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RESEARCH ARTICLE

Differential gene expression analysis of some least studied transcription factors/regulators in rice during abiotic stresses

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Abstract

Differential gene expression is an essential response that is reported in crop plants under abiotic stresses. And differential expressions of transcription factors/regulators are another important mechanism that further regulates the expression of various genes under these environments. Although, expression studies on various genes and transcription factors in rice under abiotic stresses have already been reported. However, still there are some transcription factors/regulators which have not been studied extensively. In present study we have selected eight transcription factors/regulator genes and studied their differential expression pattern in some susceptible and tolerant rice cultivars by imposing different abiotic stresses (drought and submergence). For this experiment growing of plant and stress treatment, RNA isolation and cDNA preparation, expression analysis by RT-PCR and Real Time PCR followed by data analysis was performed. Our result revealed that these TF/regulators are differentially expressed under abiotic stresses as well as plant tissues. Expression of *Arabidopsis* RESPONSE REGULATORS Type-B (ARR-B), Vascular plant One Zinc finger protein (VOZ) and GLABROUS1 enhancer-binding protein (GeBP) genes families varies during the different stress conditions. This is the first report that indicates the Real-Time PCR based differential expression analysis of above mentioned TFs/regulators. Obtained data might be useful in determining their role in regulating the expression of various genes as well as in co-expression networking analysis.

Introduction

Drought, cold, high-salinity and heat are major abiotic stresses that severely reduce the yield of crop plant worldwide. Most plants acquire drought, submergence, anoxia, salinity and chilling and freezing tolerance upon prior exposure to different abiotic stresses, a process called stress acclimation, although many agronomically important crops are incapable of stress acclimation (Pasqualina et al. 2012, Viswanathan et al. 2006). Abiotic stress acclimation contains specific regulation of expression of transcription factors and effector genes (Duarte et al. 2012, Shiv et al. 2012). Traditional plant breeding methods to develop abiotic stress tolerance of crops had restricted success due to multigenic nature of stress tolerance (Cattivellia et al. 2008). From the last few years, molecular techniques have been used to understand the role by which plants recognize environmental signals and additional their transmission to cellular mechanism to stimulate adaptive reactions (Oh et al. 2005). This information is crucial for the improvement of normal breeding and transgenic approaches to impart stress tolerance in crops. The characterization of a various genes associated with stress adaptation have done on the basis of physiological and molecular mechanisms of abiotic stress tolerance (Goswami et al. 2013, Garg et al. 2002, Ndamukong et al. 2010).

Rice feeds more than two billion people worldwide and is an important staple food in Asia, where it provides 40–70% of the total food calories consumed. Rice plants are sensitive to various abiotic stresses. Under abiotic stress

conditions, expression of many genes is induced, and their products have important roles in stress responses and tolerance. Progress has been made in understanding the biological roles of regulons especially in submergence and drought stress responses in rice. A number of transcription factors/regulators (TFs) regulate the expression of stress-responsive genes. The role of TFs on gene expression analysis is very important in genome-wide transcript expression profiling for understanding the gene function as well as gene regulation (Schena et al. 1995). Gene expression data are used in forming the gene expression network that is quite complex.

Researchers from all over the world have made great efforts in understanding the mechanisms of responses to abiotic stresses in rice. Here we report some recent studies of abiotic stress responses in rice. A greater understanding of the physiology and molecular biology of stress tolerance may provide a useful platform to improve stress tolerant rice varieties.Reports for differential expression studies on various genes and transcription factors in rice under abiotic stresses are available. It was reported that GeBP is positively regulating the cytokinin pathway in shoot apical meristem (Chevalier et al. 2008) whereas VOZ regulate the genes which involved in pollen development in plant (Mitsuda et al. 2004). ARR-B is also directly involved in various signaling pathways in plants (Tajima et al. 2004). There has been foremost improvement in the past few years connecting explicit members of these families with plant stress responses. Over expression of some transcription factors enhances salt, drought, and cold tolerance in rice. However, there are some transcription factors/regulators in rice about which expression studies have not been studied extensively. Previously we have reported the set of least studied TFs/regulators in rice for which gene expression data are scanty (Kumari et al. 2013). Therefore, expression analysis of these TFs/regulators in rice under abiotic stresses is very much important. In present study we have selected eight transcription factors/regulator genes and studied their differential expression pattern in some susceptible and tolerant rice cultivars by imposing different abiotic stresses (drought and submergence). In this study we focused on three families of transcription factors**:** GeBP (GLABROUS1 enhancer-binding protein), VOZ (Vascular plant One Zinc finger protein) and ARR-B (*Arabidopsis* RESPONSE REGULATORS Type-B).

Materials and Methods

Selection of TFs/regulators

Previously we have reported that still there are some TFs/regulators in rice about which gene expression analysis under abiotic stresses is scanty (Kumari et al. 2013). For gene expression analysis of some TFs/regulators, we have selected only eight TFs/regulators (Table-1) belonging to the Group-3 from phylogeny of nucleotide reported by Kumari et al. (2013).

Growing of Rice Seedlings

Seeds of rice varieties such as IR-64 (submergence susceptible), FR-13A (submergence tolerant), Swarna (Drought susceptible) and Birsa Gora (Drought tolerant) were selected. Seeds were dehusked and surface sterilized in 0.1% $HgCl_2$ for 40 minutes. Sterilized seeds were placed in a filter paper and washed thrice with sterile distilled water and then incubated at 37^0C for 48 h on wet filter paper under dark condition. The growth of germinated rice seedlings was observed after 48 h. These germinated seeds were transplanted into four different soil pots and grown for two weeks. Two weeks old seedlings were subjected to stress treatment. Sampling was done from control as well as stress treated plants.

Submergence treatments

For submergence treatment two weeks old rice seedlings in soil-containing pot were completely submerged in a plastic tank filled with water for 30 h. Sampling of leaf and stem tissues from control and treated rice seedling was done. Samples were stored in liquid nitrogen for further use.

Drought treatment

Two weeks old rice seedlings were used for drought treatment. Drought stress was applied by keeping the leaf and stem tissues on filter paper at room temperature for 30 h. Samples were stored in liquid nitrogen for further use.

Primer Design

3'UTR of above selected eight TFs/regulators were used to design the primer using online software Primer3 (http://frodo.wi.mit.edu/) having amplicon size (≤ 210 bp) (Table-1).

Table-1. Detail list of eight selected TFs/regulator genes and their primer sequences.

Gene name	Locus IDs	Primer	Sequence(5'--3')	Amplicon size	Tm	Cycle
GeBP 1	LOC_Os01g14720.1	1F	TTGTTTCATAGTCCCGTCGAT	199	58	35
		1R	TCAGCTTTGATCCTCATTTCA			
GeBP 2	LOC_Os03g25430.1	2F	TTAGCCTGACAGGGAAGGAA	153	60	35
		2R	AAGCAGAGAATGTGAGGGAGA			
GeBP 3	LOC_Os03g50110.1	3F	GCGGACATACTGGTGGTTTT	183	60	35
		3R	AAAAGGCAATATCCGTAGACGA			
GeBP 4	LOC_Os07g44200.1	4F	CAGGACCAGGTGAAGGCTAC	192	53	35
		4R	GCTAGTTCCAAGCCCAAACA			
GeBP 5	LOC_Os08g36450.1	5F	GTTTTGGAGGGCATTGTCTG	164	51	35
		5R	GCTTCGACATGCCTAATTTTG			
GeBP 6	LOC_Os09g27850.1	6F	GGACAAGGTTGCACATTCCT	154	56	35
		6R	TTGGCGCACATTTAAACAAA			
ARR-B_2	LOC_Os07g49460.1	7F	TGTGTTTGTTGGGTCACTGG	209	55	35
		7R	ACGAGCCTTGTCAATCACCT			
VOZ_2	LOC_Os05g43950.1	8F	AGCTCCTGTTGATGGCAAGT	190	58	35
		8R	GGAAACGGAAAGGCAAATCT			

RNA isolation, cDNA synthesis and reverse transcriptase PCR analysis

Total RNA was isolated from all samples using TRIZOL reagent. RNA quantification was performed using Eppendorf Bio-photometer (Eppendorf, USA) and quality of the RNA was also checked by running the RNA on the gel. Isolated RNA was further subjected to DNAse treatment. DNaseI-treated RNA samples were then reverse transcribed with Oligo (dT) primers using Ominiscript cDNA preparation kit for 60 minutes at 37°C. The cDNA samples were diluted with MilliQ water.

For reverse transcriptase polymerase chain reaction (RT-PCR) analysis, rice actin gene (LOC_Os05g36290) was used as a reference gene. Table-1 shows the list the primer sequences for each gene. For PCR analysis, the reaction mixtures were initially denatured at 94°C for 2 minutes, 94°C for 30 seconds, 53–60°C for 30 seconds, 72°C for 60 seconds, and final extension for 10 min at 72°C (Table-1). The amplified PCR products were electrophoresed in a 2% ethidium bromide stained agarose gel. The images were recorded using a Gel Doc1000Analyzer (Bio-Rad, Richmond, USA).

Real Time PCR for Gene Expression Analysis

To see the differential expression of above selected eight TFs/regulators genes SYBR® Green (Sigma-Aldrich) based Real time-PCR analysis was also performed. PCR reaction was optimized for these genes in a total volume of 10 µl (5 µl of SYBR® Green Mix, 50 ng of cDNA and 0.125 pmol/µl of forward and reverse primer). PCR amplification was carried out at 94°C for 3 minutes; 40–50 cycles of 15 seconds at 94°C, 15 seconds at 60°C, and 35 seconds at 72°C. Here also actin was used as a reference gene. Real-time PCR analysis was performed in Applied Biosystems 7500 Fast Real-Time PCR Systems, USA.

Results and Discussion

Semi-quantitative PCR for the expression analysis of TF/regulator genes under submergence

Gene expression analysis showed that the eight TF/regulator genes have distinct profiles of expression in different tissues under control conditions and also in different abiotic stress conditions (Figure 1(a)). In our research we found that GeBP 1 gene has expressed only in tolerant cultivars like FR-13A either in control or treated both stems and leaves tissues. It indicates that this gene do not shows any response to these stresses rather has some role in making these cultivars tolerant. In GeBP 2 and GeBP 3 have no expression in IR-64 control stems and leaves and uniform expression in FR-13A under stress condition indicating that this gene is up-regulated in all varieties of submergence.

But GeBP 4 there is uniform expression in all samples. Similarly, GeBP 5 and GeBP 6 have variably expressed in all tissues under submergence stress. Curaba et al. (2003) reported that GeBP gene family encodes a nuclear protein with DNA binding activity in *Arabidopsis* (Curaba et al. 2003). Differential expression pattern was observed in ARR-B_2 transcription regulator gene (Figure 1(a)). However, ARR-B_2 was down-regulated under submergence stress condition. Various reports have also confirmed that ARR-B involved in various signalling pathway for gene regulation (Tajima et al. 2004). Similarly, variation in the expression of VOZ_2 TF gene was noticed during the submergence stress. Similarly, expression of genes in rice under various abiotic stresses has been reported previously (Singh et al. 2010, Hazen et al. 2005, Hu et al. 2006, and Jain et al. 2010).

Semi-quantitative PCR for the expression analysis of TF/regulator genes under drought

In drought (Figure 1(b)) we found that GeBP 1 gene has expressed only in tolerant cultivar i.e. in Birsa Gora either in control or treated both stems and leaves tissues. It indicates that this gene do not shows any response to these stresses rather has some role in making these cultivars tolerant. In GeBP 2 and GeBP 3 have uniform expression in shoot tissues and no expression in Swarna-Treated leaves in drought indicating that this gene is up-regulated in shoot part. But GeBP 4 there is uniform expression in shoot tissue samples and no expression in leaves tissues which indicates that this gene do not show drought stress induced expression capacity in leaves. Similarly, GeBP 5 and GeBP 6 have variably expressed in all tissues under drought condition. However, ARR-B_2 was down-regulated and variation in the expression of VOZ_2 TF gene was also noticed during drought.

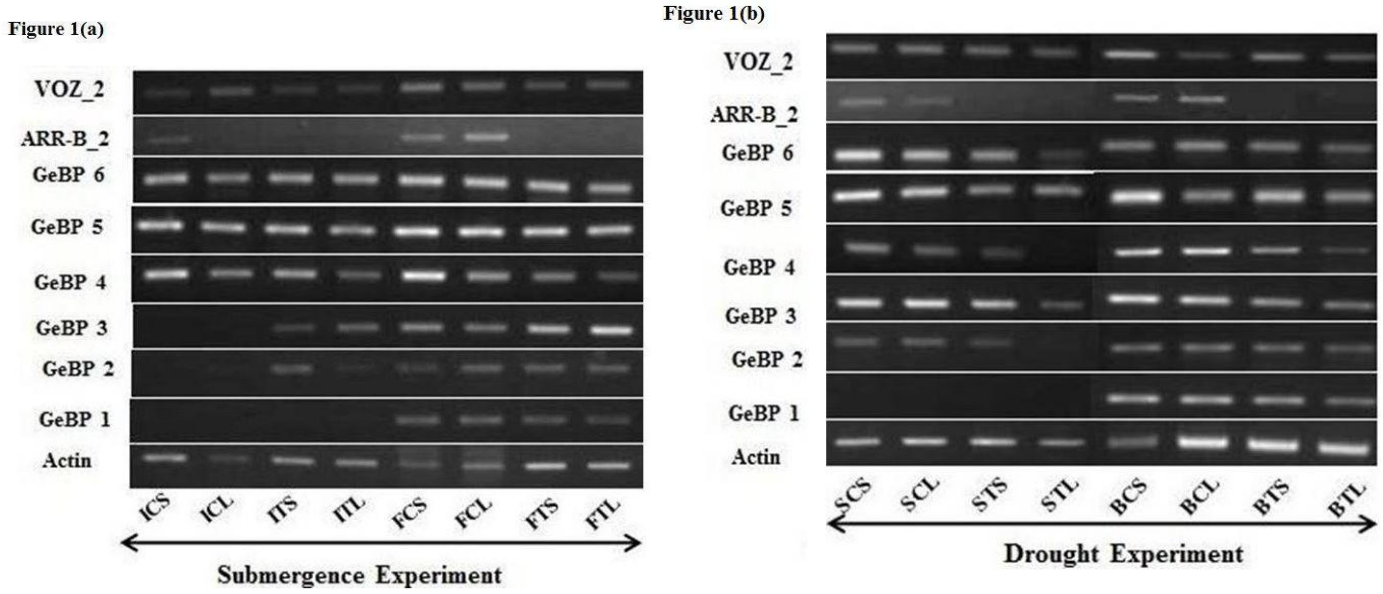

Figure 1(a). Tissue specific expression patterns of eight TFs/regulators genes under submergence. Expression analysis of shortlisted genes in IR-64 (submergence susceptible) and FR-13A (submergence tolerant). The condition for amplification is similar to all genes i.e. at 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 51–60°C (Table1) for 30sec and 72°C for 1 min and ended at 72°C for 5 min and stored at 4°C. Abbreviations: ICS-IR 64 control stem, ITS- IR 64 treated leaves, ICL- IR 64 control leaves, ITL- IR 64 treated leaves, FCS- FR13A control stem, FTS- FR13A treated stem, FCL- FR13A control leaves, FTL- FR13A treated leaves. And **Figure 1(b).** Tissue specific expression patterns of eight TFs/regulators genes under drought. Expression analysis of shortlisted genes in Swarna (Drought susceptible) and Birsa Gora (Drought tolerant). The condition for amplification is similar to all genes i.e. at 94°C for 2 min, followed by 35 cycles of 94°C for 30s, 51–60°C (Table1) for 30sec and 72°C for 1 min and ended at 72°C for 5 min and stored at 4°C. Abbreviations: SCS- Swarna control stem, STS- Swarna treated leaves, SCL- Swarna control leaves, STL- Swarna treated leaves, BCS- Birsa Gora control stem, BTS- Birsa Gora treated stem, BCL- Birsa Gora control leaves, and BTL- Birsa Gora treated leaves.

Real Time PCR analysis for the expression analysis of TF/regulator genes under submergence

Information about differential gene expression studies on above selected TFs/regulators under abiotic stresses is not available. For differential gene expression analysis SYBR® Green based quantitative real-time PCR has been extensively used (Sakamoto et al. 2013). Therefore, comparative differential expression analysis of actin along with

all TFs/regulators genes in leaf and shoot tissues were done on IR64-Control, IR64-Treatment, FR13A-Control and FR13A-Treatment for submergence experiment by calculating delta delta Ct value (ΔΔCt) (Pfaffl et al. 2001). To find statistical significance of data Student t-test was performed for calculating P-value (Pvalue <0.05; t-Test) using R software. Dissociation curves analysis was also performed for all genes showing the gene-specific amplification and no primer dimer formation, contaminating DNA, or PCR product from mis-annealed primer.

Normalization of real time PCR data is a pre-requisite to know the differential expression of TFs genes. Q-Gene tool has been used for data normalization (Simon et al. 2003). Our normalized quantitative Real Time-PCR data analysis showed that eight selected TF/regulator genes have distinctive expression profiles in different tissues under submergence stress (Figure 2(a) and 2(b)). Expression of GeBP 1 was noticed in FR13A (stems and leaves) that indicate that this gene is not submergence-responsive genes. On the other hand GeBP 2 also shows high expression only in FR13A control stem and leaves. Differential expression of GeBP 3 was noticed in IR-64 treated stem and IR-64 control leaves whereas in GeBP 4 expression was noticed in FR-13A control and treated stem. Very low level expression of GeBP 5 gene was noticed in all submergence conditions. Expression of GeBP 6 was seen in the leaves of submergence susceptible and tolerant cultivar indicating that this gene is submergence responsive in the leaves. Similarly, ARR-B_2 gene was expressed in all the samples of FR13A. Some scientist reported that in Arabidopsis ectopically expressing the constitutively active B-type ARR showed alterations in the activity of several plant signaling pathways (Tajima et al. 2004). However, VOZ_2 gene was expressed in stem part of IR-64 (control and treated) as well as FR-13A leaves (control and treated).

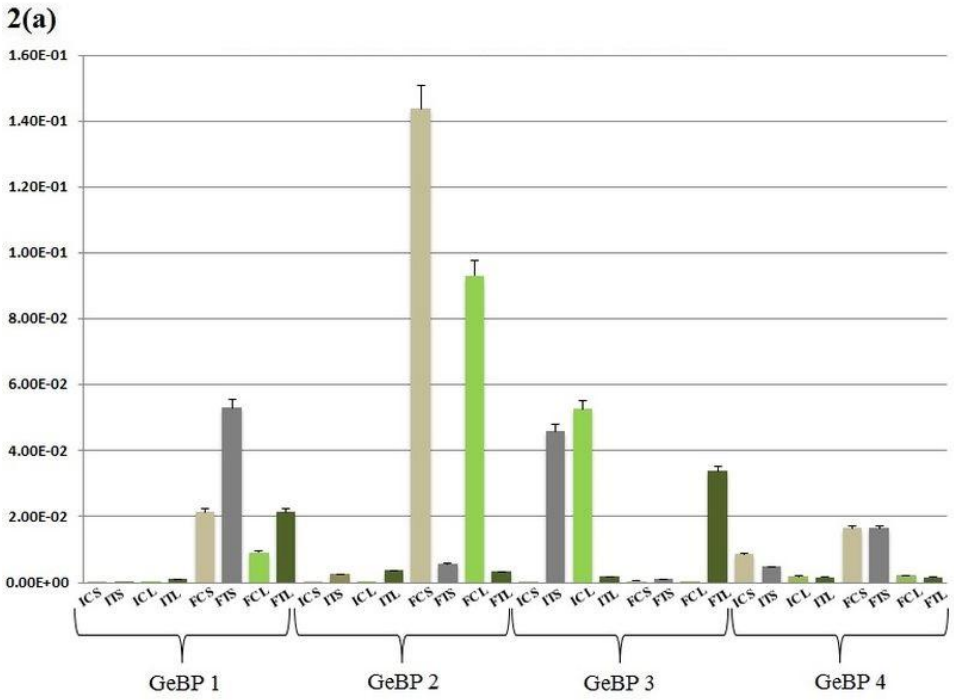

Figure 2(a). Relative expression pattern of four TFs/regulator genes (GeBP 1, GeBP 2, GeBP 3 and GeBP 4) in shoot and root of IR-64 (submergence susceptible) and FR-13A (submergence tolerant) under control and submergence treated condition. Data shown is an average of n = 3 biological replicates ± standard error. There were three technical replicates performed for each biological sample. Abbreviations: ICS-IR 64 control stem, ITS- IR 64 treated leaves, ICL- IR 64 control leaves, ITL- IR 64 treated leaves, FCS- FR 13A control stem, FTS- FR 13A treated stem, FCL- FR 13A control leaves, FTL- FR 13A treated leaves.

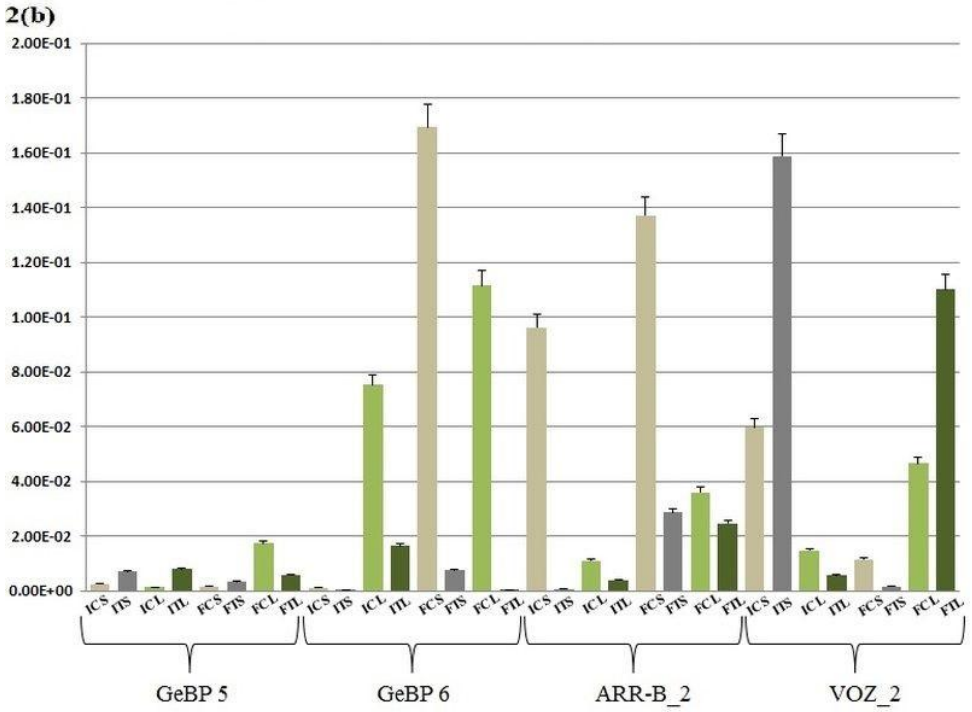

Figure 2(b). Relative expression pattern of four (GeBP 5, GeBP 6, ARR-B_2 and VOZ_2) TF/genes in shoot and leaves of IR-64 (submergence susceptible) and FR-13A (submergence tolerant) under control and submergence treated condition. Data shown is an average of n = 3 biological replicates ± standard error. There were three technical replicates performed for each biological sample. Abbreviations: ICS-IR 64 control stem, ITS- IR 64 treated leaves, ICL- IR 64 control leaves, ITL- IR 64 treated leaves, FCS- FR 13A control stem, FTS- FR 13A treated stem, FCL- FR 13A control leaves, FTL- FR 13A treated leaves.

Real Time PCR analysis for the expression analysis of TF/regulator genes under drought

It is already reported that various environmental stresses induced the expression of a variety of genes in many plant species (Xiong et al. 2002, Bartels et al. 2005, Shinozaki et al. 2003, and Pandey et al. 2012). Similarly, in present study also we observed that in drought condition eight TFs/regulator genes showing distinctive expression pattern (Figure 3(a) and 3(b)). Here we observed slight expression of GeBP 1 gene in Birsa Gora (either in control and treated tissues) whereas no expression in Swarna. GeBP 2 has shown expression in both tissue samples of Birsa Gora and Swarna except Swarna treated leaves which indicate that this gene may have tolerant capacity for drought stress. GeBP 3 gene has shown higher expression in control tissues of either Swarna or Birsa Gora cultivars as compared to the stress treated tissues. Surprisingly, among all eight TFs/regulator genes, GeBP 4 was expressed in both the tissue samples of drought susceptible and drought tolerant cultivar. GeBP 5 gene was highly expressed in control tissue as compared to treated one in both cultivar (Swarna and Birsa Gora) but no expression was seen in Swarna control and treated leaves. GeBP 6 gene was expressed only in Swarna stem (control and treated) while no expression in remaining tissues. ARR-B_2 gene was expressed in only control tissues but no expression in treated tissues. Importantly, VOZ_2 gene show very low level of expression in all tissue samples.

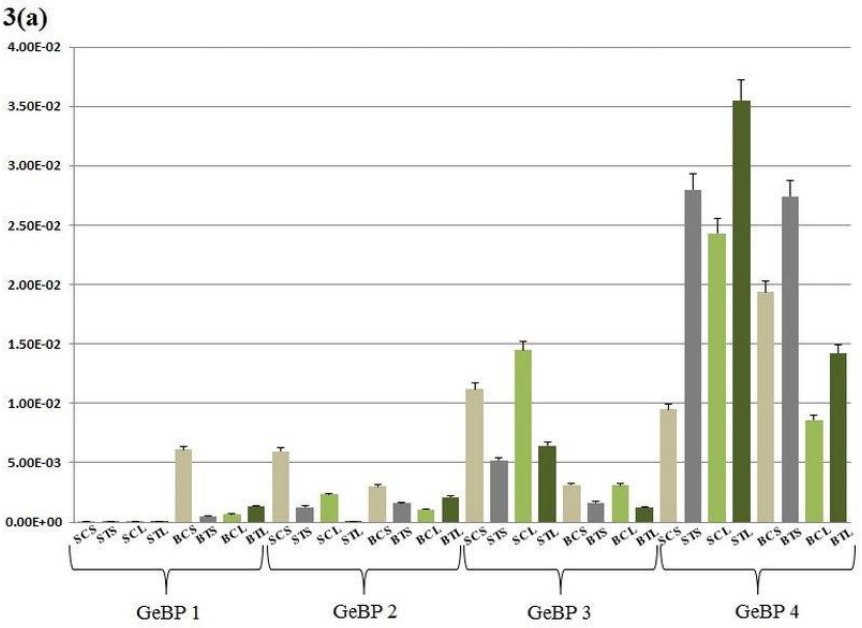

Figure 3(a). Relative expression pattern of four TFs/regulator genes (GeBP 1 to GeBP 4) in shoot and root of Swarna (Drought susceptible) and Birsa Gora (Drought tolerant) under control and drought treated condition. Data shown is an average of n = 3 biological replicates ± standard error. There were three technical replicates performed for each biological sample. Abbreviations: SCS- Swarna control stem, STS- Swarna treated leaves, SCL- Swarna control leaves, STL- Swarna treated leaves, BCS- Birsa Gora control stem, BTS- Birsa Gora treated stem, BCL- Birsa Gora control leaves, and BTL- Birsa Gora treated leaves.

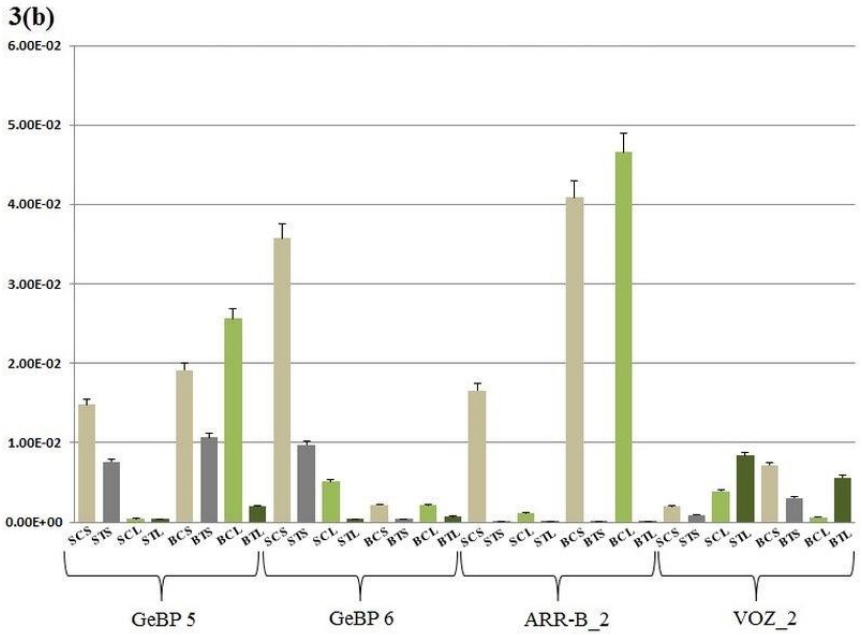

Figure 3(b). Relative expression pattern of four (GeBP 5, GeBP 6, ARR-B_2 and VOZ_2) TF/genes in shoot and leaves of Swarna (Drought susceptible) and Birsa Gora (Drought tolerant) under control and drought treated condition. Data shown is an average of n = 3 biological replicates ± standard error. There were three technical replicates performed for each biological sample. Abbreviations: SCS- Swarna control stem, STS- Swarna treated leaves, SCL- Swarna control leaves, STL- Swarna treated leaves, BCS- Birsa Gora control stem, BTS- Birsa Gora treated stem, BCL- Birsa Gora control leaves, and BTL- Birsa Gora treated leaves.

Abiotic stresses are important factors that affect plant development and yield worldwide. The progress in molecular biology, bioinformatics and other areas of science has significantly improved our understanding of plant responses to stresses. Rice plant is very much susceptible to various abiotic stresses (Xu et al. 2006, Fukao et al. 2008, Shinozaki et al. 2003). During the developmental stages, rice plants recognizes the discreet changes of the surroundings and stimulate signal transduction cascades that in turn motivate stress responsive genes and eventual changes in the physiological and biochemical activities/parameters. The significant roles of many individual TF and their family classes in plant responses to environmental stresses have been well established by studies using genetic and molecular biology approaches that have been reported by various scientist (Singh et al. 2002, Dubouzet et al. 2003).

Varying the expression of TF genes encoding their respective protein can develop stress tolerance in Arabidopsis and rice (Yamaguchi-Shinozaki et al. 2001, Maruyama et al. 2004, Pandey et al. 2013) indicating that transcriptional regulation is one of the crucial mechanisms in defending plants from various environmental stresses. Previous studies also revealed that some genes are regulated by developmental programs (Dortje et al. 2011, Grant et al. 2011) in different abiotic stress conditions in plants. Some genes are constitutively expressed under normal physiological conditions (Hamada et al. 2011, Ramamoorthy et al. 2008).

Expression response of TFs/regulators in shoots and leaves under submergence

We further analyze the quantitative real time PCR data to see the responses of above selected TFs/regulators genes in shoot and leaves under submergence. Here we observed that in IR-64 shoots: GeBP 2, GeBP 3 and VOZ_2 are highly up-regulated whereas GeBP 6 and ARR-B_2 are highly down-regulated. However, in FR13A shoots: GeBP 4, GeBP 6, ARR-B_2 and VOZ_2 are highly down regulated. In FR-13A out of eight genes, six genes are down regulated. GeBP 2 is highly up regulated in IR-64 and moderately down regulated in FR-13A whereas GeBP 4 is moderately up regulated in IR-64 but highly down regulated in FR-13A. Similarly, VOZ-2 is moderately up regulated in IR-64 and moderately down regulated in FR-13A. GeBP 6 and ARR-B_2 both are down regulated in both cultivars. In leaves of IR-64, expression of GeBp 2 and GeBP 5 are highly up-regulated but in FR-13A leaves they are down regulated, whereas GeBP 3 and VOZ_2 are up-regulated in FR-13A and down regulated in IR-64. Only in both cultivars GeBP 1 gene expression showed up-regulation and remaining of the genes are down-regulated (Figure 4(a) and 4(b)). It was reported that GeBP (GLABROUS1 enhancer-binding protein) gene is the founding member of a new plant-specific Arabidopsis TF family. Recently, GeBP and GeBP-like transcription factors were identified that influence the cytokinin response by indirectly affecting the expression levels of type-A ARRBs (Chevalier et al. 2008).

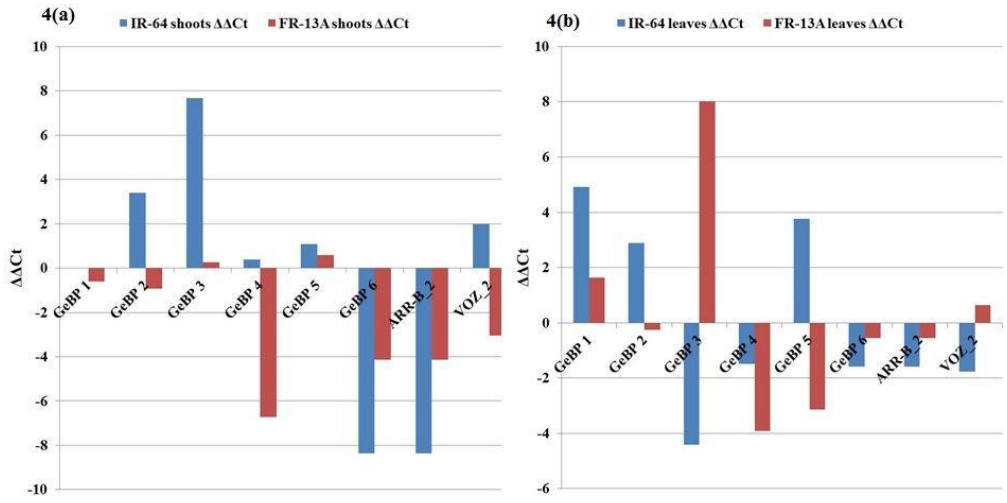

Figure 4(a). Comparative expression analysis of all eight selected TFs/regulators genes in the shoot of IR-64 and FR-13A based on their ΔΔCt values of Real time PCR. And **Figure 4(b)**. Comparative expression analysis of all eight selected TFs/regulators genes in the leaves of IR-64 and FR-13A based on theirΔΔCtvalues of Real time PCR.

Expression response of TFs/regulators in shoots and leaves under drought

We also analyze the quantitative real time PCR data to see the responses of above selected TFs/regulators genes in shoot and leaves under drought. In the shoot of both cultivars i.e. Swarna (Venuprasad et al. 2009) and Birsa Gora (Verulkara et al. 2010) GeBP 4 is up-regulated and in Swarna GeBP 1 is up-regulated and down regulated in Birsa Gora. On the other hand other TFs/regulators genes were down regulated in both the cultivars. The expressions of GeBP 1 and VOZ-2 genes are up-regulated in Swarna. While in Birsa Gora GeBP 2, GeBP 4 and VOZ_2 are up-regulated see the Figure 5 (a) and 5(b). Tyagi et al. (2011) showed the transcriptome analysis of two week-old rice (*Oryza sativa* L. var. IR64) seedling under water deficit stress condition with respect to different TF families like GeBP, jumonji, MBF1 and ULT express differentially under water-deficit conditions (Tyagi et al. 2011).

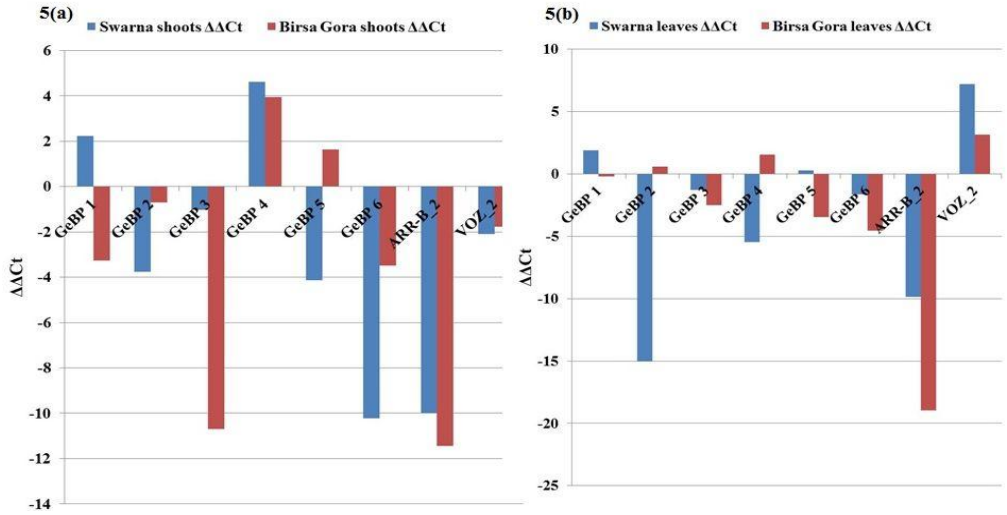

Figure 5(a). Comparative expression analysis of all eight selected TFs/regulators genes in the shoot of Swarna and Birsa Gora based on their ΔΔCtvalues of Real time PCR. And **Figure 5(b)**. Comparative expression analysis of all eight selected TFs/regulators genes in the leaves of Swarna and Birsa Gora based on their ΔΔCt values of Real time PCR.

Hence, from literature it is confirmed that GeBP and VOZ TFs are the stress tolerance mechanism in rice cultivars (Rabbani et al. 2003). Also, ARR-B transcription regulator is affecting the stress tolerance mechanism in rice plant. These three gene families are co-regulating there expression.

Conclusions

The present expression analysis study revealed that the comparative analysis of eight TFs/regulator genes in two different stresses. The selected eight TFs/regulator genes are expressed under normal condition with tissue preference. In the present study, transcripts of the eight genes were accumulated in every tissue under normal condition. It also helped to identify genes which are up regulated in stress conditions. The differential expression patterns of genes are suggesting a highly significant effect on submergence and drought stress. Thus, differential gene expression analysis play key role in providing an excellent resource for stress responsible gene discovery in different abiotic stresses. In the present study, eight screened genes were accumulated in every tissue under normal and abiotic stress conditions, although distinct expression pattern of were reported in various in tissues. Here first time we described the Real-Time PCR based differential expression analysis of above selected TFs/regulators under abiotic stresses. These findings might be useful in determining their role of these TFs/regulators in regulating the expression of various genes as well as in co-expression networking analysis.

Acknowledgements

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