Systemic Signalling in Plant-Aphid Interactions

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Introduction

Phloem-feeding aphids are major agricultural pests worldwide, depriving plants of photoassimilates and vectoring plant pathogenic viruses. For example, *Myzus persicae* (Fig. 1) transmits more than 100 viral diseases to in excess of 400 host plants[1]. Aphids locate their feeding site by guiding their slender, flexible stylets intercellularly, causing little cellular damage.

Figure 1 *Myzus persicae* on oilseed rape (*Brassica napus*).

Over the last decade, the commonly held view that this precise mode of feeding enabled evasion of plant defence responses has been challenged using a variety of experimental approaches. It is now clear that aphid saliva contains elicitors[2] recognised by coiled-coil, nucleotide-binding, leucine-rich repeat (CC-NB-LLR) proteins that mediate plant resistance[3]. Microarray studies have shown a wide range of signalling events primarily mediated through reactive oxygen, calcium and hormone pathways[4]. Despite the huge advances made in recent years, systemic signalling pathways have not been examined in relation to plant-aphid interactions. Here we present a transcriptomic analysis of local and systemic gene expression following infestation of *A. thaliana* by *M. persicae*.

Materials and methods

A. thaliana (col 0) were grown in controlled environment cabinets (20°C, RH 70%) under short days (8h). *M. persicae* genotype G[5] was reared on *Solanum tuberosum* (cv. Maris Piper) at 18°C under a 16:8h light:dark regime.

For microarray experiments four biological replicates were used per treatment. Ten week old *A. thaliana* plants were transferred to the insect growth facility and 60 aphids were caged onto a single rosette leaf for 6, 24 or 48h (Fig. 2). Following feeding, both the infested (local) and a single fully expanded non-infested (systemic) leaf were harvested and immediately frozen in liquid N_2. Experiments were conducted such that all plants were transferred, caged and harvested simultaneously with aphids applied at the appropriate time prior to harvest.

Figure 2 Aphid caging on *Arabidopsis* plants 60 aphids were caged onto a single rosette leaf for up to 48h. Following infestation both the infested (local) leaf (black arrow) and uninfested (systemic) leaf (red arrow) were harvested and subjected to transcriptome analysis.

Control treatments were caged but aphids were not applied. RNA was extracted using the Qiagen® RNeasy Plant Mini kit and quantified spectrophotometrically. The quality of RNA was assessed using Agilent 2100 Bioanalyzer. Agilent Quick Amp Labelling Kit was used to amplify and label target RNA. Microarray slides manufactured by Agilent (V4) contained 43,803 *A. thaliana* probes. Samples from infested and non-infested leaves on the same plant were hybridized together and the data analyzed using GeneSpring 7.0 as a two-colour microarray.

Results

Transcriptional changes differ in magnitude between local and systemic tissue

More than one hundred transcripts were differentially expressed within 6h of aphid infestation in both local and systemic leaves (Fig. 3A). Following 24h infestation, transcriptional reprogramming in local leaves was more extensive with in excess of 1800 transcripts showing differential expression compared to uninfested plants (Fig. 3B). Transcriptional responses remained high after 48h infestation with almost 1500 genes showing up- or down-regulation. On the contrary, transcriptional changes in systemic leaves were less extensive applying to only 326 and 226 transcripts at 24 and 48h, respectively (Fig. 3). Local and systemic responses also differed in the direction of transcriptional response with the majority of transcripts up-regulated in local tissues as opposed to a more even balance between up- and down-regulation in systemic tissues.

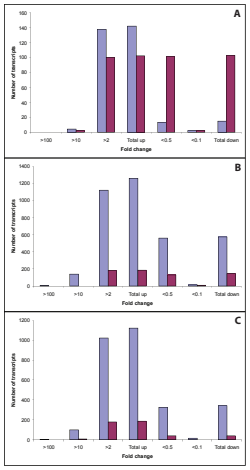

Figure 3 Transcriptional change following aphid infestation in local and systemic leaves
Charts represent the total number of transcripts up-regulated by 2-10 (>2), 10-100 (>10) or more than 100-fold (>100). Genes down regulated 2-10 (<0.5) or more than 10-fold (<0.1) are also represented. A, 6h post-infestation; B, 24h post-infestation; C, 48h post-infestation. □, local leaves; ▨, systemic leaves.

Transcriptional changes are qualitatively different between local and systemic tissue

In order to gain insight into the primary signalling events in response to aphid infestation, transcripts that were differentially expressed at 6h in both local and systemic tissues were classified according to gene ontology. In both tissues, approximately 20% of differentially regulated transcripts had no known function representative of the *A. thaliana* genome as a whole[6] (Fig. 4). In local tissues, transcriptional modification was dominated by changes in the expression of genes involved in signalling and transcriptional regulation (RNA). Genes encoding proteins involved in redox signalling, calcium signalling and ethylene signalling were highly represented. Stress responsive genes that were up-regulated included many disease resistance genes including R-genes and several genes encoding the TIR-NBS class of disease resistance proteins. Inferred downstream effects included cell wall modification (e.g. TCH4 up-regulation) and glucosinolate biosynthesis (e.g. MYB51 up-regulation). Transcriptional re-alignment covered a broader range of biological processes in systemic tissues and included pathogen-responsive WRKY transcription factors (WRKY33, WRKY40) and down-regulation of kinase and ethylene signalling. Downstream responses impacted on transport, cell wall modification and culticle biosynthesis. A model summarising transcriptional reprogramming following aphid infestation in local and systemic tissues is outlined in figure 5.

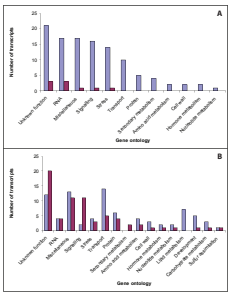

Figure 4
Gene ontology for transcripts differentially regulated following aphid infestation
Charts represent the number of genes within different functional classes that were up- (▨) or down-regulated (□) in local (A) or systemic (B) leaves 6h following aphid infestation.

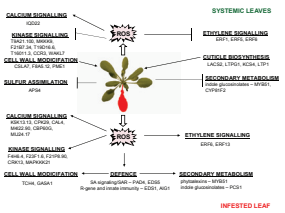

Figure 5 Proposed model for local and systemic plant signalling following aphid infestation
Aphid infestation is proposed to cause local ROS bursts as inferred from up-regulation of the redox responsive transcription factors RRTF1 (16.8 fold increase) and HSFA2 (11 fold increase) resulting in the activation of Ca^{2+}, kinase and ethylene signalling pathways. In addition, various defence pathways are activated resulting in downstream cell wall modification and changes in secondary metabolism. It is proposed that the ROS signal is propagated via RBOHD[7] impacting on systemic signalling pathways. Further systemic effects include up-regulation of cell wall modification, enhanced cuticle biosynthesis and down-regulated sulphur assimilation and glucosinolate metabolism. Key regulated proteins are listed.

Conclusions and future work

This microarray study has for the first time revealed different strategies in local and systemic responses to aphid infestation.

The role of various signalling pathways in local and systemic defence against aphids will be further examined in a series of mutant backgrounds.

References
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