Testing the importance of jasmonate signalling in induction of plant defences upon cabbage aphid (Brevicoryne brassicae) attack

Anna Kuśnierzcyk1, Diem HT Tran1, Per Winge1, Tommy S Jørstad2, John C Reese3, Joanna Troczyńska4 and Atle M Bones1*

Abstract

Background: Phloem-feeding aphids deprive plants of assimilates, but mostly manage to avoid causing the mechanical tissue damage inflicted by chewing insects. Nevertheless, jasmonate signalling that is induced by infestation is important in mediating resistance to phloem feeders. Aphid attack induces the jasmonic acid signalling pathway, but very little is known about the specific impact jasmonates have on the expression of genes that respond to aphid attack.

Results: We have evaluated the function that jasmonates have in regulating Arabidopsis thaliana responses to cabbage aphid (Brevicoryne brassicae) by conducting a large-scale transcriptional analysis of two mutants: aos, which is defective in jasmonate production, and fou2, which constitutively induces jasmonic acid biosynthesis. This analysis enabled us to determine which genes’ expression patterns depend on the jasmonic acid signalling pathway. We identified more than 200 genes whose expression in non-challenged plants depended on jasmonate levels and more than 800 genes that responded differently to infestation in aos and fou2 plants than in wt. Several aphid-induced changes were compromised in the aos mutant, particularly genes connected to regulation of transcription, defence responses and redox changes. Due to jasmonate-triggered pre-activation of fou2, its transcriptional profile in non-challenged plants mimicked the induction of defence responses in wt. Additional activation of fou2 upon aphid attack was therefore limited. Insect fitness experiments revealed that the physiological consequences of fou2 mutation contributed to more effective protection against B. brassicae. However, the observed resistance of the fou2 mutant was based on antibiotic rather than feeding deterrent properties of the mutant as indicated by an analysis of aphid feeding behaviour.

Conclusions: Analysis of transcriptional profiles of wt, aos and fou2 plants revealed that the expression of more than 200 genes is dependent on jasmonate status, regardless of external stimuli. Moreover, the aphid-induced response of more than 800 transcripts is regulated by jasmonate signalling. Thus, in plants lacking jasmonates many of the defence-related responses induced by infestation in wt plants are impaired. Constant up-regulation of jasmonate signalling as evident in the fou2 mutant causes reduction in aphid population growth, likely as a result of antibiotic properties of fou2 plants. However, aos mutation does not seem to affect aphid performance when the density of B. brassicae populations on plants is low and aphids are free to move around.

Keywords: aphid, gene expression, infestation, jasmonic acid signalling, microarrays, plant defence, EPG

* Correspondence: atle.bones@bio.ntnu.no
1Department of Biology, The Norwegian University of Science and Technology, Realfagbygget, 7491 Trondheim, Norway
Full list of author information is available at the end of the article

© 2011 Kuśnierzcyk et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Background

Jasmonates, including jasmonic acid (JA) and the biologically active intermediates and derivatives of the JA biosynthetic pathway, are powerful regulators of plant development and inducible resistance. By mediating signal transduction they influence changes in expression profiles of a wide range of genes involved in plant defence [1]. Induction of JA-related response has often been linked to tissue damage, and the important roles of JA signalling in defence against bacterial and fungal infections or caterpillar attack are well documented (for reviews [2-4]). More recent research, however, provides evidence for the activation of JA-mediated defence upon attack by phloem-sucking insects, such as aphids and silverleaf whitefly nymphs, which try to avoid tissue damage during feeding [5-10]. Phloem feeders possess stylet-like mouthparts, which they use to ingest phloem sap. During penetration of plant tissue the stylet is manoeuvred through plant tissue until it is finally anchored in a sieve tube element. Here it can stay for several hours or even days, facilitating a continuous sap supply. By avoiding extensive tissue wounding, aphids minimize the risk of inducing defence responses in the attacked plant while depriving it of assimilates. In the case of a massive infestation, the loss of nutrients interferes with plant growth and development, and may eventually lead to plant death. Constitutive or transient activation of JA-related responses is known to enhance a plant’s resistance to phloem feeders, including aphids [11-13].

JA is biosynthesized from polyunsaturated fatty acids released from chloroplast membranes via a series of enzymatic reactions usually referred to as the octadecanoid pathway. In pathogen-free laboratory conditions, a non-functional JA pathway does not result in any disturbance in normal vegetative growth. In a more natural environment, however, mutant plants that do not synthesize JA are more susceptible to pathogen attack because they fail to activate JA-dependent defences [14]. A knock-out mutation of the allene oxide synthase (AOS) gene, whose product is an enzyme essential for the synthesis of 12-oxophytodienoic acid (OPDA), a precursor for the synthesis of JA, results in a phenotype unable to produce JA or any JA derivatives [15] (Additional file 1 Figure S1). AtAOS is a single-copy gene, and no alternative enzymes possessing the same catalytic activity have been found in Arabidopsis [16]. Thus, the induction of JA-dependent genes is impaired in the aos mutant [15].

The fatty acid oxygenation up-regulated 2 (fou2) mutant was isolated by Bonaventure and co-workers in a search for plants with increased activity of two key JA biosynthetic enzymes: lipoxygenase (LOX) and AOS. JA and OPDA levels are almost doubled in non-challenged fou2 plants compared to wt [17] (Additional file 1 Figure S1). The fou2 allele carries a missense mutation resulting in an amino acid substitution in the Two Pore Channel 1 (TPC1) protein (encoded by At4g03560) [17]. TPC1 forms a non-specific, slowly activating, Ca\(^{2+}\)-regulated cation channel in vacuolar membranes [18]. In fou2 the TPC1 channel has different electrophysiological properties: lower voltage is required for its activation and its time-dependent conductance is higher than in wt [17]. Probably due to the increased sensitivity of voltage sensors in the mutated TPC1, the activation of the JA biosynthetic pathway upon wounding is stronger in fou2 plants and the levels of free JA and OPDA are higher in the mutant relative to wt [17].

Transcriptional analyses of aphid-infested Arabidopsis plants have revealed substantial changes in the expression profiles of many defence-related genes [7,9,19-21]. Several genes whose products are involved in JA synthesis or JA-dependent signalling have been reported to be up-regulated, indicating that JA-derived compounds play a role in the regulation of expressional changes. As a result of transcriptional reprogramming, the production of proteins involved in defence is promoted [22] and the metabolite profiles of plants are changed [7,23-25]. Despite significant progress in our understanding of plant responses triggered by phloem feeders attack (for reviews: [26-30]), it is largely unknown how much the induction of these defences relies on JA signalling.

In this study, we provide new insights into the role of jasmonates in the regulation of defence responses upon aphid attack. A specialized phloem feeder is represented by the cabbage aphid, Brevicoryne brassicae, for which a model of Arabidopsis-aphid interactions has been well established [8]. Our aim is to identify the genes whose expressional changes are controlled by JA signalling. The subsequent parts of this work concentrate on the following problems: Which genes are primarily dependent on jasmonates for their expression? How is the aphid-induced plant defence affected by the absence of JA or the constitutive up-regulation of the JA pathway? How does the impact of the aos and fou2 mutations affect aphid performance? To address these problems we have performed transcriptional profiling of both aphid-challenged and non-challenged wild type plants as well as aos and fou2 mutants using full genome oligonucleotide microarrays. Further, insect fitness experiments and Electrical Penetration Graph analysis have been undertaken to determine how the JA status of the host plants influences the survival and behaviour of insects.

Results

To investigate the importance of JA signalling in transcriptional reprogramming of A. thaliana triggered by aphid attack, we designed an experiment that included
comparisons of genome-wide transcription profiles at three levels (Figure 1). Each level was comprised of a series of microarray hybridizations exploring transcriptional changes in at least three biological replicates per comparison. At the first level, which we regard as the basic comparison, we aimed to identify and classify genes that are dependent on jasmonates for their basic expression. This was done by comparing the transcription profiles of non-challenged wt plants and the two mutants, aos and fou2. At the second level the changes in transcriptional activity resulting from 72 h of aphid infestation of wt, aos and fou2 plants were analysed in each of the three lines independently. At the third level we directly compared aphid-induced transcriptional changes in each of the mutants with the corresponding changes in wt plants. The microarray data generated at all three levels were used in the statistical analysis. Twelve genes that were particularly interesting due to their involvement in JA signalling and/or their association with plant defence responses were further selected for qRT-PCR analysis. The gene expression profiles revealed by qRT-PCR analysis seem to correspond well to the profiles obtained from microarray data (Additional file 2 Figure S2).

Identification of genes regulated by the JA signalling pathway

Both aos and fou2 mutations have a great impact on the regulation of the JA biosynthesis pathway regardless of environmental conditions (Figure 2). Therefore, before investigation of genes whose transcriptional regulation in response to B. brassicae attack is controlled by JA signalling, we aimed to identify the genes whose basic expression in non-challenged plants is modified according to endogenous JA levels. The following criteria have been adopted to identify jasmonate-dependent genes. To be considered positively regulated by jasmonates, a gene had to be down-regulated in aos (log2 ratio < -0.5) and up-regulated in fou2 (log2 ratio > 0.5) as compared to wt. Conversely, the expression of genes classified as negatively regulated by jasmonates was positively affected in aos and negatively affected in fou2, respectively. One-hundred seventy-two genes were found to be positively regulated by jasmonates and have been classified into the following functional gene classes: transcripts involved in JA synthesis and JA signalling, defence-related proteins including myrosinases and myrosinase binding or associated proteins, genes whose products are involved in the regulation of transcription, redox balance, cell wall modification, protein modification, nucleoside/nucleotide metabolism, transport and lipid metabolism (Additional file 3 Table S1). Among the 39 genes whose expression was negatively regulated by jasmonates were several transcription regulators, genes coding for proteins with ankyrin repeats and connected to redox status. Except for genes with unknown functions, other categories were represented by only 1-2 members (Additional file 4 Table S2).

As JA signalling is important in the regulation of plant defensive responses triggered by aphid attack we expected to observe the effect of the changed JA status on the expression of aphid-responsive genes. It should be noted that not all genes classified by us as JA dependent were found to be responsive to B. brassicae attack. Although a number of JA-dependent genes were induced
Figure 2 Regulation of genes involved in the JA biosynthesis pathway. The colour code in squares indicates gene expression changes in aos and fou2 mutants in comparison to wt and changes in B. brassicae infested wt, aos and fou2 versus aphid free control plants of the corresponding genotype. A diagonal line inside a square indicates that gene regulation was not statistically significant. Two crossing diagonal lines indicate that the AOS gene is knockout in the aos mutant. The colour scale represents log2 transformed gene expression ratios.

Abbreviations: aos, gene expression profiles of aos mutant in comparison to wt; fou2, gene expression profiles of fou2 mutant in comparison to wt; [wt, aos, fou2] + aphids, aphid-mediated changes in gene expression profiles in wt, aos and fou2, respectively. LOX2, LIPOXYGENASE 2; AOS, ALLENE OXIDE SYNTHASE; AOC, ALLENE OXIDE CYCLASE; OPDA, (9S,13S)-12-oxo-cis-10,15-phytodienoic acid; OPR3, OPDA REDUCTASE3; OPC-8:0, 3-oxo-2-(cis-2'-pentenyl)-cyclopentane-1-octanoic acid (OPC-8:0); OPC1, OPC-8:0 CoA LIGASE; ACX, OPC-8:0 CoA OXIDASE; MYC2, JASMONATE INSENSITIVE1 transcription factor; JAZ, JASMONATE-ZIM-DOMAIN PROTEIN; SCF^{COI} , SCF ubiquitin E3 ligase-CORONATINE INSENSITIVE1 (COI1) complex.
by *B. brassicae* in wt plants, their aphid-mediated induction was impaired not only in *aos*, as expected, but also in *fou2* plants. This was the case for several transcripts whose products are involved either in the biosynthesis of JA or in JA-mediated signalling (Figure 2), defence-related genes, transcription factors and redox homeostasis. Table 1 summarizes expression profiles of all genes that have been classified by us as JA dependent and whose responsiveness to *B. brassicae* attack was changed in *aos* or *fou2* mutants relative to wt.

**Table 1** Jasmonate-dependent genes whose responsiveness to *B. brassicae* attack was changed in *aos* or *fou2* mutants relative to wt.

| Gene                                      | Accession | Infested with *Brevicoryne brassicae* |
|-------------------------------------------|-----------|--------------------------------------|
| **JA synthesis**                          |           |                                      |
| LOX2                                      | At3g45140 | aos/wt -1.97 focal2/wt 2.09 wtB/wt 0.55 aosB/aos NS fou2B/fou2 -0.42 |
| AOC3                                      | At3g25780 | aos/wt -1.26 focal2/wt 2.36 wtB/wt 2.19 aosB/aos NS fou2B/fou2 NS |
| OPR3                                      | At2g06050 | aos/wt -1.51 focal2/wt 1.62 wtB/wt 0.52 aosB/aos NS fou2B/fou2 -0.47 |
| OPR1                                      | At1g20510 | aos/wt -0.77 focal2/wt 1.13 wtB/wt 1.08 aosB/aos NS fou2B/fou2 0.29 |
| **JA signalling**                         |           |                                      |
| CORI2                                     | At4g23600 | aos/wt -1.60 focal2/wt 2.42 wtB/wt 0.83 aosB/aos NS fou2B/fou2 NS |
| MYC2 (JIN1)                               | At1g32640 | aos/wt -1.45 focal2/wt 1.86 wtB/wt 0.91 aosB/aos NS fou2B/fou2 -0.43 |
| JAZ1                                      | At1g19180 | aos/wt -2.31 focal2/wt 2.62 wtB/wt 2.10 aosB/aos NS fou2B/fou2 NS |
| JAZ2                                      | At1g74950 | aos/wt -0.55 focal2/wt 1.89 wtB/wt 0.69 aosB/aos NS fou2B/fou2 NS |
| JAZ6                                      | At1g72450 | aos/wt -1.41 focal2/wt 2.00 wtB/wt 0.58 aosB/aos NS fou2B/fou2 NS |
| JAZ9                                      | At1g70700 | aos/wt -1.99 focal2/wt 2.65 wtB/wt 0.79 aosB/aos NS fou2B/fou2 NS |
| JAZ10                                     | At5g13220 | aos/wt -0.85 focal2/wt 3.69 wtB/wt 1.10 aosB/aos NS fou2B/fou2 0.91 |
| **Defence**                               |           |                                      |
| PDF1.2                                    | At5g44420 | aos/wt -3.33 focal2/wt 3.53 wtB/wt 2.99 aosB/aos NS fou2B/fou2 NS |
| PDF1.2b                                   | At2g26020 | aos/wt -3.53 focal2/wt 3.31 wtB/wt 3.00 aosB/aos NS fou2B/fou2 NS |
| PDF1.3                                    | At2g26010 | aos/wt -3.46 focal2/wt 3.23 wtB/wt 2.80 aosB/aos NS fou2B/fou2 NS |
| PDF1.2c                                   | At5g44430 | aos/wt -3.32 focal2/wt 3.23 wtB/wt 2.70 aosB/aos NS fou2B/fou2 NS |
| AFP1                                      | At1g75830 | aos/wt -3.04 focal2/wt 3.40 wtB/wt 2.64 aosB/aos NS fou2B/fou2 NS |
| S-adenosylmethionine-dependent methyltransferase | At3g44870 | aos/wt -1.45 focal2/wt 3.13 wtB/wt 1.91 aosB/aos NS fou2B/fou2 NS |
| MBP1                                      | At1g52040 | aos/wt -2.53 focal2/wt 4.98 wtB/wt 0.73 aosB/aos NS fou2B/fou2 NS |
| S-adenosylmethionine-dependent methyltransferase | At3g44860 | aos/wt -1.27 focal2/wt 2.60 wtB/wt 0.98 aosB/aos -0.86 |
| arginase                                  | At4g08870 | aos/wt -1.44 focal2/wt 4.05 wtB/wt 0.94 aosB/aos NS fou2B/fou2 -0.63 |
| strictosidine synthase                    | At3g51450 | aos/wt -1.18 focal2/wt 1.70 wtB/wt 0.55 aosB/aos -1.01 NS fou2B/fou2 NS |
| ED5S                                      | At4g39030 | aos/wt -0.52 focal2/wt 0.87 wtB/wt 1.87 aosB/aos 1.15 NS fou2B/fou2 NS |
| ASA1                                      | At5g05730 | aos/wt -0.70 focal2/wt 0.73 wtB/wt 1.08 aosB/aos 0.64 0.53 fou2B/fou2 NS |
| TAT3                                      | At2g24850 | aos/wt -1.58 focal2/wt 3.52 wtB/wt 4.20 aosB/aos 2.03 NS fou2B/fou2 NS |
| CYP79B2                                   | At4g3995 | aos/wt -0.83 focal2/wt 1.17 wtB/wt 1.41 aosB/aos 1.15 0.47 fou2B/fou2 NS |
| PR4                                       | At3g04720 | aos/wt -0.81 focal2/wt 1.35 wtB/wt 2.32 aosB/aos 0.76 1.13 fou2B/fou2 NS |
| trypsin inhibitor 1 (ATTII1)              | At2g43510 | aos/wt -1.08 focal2/wt 3.71 wtB/wt 1.74 aosB/aos 0.86 1.14 fou2B/fou2 NS |
| trypsin inhibitor                         | At1g73260 | aos/wt -1.65 focal2/wt 2.85 wtB/wt 1.00 aosB/aos 1.44 1.62 fou2B/fou2 NS |
| protease inhibitor (LTP)                  | At5g48490 | aos/wt -0.64 focal2/wt 0.79 wtB/wt -0.79 aosB/aos -0.67 -0.98 fou2B/fou2 NS |
| HSP17.4-CIII                              | At1g54050 | aos/wt -0.95 focal2/wt 0.81 wtB/wt -0.67 aosB/aos -0.59 -0.55 fou2B/fou2 NS |
| **Transcription factors**                 |           |                                      |
| WRKY75                                    | At5g13080 | aos/wt -1.82 focal2/wt 2.52 wtB/wt 3.23 aosB/aos 3.18 1.31 fou2B/fou2 NS |
| ERF2                                      | At5g47720 | aos/wt -1.13 focal2/wt 0.88 wtB/wt 2.06 aosB/aos 1.02 0.42 fou2B/fou2 NS |
| RHL41/ZAT12                               | At5g59820 | aos/wt -1.39 focal2/wt 1.85 wtB/wt 3.02 aosB/aos 2.26 NS fou2B/fou2 NS |

JA signalling has an overall significant impact on the regulation of *Arabidopsis thaliana* responses to *Brevicoryne brassicae* attack

Among all aphid responsive genes that have been classified as JA dependent in non-infested plants, the majority were found to have altered responsiveness to *B. brassicae* attack in the mutants compared to wt (Table 1). However, several other genes that did not change expression in non-challenged *aos* and *fou2* displayed unique responses to aphid infestation in the mutant.
Table 1: Jasmonate-dependent genes whose responsiveness to *B. brassicae* attack was changed in *aos* or *fou2* mutants relative to *wt* (Continued)

| Cat.                        | Genes                                           | aos/wt | fou2/wt | aos/fou2 | fou2/aos | NS    |
|-----------------------------|-------------------------------------------------|--------|---------|----------|----------|-------|
| **Redox**                   |                                                 |        |         |          |          |       |
| Atperox P37                 | At4g08770                                       | -1.46  | 1.08    | 1.57     | 1.36     | 1.41  |
| GST22/ATGSTU4               | At2g29460                                       | -0.73  | 0.99    | 1.87     | 1.20     | 1.05  |
| MDAR4                       | At5g03630                                       | -0.75  | 0.62    | 0.67     | 0.44     | NS    |
| Atperox P32                 | At3g32980                                       | -1.49  | 1.38    | -0.82    | 0.43     | NS    |
| FRO6                        | At5g49730                                       | -0.84  | 0.70    | -0.76    | 0.46     | -1.25 |
| copper amine oxidase        | At1g31710                                       | -1.56  | 1.00    | -1.17    | 0.65     | NS    |
| **Auxin synthesis**         |                                                 |        |         |          |          |       |
| ILL4                        | At1g51760                                       | -0.70  | 1.71    | 1.84     | NS       | NS    |
| NIT2                        | At3g44300                                       | -0.82  | 2.10    | 1.20     | 0.95     | 1.68  |
| **cell wall modification**  |                                                 |        |         |          |          |       |
| PGIP2                       | At5g06870                                       | -0.90  | 2.72    | 0.63     | NS       | 0.70  |
| AGP                         | At1g03820                                       | -0.55  | 2.81    | NS       | 0.57     | 1.53  |
| FLR1                        | At3g12145                                       | -0.91  | 0.24    | 0.58     | 0.53     | 0.60  |
| invertase/pectin methylesterase inhibitor | At1g62770 | -1.30 | 0.87 | -1.01 | 1.63 | 1.18 |
| **lipid metabolism**        |                                                 |        |         |          |          |       |
| esterase/lipase/thioesterase family protein | At2g39420 | -0.77 | 2.29 | 1.60 | NS | NS |
| **unknown**                 |                                                 |        |         |          |          |       |
| unknown plant specific protein (AR781) | At2g26530 | -0.53 | 1.36 | 1.31 | 0.39 | NS |

The values in the table represent log2 transformed gene expression changes for the following comparisons: *aos/wt*, change in a gene expression level in *aos* mutant in comparison to *wt*; *fou2/wt*, change in a gene expression level in *fou2* mutant in comparison to *wt*; *wtB/wt*, change in a gene expression level in *wt* plants attacked by aphids in comparison to aphid-free *wt* controls; *aosB/aos*, change in a gene expression level in *aos* plants attacked by aphids in comparison to aphid-free *aos* controls; *fou2B/fou2*, change in a gene expression level in *fou2* plants attacked by aphids in comparison to aphid-free *fou2* controls; NS, not statistically significantly regulated.
among genes less responsive to aphid attack both in aos and fou2 mutants (Figure 4A, C). These categories taken together contributed almost half of the genes whose responsiveness was negatively affected in aos and fou2 plants (Figure 3). Although the majority of the genes that responded to B. brassicae infestation in wt plants were induced in the challenged aos as well, their regulation was weaker in the mutant than in wt (Additional file 5 Table S3). Twenty two genes, whose products are involved in regulation of transcription and 34 transcripts connected to defence showed no induction or weaker up-regulation upon infestation in the aos mutant. Several

Figure 3 General overview of differences in responsiveness of aos and fou2 mutants to B. brassicae attack compared to responsiveness in wt. Bars represent contribution of different functional categories in the pool of all genes that were either less or more induced upon infestation with aphids in aos or fou2 genotype in comparison to wt. Numbers placed on the top of each bar indicate how many genes were differentially regulated in response to B. brassicae attack in each functional category in a given mutant as compared to wt.
A) Genes less induced in response to *B. brassicae* attack in *aos* and *fou2* than in wt

B) Genes more induced in response to *B. brassicae* attack in *aos* and *fou2* than in wt

C) Genes less induced in response to *B. brassicae* attack in *aos* and *fou2* than in wt

Figure 4 Simplified graphic representation of enriched GO terms connected to biological process or molecular function in genes that were less (A, C) or more (B) induced compared to wt in their response to *Brevicoryne brassicae* attack in *aos* and *fou2* mutants. The graph is based on results generated by AmiGO Term Enrichment [31] of functional gene networks. Functionally connected GO categories are represented with the same colour code. Streaked lines indicate that GO terms that exist between the two connected GO terms were omitted from presentation for clarity reasons. Only GO terms classified as enriched according to AmiGO Term Enrichment (with \( p \) value < 0.05) are presented in the graph. The numbers of genes attributed to a given GO term for *aos* and *fou2* mutants are indicated in the left and right boxes under given GO terms, respectively. A darker shade of grey corresponds to higher significance of enrichment (lower \( p \) value) of a given GO term according to the AmiGO Term Enrichment analysis.
transcription factors and defence-related proteins were, in contrast to wt, either not induced or down-regulated in the aphid-challenged aos plants; i.e. BTB and TAZ domain protein 5 (BT5), dehydration-responsive element-binding protein 2A (DREB2A), ethylene-responsive transcription factor ERF11 and ERF13, mb family transcription factor (MYBS5), C2H2 type family protein, DARK INDUCIBLE 11 (DIN11), sulfotransferase family protein (At5g07010), strictosidine synthase, plant defensive 1 (PDF1), cysteine-rich antifungal protein 1 precursor (AFP1), heat shock protein 81-1 (HSP81-1) and arginase. These observations clearly show that JA signalling is important in the activation of defensive responses triggered by B. brassicae attack. However, the fact that some genes were up-regulated during infestation despite of the lack of AOS enzyme activity indicates that JA signalling is, as expected, not the only system controlling gene regulation. Interestingly, some of the defence-related transcripts (e.g. PR1, HR3, disease resistance genes: At1g57630, At3g25010, At2g47800) accumulated in the non-challenged aos plants as compared to wt, probably as a result of stress connected to the lack of JA or an imbalance between JA and SA signalling pathways.

In the fou2 mutant, several transcription factors and defence-related genes were already up-regulated in non-challenged plants compared to wt, indicating constant activation of Defence caused by the increased endogenous JA levels [e.g. WRKY, ethylene responsive transcription factors, zinc finger family proteins, pathogenesis related proteins PRI1 and PR2, enhanced disease susceptibility 5 (EDS5), protease inhibitors, cysteine-rich antifungal proteins: PDF1.1, PDF1.2, PDF1.2b PDF1.2c, PDF1.3, DARK INDUCIBLE 11 (DIN11)]. Often the induction of these genes was stronger in non-challenged fou2 mutants in comparison to wt than in the infested wt compared to aphid free wt. In such cases no additional induction was noted in the aphid-attacked fou2 mutant compared to the aphid-free fou2 control. For other genes a slight additional induction of already up-regulated transcripts was observed in fou2 plants attacked by B. brassicae (Additional file 7 Table S3). Out of 41 transcription factors and 74 defence-related genes up-regulated upon B. brassicae infestation in wt, but having changed aphid-triggered regulation in one or both mutants, 37 and 69 genes, respectively, were less up-regulated or not induced in the fou2 mutant in response to infestation. These results indicate that the activation of defence responses may have an overall induction threshold. A potential for an additional, aphid-triggered induction is likely limited when the basal activation of transcripts in non-challenged fou2 plants is already very high.

Several senescence-associated genes responded to aphid attack with strong induction. Overall, the intensity of aphid-induced changes in this group of genes was similar in wt and aos plants, but slightly weaker in the fou2 mutant. Thus JA signalling seems not to be the key factor controlling the expression of senescence-associated genes upon infestation.

**Stress signalling in aphid-attacked plants is moderately weaker in the JA-deficient mutant**

Proteins involved in the perception of stress and transduction of signals play an important role in the initiation of defence responses [7]. After 72 h of sustained aphid infestation a large number of genes coding for proteins involved in calcium signalling, signal transduction and redox changes were up-regulated in the aphid-attacked wt plants.

Similar responses were also triggered in the aos mutant but the average intensity of gene regulation was slightly lower compared to wt. Only transcripts associated with redox processes responded to infestation with higher average induction in aos than in wt plants. These observations indicate that the JA-deficient mutant is not impaired in the perception and transduction of signals during infestation and that JA signalling plays only a partial role in the activation of these processes.

In contrast, the aphid-triggered responsiveness of genes connected to stress signalling was reduced in the fou2 mutant. The GO category denoted “regulation of biological processes”, which included “regulation of response to stimuli” and “signal transduction”, was statistically significantly enriched as indicated by the GO Term Enrichment analysis of genes that were less responsive to infestation in the fou2 mutant (Figure 4A). Signal transduction, calcium signalling and redox gene categories were also abundantly represented among genes that were less induced by infestation in fou2 than in wt (Figure 3). The expression of 45, 20 and 16 genes related to respective functional categories were either not changed, changed to a lesser extent than in wt or were oppositely regulated in response to infestation in fou2 plants (Additional file 7 Table S5). However, some of these genes were up-regulated in the non-challenged fou2 mutant in comparison to wt [e.g. calcium-binding EF-hand: CML38, CML41, CaMBP25, blue copper-binding, DSBA oxidoreductase family gene, monodehydroascorbate reductase MDAR1, glutaredoxin family protein (GRX480), thioredoxin H-type 5 (TRX5), FAD-linked oxidoreductase, peroxidase 32 precursor (PER32)]. Thus, processes connected to the perception and transduction of signals seem to be imbalanced in the non-challenged fou2 mutant and their activation upon aphid infestation might be impaired.

**Changed JA status leads to the induction of genes connected to transport and cell wall modifications**

Both aos and fou2 mutants responded to infestation by up-regulation of genes linked to transport, while the
average expression profile of these genes in wt plants remained unchanged after B. brassicae attack. GO Term Enrichment analysis indicated that mainly GO terms connected to boron and lipid transport were effected in fou2 (Figure 4B). It is possible that in response to infestation, plants in which the JA synthesis rate is somehow disturbed (either from a lack of JA in aos or an overproduction of JA-related compounds in fou2) try to compensate for unbalanced JA signalling by induction of cellular transport.

Interestingly, some genes whose products are involved in cell wall modification were differentially regulated upon infestation in the mutant plants in comparison to wt. These genes also make a considerable contribution to the set of all genes that were more induced by aphid attack in aos and fou2 mutants than in wt (Figure 3). As revealed by AmiGO Term Enrichment analysis, GO terms connected to cell wall organization and aminoglycan and polysaccharide metabolic processes are overrepresented in the set of genes that were more induced by aphid attack in the fou2 mutant (Figure 4B). Generally these genes were slightly down-regulated in the aphid-challenged wt plants, not responsive in infested aos and slightly up-regulated in infested fou2. Their expression was not changed in aphid-free mutants as compared to wt. Thus, it seems that hyper-activation of the JA signalling pathway in the fou2 mutant might cause some changes in cell walls that do not occur in the infested wt plants.

The fou2 mutation increases plant resistance to Brevicoryne brassicae by a mechanism other than feeding deterrence

The relative susceptibility of aos, fou2 and wt plants to infestation with B. brassicae was evaluated in aphid fitness experiments. First instar nymphs were placed on each of the three genotypes and their asexual fecundity was monitored simultaneously. After 13 days the number of offspring did not differ significantly between aos and Col-0 plants. However, aphid fecundity on the fou2 mutant was significantly lower when compared to the fecundity observed on aos and wt plants (Figure 5). To further investigate whether some anti-xenotic (feeding deterrent) factors are involved in the observed resistance of fou2 to B. brassicae, we employed the Electrical Penetration Graph (EPG) technique. EPG allowed us to monitor and compare the amount of time the aphids spent on various activities connected to the penetration of plant tissue and ingestion of phloem sap on fou2 mutants and wt plants. The electrical waveforms, corresponding to non-probing (when the stylet does not have any contact with plant tissue), pathway (where the stylet is manoeuvred through plant tissue accompanied by sheath salivary discharges), the sieve element phase (called SEP, when the stylet is located in a sieve element), and xylem phase (when the stylet is located in a xylem cell) were recorded for 8 h and categorized according to known wave patterns corresponding to each activity. The average time spent on each activity was calculated separately for feeding on fou2 and wt plants. The time aphids spent on non-probing, pathway, and SEP was similar in the case of fou2 and wt plants (Figure 6). As phloem sap uptake from fou2 mutants was not restricted, we conclude that feeding

![Figure 5 Asexual fecundity of Brevicoryne brassicae on wt Col-0, aos and fou2 plants](http://www.biomedcentral.com/1471-2164/12/423)

![Figure 6 The relative amount of time spent on different feeding behaviours by Brevicoryne brassicae on fou2 and wt plants as revealed by Electrical Penetration Graph recordings during 8 hours-long experiments](http://www.biomedcentral.com/1471-2164/12/423)
deterrence was not the factor limiting *B. brassicae* population size on *fou2* plants.

**Discussion**

**JA signalling contributes to aphid-triggered regulation of a wide range of genes**

Several experiments have proven that infestation with phloem feeders leads to extensive transcriptional reprogramming of the attacked plants. Gene expression changes manifested in the current experiment in *wt* plants 72 h after infestation with *B. brassicae* correspond well to the changes previously observed in different *A. thaliana* ecotypes attacked by green peach aphid (*Myzus persicae*) or *B. brassicae* [9]. Although such a long period of infestation may cause secondary effects linked to withdrawal of significant amounts of amino acids and sugars contained in the phloem sap, most of the transcriptional changes were similar to those observed in earlier phases of infestation (e.g. 48 h, 24 h, or even 12 h) [7]. This indicates that there is no dramatic change in the type of responses activated 72 h after aphid attack as compared to earlier stages of infestation.

Jasmonates are physiological signals for defence. The enhanced production of JA in response to pathogen and insect attack regulates expression of many defence-related genes and may induce broad-spectrum resistance [32]. Interestingly, many of the genes that were up-regulated in response to infestation in *wt* plants have shown similar induction in the non-challenged *fou2* mutant. Characterization of *fou2* by Bonaventure and co-workers revealed strong induction of defensive mechanisms resulting from overproduction of JA [33]. Other studies have demonstrated that the application of methyl jasmonate also causes activation of the JA pathway and similar up-regulation of genes connected to defence, responses to oxidative stress, and cell wall modification [34-36]. Similar changes have also been detected at the protein level [37]. Although plants that are deficient in the production of JA do not show any symptoms of disease when grown under laboratory conditions, our study clearly shows that lack of JA negatively influences the basal expression of a wide range of genes. As expected, many of these genes encode proteins that are directly or indirectly involved in plant defence. A number of JA-dependent defence-related transcripts were induced in *wt* plants during *B. brassicae* attack, but only a few of these were activated in the challenged *aos* mutant, which showed that the regulation of these genes upon aphid attack is primarily controlled through JA signalling. Aphid-mediated induction of many other genes was clearly affected by the *aos* mutation as well. Although the transcription of many of these genes was apparently not dependent on the JA status in non-challenged plants, JA-derived signals comprised a significant contribution to their regulation in infested plants. Aphid-induced changes in the expression of a number of transcription factors such as WRKY, C2H2 zinc fingers, BTB and TAZ domain containing proteins and ERFs were weaker in *aos* than in *wt*, indicating the importance of JA for their induction. WRKY transcription factors are important in SA-dependent defence and some are implicated in cross-talk between JA and SA signalling [38]. Transcription factors containing ethylene responsive domains have been shown to be regulated by JA [39,40] and to participate in plant stress responses [41-43]. They may integrate ET- and JA-derived signals, possibly by interaction with the GCC box in the promoter region of JA-regulated genes [44] and act as both positive and negative regulators of transcriptional changes [39]. Transcription factors such as AP2-domain protein ERF018/ORA47, ZAT10 and AZF2 have been previously identified as both positive and negative regulators of JA signalling [45]. However, their involvement in the activation of plant defence has not been assessed yet. Strong up-regulation of these genes in *wt* plants attacked by *B. brassicae* suggests that they play an important role in defence against aphids. The regulatory function of BTB and TAZ domain containing proteins has not been established yet, but BTB and TAZ domain protein (BT2) have been identified as essential components of the TELOMERASE ACTIVATOR1 (TAC1)-mediated telomerase activation pathway [46]. Telomerase activity is high in plants in rapidly dividing cells and reproductive organs. The induction of BT2 and BT5 in the non-challenged *aos* plants suggests that these genes are under negative regulation of JA. All five BTB and TAZ proteins (BT1-BT5) are known to be readily induced by H$_2$O$_2$ and SA treatments [47].

The glutaredoxin family protein GRX480, whose induction was eliminated in the infested *aos* plants, was recently identified as a regulator of JA/SA cross talk. It interacts with TGA transcription factors to antagonize expression of JA-responsive genes in an NPR1-dependent manner [48]. Our results indicate that the induction of GRX480 upon *B. brassicae* attack is dependent on JA levels.

The expression of *EDS5* in both non-challenged and aphid-attacked plants shows that JA levels also influence it. This is in contrast to previous reports, which describe solitary SA signalling based regulation of the *EDS5* gene [49]. Our results suggest that regulation of *EDS5* is more complex than previously thought.

**Additional signals are involved in regulation of the response to *B. brassicae* infestation**

Some genes, whose expression in non-challenged plants was clearly dependent on JA responded to infestation in the *aos* mutant despite the lack of JA-derived signals,
even though their induction was not as extensive as the induction observed in wt plants. This indicates that, in addition to JA, some other signalling mechanisms are involved in the regulation of these transcripts upon *B. brassicae* infestation. It is well established that the activation of invader-specific responses in plants attacked by insects is mediated by cross-talk between different signalling pathways [38]. In the case of insect infestation, in addition to JA, phytohormones such as salicylic acid (SA), ethylene (ET) and abscisic acid (ABA) play major roles in coordinating the induction of appropriate defences [26,50]. Thus SA, ET or ABA are likely regulators of the defence responses in the absence of JA for genes such as trypsin inhibitors (*ATT11* and *At1g73260*), TAT3, CYP79B2, PR4 or ASA1.

### Induction of JAZ repressors desensitizes fou2 response to *B. brassicae* attack

The transcriptional profile of the non-challenged *fou2* genotype mimics the profile of wt plants that manifest induced defence [33]. In our studies many of the genes that have been shown to be involved in the response to aphid attack in wt plants were up-regulated in the non-challenged *fou2* mutant, often showing similar or stronger intensity of changes compared to attacked wt plants (Table 1 and Additional file 7 Table S5). A similar induction of transcription factors and defence-related genes was observed by Bonaventure and co-workers [33]. However, in contrast to the previously observed reaction of *fou2* to wounding [17], further induction of these transcripts upon infestation was much weaker than observed in wt plants. A similar lack of stress responses resulting from prolonged high endogenous JA levels was observed in potato plants subjected to wounding and water stress. Although several of the genes involved in JA biosynthesis are induced by JA thereby creating a positive feedback loop [51], there exists also a negative regulatory feedback loop protecting the plants from the adverse effects of their own defence. The constitutive up-regulation of the JA synthesis pathway in the *fou2* mutant probably triggers this negative feedback loop, leading to desensitization of processes involved in the activation of the aphid-induced defence. JAZ family proteins act to repress transcription of JA-inducible genes and thus modulate JA-mediated plant responses [52,53]. The high induction of several JAZ genes in the *fou2* mutant (Additional file 3 Table S1) indicates activation of the desensitization mechanism and may explain the reduced responsiveness of *fou2* plants challenged with *B. brassicae*. The negative regulation of JA responses is delayed and takes effect some time after the proceeding induction [45]. The hyper activation of JA biosynthesis genes in *fou2* plants shortly after mechanical wounding that was observed by Bonaventure and co-workers [17] was not observed by us after 72 h of sustained *B. brassicae* infestation. This might be due to a stealthy manner of aphid feeding that causes only minimal tissue damage. The induction of the wound-specific JA responses in aphid-infested plants is therefore much weaker than in mechanically wounded plants. In addition, the high level of JAZ repressors may also tune the JA-regulated transcriptional changes in the aphid-attacked *fou2* plants after 72 h.

### Aphid fitness is comparable on wt and aos genotypes but reduced on *fou2*

Despite the reduced responsiveness of a wide range of defence-linked genes in the *aos* mutant, we did not observe any improvement in aphid fitness in comparison to wt plants. This may seem surprising as JA signalling seems to be important for plant defence mechanisms induced upon infestation. In contrast to our results, Ellis and co-workers observed increased growth of green peach aphid (*Myzus persicae*) populations on the *coi1-16* mutant that had defects in JA signalling [13]. However the *coi1-16* line carries an additional mutation that might have influenced *M. persicae* responses observed by Ellis and co-workers. This mutation lies in the PENETRATION2 (*PEN2*) gene encoding a glycoside hydrolase and renders the PEN2 protein with highly reduced stability [54]. *PEN2* is required for indole glucosinolate-dependent pathogen-induced callose deposition [55]. 

As accumulation of callose is one of the defence mechanisms against aphid infestation [7], the *pen2-4* mutation, present in *coi1-16* line, may contribute to the increased susceptibility of *coi1-16* plants to infestation with *M. persicae*.

It is also conceivable that the expressional changes of JA-regulated genes observed by us in the aphid-infested *aos* mutant were sufficient to sustain the same level of aphid resistance/susceptibility as is present in wt plants. It should be noted that many genes known to be regulated by SA, ABA or auxin signalling were up-regulated in *aos* plants. Several of these can be involved in defence against *B. brassicae* infestation and influence aphid fitness.

As revealed by the insect fitness tests, physiological changes resulting from the *fou2* mutation render plants more resistant to infestation than wt, despite the reduced intensity of the aphid-induced responses. As the observed resistance was not based on feeding deterrence, it is most probably based on antibiosis. Various defence-related responses that are constitutively activated in *fou2* plants, e.g. high expression of plant defensin proteins (PDFs), pathogenesis-related proteins (PR) or protease inhibitors, can exhibit an antibiotic effect on insect pests. The latter, for example, can disturb digestion and absorption of food in the insect gut [27]. Moreover, the high activity of LOX enzyme in *fou2* plants can increase production of reactive lipid peroxides, cause oxidative damage to the insect gut.
and significantly decrease the nutritive quality of dietary proteins [56]. It should be noted, however, that the mechanism responsible for the manifestation of the fou2 phenotype is not fully understood. Therefore, we cannot eliminate the possibility that other, unknown, features of fou2 could play a role in mediating aphid resistance.

Conclusions
A comparison of transcriptional profiles of non-challenged aos, fou2 and wt plants allowed us to identify more than 200 genes whose expression profiles in non-challenged plants were dependent on endogenous jasmonate status. Most of these transcripts were up-regulated in fou2 and down-regulated in aos mutants, which points to a positive regulatory function of JA-derived compounds. Many of the jasmonate-dependent genes were connected to regulation of transcription, defence responses, redox balance and cell wall modification.

Upon infestation with Brevicoryne brassicae, the responsiveness of many genes was changed in aos and fou2 plants. Genes attributed to GO categories connected to the regulation of transcription and responses to stress were generally less induced in both mutants. In contrast, transcripts classified as involved in cell division and development, cell wall modification and transport were more induced or not as much down-regulated in the mutants compared to wt. The observed changes in aphid-mediated responsiveness of aos had, however, no noticeable impact on aphid fitness. This may indicate that the induced responses, although weaker than in wt, were strong enough to keep the same level of resistance. Alternatively, responses were mainly induced locally, so that the aphids could benefit from frequent changes of feeding places. In the fou2 mutant, several genes involved in defence against B. brassicae were induced in non-challenged plants. As a consequence, the transcriptional profile of non-challenged fou2 resembled the aphid-induced profile of wt. Although additional B. brassicae mediated regulation of non-induced genes was limited, the aphids’ reproduction rate was negatively influenced by the fou2 mutation. As an array of defensive responses is constitutively activated in fou2 plants, the feeding aphids could not move to a leaf area where the response was not induced, as they could in the case of wt plants.

Our results indicate that JA-regulated responses are important in defining susceptibility of a plant to infestation with aphids. As shown in this study, JA-derived compounds are powerful regulators of a range of defensive responses exhibited by plants attacked by aphids.

Methods

Plant material
The Arabidopsis thaliana Columbia-0 ecotype (Col-0) single seeds line used in the experiment has been derived from seeds produced by Lehle Seeds (Round Rock, USA; Catalogue No. WT-2-8, Seed Lot No. GH195-1). The aos mutant was the one described in [15]. The fou2 mutant was kindly donated by Prof. Edward Farmer (University of Lausanne, Switzerland). Both mutants are in Col-0 background. Seeds were sterilized according to standard procedures and plants were initially grown aseptically on agar medium containing MS basal salt mixture (Sigma), 3% (v/w) sucrose, and 0.7% (v/w) agar (pH 5.7) to assure uniform germination. After 15 days, seedlings were moved to 6 cm diameter pots (3 seedlings per pot) filled with a sterile soil mix (1.0 part soil, and 0.5 part horticultural perlite). Plants were kept in growth chambers Vötsch VB 1514 (Vötsch Industrietechnik GmbH, Germany) under the following conditions: a 8/16 h (light/dark) photoperiod at 22°C/18°C, 40%/70% relative humidity, and 70/0 μmol m⁻² s⁻¹ light intensity. A short time day was applied to prevent plants from bolting. For aphid fitness experiments, plants were sown directly to pots with soil (one plant per pot) and kept in chambers under a 16/8 h (light/dark) photoperiod.

Insects
Brevicoryne brassicae was reared on Brassica napus or Brassica oleracea plants in a growth chamber with a 16/8 h (light/dark) photoperiod at 22°C/18°C, 40%/70% relative humidity, and 70/0 μmol m⁻² s⁻¹ light intensity.

Infestation experiments
Thirty-two-day-old plants (17 days after transferring to soil) had 8 fully developed leaves. Each plant was infested with 32 wingless aphids (4 per leaf), which were transferred to leaves with a fine paintbrush. Infested plants and aphid-free controls were kept in plexiglass cylinders as described in [9]. Plants were harvested 72 h after infestation between the 6th and 8th hour of the light photoperiod. Four biological replicates were run, each sampled from 15 individual plants. Whole rosettes were cut at the hypocotyls and aphids were removed by washing with Milli-Q filtered water. Harvested material was immediately frozen in liquid nitrogen.

RNA isolation, cDNA synthesis and microarray experiments
All procedures were done as described in [7]. Custom-designed, full genome Arabidopsis oligonucleotide microarrays printed at the Norwegian Microarray Consortium (Trondheim, Norway) were used in all experiments.

Quantitative real-time PCR
For qRT-PCR analysis, the total RNA was DNase treated using DNA-free™ Kit (Applied Biosystems), while the QuantiTect® kit (QIAGEN) was used for cDNA
synthesis. A LightCycler 480 System and the corresponding SYBR Green I Master mix (Roche Diagnostics GmbH) were used in a three-step programme including (1) preincubation at 95°C for 5 min; (2) 40 cycles of amplification consisting of 95°C for 10 s, 55°C or 60°C for 10 s and 72°C for 10 s; and (3) melting curve analysis by heating from 65°C to 97°C with a ramp rate of 2.2°C/s. Each 20 μl reaction contained 0.5 μM of each forward and reverse primer (for gene-specific primer sequences used in qRT-PCR, see Additional file 9 Table S7), and cDNA quantity corresponding to 50 ng of RNA. LinRegPCR software [57] was used to determine the PCR reaction efficiency for each sample and the efficiencies for each primer set were calculated by averaging the efficiency values obtained from the individual samples. Relative expression ratios of the targeted genes were calculated and normalized to TIP41-like gene (At4g34270) [58] with the use of REST 2008 software [59]. The qRT-PCR analysis was performed with the use of three biological replicates.

Statistical analysis of microarray data

The microarray experiment was a 2-by-3 factorial, with the factors as plant type (wt, aos mutant or fou2 mutant) and treatment (infested or not infested). Each experimental condition, i.e. each combination of factors, was represented by four biological replicates. Seven different direct comparisons of the experimental conditions, using four replicates (each representing 15 individual plants) for each comparison, were made with the use of microarray data sets. However, only data from microarrays with very good technical quality were used for further analyses. (Figure 1 shows the direct comparisons that were made and the comparisons for which only three replicates were of good enough technical quality). Note that using this setup means that the same biological replicate will occur on two different microarrays. Also note that experimental conditions that were not compared directly can still be contrasted, but with lower efficiency than the direct comparisons.

The microarray data for each array were filtered and normalized as discussed in [7]. To make statistical inferences about differential regulation between experimental conditions, the limma package [60] was used. In each comparison of experimental conditions a q-value [61] was calculated for each gene. For a gene to be considered differentially regulated at a statistically significant level, its q-value had to be lower than 0.05. In effect this controlled the false discovery rate (FDR) [62-64] of the comparison at a 0.05 level.

Aphid fitness experiments

B. brassicae fitness on aos and fou2 mutants in comparison to wt Col-0 was evaluated in experiments assessing aphid asexual fecundity. Two first instar nymphs were placed on each plant and plants were placed in plexiglass cages (3 plants per cage). Eleven cages (33 plants) were used for each genotype tested. After 13 days, aphid progeny numbers in each cage were counted. To compare aphid counts for the different plant types, a two-tailed Wilcoxon rank sum test was used with a significance level of 0.05.

Electrical Penetration Graph

The EPG technique was used to monitor aphid feeding behaviour [65]. An eight-channel GIGA-8 direct current amplifier (Wageningen University, The Netherlands) was used for simultaneous recordings of eight individual wingless Brevicoryne brassicae aphids feeding on eight plants (4 wt plants and 4 fou2 mutants). The aphids originated from a colony kindly donated by Prof. Gary Thompson (Oklahoma State University) propagated on Brassica oleracea plants. Before the start of an experiment, the aphids were starved for 4 h and immediately before wiring, an individual aphid’s dorsum was cleaned of wax with the help of a paintbrush hair, and a thin gold wire (12.7 μm diameter, 2-4 cm long) was glued to the dorsum with silver paint (Ted Pella). The other end of the wire was connected to an EPG probe and an output wire from the EPG monitor was inserted into the soil in which the plant was rooted. Plants used in EPG experiments were 3 to 4 weeks old, and did not reach the bolting stage. During experiments plants and insects were kept inside a Faraday cage at constant light conditions and 22°C. The waveform recordings were analysed using the EPG analysis software PROBE 3.0 (W.F. Tjallingii, Wageningen University, The Netherlands). The experiments were repeated several times to obtain a total of 30 biological replicates for fou2 and 34 for wt. A Wilcoxon rank sum test was used to compare the amount of time B. brassiae spent on different feeding behaviours as measured with EPG.

Additional material

Additional file 1: Figure S1. Consequences of the aos and fou2 mutations on jasmonic acid biosynthesis in planta.

Additional file 2: Figure S2. Verification of microarray data by quantitative RT-PCR.

Additional file 3: Table S1. List of genes whose expression in non-challenged plants was positively influenced by jasmonates. Gene expression values for which regulation was not statistically significant are shaded in grey.

Additional file 4: Table S2. List of genes whose expression in non-challenged plants was negatively influenced by jasmonates. Gene expression values for which regulation was not statistically significant are shaded in grey.

Additional file 5: Table S3. List of genes that were less induced in response to B. brassicae infestation in aos than in wt plants. Gene

Additional file 6: Table S4. List of genes whose expression was significantly regulated in response to B. brassicae infestation in aos compared to non-challenged plants. Gene expression values for which regulation was not statistically significant are shaded in grey.
expression values for which regulation was not statistically significant are shaded in grey.

Additional file 6: Table S4. List of genes that were more induced in response to *B. brassicae* infestation in *fou2* than in wt plants. Gene expression values for which regulation was not statistically significant are shaded in grey.

Additional file 7: Table S5. List of genes that were less induced in response to *B. brassicae* infestation in *fou2* than in wt plants. Gene expression values for which regulation was not statistically significant are shaded in grey.

Additional file 8: Table S6. List of genes that were more induced in response to *B. brassicae* infestation in *fou2* than in wt plants. Gene expression values for which regulation was not statistically significant are shaded in grey.

Additional file 9: Table S7. Primers used in quantitative RT-PCR analysis.

Acknowledgements

The authors thank Torfinn Sparstad, Bente Halvorsen, Elisabeth Hyldbakk and Marianne Bakke Hilsen for excellent technical assistance, Wacek Kuśnierzcyk for statistical analysis of EPG data and Ralph Kiss for comments on the manuscript. We are grateful to Prof. Edward Farmer (University of Lausanne, Switzerland) for *fou2* seeds and Prof. Gary Thompson (Oklahoma State University) for donating a *B. brassicae* colony for EPG experiments. We also thank in particular Dr. Murugan Marimuthu (Kansas State University) for the help with establishing and learning the EPG technique, Prof. Kathrin Schrick (Klaus University) for offering space in growth chambers and Predesh Chandran (Kansas State University) for help with maintaining the plants. This work was supported by the Biotechnology and Functional Genomics (FUGE) programmes of the Norwegian Research Council through grants NFR 184146, 164583, 186903, 185173 and US North Central Soybean Research Program.

Author details

1. Department of Biology, The Norwegian University of Science and Technology, Realfagbygget, 7491 Trondheim, Norway. 2. Scanpower AS, 7462 Trondheim, Norway. 3. Department of Entomology, Kansas State University, 175 Skyline Dr, Manhattan, KS 66506, USA. 4. Department of Plant Physiology, University of Technology and Agriculture, Bernardyńska 6, 85-029 Bydgoszcz, Poland.

Authors’ contributions

AK, PW, TSJ and AMB designed the study. AK, DHTT and JT performed infestation experiments for microarray analyses. TSJ performed statistical analysis of transcriptional data. AK, DHTT and PW analysed the transcriptional data. AK performed affinity purification and EPG experiments and analysed EPG data. ICR contributed with EPG equipment and coordinated EPG experiments. AK, DHTT and PW wrote the manuscript. AMB coordinated the study and completed the submission. All authors read and approved the final version of manuscript.

Received: 9 November 2010 Accepted: 19 August 2011 Published: 19 August 2011

References

1. Turner JG, Ellis C, Devoto A: The jasmonate signal pathway. *Plant Cell* 2002, 14:S153-S164.
2. Howe GA: Jasmonates as signals in the wound response. *Journal of Plant Growth Regulation* 2004, 23:223-237.
3. Hálschtsche R, Baldwin IT: Jasmonates and related compounds in plant-insect interactions. *Journal of Plant Growth Regulation* 2004, 23:238-246.
4. Pozo MJ, Van Loon LC, Pieterse CMJ: Jasmonates-signals in plant-microbe interactions. *Journal of Plant Growth Regulation* 2004, 23:211-222.
5. Gao LL, Anderson JP, Klingler JP, Nair RM, Edwards DR, Singh KB: Involvement of the octadecanoid pathway in bluegreen aphid resistance in *Medicago truncatula*. *Molecular Plant-Microbe Interactions* 2007, 20:92-93.
6. Gosset V, Harmel N, Gobel C, Francis F, Haubруge E, Wathelet JP, du Jardin P, Feussner L, Faouconnier ML: Attacks by a piercing-sucking insect (*Myzus persicae Sulfur*) or a chewing insect (*Leptinotarsa decemlineata Say*) on potato plants (*Solanum tuberosum* L.) induce differential changes in volatile compound release and oxylipin synthesis. *Journal of Experimental Botany* 2009, 60:1231-1240.
7. Kuśnierzcyk A, Winge P, Jørstad TS, Troczyńska J, Rossiter JT, Bones AM: Towards global understanding of plant defence against aphids-timing and dynamics of early Arabidopsis responses to cabbage aphid (*Brevicoryne brassicae*) attack. *Plant, Cell and Environment* 2008, 31:1097-1115.
8. Park SJ, Huang YH, Ayoubi P: Identification of expression profiles of sorghum genes in response to greenbug phloem-feeding using cDNA subtraction and microarray analysis. *Planta* 2006, 223:932-947.
9. Kuśnierzcyk A, Winge P, MidFart H, Aramberu WS, Rossiter JT, Bones AM: Transcriptional responses of *Arabidopsis thaliana* ecotypes with different glucosinolate profiles after attack by polyphagous *Myzus persicae* and oligophagous *Brevicoryne brassicae*. *Journal of Experimental Botany* 2007, 58:2537-2552.
10. Kempema LA, Cui XP, Holzer FM, Walling LL: *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology* 2007, 143:849-865.
11. Zhu-Salzman K, Salzman RA, Ahn JE, Koivu H: Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. *Plant Physiology* 2004, 134:430-431.
12. Zarate SJ, Kempema LA, Walling LL: Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiology* 2007, 143:866-875.
13. Ellis C, Carafyllidis L, Turner JG: Constitutive activation of jasmonate signaling in an Arabidopsis mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. *Molecular Plant-Microbe Interactions* 2002, 15:1025-1030.
14. Xie DX, Feys BF, James S, Nieto-Rostro M, Turner JG. COII: An Arabidopsis gene required for jasmonate-regulated defense and fertility. *Science* 1998, 280:1091-1094.
15. Park JH, Hálschtsche R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R: A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis. *Plant Journal* 2002, 31:1-12.
16. Laubert D, Pfannschmidt U, Lottspeich F, Hollander-Czytko H, Weiler EW: Cloning, molecular and functional characterization of *Arabidopsis thaliana* allene oxide synthase (CYP74), the first enzyme of the octadecanoid pathway to jasmonates. *Plant Molecular Biology* 1996, 31:323-335.
17. Bonaventure G, Gehler A, Proebsting WM, Hortenstener S, Chetelat A, Martinova E, Farmer EE: A gain-of-function allele of TPC1 activates oxylipin biogenesis after leaf wounding in *Arabidopsis*. *Plant Journal* 2007, 49:889-898.
18. Peiter E, Maathuis FJM, Mills LN, Knight H, Pelloux M, Hetherington AM, Sanders D: The vacuolar Ca2+-activated channel TPC1 regulates germination and stomatal movement. *Nature* 2005, 434:404-408.
19. Moran PJ, Cheng YF, Cassell JL, Thompson GA: Gene expression profiling of *Arabidopsis thaliana* in compatible plant-aphid interactions. *Archives of Insect Biochemistry and Physiology* 2002, 51:182-203.
20. Moran PJ, Thompson GA: Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiology* 2001, 125:1074-1085.
21. De Vos M, Van Oosten VR, Van Poecke RMP, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Meteaux JP, Van Loon LC, Dicke M, Pieterse CMJ: Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Molecular Plant-Microbe Interactions* 2005, 18:923-937.
22. Kehr J: Phloem sap proteins: their identities and potential roles in the interaction between plants and phloem-feeding insects. *Journal of Experimental Botany* 2006, 57:767-774.
23. Kim JH, Jander G: *Myzus persicae* (green peach aphid) feeding on *Arabidopsis* induces the formation of a deterrent indole glucosinolate. *Plant Journal* 2007, 49:1008-1019.
24. Mewis I, Appel HM, Horn A, Raina R, Schultz K: Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. *Plant Physiology* 2006, 138:1149-1162.
25. Mewis I, Tokuhisa JG, Schultz JC, Appel HM, Ulrichs C, Gershenson J: Gene expression and glucosinolate accumulation in Arabidopsis thaliana in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. Phytochemistry 2006, 67:2345-2362.

26. Thompson GA, Goggins FL: Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. Journal of Experimental Botany 2006, 57:755-766.

27. Zhu-Salzman K, Luhe DS, Fetzer GW: Arthropod-inducible proteins: broad spectrum defenses against multiple herbivores. Plant Physiology 2008, 146:852-868.

28. Smith CM, Boyko EV: The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata 2007, 122:1-16.

29. Goggins FL: Plant-aphid interactions: molecular and ecological perspectives. Current Opinion in Plant Biology 2007, 10:399-408.

30. De Vos M, Kim JH, Jander G: Biochemistry and molecular biology of Arabidopsis-aphid interactions. Bioessays 2007, 29:871-883.

31. Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S, the AmiGO Hub, the Web Presence Working Group: AmiGO: online access to ontology and annotation data. Bioinformatics 2009, 25:288-289.

32. Devato A, Turner JG: Regulation of jasmonate-mediated plant responses in Arabidopsis. Annals of Botany 2003, 92:109-137.

33. Bonaventure G, Gisler A, Rodriguez VM, Armand F, Farmer EE: The JAS gain-of-function plants and the wild-type allele of Two Pore Channel 1 contribute to different extents or by different mechanisms to defense gene expression in Arabidopsis. Plant and Cell Physiology 2007, 48:1775-1789.

34. Jung J, Lyou SH, Yeu S, Kim MA, Rhee S, Kim M, Lee JS, Choi YD, Cheong J: Microarray-based screening of jasmonate-responsive genes in Arabidopsis. Plant Cell Rep 2007, 26:1053-1068.

35. Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N, Sasaki H, Hirai MY, Noji M, Saito K, Masuda T, Takamiya K, Shibata D, Ohto H: Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis. The Plant Journal 2003, 44:653-668.

36. Reymond P, Bodenhanser N, Van Poecie RPM, Krishnamurthy V, Dicke M, Farmer EE: A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 2004, 16:5312-5317.

37. Chen Y, Pang Q, Dai S, Wang Y, Chen S, Yan X: Proteomic identification of differentially expressed proteins in Arabidopsis in response to methyl jasmonate. Journal of Plant Physiology 2011, 168:995-1008.

38. Koornneef A, Pieters MJ: Cross talk in defense signaling. Plant Physiology 2008, 146:839-844.

39. McGrath KC, Dombrecht B, Manners JM, Schenk PM, Edgar CI, Macdonal DJ, Scheible WR, Udvardi MK, Kazan K: Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. Plant Physiology 2005, 139:949-959.

40. Nakano T, Suzuki K, Ohtsuki N, Tsujimoto Y, Fujimura T, Shinshi H: Identification of genes of the plant-specific transcription-factor families cooperatively regulated by ethylene and jasmonate in Arabidopsis thaliana. Journal of Plant Research 2006, 119:407-413.

41. Jung J, Won SY, Suh SC, Kim H, Wing R, Jeong Y, Huang L, Kim M: The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in Arabidopsis. Plantos 2007, 225:575-588.

42. Onate-Sanchez L, Anderson JP, Young J, Singh KB: ATRF14, a member of the ERF family of transcription factors, plays a nonredundant role in plant defense. Plant Physiology 2007, 143:400-409.

43. Weiste C, Iven T, Fischer U, Onate-Sanchez L, Droge-Laser W: In planta ORFeome analysis by large-scale over-expression of GATEWAY(R)-compatible CDNA clones: screening of ERF transcription factors involved in abiotic stress defense. Plant Journal 2007, 52:382-390.

44. Brown RL, Kazan K, McGrath KC, Macdonal DJ, Manners JM: A role for the GCC-box in jasmonate-mediated activation of the PDF1.2 gene of Arabidopsis. Plant Physiology 2003, 132:1020-1032.

45. Pauwels L, Morello K, De Witte E, Lammertyn F, Van Montagu M, Boerjan W, Inze D, Goossens A: Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. Proceedings of the National Academy of Sciences of the United States of America 2008, 105:1380-1385.

46. Ren SX, Mandadi KK, Boedeker AL, Rathore KS, McKnight TD: Regulation of telomerase in Arabidopsis by BT2, an apparent target of TELOMERASE ACTIVATOR1. Plant Cell 2007, 19:23-31.

47. Du LQ, Pooiasah BH: A novel family of Ca2+-calmodulin-binding proteins involved in transcriptional regulation: interaction with fish/Ring3 class transcription activators. Plant Molecular Biology 2004, 54:549-569.

48. Ndamukong I, Al Abdallat A, Thuvour C, Fode B, Zander M, Weigel R, Gatz C: SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. Plant Journal 2007, 50:126-139.

49. Nawrath C, Heck S, Pannthawong N, Mettraux JP: EDSS, an essential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. Plant Cell 2002, 14:275-286.

50. Bodenhanser N, Reymond P: Signaling pathways controlling induced resistance to insect herbivores in Arabidopsis. Molecular Plant-Microbe Interactions 2007, 20:1406-1420.

51. Wasternack C: Jasmonates: An update on biosynthesis, signal transduction and action in plant stress response, growth and development. Annals of Botany 2007, 100:681-697.

52. Chinn A, Fonseca S, Fernandez G, Adie B, Chico JM, Lorenzo O, Garcia- Casado G, Lopez-Vidriero I, Lozano FM, Ponce MR, Miclo JL, Solano R: The JAZ family of repressors is the missing link in jasmonate signalling. Nature 2007, 448:666-671.

53. Thines B, Katsir L, Melotto M, Ni, Y, Mandaokar A, Liu GH,Nomura K, He SY, Howe GA, Brouje V: JAZ repressor proteins are targets of the SCFCO11 complex during jasmonate signalling. Nature 2007, 448:661-665.

54. Westphal L, Scheel D, Rosahl S: The coI-16 mutant harbors a second site mutation rendering PEN2 nonfunctional. The Plant Cell 2008, 20:824-842.

55. Clay NK, Adewale MA, Denoux C, Jander G, Ausubel FM: Glucosinolate metabolites required for an Arabidopsis innate Immune Response. Science 2009, 323:95-101.

56. Felton GW, Bl JI, Summers CB, Mueller AJ, Dufey SS: Potential role of lipoxygenases in defense against insect herbivory. Journal of Chemical Ecology 1994, 20:651-666.

57. Ramakers C, Ruiters RM, Hoffman AFM: Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neuroscience Letters 2003, 339:62-66.

58. Czechowski T, Stitt M, Altmann T, Udvardi MK, Scheible WR: Genome-wide identification and testing of superior reference genes for transcript normalization in Arabidopsis. Plant Physiology 2005, 139:5-17.

59. Paffi MW, Horgan GW, Dempfle L: Relative expression software tool (REST™) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic acids research 2002, 30:836.

60. Smyth GK: Limma: linear models for microarray data. Bioinformatics and Computational Biology Solutions using R and Bioconductor New York: Springer; 2005.

61. Steffey JD: A direct approach to false discovery rates. Journal of the Royal Statistical Society Series B-Methodological 1995, 57:479-498.

62. Benjamini Y, Hochberg Y: Controlling the false discovery rate-a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological 1995, 57:289-300.

63. Jarstad TS, Langaa M, Bones AM: Understanding sample size: what determines the required number of microarrays for an experiment? Trends in Plant Science 2007, 12:46-50.

64. Jarstad TS, Melfar J, Bones AM: A mixture model approach to sample size estimation in two-sample comparative microarray experiments. BMC Bioinformatics 2008, 9:117.

65. Tjallingii WF: Electrical recording of stylen penetration activities. In Aphids: Their Biology, Natural Enemies and Control. Volume 2B. Edited by: Minks AK, Harrewijn P. Amsterdam: Elsvier; 1988:95-108.

Cite this article as: Kušnierczyk et al. BMC Genomics 2011, 12:423

http://www.biomedcentral.com/1471-2164/12/423

doi:10.1186/1471-2164-12-423

Cite this article as: Kušnierczyk et al. Testing the importance of jasmonate signalling in induction of plant defences upon cabbage aphid (Brevicoryne brassicae) attack. BMC Genomics 2011 12:423.