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Plant Defense-related Transcription Factor WRKY6 Plays Both Supportive and Inhibitory Roles in *Tobacco mosaic virus* Infection

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ABSTRACT

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The initial stage of crosstalk between a virus and host may determine the successful replication and further infection by the virus. To understand the early stage of host gene regulation in response to viral infection, we used a microarray assay to analyze protoplasts of *Arabidopsis thaliana* 4 hr after their inoculation with *Tobacco mosaic virus* (TMV). Approximately only 2% of the host genes showed greater than 2-fold changed expression, and only 8 genes showed greater than 3-fold changed expression. Four of the 8 genes are involved in transcriptional regulation. We selected a versatile transcription factor, WRKY6, involved in plant resistance, development and plant sensitivity in response to phosphorus and boron deficiency for further analysis. Protoplasts derived from WRKY6-overexpressed or -knockout plants were inoculated with TMV, and the accumulation of TMV was not affected 24 hr post-inoculation. However, TMV accumulation was inhibited in both WRKY6-overexpressed and -knockout plants as compared with wild-type inoculated plans. Therefore, WRKY6 may not be essential in the initial establishment of virus infection but may have dual roles in support and inhibition of virus infection.

Keywords: *Tobacco mosaic virus*, *Arabidopsis thaliana*, Microarray, WRKY6

INTRODUCTION

Plant resistance pathways are arranged in complex networks. Plants protect against pathogens through host innate immunity and induced resistance. The physical structures such as cell wall, wax and trichome provide constitutive barriers in plants against pathogen attack. Plant surface receptors that recognize pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs, respectively) also contribute basal resistance. Furthermore, a series of defense responses can be induced after pathogen infection. These defense mechanisms can be divided into local and systemic responses. Local resistant responses induce a hypersensitive response in the infected tissue to block pathogen movement and further infection. Systemic resistant responses consist of systemic acquired resistance and induced systemic resistance [(13)].

Recognition of the PAMPs or MAMPs initiates signalling pathways involving mitogen-activated protein

kinase (MAPK) cascades and global transcriptional changes to rapidly deploy various defense responses[4, 53]. This activity results in extensive remodeling of cellular metabolism and intracellular compartmentation [8]. Despite these series of defense mechanisms in hosts, plant pathogens have developed various strategies to overcome the plant defense response. Successful pathogens can deploy PAMP-triggered immunity (PTI)-suppressing pathogen effectors, which can interfere with PTI [20]. Thus, the early interaction between the plant and pathogen is a detrimental step for pathogen infection. Among the pathogens, only plant viruses can achieve whole infection within cells. Besides suppressing the plant immunity response and inducing host gene downregulation persistently in infected tissues [17], plant viruses need to reorganize the cellular compartment and recruit host factors for successful infection. Virus infection modulates a number of host factors. Current studies revealed that the viral encoded proteins cannot support successful viral infection without the help of host factors. As the main plant viruses, single-strand positive RNA [ss(+)RNA] virus, must induce the biogenesis of unique intracellular membranous structures to form virus replication complexes (VRCs) for replication [1, 24, 32, 37]. The origins of membranes and cytoplasmic vesicles are diverse in different type of invading virus [23, 24, 38]. In addition, increasing host factors are known to involve in this process; examples are secretory/endocytosis pathway-related coat protein complex I (COPI), COPII[47], endosomal sorting complexes required for transport [3], Hsp70 (homolog of yeast *SSA1* or *SSA2* in *Nicotiana benthamiana*) [46] and *Arabidopsis TOM* genes [16, 43, 49, 50]. These showed that virus infection involves in elaborate alteration of host transcriptome. VRC formation requires only a few hours. Viruses encode only a few proteins. How viruses use this limited genetic material to overcome the host defense response, recruit host factors, and rearrange cellular compartments for infection is not completely understood.

In this study, we used the Arabidopsis-*Tobacco mosaic virus* (TMV) system to investigate the host transcriptome in response to the early stage of virus infection. We identified a plant defense-related transcription factor (TF), WRKY6, which is upregulated early in virus infection. Overexpressed WRKY6 can reduce the accumulation of TMV but its adequate expression is needed for efficient TMV accumulation.

MATERIALS AND METHODS

Clones used in this study

Clones used for cDNA microarray assay: The *Tobacco mosaic virus* wild-type infectious clone (pTMV) [41] and pTMV*CP.MP were as described (Chen *et al*. unpublished data). Two stop codons were introduced in the coat protein (CP, at the 14 amino acid position) and movement protein (MP, at the 24 amino acid position) by site-directed mutagenesis to generate pTMV*CP.MP.

Clones used for RNA probe preparation: The clone (pGEMT-CP) used for TMV RNA probe preparation was as described (Chen *et al*. unpublished data). The WRKY6 fragments were amplified by RT-PCR, with *Arabidopsis* total RNA used as a template and the primer pair WRKY6 R-T-F/WRKY6 R (Table 1). The fragment was cloned into pGEM-T Easy vector (Promega; Madison, WI, USA) by use of T4 DNA ligase (Promega) to generate pGEMT-WRKY6.

Plants

Plant lines for wild-type *Arabidopsis thaliana* ecotype Col-0, WRKY6 overexpression (*CaMV* 35S::*WRKY6*) and the WRKY6 homozygous mutant (*wrky6*) were all grown at 22°C under long-day conditions (16-hr light/8-hr dark, 100-150 μE). The *CaMV* 35S::*WRKY6* lines were kindly provided by Dr. Imre E. Somssich [35]. The *wrky6* lines (SALK_012997C) were obtained from the SALK collection (http://signal.salk.edu/).

In vitro transcription

Capped transcripts corresponding to the wild-type virus (TMV) and the TMV*CP.MP were synthesized by use of the mMESSAGE mMACHINE T7 Kit (Ambion, Austin, Texas, USA) as described [22, 36, 51], except that TMV and TMV*CP.MP were linearized with *Kpn*I.

Protoplast isolation and PEG transfection

Six- to 7-week-old *A. thaliana* expanded leaves were used for protoplast isolation. The procedures were as previously described [52] with some modifications. Leaves were cut into 0.5- to 1-mm strips with use of a clean razor. The leaves were incubated in a Petri dish with enzyme solution containing 1% cellulose R10 (Yakult Honsha Co.,

Table 1. Primers used in this study

Primer	Nucleotide sequence (5'-3')	Purpose
WRKY6 R-T-F	5'-ACCCCGACGACATTTGATCA-3'	Real-time RT-PCR for WRKY6 and construction of pGEMT-WRKY6.
WRKY6 R-T-R	5'-TGACATCCATCGCTTATCATCG-3'	Real-time RT-PCR for WRKY6.
WRKY6 R	5'-ATTCCCGGAGGTAAGTTCGT-3'	Construction of pGEMT-WRKY6.
UBQ10 R-T-F	5'-AGAAGTTCAATGTTTCGTTTCATGTAA-3'	Real-time RT-PCR for UBQ10.
UBQ10 R-T-R	5'-GAACGGAAACATAGTAGAACACTTATTCA-3	Real-time RT-PCR for UBQ10.

Tokyo, Japan), 0.2% macerozyme R10 (Yakult Honsha), 0.4 M mannitol, 20 mM KCl, 20 mM MES, 10 mM $CaCl_2$, and 0.1% BSA, pH 5.7, and incubated for 3 hr in the dark. The protoplasts were harvested by spinning the enzyme solution at 100 × g to pellet the protoplasts. The protoplasts were washed twice with W5 solution (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl, 2 mM MES, pH adjusted to 5.7). Then protoplasts were pelleted and resuspended in MMg solution (0.4 M mannitol, 15 mM $MgCl_2$, 4 mM MES, pH 5.7). Protoplasts were transfected by use of the PEG method as described [52] with some modifications. Protoplasts (1×10^5 cells) were collected in a round-bottomed tube. Ten μg RNA transcripts were used for virus inoculation, and 110 μl PEG/Ca solution (4 g PEG 4000, 3 ml H_2O, 2.5 ml 0.8 M mannitol, 1 mM $CaCl_2$) were added to the tube smoothly for incubation at 23°C for 20 min, then the tube was diluted with 0.44 ml W5 solution. The solution was gently mixed and centrifuged for 1 min to remove PEG. The protoplasts were resuspended in 10 ml W5 solution and incubated at 25°C in dark.

RNA extraction

RNA used in northern blot analysis, real-time quantitative RT-PCR and cDNA microarray analysis was extracted from plant tissues or protoplasts by the Pine Tree Method [5] and dissolved in diethyl pyrocarbonate-treated water. For cDNA microarray analysis, the quality of RNA was checked by use of the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

cDNA microarray fabrication and hybridization

The cDNA microarray glass slides (including 11,500 *Arabidopsis* cDNA clones corresponding to 10,452 unique genes) and techniques used in cDNA microarray screening were provided and supported by the DNA Microarray Core Laboratory, Institute of Plant and Microbial Biology, Academia Sinica (Taipei, Taiwan). Total RNA extracted from TMV*CP.MP-inoculated protoplasts at 0.5 and 4 hr post-inoculation (hpi) was labeled with Cy5 and Cy3, respectively. Methods for preparing the fluorescent probe and hybridization were as described (http://ipmb.sinica.edu.tw/microarray/protocol.htm). The hybridization signals were acquired with the use of Axon GenePix 4000B and analyzed by GenePix 4.0 (Axon Instruments, Foster City, CA, USA).

Real-time quantitative RT-PCR

Extracted total RNA was treated with RNase-free DNase (Ambion) to remove residual DNA. An amount of 2 μg total RNA was used as a template for synthesis of cDNA by use of the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Tokyo, Japan). A one-fourth aliquot of the cDNA was used as a template, and 2 × SYBR Green PCR master mix was added (Applied Biosystems). Real-time quantitative RT-PCR involved use of the ABI Prism 7000 sequence detection system (Applied Biosystems) following the manufacturer's instructions. The primers used in quantification are in Table 1. For gene quantification, the expression of each gene was analyzed from RNA extracted from 3 repeated individual experiments. For real-time RT-PCR reactions, each sample was analyzed in triplicate. The relative quantification was calculated according to the manufacturer's instructions (Applied Biosystems). The *Arabidopsis* UBQ10 gene was used as an internal quantification control.

Northern blot hybridization

T3 RNA polymerase and *EcoR*I-digested pGEMT-CP or pGEMT-WRKY6 plasmids were used to generate negative-sense digoxigenin (DIG)-labeled minus-sense

probes (Roche Applied Science, Indianapolis, IN, USA). Northern blot hybridization was performed as described [22]. Hybridization signals were detected by use of the chemiluminescent substrate CDP STAR (Roche Applied Science), and blots were exposed to Fuji medical x-ray film (Fuji, Tokyo, Japan).

RESULTS

Transcriptome change of *A. thaliana* in the early stage of TMV infection

In order to explore the initial stage of crosstalk between a virus and host, we selected the initial stage of virus replication for our analysis. Especially, we focused on the crosstalk in the early stage of virus replication. The coat protein (CP) and movement protein (MP) are not necessary for virus replication [25, 30]. Thus, to prevent nonreplication-associated gene (s) of TMV that may induce host response, we remove the CP and MP (TMV*CP.MP; Fig. 1) for our analysis. We used microarray analysis to identify gene(s) involved in the early stage of TMV*CP.MP (Fig. 1) replication in *Arabidopsis* protoplasts. Because TMV accumulation can be detected within 4 hr [18, 39], we analyzed the transcriptome change of *Arabidopsis* at 4 hpi relative to 0.5 hpi. Only 2% (193/10,452) of *Arabidopsis* genes were changed in expression by greater than 2-fold at 4 hpi relative to 0.5 hpi. Four genes were downregulated and 189 genes were upregulated. Among the 189 upregulated genes, 8 were upregulated greater than 3-fold (Table 2). *In silico* functional analysis of these 8 genes by use of MIPS Functional Catalogue [31] revealed 4 of them (AT1G62300, AT2G15430, AT4G34730, and AT4G36990) involved in transcriptional regulation.

Real time RT-PCR and northern blot analysis of WRKY6 expression

Because transcription-related factors usually play important roles in modulation of host response, we decided to select important transcription-related factors for our initial analysis. One of the 4 transcription-related factors, AT1G62300, belonging to the WRKY family, was previously known as WRKY6. The family of WRKY TFs is known to associate with plant responses to various biotic

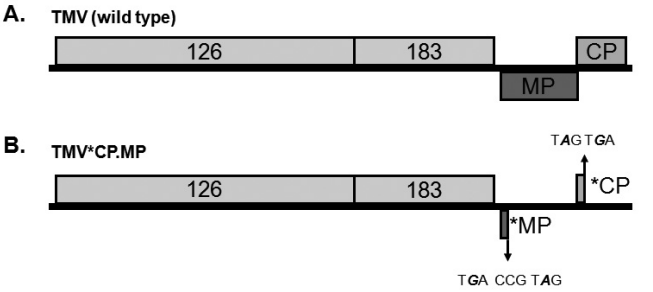

Fig. 1 Schematic representation of *Tobacco mosaic virus* (TMV) genomic RNA and mutated virus used in microarray analysis. Rectangles represent open reading frames encoded by TMV genomic RNA. Wild-type TMV (A) encodes the 126-kDa and the read-through 183-kDa replicase proteins, the movement protein (MP, 30 kDa) and coat protein (CP, 17.5 kDa). The mutated sequence in TMV*CP.MP (B) is indicated by bold italic letters.

Table 2. Genes with greater than 3-fold expression induced by *Tobacco mosaic virus* at 4 hr post-inoculation

Locus number	Description	Fold Expression [a] 4 hpi / 0.5 hpi
AT3G04130	pentatricopeptide (PPR) repeat-containing protein	6.704
AT1G50250	encodes an FTSH protease that is localized to the chloroplast	4.659
AT4G36990	encodes a protein whose sequence is similar to heat shock factors that regulate the expression of heat shock proteins	4.585
AT4G34730	ribosome-binding factor A family protein	4.469
AT2G15430	non-catalytic subunit of nuclear DNA-dependent RNA polymerases II, IV and V	3.835
AT4G37330	cytochrome P450, member of CYP81D	3.645
AT5G16620	chloroplast protein import	3.362
AT1G62300	encodes a transcription factor WRKY6	3.054

[a] Expression at 4 hpi relative to that at 0.5 hpi

and abiotic stresses [44], and the expression of WRKY6 was induced in by some virus [48]. Thus, we selected WRKY6 for further analysis. Quantitative real-time RT-PCR and northern blot analysis revealed the mRNA level of WRKY6 higher in TMV*CP.MP-infected cells at 4 hpi relative to 0.5 hpi (Fig. 2A, 2B).

Role of WRKY6 in accumulation of TMV in *Arabidopsis* protoplasts

To analyze the role of WRKY6 in TMV infection, we first inoculated wild-type TMV in protoplasts derived from WRKY6-overexpressed (*CaMV* 35S::*WRKY6*) and -knockout (*wrky6*) plants. As was previously reported, WRKY6 overexpression produced phenotypes of stunting and rosette leaves (Fig. 3A), with no obvious phenotype observed in the *wrky6* mutant (Fig. 3A) [35]. The expression of WRKY6 in *CaMV* 35S::*WRKY6* and *wrky6* plants was analyzed by northern blot hybridization (Fig. 3B). Protoplasts isolated from wild type, *CaMV* 35S::*WRKY6* and *wrky6* plants were used to inoculate wild-type TMV. Northern blot hybridization revealed no difference in virus accumulation in TMV-infected protoplasts collected at 0.5, 6, 12, 18 and 24 hpi (Fig. 3C).

Effect of WRKY6 on TMV infection in plants

To further understand the role of WRKY6 in TMV infection in whole plants, wild-type, *CaMV* 35S::*WRKY6* and *wrky6* plants were used for wild-type TMV inoculation assay. TMV accumulation was detected in inoculated leaves at 7 dpi (Fig. 4A) and in systemic leaves at 7, 14 and 21 dpi (Fig. 4B) by northern blot analysis. TMV accumulation was suppressed in inoculated and systemic leaves both in WRKY6-overexpressed and -knockout plants as compared with the wild type (Fig. 4A, 4B).

DISCUSSION

The initial interaction between a virus and host is a determinative step for virus infection. To understand the early stage of host gene regulation in response to viral infection, we used microarray assay to analyze protoplasts of *A. thaliana* 4 hr after inoculation with TMV. We identified a TF, WRKY6, upregulated in the early stage of virus infection. After exploring the roles of WRKY6 in virus infection, our data suggested that WRKY6 is not essential in the initial stage of virus infection, but plays dual roles in both support and inhibition of virus infection.

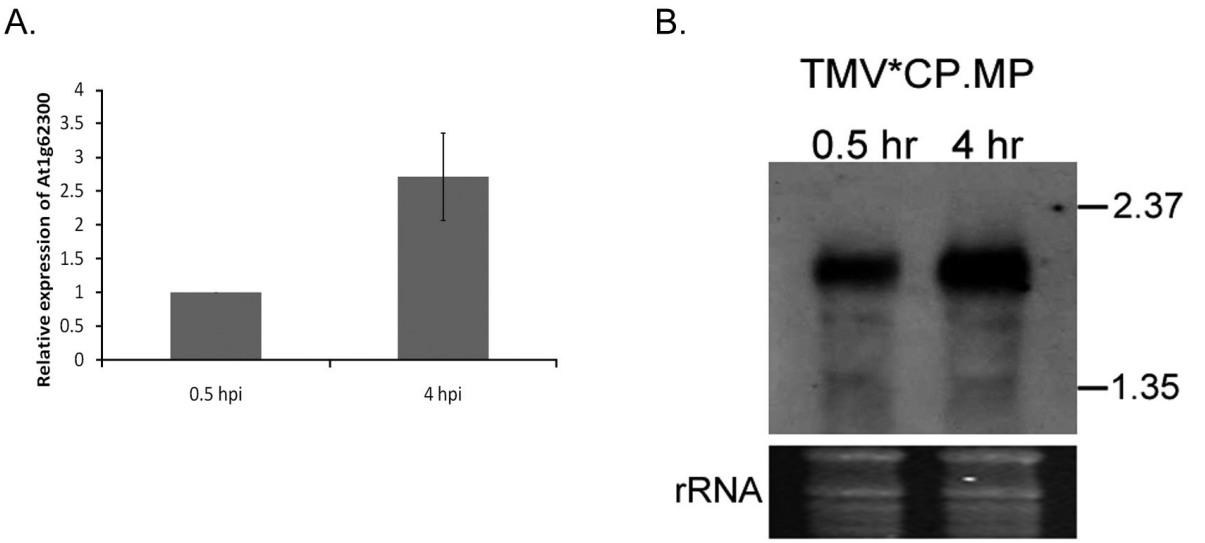

Fig. 2 (A) Relative expression of At1g62300 (WRKY6) in TMV*CP.MP-inoculated *Arabidopsis* protoplasts. Infected *Arabidopsis* protoplasts were collected at 0.5 and 4 hr post-inoculation (hpi). Total RNA was purified and the expression of At1g62300 at 4 hpi relative to that at 0.5 hpi was measured by real-time RT-PCR. The calculation is based on 3 individual experiments. Error bars represent standard deviation. (B) Expression of At1g62300 (WRKY6) detected by northern blot analysis. TMV*CP.MP-inoculated protoplasts were harvested at 0.5 and 4 hpi. Total RNA purified from the protoplasts was used for northern blot hybridization. Numbers at the right correspond to positions of marker RNAs (sizes in kb). WRKR6-predicted transcript size is 1.8 kb. Ribosomal RNA (rRNA) was used as a loading control.

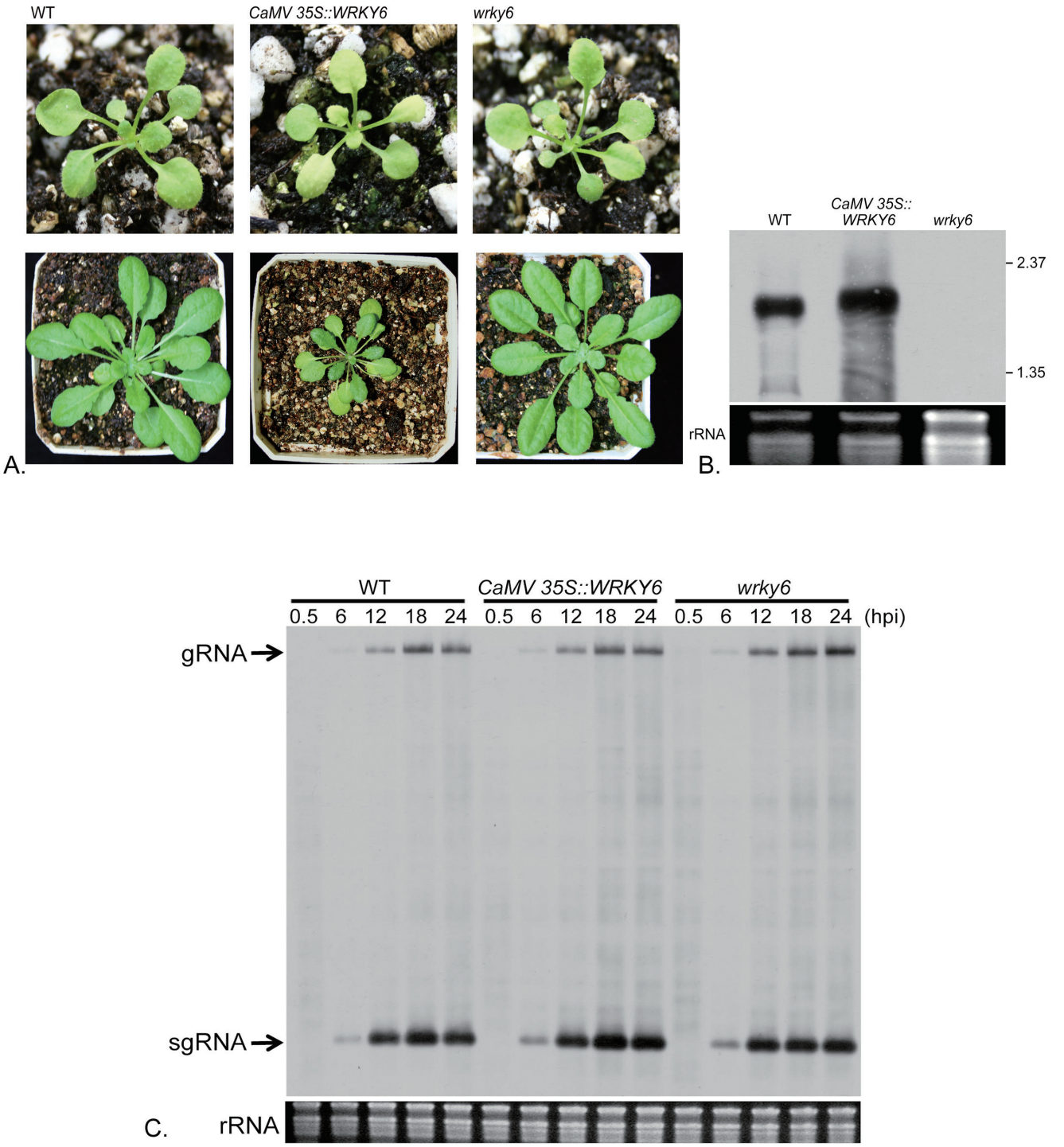

Fig. 3 (A) Phenotype of wild-type (WT), WRKY6-overexpressed (*CaMV* 35S::*WRKY6*) and -knockout (*wrky6*) plants. The morphology of 4-week-old (upper panel) and 8-week-old (lower panel) plants are shown. (B) Expression analysis of WRKY6 in WT, *CaMV* 35S::*WRKY6* and *wrky6* plants. Total RNA purified from plants was used for northern blot hybridization. Numbers at the right correspond to positions of marker RNAs (sizes in kb). WRKR6-predicted transcript size is 1.8 kb. Ethidium bromide-stained rRNA was used as a loading control. (C) TMV accumulation in protoplasts analyzed by northern blot hybridization. Protoplasts isolated from WT *Arabidopsis*, *CaMV* 35S::*WRKY6* and *wrky6* were inoculated with TMV, then collected at 0.5, 6, 12, 18 and 24 hpi. Total RNA purified from the protoplasts was used for northern blot hybridization. Viral genomic RNA (gRNA) and subgenomic RNA (sgRNA) are indicated, and rRNA was used as a loading control.

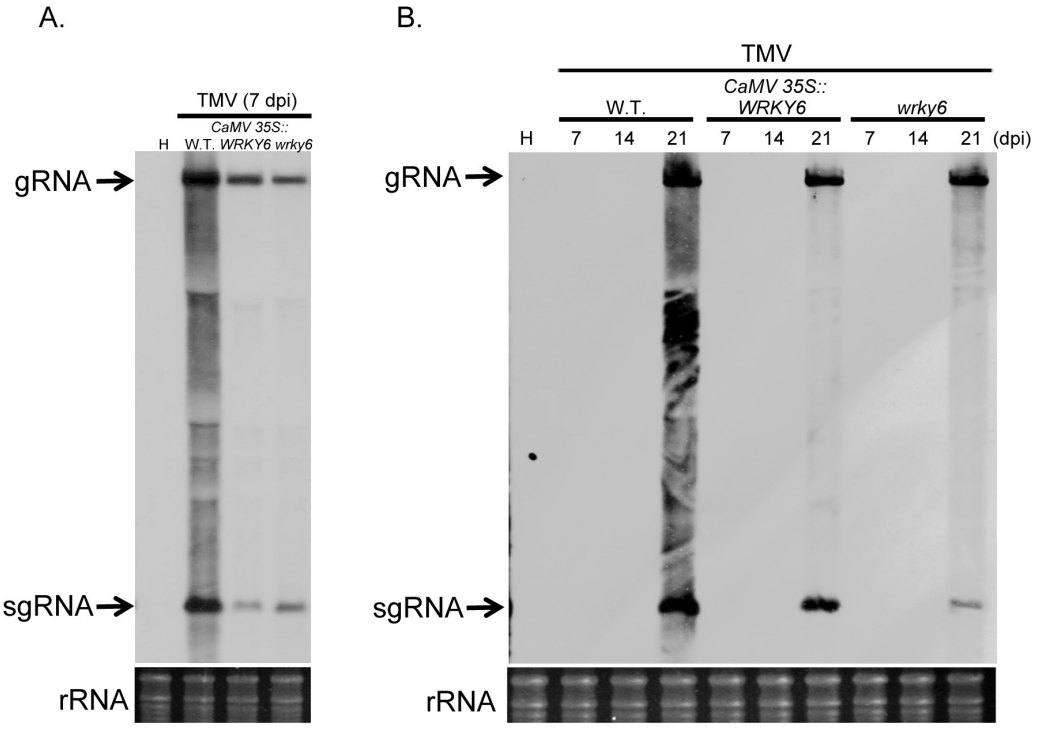

Fig. 4 TMV accumulation in plants analyzed by northern blot hybridization. WT *Arabidopsis*, *CaMV* 35S::*WRKY6* and *wrky6* plants were inoculated with TMV. Inoculated leaves were collected at 7 day post-inoculation (dpi) (A) and systemic leaves at 7, 14 and 21 dpi (B). Total RNA purified from these samples was used for northern blot hybridization. Viral genomic RNA (gRNA) and subgenomic RNA (sgRNA) are indicated, and rRNA was used as a loading control.

WRKYs are a large family of plant-specific TFs and comprise 74 members in *Arabidopsis* and more than 90 members in rice [11, 44]. WRKY family proteins contain a conserved WRKYGQK sequence, which belongs to a zinc-finger motif. WRKY TFs can recognize a specific DNA sequence [(W-box, (C/T)TGAC(C/T)] [9, 11, 44], and in general, the WRKY binding site is present in the promoter region of pathogenesis-related proteins and *NPR1* [9, 10, 28]. In rice (*Oryza sativa*), WRKY13, 45, 71, 53 and 89 can mediate defense responses to pathogens such as *Magnaporthe grisea* (rice blast) and *Xanthomonas oryzae* (bacterial blight) [7, 27, 33, 40, 45]. In *Arabidopsis*, at least 49 WRKY TFs are regulated during pathogen infection or salicylic acid (SA) treatment [9, 12], and WRKY is also associated with crosstalk between SA and jasmonic acid [26].

Although WRKYs are known to be involved in a number of plant defense mechanisms, the roles of WRKYs in the plant defense response have been investigated only in plant pathogenic bacteria [12]. Several WRKYs are also regulated by plant viruses. The expression of some WRKY family genes is altered in TMV, *cucumber mosaic virus*, *oil seed rape virus*, *turnip vein clearing virus*, *potato virus X* and *turnip mosaic virus*-infected *Arabidopsis* plants and *plum pox virus*-infected protoplasts [2, 14, 29, 48]. Furthermore, the regulation of WRKYs is observed within a few hours after virus infection [2, 29]. These data suggest that the host transcriptome may be modulated through WRKY TFs during virus infection.

We found WRKY6 upregulated during the early stage (within 4 hr) of virus infection. However, overexpression or knockout of WRKY6 did not affect the accumulation of TMV in protoplasts (Fig. 3C). The cascade regulation modulated by WRKY6 may have no role in TMV early infection.

Previously reports showed WRKY6 involved in the plant defense response and increasing the promoter activity of pathogenesis-related protein 1 (*PR1*) [34, 35], a characteristic marker of the SA-related plant defense pathway. The SA-related plant defense pathway is usually triggered by viruses and has been suggested to have major roles in defense against virus infection [15, 42]. Thus, WRKY6 may play a role in triggering the plant defense response. In

agreement with the previously known role of WRKY6, our data suggest that overexpression of WRKY6 reduces the accumulation of TMV.

Interestingly, our data also suggest that adequate expression of WRKY6 helps the accumulation of TMV (Fig. 4). Besides being involved in defense responses, WRKYs also participate in various plant physiology responses such as the development of trichomes, seed coat and senescence [19, 34]. Previously reports indicated that WRKY6 participates in plant senescence [34, 35] and is associated with plant sensitivity in response to phosphorus and boron deficiency [6, 21]. Thus, the downstream gene (s) modulated by WRKY6 may play an accessory role in virus infection.

Virus-host interaction is an important topic in plant pathology. Understanding the interaction can help in designing better strategies to combat viral disease. Our results suggested that hosts may trigger defense responses by modulating TFs in response to virus infection; however, the cascade response may help in plant defense and in the infection of viruses depending on the amount of expression of the TF. Our identification of an early-response TF involved in this complicated interaction can help resolve the sophisticated interactions between viruses and plants.

LITERATURE CITED

1. Ahlquist, P., Noueiry, A. O., Lee, W. M., Kushner, D. B., and Dye, B. T. 2003. Host factors in positive-strand RNA virus genome replication. J. Virol. 77: 8181-8186.
2. Babu, M., Griffiths, J. S., Huang, T. S., and Wang, A. 2008. Altered gene expression changes in *Arabidopsis* leaf tissues and protoplasts in response to *Plum pox virus* infection. BMC Genomics 9: 325.
3. Barajas, D., Jiang, Y., and Nagy, P. D. 2009. A unique role for the host ESCRT proteins in replication of Tomato bushy stunt virus. PLoS Pathog 5: e1000705.
4. Boudsocq, M., Willmann, M. R., McCormack, M., Lee, H., Shan, L., He, P., Bush, J., Cheng, S. H., and Sheen, J. 2010. Differential innate immune signalling via Ca^{2+} sensor protein kinases. Nature 464: 418-422.
5. Chang, S., Puryear, J., and Cairney, J. 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Reptr. 11: 113-116.
6. Chen, Y. F., Li, L. Q., Xu, Q., Kong, Y. H., Wang, H., and Wu, W. H. 2009. The WRKY6 transcription factor modulates *PHOSPHATE1* expression in response to low Pi stress in *Arabidopsis*. Plant Cell 21: 3554-3566.
7. Chujo, T., Takai, R., Akimoto-Tomiyama, C., Ando, S., Minami, E., Nagamura, Y., Kaku, H., Shibuya, N., Yasuda, M., Nakashita, H., Umemura, K., Okada, A., Okada, K., Nojiri, H., and Yamane, H. 2007. Involvement of the elicitor-induced gene OsWRKY53 in the expression of defense-related genes in rice. Biochem. Biophys. Acta 1769: 497-505.
8. Dixon, R. A., Harrison, M. J., and Lamb, C. J. 1994. Early events in the activation of plant defense responses. Annu. Rev. Phytopathol. 32: 479-501.
9. Dong, J., Chen, C., and Chen, Z. 2003. Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defense response. Plant Mol. Biol. 51: 21-37.
10. Durrant, W. E., and Dong, X. 2004. Systemic acquired resistance. Annu. Rev. Phytopathol. 42: 185-209.
11. Eulgem, T., Rushton, P. J., Robatzek, S., and Somssich, I. E. 2000. The WRKY superfamily of plant transcription factors. Trends Plant Sci. 5: 199-206.
12. Eulgem, T., and Somssich, I. E. 2007. Networks of WRKY transcription factors in defense signaling. Curr. Opin. Plant Biol. 10: 366-371.
13. Feys, B. J., and Parker, J. E. 2000. Interplay of signaling pathways in plant disease resistance. Trends Genet. 16: 449-455.
14. Golem, S., and Culver, J. N. 2003. *Tobacco mosaic virus* induced alterations in the gene expression profile of Arabidopsis thaliana. Mol. Plant Microbe Interact. 16: 681-688.
15. Gozzo, F. 2003. Systemic acquired resistance in crop protection: from nature to a chemical approach. J. Agric. Food Chem. 51: 4487-4503.
16. Hagiwara, Y., Komoda, K., Yamanaka, T., Tamai, A., Meshi, T., Funada, R., Tsuchiya, T., Naito, S., and Ishikawa, M. 2003. Subcellular localization of host and viral proteins associated with tobamovirus RNA replication. EMBO J. 22: 344-353.
17. Havelda, Z., Varallyay, E., Valoczi, A., and Burgyan, J. 2008. Plant virus infection-induced persistent host gene downregulation in systemically infected leaves. Plant J. 55: 278-288.
18. Ishikawa, M., Meshi, T., Ohno, T., and Okada, Y. 1991. Specific cessation of minus-strand RNA accumulation at an early stage of *Tobacco mosaic virus* infection. J. Virol. 65: 861-868.
19. Johnson, C. S., Kolevski, B., and Smyth, D. R. 2002. TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of *Arabidopsis*, encodes a WRKY transcription factor. Plant Cell 14: 1359-1375.
20. Jones, J. D., and Dangl, J. L. 2006. The plant immune system. Nature 444: 323-329.
21. Kasajima, I., Ide, Y., Yokota Hirai, M., and Fujiwara, T. 2010. WRKY6 is involved in the response to boron

deficiency in *Arabidopsis thaliana*. Physiol. Plant 139: 80-92.
22. Klaassen, V. A., Mayhew, D., Fisher, D., and Falk, B. W. 1996. In vitro transcripts from cloned cDNAs of the *Lettuce infectious yellows closterovirus* bipartite genomic RNAs are competent for replication in *Nicotiana benthamiana* protoplasts. Virology 222: 169-175.
23. Kopek, B. G., Perkins, G., Miller, D. J., Ellisman, M. H., and Ahlquist, P. 2007. Three-dimensional analysis of a viral RNA replication complex reveals a virus-induced mini-organelle. PLoS Biol 5: 2022-2034.
24. Laliberte, J. F., and Sanfacon, H. 2010. Cellular remodeling during plant virus infection. Annu. Rev. Phytopathol. 48: 69–91.
25. Lewandowski, D. J., and Dawson, W. O. 2000. Functions of the 126- and 183-kDa proteins of *Tobacco mosaic virus*. Virology 271: 90-98.
26. Li, J., Brader, G., and Palva, E. T. 2004. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell 16: 319-331.
27. Liu, X., Bai, X., Wang, X., and Chu, C. 2007. OsWRKY71, a rice transcription factor, is involved in rice defense response. J. Plant Physiol. 164: 969-979.
28. Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K. A., Dangl, J. L., and Dietrich, R. A. 2000. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nat. Genet. 26: 403-410.
29. Marathe, R., Guan, Z., Anandalakshmi, R., Zhao, H., and Dinesh-Kumar, S. P. 2004. Study of *Arabidopsis thaliana* resistome in response to cucumber mosaic virus infection using whole genome microarray. Plant Mol. Biol. 55: 501-520.
30. Meshi, T., Watanabe, Y., Saito, T., Sugimoto, A., Maeda, T., and Okada, Y. 1987. Function of the 30 kd protein of tobacco mosaic virus: involvement in cell-to-cell movement and dispensability for replication. EMBO J. 6: 2557-2563.
31. Mewes, H. W., Amid, C., Arnold, R., Frishman, D., Guldener, U., Mannhaupt, G., Munsterkotter, M., Pagel, P., Strack, N., Stumpflen, V., Warfsmann, J., and Ruepp, A. 2004. MIPS: analysis and annotation of proteins from whole genomes. Nucleic Acids Res. 32: D41-44.
32. Netherton, C., Moffat, K., Brooks, E., and Wileman, T. 2007. A guide to viral inclusions, membrane rearrangements, factories, and viroplasm produced during virus replication. Adv. Virus Res. 70: 101-182.
33. Qiu, D., Xiao, J., Ding, X., Xiong, M., Cai, M., Cao, Y., Li, X., Xu, C., and Wang, S. 2007. OsWRKY13 mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol. Plant Microbe Interact. 20: 492-499.
34. Robatzek, S., and Somssich, I. E. 2001. A new member of the *Arabidopsis* WRKY transcription factor family, AtWRKY6, is associated with both senescence- and defence-related processes. Plant J. 28: 123-133.
35. Robatzek, S., and Somssich, I. E. 2002. Targets of AtWRKY6 regulation during plant senescence and pathogen defense. Genes Dev. 16:1139-1149.
36. Rubio, L., Yeh, H. H., Tian, T., and Falk, B. W. 2000. A heterogeneous population of defective RNAs is associated with *Lettuce infectious yellows virus*. Virology 271: 205-212.
37. Salonen, A., Ahola, T., and Kaariainen, L. 2005. Viral RNA replication in association with cellular membranes. Curr. Top. Microbiol. Immunol. 285: 139-173.
38. Schwartz, M., Chen, J., Lee, W. M., Janda, M., and Ahlquist, P. 2004. Alternate, virus-induced membrane rearrangements support positive-strand RNA virus genome replication. Proc. Natl. Acad. Sci. U.S.A. 101: 11263-11268.
39. Shaw, J. G. 1999. *Tobacco mosaic virus* and the study of early events in virus infections. Philos. Trans. R. Soc. Lond. B Biol. Sci. 354: 603-611.
40. Shimono, M., Sugano, S., Nakayama, A., Jiang, C. J., Ono, K., Toki, S., and Takatsuji, H. 2007. Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. Plant Cell 19: 2064-2076.
41. Shivprasad, S., Pogue, G. P., Lewandowski, D. J., Hidalgo, J., Donson, J., Grill, L. K., and Dawson, W. O. 1999. Heterologous sequences greatly affect foreign gene expression in *Tobacco mosaic virus*-based vectors. Virology 255: 312-323.
42. Sticher, L., Mauch-Mani, B., and Metraux, J. P. 1997. Systemic acquired resistance. Annu. Rev. Phytopathol. 35: 235-270.
43. Tsujimoto, Y., Numaga, T., Ohshima, K., Yano, M. A., Ohsawa, R., Goto, D. B., Naito, S., and Ishikawa, M. 2003. *Arabidopsis* TOBAMOVIRUS MULTIPLICATION (TOM) 2 locus encodes a transmembrane protein that interacts with TOM1. EMBO J. 22: 335-343.
44. Ulker, B., and Somssich, I. E. 2004. WRKY transcription factors: from DNA binding towards biological function. Curr. Opin. Plant Biol. 7: 491-498.
45. Wang, H., Hao, J., Chen, X., Hao, Z., Wang, X., Lou, Y., Peng, Y., and Guo, Z. 2007. Overexpression of rice WRKY89 enhances ultraviolet B tolerance and disease resistance in rice plants. Plant Mol. Biol. 65: 799-815.
46. Wang, R. Y., Stork, J., and Nagy, P. D. 2009. A key role for heat shock protein 70 in the localization

and insertion of tombusvirus replication proteins to intracellular membranes. J. Virol. 83: 3276-3287.
47. Wei, T., and Wang, A. 2008. Biogenesis of cytoplasmic membranous vesicles for plant potyvirus replication occurs at endoplasmic reticulum exit sites in a COPI- and COPII-dependent manner. J. Virol. 82: 12252-12264.
48. Whitham, S. A., Quan, S., Chang, H. S., Cooper, B., Estes, B., Zhu, T., Wang, X., and Hou, Y. M. 2003. Diverse RNA viruses elicit the expression of common sets of genes in susceptible *Arabidopsis thaliana* plants. Plant J. 33: 271-283.
49. Yamanaka, T., Imai, T., Satoh, R., Kawashima, A., Takahashi, M., Tomita, K., Kubota, K., Meshi, T., Naito, S., and Ishikawa, M. 2002. Complete inhibition of tobamovirus multiplication by simultaneous mutations in two homologous host genes. J. Virol. 76: 2491-2497.
50. Yamanaka, T., Ohta, T., Takahashi, M., Meshi, T., Schmidt, R., Dean, C., Naito, S., and Ishikawa, M. 2000. TOM1, an *Arabidopsis* gene required for efficient multiplication of a tobamovirus, encodes a putative transmembrane protein. Proc. Natl. Acad. Sci. U.S.A. 97: 10107-10112.
51. Yeh, H. H., Tian, T., Rubio, L., Crawford, B., and Falk, B. W. 2000. Asynchronous accumulation of lettuce infectious yellows virus RNAs 1 and 2 and identification of an RNA 1 trans enhancer of RNA 2 accumulation. J. Virol. 74: 5762-5768.
52. Yoo, S. D., Cho, Y. H., and Sheen, J. 2007. *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat. Protoc. 2: 1565-1572.
53. Zhang, S., and Klessig, D. F. 2001. MAPK cascades in plant defense signaling. Trends Plant Sci. 6: 520-527.

摘 要

陳正恩[1]、葉信宏[1, 2, 3]. 2010. 與植物抗病相關之轉錄因子 WRKY6 在煙草嵌紋病毒感染過程中扮演支持與抑制病毒之雙重角色. 植病會刊 19: 31-40. ([1]國立台灣大學植物病理與微生物學系；[2]國立台灣大學植物醫學研究中心；[3]聯絡作者，電子郵件：hyeh@ntu.edu.tw)

病毒侵染寄主時，是否可以成功進行複製並完成生活史乃是取決於和寄主產生的交互作用。爲了瞭解病毒感染早期所調控的寄主反應，我們以 Microarray 系統的分析菸草嵌紋病毒 (*Tobacco mosaic virus*, TMV) 感染初期 (4 小時) 會對阿拉伯芥的轉錄體造成哪些影響？結果顯示約 2% 的寄主基因在 TMV 感染初期有被顯著的調控 (變化大於 2 倍)，而僅有 8 個基因的變化大於 3 倍，8 個基因中和轉錄調控相關的基因佔了 4 個。進一步挑選其中一個目標基因 WRKY6 transcription factor 進行分析。於先前研究已知，WRKY6 和許多植物生理功能的調節相關，包括植物的抗性反應、發育以及植物對磷、硼元素缺乏的敏感性皆會受 WRKY6 影響。在 TMV 感染 WRKY6 大量表現或抑制表現的原生質體中，TMV 於 24 小時內的累積量未受到明顯影響；但 TMV 的累積量在 WRKY6 大量表現或抑制表現的植物中皆會受到抑制。顯示 WRKY6 並非是病毒感染初期的所需因子，但 WRKY6 在植物中同時扮演了促進和抑制病毒感染的角色。

關鍵詞：菸草嵌紋病毒、阿拉伯芥、Microarray、WRKY6