# The transcription factor AtMYC2 shapes plant defense responses in *Arabidopsis* upon *Pieris rapae* herbivory

Adriaan Verhage[1], Martin De Vos[1], Marcel Dicke[2], and Corné M. J. Pieterse[1]

[1]Plant-Microbe Interactions, Utrecht University, P.O.Box 800.84, 3508 TB Utrecht, the Netherlands; [2]Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, the Netherlands

A.Verhage@uu.nl

## Introduction

Plants have to cope with a variety of biotic stresses, including insect herbivory and pathogen attack. To minimize damage caused by pathogen or insect attack, plants have evolved sophisticated defense mechanisms. Hormones such as jasmonic acid (JA), ethylene (ET), salicylic acid (SA), and abscisic acid (ABA) are important players for determining the proper defense mechanism by the plant. To understand how plants integrate pathogen- and insect-induced signals into specific defense responses, we monitored the dynamics of SA, JA, and ET signaling in *Arabidopsis* after attack by a set of microbial pathogens and herbivorous insects with different modes of attack. *Arabidopsis* plants were exposed to a pathogenic leaf bacterium (*Pseudomonas syringae* pv. *tomato*), a pathogenic leaf fungus (*Alternaria brassicicola*), tissue-chewing caterpillars (*Pieris rapae*), cell-content-feeding thrips (*Frankliniella occidentalis*), or phloem-feeding aphids (*Myzus persicae*) (De Vos et al., 2005). Monitoring the signal signature in each plant-attacker combination showed that the kinetics of SA, JA, and ET production varies greatly in both quantity and timing. Analysis of global gene expression profiles demonstrated that the signal signature characteristic of each *Arabidopsis*-attacker combination is orchestrated into a surprisingly complex set of transcriptional alterations. For instance, although *A. brassicicola, P. rapae,* and *F. occidentalis* all stimulated JA biosynthesis, the majority of the

changes in JA-responsive gene expression was attacker-specific (De Vos *et al.* 2005). A nice example is the expression of the JA-responsive genes *PDF1.2* and *VSP2* (Fig. 1). *A. brassicicola* and *F. occidentalis* both induce *PDF1.2* and not *VSP2*, while *P. rapae* induces *VSP2* and not *PDF1.2* Here, we investigated the molecular mechanism underlying this differential activation of the JA response.

## Unraveling the *Arabidopsis-Pieris rapae* interaction

Plants possess inducible defense mechanisms to cope with attack by herbivorous insects. These involve direct defenses, such as the production of proteinase inhibitors that affect insect feeding, and indirect defenses, such as the production of volatiles that attract parasitoids and predators of the herbivores that feed on the plant (Kessler and Baldwin, 2002; Van Poecke and Dicke, 2004). The plant hormone jasmonic acid (JA) and its oxylipin derivatives are key players in the regulation of these induced plant responses against herbivory (Howe, 2004). Continuing co-evolution between plants and herbivores has provided the latter with mechanisms to avoid, suppress, or eliminate host defenses (Musser et al., 2002; Zarate et al., 2007). Larvae of the specialist insect herbivore *P. rapae* (cabbage white butterfly) feed exclusively on crucifers and are well adapted to the induced defenses of Brassicaceous species. For instance, by detoxifying the glucosinolates that are released by the herbivore-triggered 'mustard oil bomb', *P. rapae* caterpillars

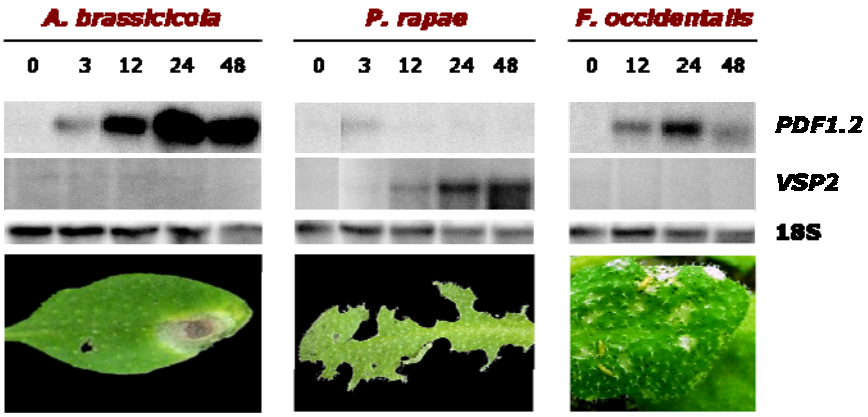


**Fig. 1.** Expression of JA-responsive *VSP2* and *PDF1.2* in wild-type Col-0 during attack by the necrotrophic pathogen *Alternaria brassicicola*, the tissue-chewing insect *Pieris rapae* and the cell-content-feeding insect *Frankliniella occidentalis*.

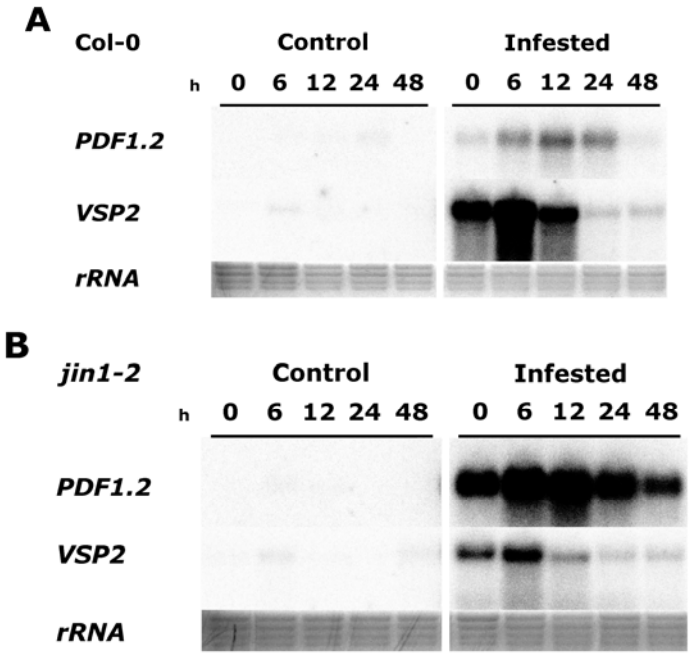


**Fig. 2.** Expression of JA-responsive AtMYC2-induced *VSP2* and AtMYC2-suppressed *PDF1.2* in wild-type Col-0 (A) and AtMYC2 impaired *jin1-2* (B) plants 0, 6, 12, 24 and 48 hours after the removal of caterpillars that had been feeding for 24 hours on these genotypes.

efficiently avoid exposure to these highly toxic chemicals that are released upon herbivory (Ratzka et al., 2002). *P. rapae* has been reported to feed on many Brassicaceous species in the field, including *Arabidopsis* (Yano and Ohsaki, 1993). In *Arabidopsis*, herbivory by *P. rapae* leads to increased production of JAs and extensive reprogramming of the expression of JA-responsive genes, many of which are associated with plant defense (Reymond et al., 2004; De Vos et al., 2005). However, the *P. rapae*-induced transcriptional changes suggest that insect-derived cues play an important role in the modulation of the plant's transcriptional response to herbivory.

## *PDF1.2* transcription is suppressed during herbivory by *P. rapae*

We selected the *Arabidopsis* marker genes *PDF1.2* and *VSP2* from a microarray data search for JA-responsive genes that are differentially

expressed upon mechanical wounding or *P. rapae* feeding, and monitored its expression in *Arabidopsis* upon herbivory. Figure 2A shows that *P. rapae* feeding results in a differential activation of the JA response, resulting a a strong activation of the *VSP2* marker gene, whereas *PDF1.2* was only mildly induced. Previously, AtMYC2 was demonstrated to differentially regulate two branches of the JA-response pathway (Lorenzo et al., 2004), which are exemplified by the JA-responsive genes *PDF1.2* (suppressed by AtMYC2) and *VSP2* (induced by AtMYC2). To investigate whether *AtMYC2* plays a role in the differential activation of the JA response during *P. rapae* feeding, we monitored the expression *VSP2* and *PDF1.2* in mutant *jin1-2*, which is impaired in the *AtMYC2* gene (Lorenzo *et al.*, 2004). Figure 2B shows that in the *AtMYC2* mutant *jin1-2*, *PDF1.2* was strongly induced upon *P. rapae* feeding, whereas *VSP2* was only mildly induced. These results indicate that during *P. rapae* feeding on wild-type plants, AtMYC2 is involved in suppressing the *PDF1.2* branch of the JA-response pathway.

## *AtMYC2* is up-regulated in *Arabidopsis* upon *P. rapae* herbivory

If AtMYC2 plays a role in the suppression of the *PDF1.2* branch of the JA response, then one would expect that *P. rapae* feeding activates the *AtMYC2* gene. To test this hypothesis we monitored the expression of *AtMYC2* during *P. rapae* feeding. In a time course experiment, in which first-instar larvae of *P. rapae* were allowed to feed on 5-week-old *Arabidopsis* plants for 24 hours, infested leaves were harvested at 0, 6, 12, 24 and 48 hours after caterpillar removal. Subsequently, *AtMYC2* gene expression was assessed using qRT-PCR. Fig. 3 shows that *AtMYC2* mRNA levels were highly up-regulated upon *P. rapae* infestation.

## *P. rapae* feeding results in enhanced resistance to secondary *P. rapae* attack, but has no effect on resistance against the necrotrophic pathogen *Alternaria brassicicola*

*P. rapae* and *A. brassicicola* are both sensitive to JA-dependent defenses. To investigate whether *P. rapae* feeding induces resistance against both *P. rapae* itself and *A. brassicicola*, induced resistance assays were performed. Interestingly, *P. rapae* feeding resulted in a systemic defense response that significantly reduced growth of *P. rapae* caterpillars. However, this type of induced resistance was not effective against *A. brassicicola* (De Vos *et al.*, 2006).

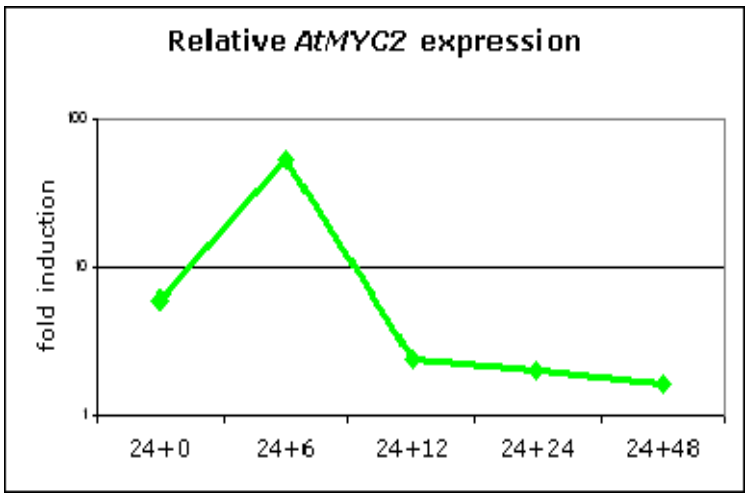


**Fig. 3.** QRT-PCR analysis of *AtMYC2* transcript levels (relative to control) in *P. rapae*-infested Arabidopsis Col-0 plants 0, 6, 12, 24 and 48 hours after the removal of caterpillars that had been feeding for 24 hours on these plants.

## Conclusion

Taken together, these results indicate that in plants attacked by the specialist herbivore *P. rapae* a branch of the JA response (exemplified by *PDF1.2*) is repressed. The master regulator protein AtMYC2 is required for this phenomenon, because suppression of *PDF1.2* was alleviated in *AtMYC2*-impaired *jin1-2* mutant plants. We hypothesize that activation of the *VSP2* branch of the JA-response, such as during herbivory by *P. rapae*, leads to enhanced defense against herbivory, while activation of the *PDF1.2* branch of the JA response confers resistance against necrotrophic pathogens such as *A. brassicicola.*

## Literature cited

De Vos, M., Van Oosten, V.R., Van Poecke, R.M.P., Van Pelt, J.A., Pozo, M.J., Mueller, M.J., Buchala, A.J., Métraux, J.P., Van Loon, L.C., Dicke, M., and Pieterse, C.M.J. 2005. Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. Mol. Plant-Microbe Interact. 18:923-937.

De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, J.P., Dicke, M., Van Loon, L.C., and Pieterse, C.M.J. 2006. Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. Plant Physiol. 142:352-363.

Howe, G.A. 2004. Jasmonates as signals in the wound response. J. Plant Growth Regul. 23:223-237.

Kessler, A., and Baldwin, I.T. 2002. Plant responses to insect herbivory: The emerging molecular analysis. Annu. Rev. Plant Biol. 53:299-328.

Lorenzo, O., Chico, J.M., Sanchez-Serrano, J.J., and Solano, R. 2004. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. Plant Cell 16:1938-1950.

Musser, R.O., Hum-Musser, S.M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J.B., and Felton, G.W. 2002. Herbivory: caterpillar saliva beats plant defences. Nature 416:599-600

Ratzka, A., Vogel, H., Kliebenstein D.J., Mitchell-Olds, T., and Kroymann, J. 2002. Disarming the mustard oil bomb. Proc. Natl. Acad. Sci. U.S.A. 99:11223-11228.

Reymond, P., Bodenhausen, N. Van Poecke, R.M.P. Krishnamurthy, V., Dicke M., and Farmer, E.E. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16:3132-3147

Van Poecke, R.M.P., and Dicke, M. 2004. Indirect defence of plants against herbivores: using *Arabidopsis thaliana* as a model plant. Plant Biol. 6:387-401.

Yano, S., and Ohsaki, N. 1993. The phenology and intrinsic quality of wild crucifers that determine the community structure of their herbivorous insects. Res. Pop. Ecol. 35:151-170.

Zarate, S.I., Kempema, L.A., and Walling, L.L. 2007. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol. 143:866-875.