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Direct regulation of *WRKY70* by *AtMYB44* in plant defense responses

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Abbreviations: SA, salicylic acid; JA, jasmonic acid; *Pst* DC3000, *Pseudomonas syringae* pv. *tomato* DC3000; MPK, mitogen-activated protein kinase

Cross-talk between hormones is required for plant response to developmental cues and environmental stresses. This cross-talk is achieved through several regulators located in convergence point of distinct hormonal signaling. In plant defense responses, salicylic acid and jasmonic acid affect each other in antagonistic manner. In a recent study we showed that *AtMYB44* transcription factor positively regulates SA-mediated defense expression and enhanced resistance to *Pst* DC3000. On the other hand, *AtMYB44* negatively regulates expression of JA-mediated defense gene expression and downregulated resistance to *Alternaria brassicicola*. Effects of *AtMYB44* in SA- and JA-mediated defense responses were achieved through direct regulation of *WRKY70* expression which acts as an integrator of cross-talk between SA and JA in plant defense responses. Here we provide further evidence that *AtMYB44* regulates defense responses by transcriptional activation of downstream gene, *WRKY70*. This result shows that *AtMYB44* is an integrator of cross-talk between SA and JA in plant defense responses.

Plants compete with pathogens for survival throughout their life cycle. To cope with attack from diverse pathogens, plants produce several phytohormones including salicylic acid (SA) and jasmonic acid (JA).[1,2] SA and JA have well defined function in the regulation of plant defense responses. SA is associated in defense response against biotrophic pathogen, whereas JA has a function in defense responses against herbivore and necrotrophic pathogen.[3,4] There is a significant cross-talk between SA- and JA-mediated defense signaling.[1,5,6] SA signaling mutants resulted in upregulation of JA-mediated defense response. On the contrary, JA signaling mutants resulted in upregulation of SA-mediated defense. Mutual antagonism between SA and JA is regarded as an effective mechanism for developing specialized defense responses.[2,6] In spite of significance of antagonistic interaction between SA and JA, its network was still not elucidated.

AtMYB44 Directly Regulates Expression of *WRKY70*

AtMYB44, a R2R3 MYB transcription factor, have been involved in development and abiotic stresses.[7,8] It is also reported that *AtMYB44* is induced by defense signals.[8-10] Previously, we reported that *AtMYB44* negatively regulated expression of JA-mediated genes such as *VSP1* and *PDF1.2*.[11,12] To understand molecular mechanism of antagonistic interaction between SA and JA, we monitored expression of JA- and SA-responsive gene expression through microarray analysis.[8] Overexpression of *AtMYB44* leads to activation of *PR* genes and T-DNA insertional knockout mutation resulted in attenuated expression of them.[12] The role of *AtMYB44* in positive modulation of SA-mediated defense responses was also reported. *AtMYB44* overexpression plants showed enhanced cell death and hydrogen peroxide accumulation against *Pst* DC3000 infection.[13]

We showed that *WRKY70,* an integrator in cross-talk between SA and JA, was constitutively expressed in *AtMYB44* overexpression plants.[12,14] Furthermore, induced expression of *AtMYB44* by the β-esradiol inducible promoter in transgenic plants resulted in epistatic expression of *WRKY70* and *PR1* in downstream. Overexpression effects of *AtMYB44* were abolished by *wrky70* mutation. *AtMYB44* directly regulates expression of *WRKY70* independently of *NPR1,* a signal transducer of SA.

Transactivation of *WRKY70* by AtMYB44

From SELEX, EMSA and ChIP experiments we demonstrated that *AtMYB44* directly binds to CNGTTA element in the promoter region of *WRKY70*.[12] There are three MYB binding elements (REs) in the promoter region of *WRKY70*. To test their function in transcriptional activation of *WRKY70* by *AtMYB44*, we performed *trans*-activation analysis by using

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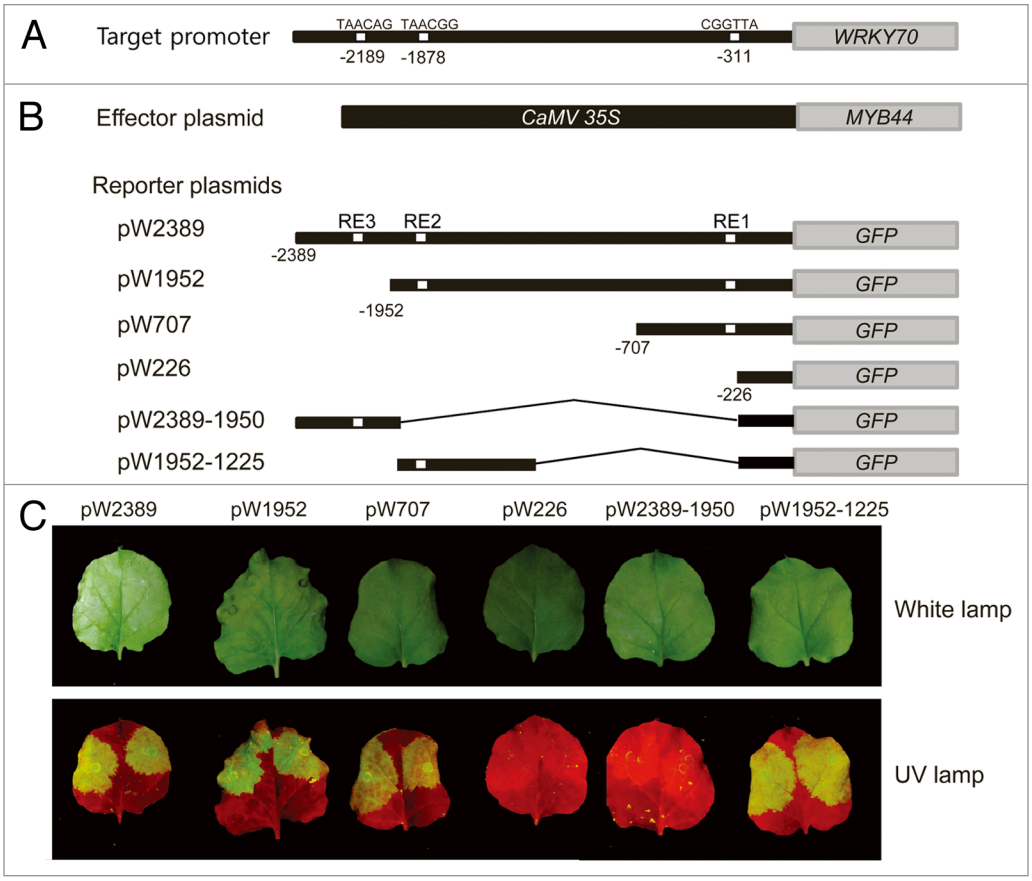

Figure 1. Transactivation of *WRKY70* by AtMYB44. (**A**) Structure of AtMYB44 binding sequences, REs, in *WRKY70* promoter. (**B**) A cDNA encoding AtMYB44 was fused to the CaMV 35S promoter as an effector and various constructs of truncated *WRKY70* promoter sequences was fused to the GFP reporter gene. (**C**) The reporter and effector constructs were infiltrated into *Nicotiana benthamiana*. Transactivation activity was detected by GFP fluorescence.

transient expression system in *Nicotiana benthamiana* (**Fig. 1**). The *WRKY70* promoter region of 2,380 bp containing three MYB binding elements was successfully activated reporter gene expression by the effector AtMYB44. To define the contribution of each MYB binding sequence to *AtMYB44* dependent activation of *WRKY70*, we co-infiltrated various constructs of truncated *WRKY70* promoter sequences with *AtMYB44* effector plasmid. Presence of RE1 or RE2 in the reporter plasmid was sufficient to activate *GFP* gene by *AtMYB44*. However, *GFP* gene fused under *WRKY70* promoter sequence containing RE3 alone was not activated by *AtMYB44*. It means that AtMYB44 binds to RE1 and RE2, and directly activates expression of *WRKY70*. Previously, we showed that *WRKY70* promoter fragment containing RE1 was sufficient for *AtMYB44* dependent activation and this activation was abolished by mutation in RE1.[12] Those findings demonstrate that AtMYB44 binds to at least two regions of *WRKY70* promoter for target gene activation.

AtMYB44 Contributes to Plant Innate Immunity

In *Arabidopsis*, the MPK3 and its upstream regulator MKK4 initiate signal cascade to abiotic and biotic stress responses. In pathogen molecular pattern (PAMP)-induced resistance, VirE2 interacting protein 1 (VIP1) is phosphorylated by MPK3 for nuclear transportation in response to bacterial invasion. In the nucleus, phosphorylated VIP1 activates expression of stress inducible genes including *PR1*.[15] *AtMYB44* is reported to be one of the targets of *VIP1*.[10] We showed that *AtMYB44* directly activates expression of *WRKY70* thus *PR1*.[12] From those data, *AtMYB44* could mediate PAMP induced defense responses through *WRKY70* and *PR1*. Even though its effect in biotic stress response is not known, there are reports that AtMYB44 is phosphorylated by MPK3 and its phosphorylation is required for abiotic stress tolerances.[16,17]

According to our previous report, *AtMYB44* is also induced by abiotic stresses such as drought, cold temperature and salt.[8] Its overexpression showed tolerance to those ABA-mediated abiotic stresses by modulating stomatal closure. In fact, *AtMYB44* is highly expressed in guard cell and its overexpression resulted in rapid closure of stomata. There are reports that ABA and SA are required for stomatal closure to biotrophic pathogen and PAMPs.[18,19] Stomatal closure triggered by bacterial invasion is considered to be a key part in the beginning of plant innate immunity. It is therefore possible that regulation of stomatal movement and defense gene expression by

AtMYB44 together could contribute to plant innate immunity against biotrophic pathogens. Further studies on the function and molecular mechanism of *AtMYB44* in plant innate immunity are required.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Thaler JS, Humphrey PT, Whiteman NK. Ecolution of jasmonate and salicylate signal crosstalk. Trens Plnat Sci 2012; 17:260-70; http://dx.doi.org/10.1016/j.tplants.2012.02.010
2. Robert-Seilaniantz A, Grant M, Jones JDG. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 2011; 49:317-43; PMID:21663438; http://dx.doi.org/10.1146/annurev-phyto-073009-114447
3. Durrant WE, Dong X. Systemic acquired resistance. Annu Rev Phytopathol 2004; 42:185-209; PMID:15283665; http://dx.doi.org/10.1146/annurev.phyto.42.040803.140421
4. Browse J, Howe GA. New weapons and a rapid response against insect attack. Plant Physiol 2008; 146:832-8; PMID:18316637; http://dx.doi.org/10.1104/pp.107.115683
5. Kunkel BN, Brooks DM. Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol 2002; 5:325-31; PMID:12179966; http://dx.doi.org/10.1016/S1369-5266(02)00275-3
6. Spoel SH, Koornneef A, Claessens SMC, Korzelius JP, Van Pelt JA, Mueller MJ, et al. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 2003; 15:760-70; PMID:12615947; http://dx.doi.org/10.1105/tpc.009159.
7. Kirik V, Kölle K, Miséra S, Bäumlein H. Two novel MYB homologues with changed expression in late embryogenesis-defective Arabidopsis mutants. Plant Mol Biol 1998; 37:819-27.; PMID:9678577; http://dx.doi.org/10.1023/A:1006011002499
8. Jung C, Seo JS, Han SW, Koo YJ, Kim CH, Song SI, et al. Overexpression of *AtMYB44* enhances stomatal closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol 2007; 146:623-35; PMID:18162593; http://dx.doi.org/10.1104/pp.107.110981
9. Kranz HD, Denekamp M, Greco R, Jin H, Leyva A, Meissner RC, et al. Towards functional characterisation of the members of the R2R3-MYB gene family from Arabidopsis thaliana. Plant J 1998; 16:263-76; PMID:9839469; http://dx.doi.org/10.1046/j.1365-313x.1998.00278.x
10. Pitzschke A, Djamei A, Teige M, Hirt H. VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. Proc Natl Acad Sci USA 2009; 106:18414-9; PMID:19820165; http://dx.doi.org/10.1073/pnas.0905599106
11. Jung C, Shim JS, Seo JS, Lee HY, Kim CH, Choi YD, et al. Non-specific phytohormonal induction of *AtMYB44* and suppression of jasmonate-responsive gene activation in *Arabidopsis thaliana.* Mol Cells 2010; 29:71-6; PMID:20016937; http://dx.doi.org/10.1007/s10059-010-0009-z
12. Shim JS, Jung C, Lee S, Min K, Lee YW, Choi Y, et al. *AtMYB44* regulates *WRKY70* expression and modulates antagonistic interaction between salicylic acid and jasmonic acid signaling. Plant J 2013; 73:483-95; PMID:23067202; http://dx.doi.org/10.1111/tpj.12051
13. Zou B, Jia Z, Tian S, Wang X, Gou Z, Lü B, et al. AtMYB44 positively modulates disease resistance to Pseudomonas syringae through the salicylic acid signalling pathway in Arabidopsis. Funct Plant Biol 2013; 40:304-31; http://dx.doi.org/10.1071/FP12253
14. Li J, Brader G, Palva ET. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell 2004; 16:319-31; PMID:14742872; http://dx.doi.org/10.1105/tpc.016980
15. Djamei A, Pitzschke A, Nakagami H, Rajh I, Hirt H. Trojan horse strategy in *Agrobacterium* transformation: abusing MAPK defense signaling. Science 2007; 318:453-6; PMID:17947581; http://dx.doi.org/10.1126/science.1148110
16. Nguyen XC, Hoang MH, Kim HS, Lee K, Liu XM, Kim SH, et al. Phosphorylation of the transcriptional regulator MYB44 by mitogen activated protein kinase regulates Arabidopsis seed germination. Biochem Biophys Res Commun 2012; 423:703-8; PMID:22704933; http://dx.doi.org/10.1016/j.bbrc.2012.06.019
17. Persak H, Pitzschke A. Tight Interconnection and Multi-Level Control of Arabidopsis MYB44 in MAPK Cascade Signalling. PLoS ONE 2013; 8:e57547; PMID:23437396; http://dx.doi.org/10.1371/journal.pone.0057547
18. Ton J, Flors V, Mauch-Mani B. The multifaceted role of ABA in disease resistance. Trends Plant Sci 2009; 14:310-7; PMID:19443266; http://dx.doi.org/10.1016/j.tplants.2009.03.006
19. Melotto M, Underwood W, Koczan J, Nomura K, He SY. Plant stomata function in innate immunity against bacterial invasion. Cell 2006; 126:969-80; PMID:16959575; http://dx.doi.org/10.1016/j.cell.2006.06.054