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Dehydration-induced WRKY genes from tobacco and soybean respond to jasmonic acid treatments in BY-2 cell culture

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ABSTRACT

Drought is one of the important environmental factors affecting crop production worldwide and therefore understanding the molecular response of plant to stress is an important step in crop improvement. WRKY transcription factors are one of the 10 largest transcription factor families across the green lineage. In this study, highly upregulated dehydration-induced WRKY and enzyme-coding genes from tobacco and soybean were selected from microarray data for promoter analyses. Putative stress-related cis-regulatory elements such as TGACG motif, ABRE-like elements; W and G-like sequences were identified by an *in silico* analyses of promoter region of the selected genes. GFP quantification of transgenic BY-2 cell culture showed these promoters direct higher expression in-response to 100 μM JA treatment compared to 100 μM ABA, 10% PEG and 85 mM NaCl treatments. Thus promoter activity upon JA treatment and enrichment of MeJA-responsive elements in the promoter of the selected genes provides insights for these genes to be jasmonic acid responsive with potential of mediating cross-talk during dehydration responses.

1. Introduction

Plants are sessile organisms and therefore need to constantly adapt their growth and architecture to cope with various abiotic and biotic stresses imposed by ever-changing environments. In doing so, plants have evolved mechanisms to discriminate prolonged signal from transient background noise so that the right endogenous developmental programs are integrated with specific environmental cues [1]. With their coping mechanisms plants elicit various physiological, biochemical and molecular responses, which enable them to survive during adverse environmental conditions. Plant responses to environmental cues can include changes in gene expression, regulation of protein amount or activity, alteration of metabolite levels and changes in the homeostasis of ions etc. [2]. Understanding how plants respond to these stresses is important since these abiotic stresses have significant impact on crop yield.

Phytohormones play a central role in the ability of plants to adapt to abiotic stresses through a wide range of adaptive mechanisms which include rapid change in the gene expression by inducing or repressing the degradation of transcriptional regulators via the ubiquitin-proteosome system [3,4]. Among the phytohormones, the role of abscisic acid (ABA) is well studied in plant's response to abiotic stress [5,6]. Other hormones such as cytokinin, salicylic acid, ethylene and jasmonic acid have been shown to play direct or indirect roles in plant's response to abiotic stresses [3].

Gene regulation at the mRNA level is considered as one of the major control point in many biological processes. Approximately 7% of the total plant genome constitutes of transcription factors and they are integral part of the gene regulation at mRNA level [7]. One of the 10 largest family of transcription factors across the green lineage are WRKY, named after the highly conserved WRKY domain in the N-terminus [8,9]. Most WRKY transcription factors bind to the W Box motif (T)(T)TGAC(C/T) that is found in the promoter regions of many stress-inducible promoters of their target genes [8–10]. Several studies had reported the role of WRKY transcription factors (TFs) in response to biotic stress but their role in abiotic stress responses has relatively lagged behind [9]. Hence this study was conducted towards understanding role of the WRKY transcription factors in phytohormone signaling network during water stress.

2. Materials and methods

2.1. Phylogenetic analyses

Phylogenetic and molecular evolutionary analyses was performed using MEGAv5 [11]. The 60 amino acid sequences of the WRKY domains of the different tobacco and soybean WRKY TFs

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were generated and used to construct multiple sequence alignments using Clustal W. Using the aligned sequences, phylogenetic tree was produced following neighbor-joining method [12] with settings described in Tripathi et al. [13]. Bootstrap statistical method was also performed using 1000 replicates to provide confidence limits on the generated phylogenetic tree [14].

2.2. Culture of BY-2 and Agrobacterium

Cell suspension culture of tobacco (*Nicotiana tabacum* L.) cv. Bright Yellow-2 (BY-2) were obtained from the University of Virginia (Charlottesville, VA). The suspension cell culture was grown at 26 °C on a rotary shaker in modified Murashige and Skoog (MS) medium (Caisson Labs, USA) supplemented with 0.15 mM KH_2PO_4, 2,4 dichlorophenoxy acetic acid, thiamine, myoinositol and 90 mM sucrose. Cells were subcultured weekly by transferring 1 mL cell culture into 50 mL of fresh medium. Stocks of BY-2 calli were maintained in petri plate with MS medium solidified with 1.5% (w/v) agar and subcultured each month. *Agrobacterium tumefaciens* strain LBA4404 was cultured in yeast extract bactotryptone (YEB) liquid medium and was used for BY-2 transformation.

2.3. Gene selection and promoter constructs

Highly up regulated drought-inducible genes from tobacco and soybean (*Glycine max* L.) oligoarray were selected (Table 1). The promoter region of the selected genes were excised as SacI-HindIII fragments and ligated into the pGPTV-GUS-KAN binary vector [15,16]. The GUS reporter gene was removed from the vector and replaced with a green fluorescent protein (GFP) reporter gene. This vector confers kanamycin resistance to transformed cells.

2.4. Cell transformation

Overnight culture of promoter constructs in *A. tumefaciens* strain LBA4404 was used in the cell transformation. The culture was washed with fresh medium and resuspended in 0.5 mL of MS medium. Three transformation events were done for each constructs using different amounts of *Agrobacterium* (50 μL, 150 μL and 350 μL) added to the BY-2 cell culture. The cell cultures were transformed by co-cultivation with *A. tumefaciens* in a petri dish in the dark for 2 d. The cells were then washed two to three times using 15 mL fresh MS medium. A 100 μL of the cell culture was plated to obtain stable and independent transformants. The cells were plated at low density onto solidified MS agar medium supplemented with vitamins, 500 mg/l cefotaxime and 50 mg/l kanamycin (Gold Biotechnology, USA). Independent cell lines were selected from the microcalli growing on the transformed cell culture and grown in a fresh solidified MS agar medium supplemented with antibiotics and vitamins.

2.5. Cell treatment

Transformed cells grown in solidified agar medium were subcultured into fresh liquid medium for maintenance. After 5–6 d of subculture, each line of transformed cells was subjected to 10% polyethylene glycol (PEG) and 85 mM NaCl (Fisher Scientific, USA). A 0.5 mL of the cells was placed in 4.5 mL of MS medium supplemented with either PEG or NaCl and incubated at 26 °C. Then the cells were harvested by vacuum filtration 6 d after treatment. Treatment with ABA and JA was done following the protocol outlined by Zhang et al. [17]. The different plant hormones were added to the final concentration of 100 μM after 4 d subculture in a medium without 2,4-D and incubated for 24 h. The treated and untreated cells were harvested using vacuum filtration.

2.6. GFP visualization and assay

Slide mounts of the transgenic cell cultures were observed under Olympus AX70 compound microscope attached to Olympus AX70 digital camera (Olympus, USA). All the images of the cells were taken at 20× lens objective with automatic exposure setting for bright field and 5 s exposures setting for the fluorescence. Camera sensitivity was set at ISO 200 and the filter used for GFP viewing of the cells was FITC filter set.

GFP quantification was done using fluorescence assay. Harvested cells were grounded in lysis buffer (Cell BioLabs Inc., USA) supplemented with protease inhibitor (Roche, USA). The standard curve and sample preparations were performed according to the manufacturer's instructions. A 100 μL of lysate was placed in a microplate and the fluorescence was determined using Synergy 2 microplate reader (BioTek, USA) using 485 nm excitation and 507 nm emission filters.

3. Results and discussion

3.1. Phylogenetic analysis of the candidate genes

The WRKY family of transcription factor is divided into three groups (GroupII is subdivided into five groups) depending on the number of WRKY signature sequence at N-terminus and the structure of zinc finger motif at C-terminus [9]. The phylogenetic analysis of members of the WRKY TF family in tobacco and soybean was performed using MEGAv5 [11]. WRKY genes that showed higher fold induction in both crop species were highlighted in the phylogenetic tree to identify possible 'hotspots' that could be responsible to water-stress related response in plants. Student's *t*-test with false discovery rate (FDR) correction using Benjamini-Hochberg method [18] was performed to identify differentially regulated genes with >8-fold induction ($p < 0.05$). Fig. 1 shows that the dehydration-induced differentially regulated genes were present in all groups in the phylogenetic tree. This indicates that no

Table 1
Gene expression in roots and leaves of selected tobacco and soybean genes under different dehydration time course. Values in bold are downregulated fold induction.

Gene	Roots					Leaf				
	20 min	40 min	1 h	2 h	4 h	20 min	40 min	1 h	2 h	4 h
NtWRKY3	2.0	1.6	1.3	1.5	1.7	3.9	8.0	7.1	6.1	3.3
NtWRKY69	2.2	1.8	1.3	1.4	1.4	2.8	8.7	8.6	8.6	3.0
NtWRKY70	2.7	2.8	3.0	3.7	4.0	1.3	5.6	7.5	19.1	32.8
NtWRKY95/21	6.2	8.4	8.3	9.4	9.9	3.3	12.4	9.1	5.5	4.0
NtGolS	2.0	1.9	3.0	5.2	4.5	2.1	1.3	4.2	39.3	143.4
NtRS	1.4	1.3	3.1	14.7	16.3	1.4	1.5	2.3	32.8	170.3
NtUPLL2	8.8	9.5	27.1	66.9	60.6	6.8	44.2	117.0	178.3	254.3
	30 min	*1 h*	*2 h*	*3 h*	*5 h*	*30 min*	*1 h*	*2 h*	*3 h*	*5 h*
GmWRKY-112	2.7	1.6	2.3	3.8	2.9	1.5	5.3	21.6	19.2	6.4

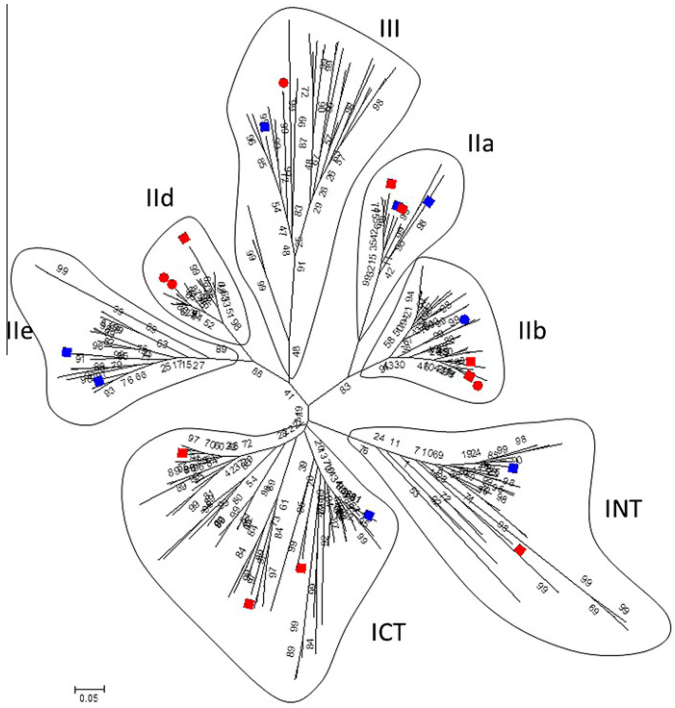

Fig. 1. Evolutionary relationships of WRKY genes in tobacco and soybean. The evolutionary history was inferred using the neighbor-joining method [12]. The bootstrap [14] consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Evolutionary analyses were conducted in MEGA5 [11]. For simplicity, only WRKY genes that showed 8-fold induction were highlighted. Tobacco is shown in red and soybean in blue. Round figures in both colors are the selected WRKY genes for promoter analysis. (For interpretation of color in Fig. 1, the reader is referred to the web version of this article.)

particular group is specifically responsive to dehydration in both crops. The same trend was observed by Ling et al. in cucumber seedlings that were subjected to dehydration [19].

3.2. In silico Promoter analysis of selected genes

Four WRKY genes from tobacco and one from soybean that were highly up regulated in either roots or leaves and also represent the different groups in the WRKY phylogenetic tree were selected for *in silico* analyses of their promoter regions (approx. 1–1.2 kb upstream region from the start of translation). *NtWRKY70* and *GmWRKY112* were selected for analysis since they belong to the same group (GroupIIb) and could be potential orthologs, hence are good candidates for the comparative studies across the two crop species. Also selected were three genes which encode for metabolically important enzymes: galactinol synthase (*NtGolS*; EC 2.4.1.123), raffinose synthase (*NtRS*; EC 2.4.1.82) and ubiquitin protein ligase-like (*NtUPLL2*). These genes were highly up regulated during dehydration in tobacco plants (Table 1). Galactinol and raffinose synthases had been established to be involved in plant response to drought in Arabidopsis [20,21]. *In silico* analyses of the promoter region to identify potential cis-elements were performed using the Plant Cis-acting Regulatory DNA Elements (PLACE) database (http://www.dna.affrc.go.jp/PLACE/) [22]. Supplementary Table 1 shows the different putative cis-regulatory DNA elements identified in the promoter regions of the selected genes. Core promoter elements such as TATA and CAAT boxes were present in all the promoters. An interesting promoter, ASF1MOTIFCAMV (TGACG) was present in promoters of *NtWRKY70, GmWRKY112, NtGolS* and *NtRS*. The motif TGACG has been characterized by Rouster et al. to be methyl jasmonates (MeJA)-responsive element in barley [23]. Among the promoters with TGACG motif, only *NtWRKY70* and *NtRS* have G-box element (CACGTG motif) which is another common feature of MeJA-responsive promoters [24]. Interestingly, W box (TGAC) was found to be present in all the promoters which could indicate a possible protein–protein interaction by another WRKY transcription factor. It has been reported by Wang et al. that a WRKY gene (*BhWRKY1*) induces dehydration tolerance in a resurrection plant (*Boea hygrometrica*) by binding to the W-box elements of galactinol synthase (*BhGolS1*) promoter [25]. Interaction among members of WRKY transcription factors in response to stresses had been reported in several studies [9]. A complex interplay occurs between *AtWRKY18* and its structurally similar family members, *AtWRKY*60 and -80 that modulates their function during pathogen attack [26,27]. Another enrichment in all promoters is for the E-box (CANNTG)-like elements. E-box element is a potential binding site of basic helix-loop-helix (bHLH) family of transcription factors [28]. bHLH is another large family of transcription factors involve in wide array of biological processes such as light signaling [29], hormone signaling [30], and drought response [31]. An abscisic acid-responsive (ABRE) core element ACGT, which is a potential binding site for basic leucine zipper (bZIP) transcription factors [32] and possible binding site of bHLH and NAC transcription factors [5,33], was enriched in the promoter region of *NtWRKY69 NtWRKY70, NtRS, NtUPLL2* and *GmWRKY112*. bZIP transcription factors are involved in ABA-dependent signal transduction pathway in Arabidopsis response to drought and salinity stresses [32].

3.3. Promoter activity in different abiotic stress treatments

In order to validate the significance of the cis-regulatory elements identified in the promoter regions of the selected genes as well as identify other stress treatments that the selected candidates respond, the promoter region of the genes were cloned and transformed in BY-2 cell culture as described in the methods. Transgenic BY-2 cell lines were subjected to different stress treatments (NaCl and PEG) as well as to ABA and JA treatments. Stress treatments (NaCl and PEG) were optimized to identify the right concentration that would maintain healthy growth of BY-2 culture. Several concentrations of NaCl (85 mM, 128 mM, 170 mM and 256 mM) and PEG (5%, 10% and 30%) were used and thereafter 85 mM NaCl and 10% PEG were identified to be the optimum condition for BY-2 culture. Fig. 2 shows the level of GFP expression driven by the different promoters as the transgenic cell lines were subjected to the different treatments. Treatment with 10% PEG showed increase in GFP intensity in all promoters. Although the change in GFP activity after the culture were treated with 10% PEG was not that far above from the control with 3x fold change as the highest which was observed in *ProNtWRKY70:GFP* and *ProNtGS:GFP* (Fig. 2A). The same observation can be told in the response of the different promoters to NaCl treatment except for *ProGmWRKY112:GFP* which showed 9× fold induction (Fig. 2B). Fig. 3 shows the GFP in the cells when subjected to PEG and NaCl. To determine if these promoters respond to application of exogenous phytohormones, we have treated the culture with 100 μM of ABA and JA. Application of exogenous ABA did not result to significant increase in GFP activity (Fig. 2C) even in *ProNtWRKY69:GFP ProNtWRKY70:GFP, ProNtRS:GFP*, *ProNtUPLL2:GFP* and *ProGmWRKY112:GFP*, where its promoter contains ABRE element. Several studies have already established the role of ABA in plant response to drought, however, these selected WRKY genes and enzyme-coding genes seem to be not responding to ABA. This indicates that these genes might be involved in ABA-independent signaling network. Plant response to abiotic stress such as drought and high salinity involved two independent pathways:

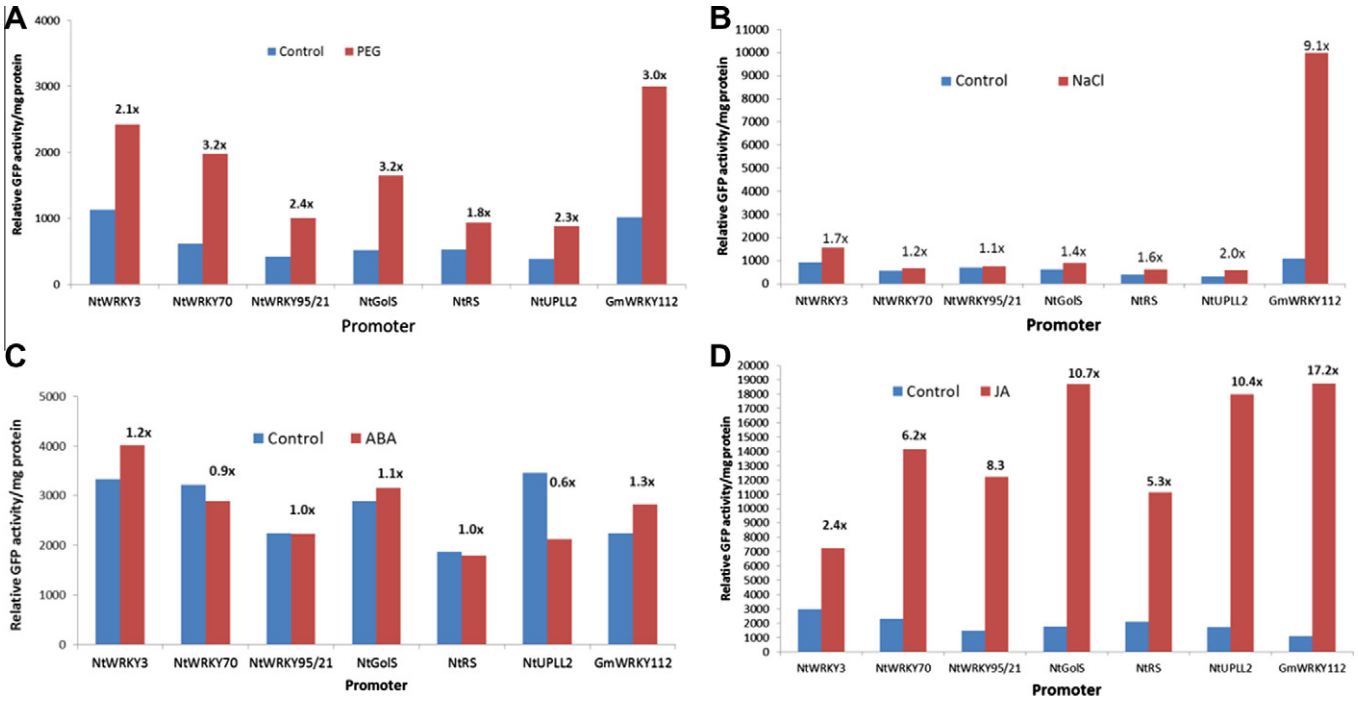

Fig. 2. GFP activity (total fluorescence) of the different promoters subjected to various treatments: (A) 10% PEG, (B) 85 mM NaCl, (C) 100 uM ABA, and (D) 100 uM JA.

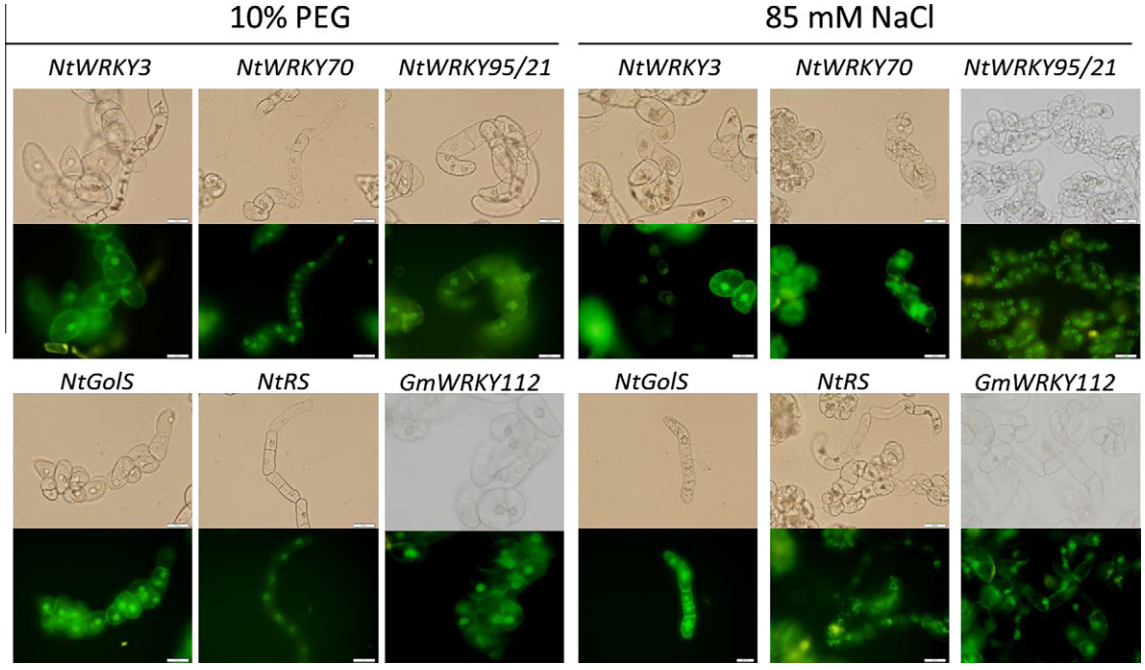

Fig. 3. GFP activity of the different promoter: GFP constructs in transgenic BY-2 cell culture subjected to 10% PEG and 85 mM NaCl.

ABA-dependent and ABA-independent pathway [20]. Another possible reason could be the absence of coupling elements (CEs) or another copy of ABRE in the promoter region. Shen et al. demonstrated that an ACGT box and CE3 are essential to have a successful ABA-induced gene expression in barley [34]. A genome-wide analysis of CE3 and ABRE elements in Arabidopsis and rice showed that CE3 is practically absent in Arabidopsis genome, however ABRE–ABRE pairs are common in both genomes which could form a functional ABA-responsive complexes (ABRC) [35]. Previous studies of Straub et al. showed that at least two copies of ABRE elements are necessary to have a successful ABA-induced gene expression of barley *HVA*1 [36]. Thus a detailed promoter dissection of these candidate genes will provide novel insights towards detailed understanding of ABA mediated gene regulation in these two crop plants.

Compared to ABA, the application of JA showed a higher response in GFP activity almost in all promoters (Fig. 3D). Except for *ProNtWRKY3:GFP* other promoters in tobacco showed at least 5× fold change with *ProNtGolS:GFP* showing highest GFP activity at 11× fold induction while in soybean *ProGmWRKY112:GFP* shows

~17× fold induction upon JA treatment. Promoter analysis of *NtGolS* showed it has TGACG motif that is MeJA-responsive element but do not contain the G-box element which is a common feature of MeJA-responsive promoters [24]. JA has been established to be the plant's hormonal response to herbivores and wounding [37].

Our results have shown that dehydration-induced WRKY transcription factors show significant response on JA treatment and hence present data suggests that there could be involvement of these candidates in biotic responses, due to potential crosstalk between biotic and abiotic stress signaling networks. However, these responses could be due to post dehydration responses as well. But, the promoter activity of the candidates being ABA-independent suggests that there is potential of cross talk to regulate the gene expression at mRNA level. Phytohormone level measurements (Tripathi et al., unpublished) showed an increase in jasmonic acid concentration in root tissue under dehydration in soybean. JA (155× fold) and JA-isoleucine (958× fold) accumulation was observed after 5 h of dehydration compared to well-watered control in soybean root tissues. These findings provide insights towards regulation of gene expression via potential promoters during dehydration with possible phytohormone crosstalk. The response of selected candidates during dehydration and enrichment in MeJA responsive elements provides support towards our hypothesis of crosstalk at mRNA level that could play an important role in regulating water-stress responses. Utilization of BY-2 cell culture provides system of rapid analysis of regulatory elements during dehydration that can be further explored for successful dissection of promoter elements playing important role in crosstalk. Detailed analysis of the promoter architecture reveals presence of putative binding sites of different potential targets suggests possibility of protein–protein interaction. These interactions could play an integral part in regulating these responses during stress and crosstalk. The role of WRKY transcription factors in plant defense signaling is well established while their involvement in regulation and understanding its specific role in the crosstalk between biotic and abiotic stress signal transductions demands in-depth analysis of modules and regulons with promoter dissection. Thus, our study provides insights towards understanding crosstalk between abiotic and biotic stresses with some suitable candidates in two economically important crop species.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.156.

References

[1] Y. Jaillais, J. Chory, Unraveling the paradoxes of plant hormone signaling integration, Nat. Struct. Mol. Biol. 17 (2010) 642–645.
[2] F. Eyidogan, M.T. Oz, M. Yucel, H.A. Oktem, Signal transduction of phytohormones under abiotic stresses phytohormones and abiotic stress tolerance in plants, in: N.A. Khan, R. Nazar, N. Iqbal, N.A. Anjum (Eds.), Springer, Berlin Heidelberg, 2012, pp. 1–48.
[3] Z. Peleg, E. Blumwald, Hormone balance and abiotic stress tolerance in crop plants, Curr. Opin. Plant Biol. 14 (2011) 290–295.
[4] A. Santner, M. Estelle, The ubiquitin-proteasome system regulates plant hormone signaling, Plant J. 61 (2010) 1029–1040.
[5] K. Yamaguchi-Shinozaki, K. Shinozaki, Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses, Annu. Rev. Plant Biol. 57 (2006) 781–803.
[6] N. Tuteja, Abscisic acid and abiotic stress signaling, Plant Signal. Behav. 2 (2007) 135–138.
[7] M.K. Udvardi, K. Kakar, M. Wandrey, O. Montanari, J. Murray, A. Andriankaja, J.Y. Zhang, V. Benedito, J.M. Hofer, F. Chueng, C.D. Town, Legume transcription factors: global regulators of plant development and response to the environment, Plant Physiol. 144 (2007) 538–549.
[8] B. Ulker, I.E. Somssich, WRKY transcription factors: from DNA binding towards biological function, Curr. Opin. Plant Biol. 7 (2004) 491–498.
[9] P.J. Rushton, I.E. Somssich, P. Ringler, Q.J. Shen, WRKY transcription factors, Trends Plant Sci. 15 (2010) 247–258.
[10] T. Eulgem, P.J. Rushton, S. Robatzek, I.E. Somssich, The WRKY superfamily of plant transcription factors, Trends Plant Sci. 5 (2000) 199–206.
[11] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, Mol. Biol. Evol. 28 (2011) 2731–2739.
[12] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.
[13] P. Tripathi, R.C. Rabara, T.J. Langum, A.K. Boken, D.L. Rushton, D.D. Boomsma, C.I. Rinerson, J. Rabara, R.N. Reese, X. Chen, J.S. Rohila, P.J. Rushton, The WRKY transcription factor family in brachypodium distachyon, BMC Genomics 13 (2012) 270.
[14] J. Felsenstein, Confidence limits on phylogenies: an approach using the bootstrap, Evolution 39 (1985) 783–791.
[15] D. Becker, E. Kemper, J. Schell, R. Masterson, New plant binary vectors with selectable markers located proximal to the left T-DNA border, Plant Mol. Biol. 20 (1992) 1195–1197.
[16] P.J. Rushton, A. Reinstadler, V. Lipka, B. Lippok, I.E. Somssich, Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signaling, Plant Cell 14 (2002) 749–762.
[17] H.B. Zhang, M.T. Bokowiec, P.J. Rushton, S.C. Han, M.P. Timko, Tobacco transcription factors NtMYC2a and NtMYC2b form nuclear complexes with the NtJAZ1 repressor and regulate multiple jasmonate-inducible steps in nicotine biosynthesis, Mol. Plant 5 (2012) 73–84.
[18] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate-a practical and powerful approach to multiple testing, J. R. Stat. Soc., B Methodol. 57 (1995) 289–300.
[19] J. Ling, W. Jiang, Y. Zhang, H. Yu, Z. Mao, X. Gu, S. Huang, B. Xie, Genome-wide analysis of WRKY gene family in *Cucumis sativus*, BMC Genomics 12 (2011) 471.
[20] K. Shinozaki, K. Yamaguchi-Shinozaki, Gene networks involved in drought stress response and tolerance, J. Exp. Bot. 58 (2007) 221–227.
[21] T. Taji, C. Ohsumi, S. Iuchi, M. Seki, M. Kasuga, M. Kobayashi, K. Yamaguchi-Shinozaki, K. Shinozaki, Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana, Plant J. 29 (2002) 417–426.
[22] K. Higo, Y. Ugawa, M. Iwamoto, T. Korenaga, Plant cis-acting regulatory DNA elements (PLACE) database: 1999, Nucleic Acids Res. 27 (1999) 297–300.
[23] J. Rouster, R. Leah, J. Mundy, V. Cameron-Mills, Identification of a methyl jasmonate-responsive region in the promoter of a lipoxygenase 1 gene expressed in barley grain, Plant J. 11 (1997) 513–523.
[24] B. Xu, M. Timko, Methyl jasmonate induced expression of the tobacco putrescine *N*-methyltransferase genes requires both G-box and GCC-motif elements, Plant Mol. Biol. 55 (2004) 743–761.
[25] Z. Wang, Y. Zhu, L. Wang, X. Liu, Y. Liu, J. Phillips, X. Deng, A WRKY transcription factor participates in dehydration tolerance in boea hygrometrica by binding to the W-box elements of the galactinol synthase (BhGolS1) promoter, Planta 230 (2009) 1155–1166.
[26] T. Eulgem, Dissecting the WRKY web of plant defense regulators, PLoS Pathog. 2 (2006) e126.
[27] X.P. Xu, C.H. Chen, B.F. Fan, Z.X. Chen, Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors, Plant Cell 18 (2006) 1310–1326.
[28] L. Carretero-Paulet, A. Galstyan, I. Roig-Villanova, J.F. Martinez-Garcia, J.R. Bilbao-Castro, D.L. Robertson, Genome-wide classification and evolutionary analysis of the bHLH family of transcription factors in Arabidopsis, poplar, rice, moss, and algae, Plant Physiol. 153 (2010) 1398–1412.
[29] P. Leivar, E. Monte, B. Al-Sady, C. Carle, A. Storer, J.M. Alonso, J.R. Ecker, P.H. Quail, The Arabidopsis phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels, Plant Cell 20 (2008) 337–352.
[30] S. Lee, S. Lee, K.Y. Yang, Y.M. Kim, S.Y. Park, S.Y. Kim, M.S. Soh, Overexpression of PRE1 and its homologous genes activates Gibberellin-dependent responses in Arabidopsis thaliana, Plant Cell. Physiol. 47 (2006) 591–600.
[31] H. Abe, T. Urao, T. Ito, M. Seki, K. Shinozaki, K. Yamaguchi-Shinozaki, Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling, Plant Cell 15 (2003) 63–78.
[32] Y. Uno, T. Furihata, H. Abe, R. Yoshida, K. Shinozaki, K. Yamaguchi-Shinozaki, Arabidopsis basic leucine zipper transcription factors involved in an abscisic

acid-dependent signal transduction pathway under drought and high-salinity conditions, Proc. Natl. Acad. Sci. USA 97 (2000) 11632–11637.
[33] R.A. Ingle, U.G. Schmidt, J.M. Farrant, J.A. Thomson, S.G. Mundree, Proteomic analysis of leaf proteins during dehydration of the resurrection plant Xerophyta viscosa, Plant Cell Environ. 30 (2007) 435–446.
[34] Q. Shen, P. Zhang, T.H. Ho, Modular nature of abscisic acid (ABA) response complexes: composite promoter units that are necessary and sufficient for ABA induction of gene expression in barley, Plant Cell 8 (1996) 1107–1119.
[35] J.L. Gomez-Porras, D.M. Riano-Pachon, I. Dreyer, J.E. Mayer, B. Mueller-Roeber, Genome-wide analysis of ABA-responsive elements ABRE and CE3 reveals divergent patterns in Arabidopsis and rice, BMC Genomics 8 (2007) 260.
[36] P.F. Straub, Q. Shen, T.D. Ho, Structure and promoter analysis of an ABA- and stress-regulated barley gene, HVA1, Plant Mol. Biol. 26 (1994) 617–630.
[37] C. Wasternack, Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development, Ann. Bot. 100 (2007) 681–697.