, D. 2014. Dehydrin expression in seeds: an issue of maturation drying. *Frontiers in Plant Science* 5: 402.
- Koç, I., Yuksel, I., and Caetano-Anollés, G. 2018. Metabolite-centric reporter pathway and tripartite network analysis of *Arabidopsis* under cold stress. *Frontiers in Bioengineering and Biotechnology* 6.
- Koike, M., Takezawa, D., Arakawa, K., & Yoshida, S. 1997. Accumulation of 19-kDa plasma membrane polypeptide during induction of freezing tolerance in wheat suspension-cultured cells by abscisic acid. *Plant and Cell Physiology* 38(6): 707-16.
- Kumar, M., Lee, S. C., Kim, J. Y., Kim, S. J., & Kim, S. R. 2014. Over-expression of dehydrin gene, OsDhn1, improves drought and salt stress tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). *Journal of Plant Biology* 57(6):383-93.
- Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouzé, P., & Rombauts, S. 2002. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research* 30(1): 325-7.
- 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 Biotechnology Journal* 9(3): 315-27.
- Li, S. & Zachgo, S. 2013. TCP3 interacts with R2R3-MYB proteins, promotes flavonoid biosynthesis and negatively regulates the auxin response in *Arabidopsis thaliana*. *The Plant Journal* 76(6): 901-13.
- Li, C., Li, J., Chong, K., Harter, K., Lee, Y., Leung, J., Martinoia, E., Matsuoka, M., Offringa, R., Qu, L. & Schroeder, J. 2016. Toward a molecular understanding of plant hormone actions. *Molecular Plant* 9(1): 1-3.
- Liu, L., Xu, W., Hu, X., Liu, H., & Lin, Y. 2016. W-box and G-box elements play important roles in early senescence of rice flag leaf. *Scientific Reports* 6: 20881.
- Ma S., Shah S., Bohnert H. J., Snyder M., & Dinesh-Kumar S. P. 2013. Incorporating motif analysis into gene co-expression networks reveals novel modular expression pattern and new signaling pathways. *PLoS Genetics* 9(10): e1003840.
- Michalak, P. 2008. Coexpression, coregulation, and cofunctionality of neighboring genes in eukaryotic genomes. *Genomics* 91(3): 243-248.
- Nemhauser, J. L., Hong, F., & Chory, J. 2006. Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell* 126(3): 467-75.
- O'Brien, J. A. & Benková, E. 2013. Cytokinin cross-talking during biotic and abiotic stress responses. *Frontiers in Plant Science* 4:451.
- Ohme-Takagi, M. & Shinshi, H. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. *The Plant Cell* 7 (2): 173-82.

- Park, H. C., Kim, M. L., Kang, Y. H., Jeon, J. M., Yoo, J. H., Kim, M. C., ... & Yoon, H. W. 2004. Pathogen-and NaCl-induced expression of the SCA4 promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. *Plant Physiology* 135(4): 2150-2161.
- Rabbani, M. A., Maruyama, K., Abe, H., Khan, M. A., 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 Physiology* 133: 1755-1767.
- Saidi, A. & Hajibarat, Z. 2018. In silico Analysis of floral MADS-BOX gene in *Brachypodium distachyon*. *Bionature*. 366-375.
- Salehin, M., Bagchi, R., & Estelle, M. 2015. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant Cell* 27(1): 9-19.
- Sato, Y., Takehisa, H., Kamatsuki, K., Minami, H., Namiki, N., Ikawa, H., Ohyanagi, H., Sugimoto, K., Antonio, B.A. & Nagamura, Y. 2012. RiceXPro version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Research* 41: D1206-13.
- Shariatipour, N. & Heidari, B. 2018. Investigation of Drought and Salinity Tolerance Related Genes and their Regulatory Mechanisms in *Arabidopsis thaliana*. *The Open Bioinformatics Journal* 11(1).
- Sharoni, A. M., Nuruzzaman, M., Satoh, K., Moumeni, A., Attia, K., & Venuprasad, R. *et al.* 2012. Comparative transcriptome analysis of AP2/EREBP gene family under normal and hormone treatments, and under two drought stresses in NILs setup by Aday Selection and IR64. *Molecular Genetics and Genomics* 287(1): 1-19.
- Smita, S., Lenka, S. K., Katiyar, A., Jaiswal, P., Preece, J., & Bansal, K. C. 2011. QlicRice: a web interface for abiotic stress responsive QTL and loci interaction channels in rice. *Database* 2011.
- Song, Y., Wang, L., & Xiong L. 2009. Comprehensive expression profiling analysis of *OsLAA* gene family in developmental processes and in response to phytohormone and stress treatments. *Planta* 229(3): 577-91.
- Spartz, A. K., Ren, H., Park, M. Y., Grandt, K. N., Lee, S. H., Murphy, A. S., Sussman, M. R., Overvoorde, P. J., & Gray, W. M. 2014. SAUR inhibition of PP2C-D phosphatases activates plasma membrane H⁺-ATPases to promote cell expansion in *Arabidopsis*. *The Plant Cell* 26: 2129-42.
- Szklarczyk, D., Morris, J. H., Cook, H., Kuhn, M., Wyder, S., Simonovic, M., Santos, A., Doncheva, N. T., Roth, A., Bork, P., & Jensen, L. J. 2016. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Research* 18.
- Uddin, R. & Singh, S. M. 2017. Gene network construction from microarray data identifies a key network module and several candidate hub genes in age-associated spatial learning impairment. *Frontiers in Systems Neuroscience* 11: 75.
- Wang, D., Pajerowska-Mukhtar, K., Culler, A. H., & Dong, X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology* 17(20): 1784-90.
- Wilmoth, J. C., Wang, S., Tiwari, S. B., Joshi, A. D., Hagen, G., Guilfoyle, T. J., Alonso, J. M., Ecker, J. R. and Reed, J. W. 2005. NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *The Plant Journal* 43(1): 118-30.
- Wittkopp, P. J. & Kalay, G. 2012. Cis-regulatory elements molecular mechanisms and evolutionary processes underlying divergence. *Nature Reviews Genetics* 13(1): 59.
- Yazaki, J., Kishimoto, N., Nagata, Y., Ishikawa, M., Fujii, F., Hashimoto, A., Shimbo, K., Shimatani, Z., Kojima, K., Suzuki, K., & Yamamoto, M. 2003. Genomics approach to abscisic acid- and gibberellin-responsive genes in rice. *DNA Research* 10(6): 249-61.
- Zhang, X., Shang, C., Liu, Y., Hu, G., Harich, K., and Ervin, E. H. 2017. Hormone and dehydration expression responses to cold acclimation in two Zoysiagrass cultivars with contrasting freezing tolerance. *International Turfgrass Society Research Journal* 13(1): 547-555.