



**Universiteit
Leiden**
The Netherlands

Functional analysis of jasmonate-responsive transcription factors in *Arabidopsis thaliana*

Zarei, A.

Citation

Zarei, A. (2007, December 11). *Functional analysis of jasmonate-responsive transcription factors in Arabidopsis thaliana*. Retrieved from <https://hdl.handle.net/1887/12484>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/12484>

Note: To cite this publication please use the final published version (if applicable).

Chapter 6

Summary

Jasmonic acid (JA) and related oxylipins, collectively known as jasmonates (JAs), are key regulators of plant development and plant responses to abiotic and biotic challenges (Turner et al., 2002; Farmer et al., 2003). More than four decades after the discovery of methyl jasmonic acid (MeJA) as the major lipid constituent of jasmine scent (Demole et al., 1962), the octadecanoid pathway for biosynthesis of JAs from linolenic acid is now well-established (Turner et al., 2002; Wasternack and Hause, 2002). The expression of all JA biosynthesis genes is induced by wounding or treatment with exogenous JA or MeJA (Turner et al., 2002), indicating that JA signaling is amplified by a positive feedback loop initiated by JA.

In contrast to JA biosynthesis, our current understanding of JA signal transduction steps lags behind (Lorenzo and Solano, 2005). Efforts to elucidate the JA signaling pathway have defined two steps. The first comprises components and regulators of the E3 ubiquitin ligase complex SCF^{coi1} (Lorenzo and Solano, 2005). The second step is defined by transcription factors including AtMYC2, octadecanoid derivative-responsive AP2/ERF-domain transcription factors (ORAs) and ERF1, which regulate the expression of specific sets of JA-responsive genes (Lorenzo and Solano, 2005; Pré, 2006). In the case of AtMYC2, these two steps are linked via members of the JAZ family of repressors (Chini et al., 2007; Thines et al., 2007).

The studies described in this thesis are mainly focused on the transcription factors comprising the second part of JA signaling. The interaction of JA-responsive transcription factors from Arabidopsis with their target genes had not been studied in much detail, which prompted the initiation of the studies described in this thesis. In addition, part of the studies was focused on possible activating effects of JA signaling on the activity of the transcription factor ORA59 at the protein level.

In Arabidopsis, the ERF transcription factor family comprises 122 proteins. In a family-wide screening, Atallah (2005) previously characterized a set of 14 genes which were rapidly induced by JA treatment in young seedlings. Later, Pré (2006) uncovered the function of several ORAs including ORA59, ORA47 and ORA37 by modulating their expression levels and studying the effect on gene expression and plant phenotype.

In certain defense responses, JA and ethylene signaling pathways synergize to activate a specific set of defense genes including *plant defensin1.2* (*PDF1.2*). The transcription factors ORA59 and ERF1 have been suggested to act as integrators of JA and ethylene signaling pathways in Arabidopsis to control this gene subset (Lorenzo et al., 2003; Pré, 2006). Overexpression of ORA59 as well as of ERF1 activates the expression of several defense-related genes including *PDF1.2* and causes increased resistance against the necrotrophic fungus *Botrytis cinerae* (Berrocal-Lobo et al., 2002; Pré, 2006). Analysis of

plants where *ORA59* expression is knocked out by RNAi shows that the JA- and ethylene-responsive expression of defense genes including *PDF1.2* is not controlled by ERF1 as previously reported (Lorenzo et al., 2003), but instead by *ORA59* (Pré, 2006). Therefore, the *ORA59* acts as the integrator of the JA and ethylene signalling pathways and is the key regulator of JA- and ethylene-responsive *PDF1.2* expression.

In **Chapter 2** studies are described which aimed at dissecting the interaction of *ORA59* and the related transcription factor ERF1 with the *PDF1.2* promoter. Two GCC boxes in the *PDF1.2* promoter are important for trans-activation by *ORA59* and ERF1 in transient assays in protoplasts and for in vitro binding. No synergistic effect between ERF1 and *ORA59* in trans-activation of the *PDF1.2* promoter was observed, indicating that each transcription factor acts independently on the *PDF1.2* promoter. Use of the chromatin immunoprecipitation (ChIP) technique showed that *ORA59* binds to the *PDF1.2* promoter in vivo. Interestingly, mutation of a single GCC box at positions -256 to -261 previously reported by others (Brown et al., 2003) to be important for JA-responsive expression completely abolished the expression of the *PDF1.2* promoter in response to JA alone or in combination with the ethylene-releasing agent ethephon, suggesting that in vivo two functional GCC boxes are required for a switch-like effect of these defense hormones on *PDF1.2* gene expression.

The aim of the studies reported in **Chapter 3** was to determine whether JA has an activating effect on *ORA59* at the protein level. The results show that JA caused stabilization as well as nuclear localization of *ORA59*. Domain mapping of *ORA59* showed that stabilization and nuclear localization were conferred by more than one single domain. Interestingly, nuclear localization of *ORA59* did not require a functional COI1 protein. It is postulated that there is a jasmonate receptor distinct from COI1, an F-box protein that targets *ORA59* for degradation, and a repressor protein that retains *ORA59* in the cytoplasm.

ORA47 regulates a completely different subset of JA-responsive genes than *ORA59* does. Inducible overexpression of *ORA47* leads to elevated expression levels of a large number of genes encoding enzymes involved in JA biosynthesis, including AOC2, AOS and LOX2 (Pré, 2006). Oxylin measurements in plants overexpressing *ORA47* revealed strong alterations in the oxylin profile. These results suggest that *ORA47* is responsible for the regulation of the auto-stimulatory loop in JA biosynthesis.

The specific aims of the studies described in **Chapter 4** were to determine whether *ORA47* affects the expression of all four members of the small gene family encoding AOC, and whether the AOC genes are direct targets of *ORA47*. The results showed that the expression of all four members of the AOC gene family was induced by overexpression of *ORA47*. A GCC-like box in the AOC2 promoter interacted specifically with *ORA47* in vitro and

in vivo, and this GCC box is important for ORA47-mediated activity of the *AOC2* promoter. In addition the results showed that ORA47 interacted with the *AOC1* promoter in vivo, and that ORA47 can trans-activate the *AOC1* promoter in a transient assay. Mutation of the GCC-like box in the context of the 600 bp *AOC2* promoter led to a 2.5-fold reduction in the induction level by JA, but did not abolish JA-responsive promoter activity. This indicates that the GCC-like box is important for JA-responsive expression, but that additional JA-responsive sequences are present in the 600 bp *AOC2* promoter.

In *Catharanthus roseus* the AP2/ERF domain transcription factor ORCA3 controls the MeJA-responsive expression of several genes involved in terpenoid indole alkaloid biosynthesis (Memelink et al., 2001). *ORCA3* gene expression is itself induced by MeJA. A 74 bp fragment called "D" from the *ORCA3* promoter functions as an autonomous jasmonate-responsive element (JRE; Vom Endt et al., 2007). The JRE is composed of two important sequences, a quantitative sequence responsible for a high level of expression, and a qualitative sequence that acts as an on/off switch in response to MeJA. The qualitative sequence consists of a G-box with one mismatch (AACGTG). Based on the ability of members from the basic helix-loop-helix (bHLH) family of transcription factors from tomato to bind to such a G-box-like sequence in a JA-responsive promoter (Boter et al., 2004), the qualitative sequence from the *ORCA3* JRE was predicted to interact with a bHLH protein from *Catharanthus*.

The aim of the studies described in **Chapter 5** was to investigate whether the JRE is active in Arabidopsis, and if so, whether its activity depends on the bHLH transcription factor AtMYC2. The results show that the JRE element from the *ORCA3* promoter is functional in Arabidopsis. The JRE interacts in vitro and in vivo in a specific manner with AtMYC2. Analysis of JRE-mediated reporter gene expression in an *atmyc2-1* mutant background showed that the activity was strictly dependent on AtMYC2. As in *Catharanthus* cells (Vom Endt et al., 2007), the JA-responsive activity of the JRE did not depend on de novo protein synthesis in Arabidopsis. This also is consistent with the notion that the JRE is directly interacting with AtMYC2. In addition, it shows that pre-existing AtMYC2 is activated in response to JA without requirement for newly synthesized proteins, which is consistent with the recently discovered mechanism of AtMYC2 activation by COI1- and proteasome-dependent degradation of members of the JAZ family of repressor proteins (Chini et al., 2007; Thines et al., 2007).

The aim of the studies described in this thesis was the functional analysis of JA-responsive transcription factors in Arabidopsis with an emphasis on the interaction with the promoters of their target genes.

In short, the following new results were obtained. The promoter of the *PDF1.2* gene contains at least two functionally equivalent GCC boxes instead of one as reported earlier (Brown et al., 2003). The JA biosynthesis genes *AOC1* and *AOC2* are direct target genes of ORA47, corroborating the notion that ORA47 controls JA biosynthesis via regulation of the biosynthesis genes (Pré, 2006). JA controls ORA59 activity by promoting the nuclear localization and stabilization of the protein. JA-responsive nuclear localization is independent of the jasmonate receptor COI1, indicating the existence of an alternative jasmonate receptor. The JRE from the *ORCA3* promoter is active in Arabidopsis and its activity is controlled by the bHLH transcription factor AtMYC2, suggesting that a related bHLH protein controls *ORCA3* gene expression in *Catharanthus*.

Interesting follow-up questions concern the mechanisms of ORA59 stabilization and nuclear localization and the identity of the alternative jasmonate receptor. In addition, it will be interesting to identify the bHLH protein(s) from *Catharanthus* responsible for JA-responsive *ORCA3* expression, and to study their regulation and their impact on terpenoid indole alkaloid biosynthesis.

References

- Atallah, M.** (2005). Jasmonate-responsive AP2-domain transcription factors in Arabidopsis. PhD thesis. Leiden University, Leiden, The Netherlands.
- Berrocal-Lobo, M., Molina, A., and Solano, R.** (2002). Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. *Plant J.* **29**, 23-32.
- Boter, M., Ruiz-Rivero, O., Abdeen, A., and Prat, S.** (2004). Conserved MYC transcription factors play a key role in jasmonate signaling both in tomato and Arabidopsis. *Genes Dev.* **18**, 1577-1591.
- Brown, R.L., Kazan, K., McGrath, K.C., Maclean, D.J., and Manners, J.M.** (2003). A role for the GCC-box in jasmonate-mediated activation of the *PDF1.2* gene of Arabidopsis. *Plant Physiol.* **132**, 1020-1032.
- Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and Solano, R.** (2007). The JAZ family of repressors is the missing link in jasmonate signalling. *Nature.* **448**, 666-671.
- Demole, E., Lederer, E. and Mercier, D.** (1962). Isolement et détermination de la structure du jasmonate de méthyle, constituant odorant caractéristique de l'essence de jasmin. *Helv. Chim. Acta* **45**, 675-685.
- Farmer, E.E., Almeras, E., and Krishnamurthy, V.** (2003). Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Curr. Opin. Plant Biol.* **6**, 372-378.
- Lorenzo, O., Piqueras, R., Sanchez-Serrano, J.J., and Solano, R.** (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *Plant Cell* **15**, 165-178.

-
- Lorenzo, O., and Solano, R.** (2005). Molecular players regulating the jasmonate signalling network. *Curr. Opin. Plant Biol.* **8**, 532-540.
- Memelink, J., Verpoorte, R., and Kijne, J.W.** (2001). ORCAnization of jasmonate-responsive gene expression in alkaloid metabolism. *Trends Plant Sci.* **6**, 212-219.
- Pré, M.** (2006). ORA EST: Functional analysis of jasmonate-responsive AP2/ERF-domain transcription factors in *Arabidopsis thaliana*. PhD thesis. Leiden University, Leiden, The Netherlands.
- Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A., and Browse, J.** (2007). JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature.* **448**, 661-665.
- Turner, J.G., Ellis, C., and Devoto, A.** (2002). The jasmonate signal pathway. *Plant Cell* **14** Suppl., S153-S164.
- Vom Endt, D., Soares e Silva, M., Kijne, J.W., Pasquali, G., and Memelink, J.** (2007). Identification of a bipartite jasmonate-responsive promoter element in the *Catharanthus roseus* *ORCA3* transcription factor gene that interacts specifically with AT-hook DNA-binding proteins. *Plant Physiol.* **144**, 1680-1689.
- Wasternack, C., and Hause, B.** (2002). Jasmonates and octadecanoids: Signals in plant stress responses and development. *Prog. Nucleic Acid Res. Mol. Biol.* **72**, 165-221.

