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Prediction of direct targets of Arabidopsis RRTF1, a H_2O_2 responsive AP2/ERF transcription factor which mediates multiple stress responses

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REDOX RESPONSIVE TRANSCRIPTION FACTOR1 (RRTF1) is an AP2/ERF type transcription factor regulating hundreds of stress responsive genes. Previous studies revealed that regulation of *RRTF1* expression was involved in various stress responses and also ROS homeostasis. In this report, we predict direct targets of RRTF1 among genes regulated by RRTF1 overexpression and also H_2O_2, using transcriptome data of *RRTF1* overexpressors with the aid of a reported GCC-box like binding site from the *RAP2.6* promoter. Direct targets of RRTF1 included the predicted genes for jasmonic acid/ethylene signaling, biotic and abiotic stress responses, and growth control via brassinosteroid signaling.

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Introduction

Arabidopsis REDOX RESPONSIVE TRANSCRIPTION FACTOR1 (RRTF1) belongs to the group X of ERF family (Nakano et al. 2006). This transcription factor was first identified as a redox responsive gene whose activation was suppressed by 3–(3,4–dichlorophenyl)–1,1–dimethylurea (DCMU) (Khandelwal et al. 2008). Subsequent studies revealed that expression of *RRTF1* was activated by jasmonic acid (JA) (Wang et al. 2008) and also by high light stress, infection by *Altenaria brassicae*, and H_2O_2 (Matsuo et al. 2015).

Studies on upstream factors of *RRTF1* expression revealed that *RRTF1* expression was regulated by WRKY40, which targeted at a W box in the *RRTF1* promoter (Pandey et al. 2010). WRKY40 had functional redundancy with WRKY18 and WRKY60 (Xu et al. 2006), and *wrky40/wrky18* double mutants showed enhanced transcriptional activation of *RRTF1* during infection of powdery mildew (*Golovinomyces orontii*), providing evidence of WRKY18/40 as negative regulators of *RRTF1* (Pandey et al. 2010).

Downstream events after activation of *RRTF1* included accumulation of H_2O_2 and transcriptional activation of more than 800 genes, which were identified by analysis of *RRTF1* overexpressors (RRTF1ox) (Matsuo et al. 2015). The former event is supposed to have a role in amplification of the H_2O_2 signal, which is necessary for long distance signaling.

Our trials for identification of direct target genes of RRTF1 was not easy, because the over 800 genes included both direct and indirect targets. After examinations of 6 sites in the promoter of the downstream genes, one site in the *RAP2.6* promoter showed sequence-specific binding to the RRTF1 protein *in vitro*. The identified RRTF1 target site contained a GCC-box-like sequence, TGACGGCT.

In this study, we performed more precise prediction of direct targets of RRTF1 based on microarray data of the RRTF1ox and of the H_2O_2 response. Utilization of information of the identified RRTF1 binding sequence was expected to significantly enhance prediction accuracy.

Results and Discussion

A scheme for prediction of direct targets of RRTF1 is shown in Figure 1. Microarray data of RRTF1ox (Matsuo et al. 2015) was used for promoter prediction. Our prediction method used in this study (Yamamoto et al. 2011) detected overrepresented octamer sequences in the RRTF1-activated promoters over all the promoters in the genome. Positive octamers (RAR >3) were searched for 21 H_2O_2-responsive RRTF1 regulated promoters (Venn diagram in Figure 1) and then subjected to multiple alignments (Figure 1). The

resultant clusters were composed of homologous sequences with the binding sequences of RRTF1. The homologous sequences as putative direct targets of RRTF1 and the corresponding promoters of *AT1G10585*, *IGMT1*, *VQ12*, *PDF1.2b*, *AZI1*, and *TCH4,* in addition to *RAP2.6*, were shown in Table 1 and Figure 2.

VQ12 contains a VQ motif which has binding activity to WRKY transcription factors and acts as their regulator (Cheng et al. 2012). Potential binding targets of VQ12 were WRKY20, WRKY23, and WRKY24 (Dreze et al. 2011). Previous study showed that VQ12 is related to COI1-mediated to JA/ethylene (ET) signaling and acts as a negative regulator of basal defense against *Botrytis cinerea* (Wang et al. 2015). WRKY23 is a negative regulator of auxin transport and an enhancer of local flavonol production for proper root growth and stem cell specification (Grunewald et al. 2012, 2013). The functions of WRKY20 and WRKY24 have not been well studied.

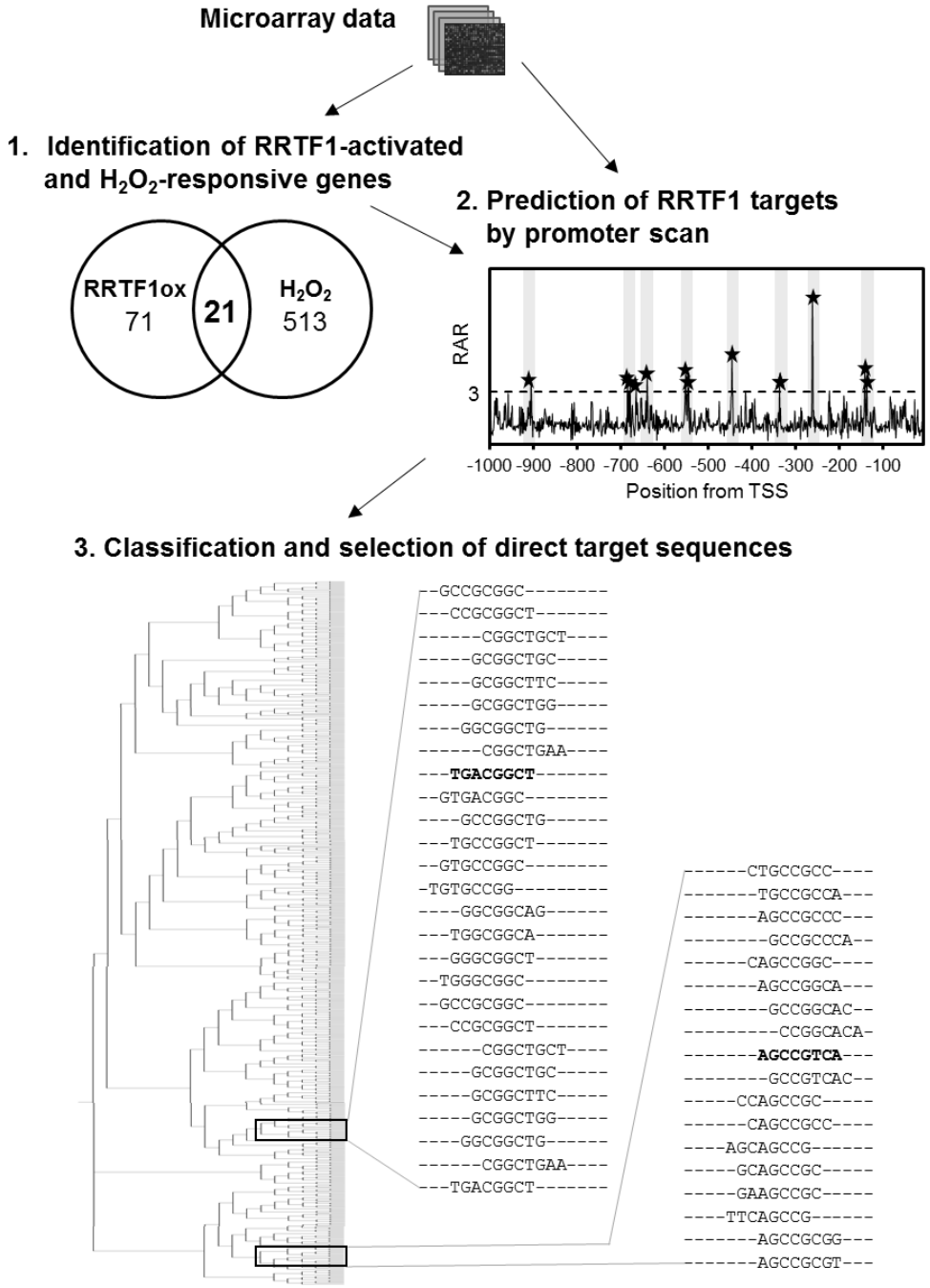

Figure 1: Outline for prediction of direct target sequences of RRTF1. 1. The full Venn diagram shows the number of expressed genes (fold change ≧5 for RRTF1 overexpressors (RRTF1ox) and ≧2.5 for H_2O_2) identified by microarray analyses (Matsuo et al. 2015). 2. The full microarray data of RRTF1ox was also used for the prediction of *cis*-elements (Yamamoto et al. 2011). 3. Predicted octamers (RAR >3) found in the 21 promoters were subjected to multiple alignment analyses. Two clusters containing a reported direct target sequence in the *RAP2.6* promoter, TGACGGCT, (Matsuo et al. 2015), and its complementary sequence, TGACGGCT and AGCCGTCA (both of which are shown in bold) were selected as putative direct targets of RRTF1.

AZI1 functions in defense priming and systemic plant immunity (Atkinson et al. 2013; Jung et al. 2009), and also in tolerance to salt (Pitzschke et al. 2014) and cold stresses (Xu et al. 2011). Because *AZI1* gene expression was elevated in *cat2* plants, accumulation of intracellular H_2O_2 was suggested to up-regulate *AZI1* expression. In *cat2 sid2* plants this activation was not detected, therefore, it was proposed that *AZI1* activation requirs not only H_2O_2 but also salicylic acid (SA) (Chaouch et al. 2010).

PDF1.2b is one of the marker genes for JA/ET signaling in defense responses (Brown et al. 2003). In a previous study, the ROSE7/GCC box was detected as a H_2O_2-responsive *cis*-element in the promoter of *PDF1.2b* (Wang et al. 2013). The ROSE7/GCC box is similar to the complementary sequence of the RRTF1 target (Matsuo et al. 2015).

INDOLE GLUCOSINOLATE O-METHYLTRANSFERASE1 (IGMT1) is an enzyme for indole glucosinolate biosynthesis which is produced in crucifer plants and required for defense against herbivorous insects, pathogens and other pests (Pfalz et al. 2011).

TCH4 has a role in growth regulation. TCH4 encodes a xyloglucan endotransglucosylase/hydrolase for modification of the cell wall structure in response to touch, darkness, cold, heat, and auxin (Iliev et al. 2002; Lee et al. 2004). The TCH4 is supposed to function in morphological modification in response to stress conditions.

AT1G10585 encodes a bHLH transcription factor, and its function is not reported.

Our results suggest a possible role of RRTF1 as a branching point from the H_2O_2 signal to the JA/ET signaling pathway for various stress tolerance responses, systemic signaling, indole glucosinolate biosynthesis, and morphological modifications through direct activation of the identified target genes predicted in this study.

Table 1: Putative direct target genes of RRTF1. Among 21 genes which were induced by both RRTF1ox and H_2O_2, these with putative direct target octamers in their promoter region -1000 to -1 are shown in Figure 1 and fully predicted RRTF1 target sequences are listed here. Position from transcription start site is the first base of each octamer sequence (5' end of the octamer). When the octamers contain GCCG, the complementary sequence of the RRTF1 target in the *RAP2.6* promoter is shown ("comp").

AGI	Description	Predicted RRTF1 target sequence (5'-3')		
AT1G10585	bHLH DNA-binding superfamily	-790	GAATGCCG	comp
AT1G21100	*IGMT1* (INDOLE GLUCOSINOLATE O-METHYLTRANSFERASE1)	-364 -363 -133 -131 -130 -129 -128	CTGCCGCC TGCCGCCA TTCAGCCG CAGCCGGC AGCCGGCA GCCGGCAC CCGGCACA	comp comp comp - - - -
AT1G43160	*RAP2.6*, AP2/ERF transcription factor	-79	TGACGGCT	*
AT2G22880	*VQ12*, VQ motif-containing protein	-954 -953 -900 -899 -896	AGCCGTCA GCCGTCAC AGCCGCGG GCCGCGGC GCGGCTTC	comp comp comp - -
AT2G26020	*PDF1.2b* (PLANT DEFENSIN1.2b)	-256 -255 -254 -253 -234 -233	CCAGCCGC CAGCCGCC AGCCGCCC GCCGCCCA AGCAGCCG GCAGCCGC	comp comp comp comp comp comp
AT4G12470	*AZI1* (AZELAIC ACID INDUCED1)	-366	AGCCGTCA	comp
AT5G57560	*TCH4* (TOUCH4), xyloglucan endotransglucosylase	-109 -107	ACGCGGCT GCGGCTTC	- -

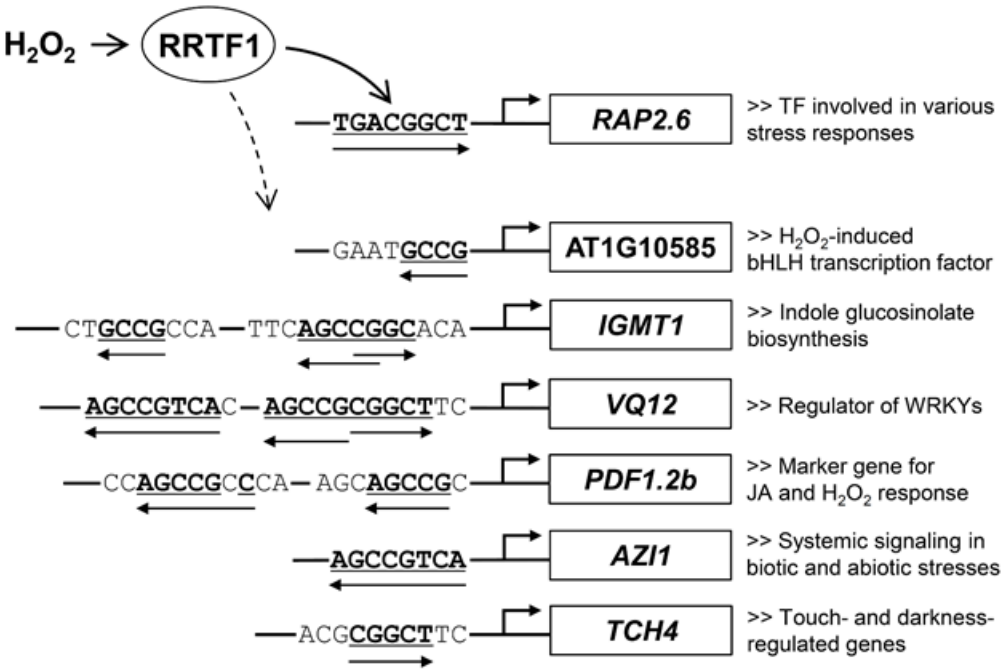

Figure 2: Putative direct targets of RRTF1 and their function. Target sequences of RRTF1 in the promoters of downstream genes are shown. The target sequences contained either CGGC or GCCG as a core sequence, and those with a CGGC motif are marked by an arrow from left to right, while those with a GCCG motif by an arrow from right to left. Bold bases are underlined and indicate a match with TGACGGCT, the RRTF1 target in the *RAP2.6* promoter.

References

Atkinson NJ, Lilley CJ, Urwin PE. (2013) Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stresses. *Plant Physiol.* 162:2028–2041.

Brown RL, Kazan K, McGrath KC, Maclean DJ, Manners JM. (2003) A role for the GCC-box in jasmonate-mediated activation of the *PDF1.2* gene of Arabidopsis. *Plant Physiol.* 132:1020–1032.

Chaouch S, Queval G, Vanderauwera S, Mhamdi A, Vandorpe M, Langlois-Meurinne M, Van Breusegem F, Saindrenan P, Noctor G. (2010) Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. *Plant Physiol.* 153:1692–1705.

Cheng Y, Zhou Y, Yang Y, Chi YJ, Zhou J, Chen JY, Wang F, Fan B, Shi K, Zhou YH, Yu JQ, Chen Z. (2012) Structural and functional analysis of VQ motif-containing proteins in Arabidopsis as interacting proteins of WRKY transcription factors. *Plant Physiol.* 159:810–825.

Dreze M, Carvunis AR, Charloteaux B, Galli M, Pevzner SJ, Tasan M, Ahn YY, Balumuri P, Barabasi AL, Bautista V, Braun P, Byrdsong D, Chen H, Chesnut JD, Cusick ME, Dangl JL, de los Reyes C, Dricot A, Duarte M, Ecker JR, Fan C, Gai L, Gebreab F, Ghoshal G, Gilles P, Gutierrez BJ, Hao T, Hill DE, Kim CJ, Kim RC, Lurin C, MacWilliams A, Matrubutham U, Milenkovic T, Mirchandani J, Monachello D, Moore J, Mukhtar MS, Olivares E, Patnaik S, Poulin MM, Przulj N, Quan R, Rabello S, Ramaswamy G, Reichert P, Rietman EA, Rolland T, Romero V, Roth FP, Santhanam B, Schmitz RJ, Shinn P, Spooner W, Stein J, Swamilingiah GM, Tam S, Vandenhaute J, Vidal M, Waaijers S, Ware D, Weiner EM, Wu S, Yazaki J. (2011) Evidence for network evolution in an Arabidopsis interactome map. *Science.* 333:601–607.

Grunewald W, Smet ID, Lewis DR, Löfke C, Jansen L, Goeminne G, Bossche RV, Karimi M, Rybel BD, Vanholme B, Teichmann T, Boerjan W, Montagu MCEV, Gheysen G, Muday GK, Friml J, Beeckman T. (2012) Transcription factor WRKY23 assists auxin distribution patterns during Arabidopsis root development through local control on flavonol biosynthesis. *Proc Natl Acad Sci USA.* 109:1554–1559.

Grunewald W, De Smet I, De Rybel B, Robert HS, van de Cotte B, Willemsen V, Gheysen G, Weijers D, Friml J, Beeckman T. (2013) Tightly controlled *WRKY23* expression mediates Arabidopsis embryo development. *EMBO Rep.* 14:1136–1142.

Iliev EA, Xu W, Polisensky DH, Oh MH, Torisky RS, Clouse SD, Braam J. (2002) Transcriptional and posttranscriptional regulation of Arabidopsis *TCH4* expression by diverse stimuli. Roles of cis regions and brassinosteroids. *Plant Physiol.* 130:770–783.

Jung HW, Tschaplinski TJ, Wang L, Glazebrook J, Greenberg JT. (2009) Priming in systemic plant immunity. *Science.* 324:89–91.

Khandelwal A, Elvitigala T, Ghosh B, Quatrano RS. (2008) Arabidopsis transcriptome reveals control circuits regulating redox homeostasis and the role of an AP2 transcription factor. *Plant Physiol.* 148:2050–2058.

Lee D, Polisensky DH, Braam J. (2004) Genome-wide identification of touch- and darkness-regulated Arabidopsis genes: a focus on calmodulin-like and *XTH* genes. *New Phytol.* 165:429–444.

Matsuo M, Johnson JM, Hieno A, Tokizawa M, Nomoto M, Tada Y, Godfrey R, Obokata J, Sherameti I, Yamamoto YY, Bohmer FD, Oelmuller R. (2015) High REDOX RESPONSIVE TRANSCRIPTION FACTOR1 levels result in accumulation of reactive oxygen species in *Arabidopsis thaliana* shoots and roots. *Mol Plant.* 8:1253-1273.

Nakano T, Suzuki K, Fujimura T, Shinshi H. (2006) Genome-wide analysis of the *ERF* gene family in Arabidopsis and rice. *Plant Physiol.* 140:411–432.

Pandey SP, Roccaro M, Schon M, Logemann E, Somssich IE. (2010) Transcriptional reprogramming regulated by WRKY18 and WRKY40 facilitates powdery mildew infection of Arabidopsis. *Plant J.* 64:912–923.

Pfalz M, Mikkelsen MD, Bednarek P, Olsen CE, Halkier BA, Kroymann J. (2011) Metabolic engineering in *Nicotiana benthamiana* reveals key enzyme functions in Arabidopsis indole glucosinolate modification. *Plant Cell.* 23:716–729.

Pitzschke A, Datta S, Persak H. (2014) Salt stress in Arabidopsis: lipid transfer protein AZI1 and its control by mitogen-activated protein kinase MPK3. *Mol Plant.* 7:722–738.

Wang H, Hu Y, Pan J, Yu D. (2015) Arabidopsis VQ motif-containing proteins VQ12 and VQ29 negatively modulate basal defense against *Botrytis cinerea*. *Sci Rep.* 5:14185.

Wang P, Du Y, Zhao X, Miao Y, Song CP. (2013) The MPK6-ERF6-ROS-responsive *cis*-acting Element7/GCC box complex modulates oxidative gene transcription and the oxidative response in Arabidopsis. *Plant Physiol.* 161:1392–1408.

Wang Z, Cao G, Wang X, Miao J, Liu X, Chen Z, Qu LJ, Gu H. (2008) Identification and characterization of COI1-dependent transcription factor genes involved in JA-mediated response to wounding in Arabidopsis plants. *Plant Cell Rep.* 27:125–135.

Xu X, Chen C, Fan B, Chen Z. (2006) Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. *Plant Cell.* 18:1310–1326.

Xu ZY, Zhang X, Schlappi M, Xu ZQ. (2011) Cold-inducible expression of *AZI1* and its function in improvement of freezing tolerance of *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. *J Plant Physiol.* 168:1576–1587.

Yamamoto YY, Yoshioka Y, Hyakumachi M, Maruyama K, Yamaguchi-Shinozaki K, Tokizawa M, Koyama H. (2011) Prediction of transcriptional regulatory elements for plant hormone responses based on microarray data. *BMC Plant Biol.* 11:39.