Bulletin of Entomological Research (2007) **97**, 523–532 doi:10.1017/S0007485307005160

Exploring plant responses to aphid feeding using a full *Arabidopsis* microarray reveals a small number of genes with significantly altered expression

C. Couldridge, H.J. Newbury, B. Ford-Lloyd, J. Bale and J. Pritchard*

School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Abstract

The aim of this study was to determine which *Arabidopsis thaliana* (L.) genes had significantly altered expression following 2–36 h of infestation by the aphid *Myzus persicae* (Sulzer). Six biological replicates were performed for both control and treatment at each time point, allowing rigorous statistical analysis of any changes. Only two genes showed altered expression after 2 h (one up- and one down-regulated) while two were down-regulated and twenty three were up-regulated at 36 h. The transcript annotation allowed classification of the significantly altered genes into a number of classes, including those involved in cell wall modification, carbon metabolism and signalling. Additionally, a number of genes were implicated in oxidative stress and defence against other pathogens. Five genes could not currently be assigned any function. The changes in gene expression are discussed in relation to current models of plant-insect interactions.

Keywords: aphid feeding, *Myzus persicae*, phloem, sieve element, microarray *Arabidopsis thaliana*, functional genomics, defence

Introduction

The interrelationship between plants and herbivorous organisms, particularly insects, is one of the most important factors determining the stability of ecosystems, both natural and man-managed. One important guild of herbivores includes sap-sucking insects such as aphids, which are pests of many temperate and glasshouse crops (Slosser, 1993; Abate *et al*., 2000; Douglas, 2003). These insects cause direct feeding damage by the removal of large quantities of plant sap and, in some species, also transmit plant virus diseases. Physiological studies have revealed important information that describes the plant-aphid interaction (Tjallingii, 2006); but, currently, little is known about the underlying biochemical and molecular mechanisms involved.

Optimal growth and reproduction of aphids is facilitated by sustained sap ingestion from the phloem sieve element. There are many potential barriers that aphids must overcome before the sieve element is successfully accessed. The electrical penetration graph system (EPG) and microscopy studies have revealed that the pathway to the sieve element is predominantly intercellular, passing between cells; however, individual cells may be punctured *en route* (Tjallingii & Hogen Esch, 1993). Passage of the stylets between and within cells may be facilitated by the secretion of a range of cell wall degrading enzymes (Cherqui & Tjallingii, 2000). During the pathway phase, aphids produce a gelling saliva composed of lipoprotein, which forms a sheath and supports and protects the stylets as they penetrate the plant tissues (Douglas, 2003).

*Author for correspondence
Fax: 0121 414 5925
E-mail: j.pritchard@bham.ac.uk

A variety of plant chemical cues and physical barriers facilitate or restrict location of the phloem tissue (Pritchard *et al*., 2007). Once the sieve element has been accessed, a watery saliva is injected (Tjallingii, 2006), which may overcome phloem-localised defence proteins mediated by calcium (Will & van Bel, 2006). Both watery and gelling saliva are potential elicitors of plant responses to insect feeding (Moran *et al*., 2002).

Phloem sap consists of water, carbohydrate (often as sucrose), amino acids, inorganic ions, proteins, hormones, mRNA, lipids and various secondary compounds (van Bel, 2003). Considerable amounts of phloem sap can be extracted from plants by aphids; for example, the willow aphid (*Tuberolachnus salignus*, Gmelin) produces around 2 µl of honeydew per hour (Mittler, 1958). Aphids are, therefore, a considerable sink on the plant and additionally can alter phloem sap composition to increase the quality of their diet (Sandström *et al*., 2000).

The EPG technique has provided considerable detail about the location and acceptance of sieve elements by aphids and modification of the process by altered environments (Ponder *et al*., 2000, 2001; Hale *et al*., 2003). This information is now being supplemented by genetic techniques to reveal the molecular mechanisms that underlie the plant-insect interaction (Qubbaj *et al*., 2005; Hunt *et al*., 2006). Recently, this approach has been further developed by a number of studies using gene expression array technology to examine the response of a subset of plant defence genes to sap-feeding pests (Moran *et al*., 2002; Voelckel *et al*., 2004; Zhu-Salzman *et al*., 2004). However, these methods reveal the response of only those genes that are already implicated in the interaction. Alternative strategies have used subtraction cDNA libraries to identify aphid responsive genes in phloem-enriched tissue of celery (Divol *et al*., 2005). Importantly, the availability of whole genome arrays for *A. thaliana* provides an opportunity to examine the response of the whole plant genome to pathogen attack. Currently, studies on nematodes (Jammes *et al*., 2005), whitefly (Kempema *et al*., 2007) and aphids (De Vos *et al*., 2005) have suggested that very large numbers of *Arabidopsis* genes (i.e. ranging from 832 to 1349 in the De Vos study) are up- or down-regulated in response to pest infestation. While these studies have produced tantalising clues as to the complexity of the plant response to biotic stress, they are limited by having used only one or two biological replicates. The paucity of replication reduces considerably the statistical confidence that can be placed on changes in gene expression, and, therefore, the models of the molecular regulation of the interaction that can be constructed from the data.

The aim of the present study was to identify, with rigorous analysis, *Arabidopsis* genes that were up- or down-regulated in leaf tissue during the first 2 h (when EPG experiments indicate that aphids predominantly exhibit the pathway phase of stylet penetration) and the following 34 h of aphid infestation (when the predominant activity is sustained phloem sap ingestion). Disseminating information on such a list of responsive genes will be important for stimulating further studies by other researchers in this area. To achieve this objective, six independent biological replicates were utilised for infested and uninfested tissue at each time point. Each leaf was infested with ten fourth instar aphids to ensure that infestation levels were constant throughout the experimental period (i.e. there was no increase in aphid numbers through reproduction).

Materials and methods

Plant and insect cultivation

Arabidopsis thaliana (Col-0) were grown individually in soil (four parts loam-based John Innes Compost Number 2, four parts peat-based compost, one part Silvaperl, Osmocote) and maintained at 22°C in an 18 : 6 L : D cycle with unregulated relative humidity (≈70%). Plants were used when rosette growth was complete, just prior to inflorescence bolt production at principal growth stage 3.90, which was usually 3–4 weeks after sowing (Boyes *et al*., 2001).

Myzus persicae (Sulzer) were cultured on mature rosette stage or older *A. thaliana* (Col-0) plants grown under the conditions described above. Insects were obtained from a long-term clonal culture maintained at the University of Birmingham. Only apterous aphids were used for experimental purposes.

Insect bioassays

For both the 2 and 36 h time points, six independent biological replicates were performed; control and infested treatments at each time point, therefore, required a total of 24 chips. For each replicate, 20 plants were selected and paired according to rosette size and leaf number. Each pair of plants was assigned to a control or aphid infestation treatment, making ten plants per treatment or control. Two fully-expanded rosette leaves were chosen; however, the size of the clip ages prevented use of adjacent leaves. Each of the chosen leaves was infested on the upper surface with ten, fourth instar nymphs; this ensured that no reproduction would take place during the experiment, thus maintaining a constant level of infestation. Aphids were confined within a 2 cm diameter clip cage on each leaf. Leaves on control plants were brushed on the upper surface with a fine paintbrush to simulate manipulation and also enclosed within a clip cage. Control and experimental plants were placed in a randomised block design and maintained at 22°C (light intensity ~100 µmole $m^{-2} s^{-1}$, relative humidity ~63%) for 2 or 36 h. Experiments were always started at 08:00 h, at the start of the light period, to minimise any effects of diurnal variation. Clip cages and aphids were removed and the 20 treated or control leaves were collected and immediately frozen. This procedure was repeated 12 times, six times for the 2 h and 6 times for the 36 h time points, producing 24 batches of leaf samples for subsequent RNA extraction.

RNA isolation

Total RNA was extracted from leaf samples using Qiagen® RNeasy Plant Mini Kits according to the manufacturer's recommended protocol. Each replicate sample consisted of 20 leaves. However, due to technical limitations, these were extracted as leaf pairs and the RNA samples pooled for subsequent expression profiling.

Expression profiling

cDNA, and subsequently cRNA, was synthesised from total RNA (5 µg) for each sample according to the protocol previously described by Zhu *et al*. (2001). Each labelled cRNA sample was hybridised to an individual *Arabidopsis* whole genome ATH1 GeneChip (Affymetrix) (Zhu *et al*.,

https://doi.org/10.1017/S0007485307005160 Published online by Cambridge University Press

2001). Design features of this custom chip are reviewed by Zhu (2003).

Gene expression analysis

The expression data were subjected to per chip normalisation (to the 50th percentile) and per gene normalisation (to the median) using GeneSpring 6.2 (Silicon Genetics: www.silicongenetics.com). The data were then filtered on flags (present only in at least six out of the 12 samples). Secondarily, a two-fold change in expression filter was applied. Analysis of variance on log transformed expression values was carried out separately for treated versus control for each time point with GeneSpring, applying the Benjamini & Hochberg (1995) method for controlling the false discovery rate.

Results and discussion

Following 2 or 36 h of exposure to aphids, only 27 genes from the 24 K in the whole *Arabidopsis* genome showed significantly altered expression in the leaf on which the aphid was feeding. After 2 h, one gene was up-regulated and one down-regulated, while after 36 h, 23 genes were up-regulated and two were down-regulated (fig 1). There were no genes in common between 2 and 36 h. The number of genes affected by aphid feeding was markedly lower than that reported in a study of *M. persicae* infesting *Arabidopsis* for 48 and 72 h, where 832 genes were reported to be up-regulated, and 1349 down-regulated (De Vos *et al*., 2005). In our study, nine of the genes that were up-regulated after 36 h were also reported to be up-regulated in the De Vos study. These included the lectin-like protein gene At3g16530 and two of the three glutathione transferases (At1g02930 and At2g02930) (table 1). Interestingly, one gene, At5g55730, coding for a fasciclin-like protein, was down-regulated in both studies. The *Arabidopsis* gene chip has recently been used to analyse the effect of the phloem-feeding whitefly, *Bemisia tabaci* (Gennad) (Kempema *et al*., 2007). This study seemingly identified 700 genes up-regulated and 556 down-regulated. Only 17% of these were common to the De Vos study and one gene (*PAD4*) was common with the present study. Currently, there are few published studies using full genome arrays to investigate plant-pest interactions, the current status of which has been recently reviewed (Thompson & Goggin, 2006).

While common features between these data sets may be viewed as encouraging, the lack of adequate biological replication (one and two, respectively), and resultant higher numbers of genes identified in both studies, make it difficult to determine the significance of the overlap. It is possible that the longer exposure times and heavier infestation (72 h and 40 reproducing aphids in the De Vos *et al*. (2005) study and 100 whitefly for 21 days in the Kempema *et al*. (2007) study) may account for the much higher numbers of genes showing altered expression (2181 in the De Vos *et al*. (2005) study and 1256 in the Kempema *et al*. (2007) study, compared to 27 in the present investigation). It must also be likely that our statistical stringency and a bias towards higher transcript levels may have led to type II errors. However, it is more likely that the inadequate biological replication and inherent lack of statistical power is the major contributing factor and that at least three (Lee *et al*., 2000) and probably more than five (Pavlidis *et al*., 2003) biological replicates are required to achieve stable results. Analysing one chip per treatment or not accounting for the false discovery rate using our data yielded a list of genes showing different expression levels similar in size to those of De Vos *et al*. (2005) but with no statistically significant differences. Such comparison highlights the importance of an appropriate level of replication and statistical analysis in transcriptomics experiments. The need for higher numbers of replicates is also demonstrated by the high number of genes showing significantly altered expression following nematode infestation when using only two biological replicates (Jammes *et al*., 2005). It is, therefore, vital that the emerging MIAME protocols take account of replication to ensure comparability between different transcriptomics experiments and also to encourage standardisation of statistical analysis. Without such rigour, comparison between different studies remains speculative at best.

Since aphids are specialist herbivores targeting the phloem sieve element, it is reasonable to expect that changes in gene expression may be phloem-specific. Currently, interest is focusing on the role of forisomes in such phloem-specific defence in legumes (Fabaceae) (Will & van Bel, 2006). Alternatively (or additionally), alterations in the nutritional quality of the sieve element sap may have a role in plant-aphid interactions (Sandström *et al*., 2000; Hunt *et al*., 2006). In this study, we extracted mRNA from whole leaves, where changes in gene expression will be biased toward higher transcript numbers. By comparison, phloem constitutes a small fraction (< 0.5% of total leaf volume) of total plant tissue (Sjölund, 1997). In the present study, where changes in gene expression may be restricted to a single cell type, such low transcript numbers may well be undetected. In this regard, none of the genes identified in this work were homologous with those previously reported in either phloem-enriched protein (Barnes *et al*., 2004) or cDNA (Doering-Saad *et al*., 2002, 2006) libraries.

Despite the cell-specific site of aphid feeding, EPG experiments reveal other interactions as the aphid locates the sieve element. Thus, changes in gene expression induced by aphid infestation may be responses of the plant during the pathway phase of the interaction. Alternatively, gene expression changes may be induced by the insect to manipulate the plant so as to produce a more favourable food source (Prado & Tjallingii, 1997) or to overcome plant defence (Urbanska *et al*., 1998).

The annotation of the *Arabidopsis* genome provides an objective way to assign function to the genes that have been identified. However, since the annotation is not complete, it is still useful to analyse altered expression profiles subjectively, combining the putative function (if known) with current knowledge of the physiology and biochemistry of aphid feeding. Taking this approach, the gene transcripts identified in the present study can be placed into a number of functional classes.

Oxidative stress

A common feature of plants under stress is the induction of genes associated with oxidative stress. Aphids are known to induce oxidative stress (Ni *et al*., 2001; Moran *et al*., 2002); for example, reactive oxygen species were increased in tomato (*Solanum lycopersicum* L.) during infestation by the potato aphid, *Macrosiphum euphorbiae* (Thomas) (de Ilarduya *et al*., 2003). Four genes with significant up-regulation

https://doi.org/10.1017/S0007485307005160 Published online by Cambridge University Press

Table 1. Putative annotation of *Arabidopsis* genes showing significant changes in expression following 2 or 36 h infestation of mature rosette leaves by 4th instar nymphs of *Myzus persicae*. Each leaf was infested with ten aphids; two leaves per plant were infested and mRNA was extracted from infested leaves only. Average spot fluorescence (arbitrary units) of the six independent replicates is shown, plus the significance of difference in fluorescence of control and infested leaves determined by paired Students *t*-test. Note that these 27 genes were identified as having significantly altered expression by genome wide analysis using Genespring (see text for further details).

Gene	Putative function/annotation	Time of altered expression	Uninfested leaf fluorescence (arbitrary units)	Infested leaf fluorescence (arbitrary units)	*P* value
At4g29650	Cytidine Aminohydrolase	36 h	49 ± 10	31 ± 7	0.005
At5g55730	Fasciclin-like arabinogalactan-protein	36 h	79 ± 13	49 ± 4	0.001
At3g62860	Esterase family protein	2 h	49 ± 5	32 ± 9	0.005
At5g54710	Ankyrin repeat family protein	36 h	33 ± 4	49 ± 11	0.004
At1g21000	Zinc-binding family protein	36 h	193 ± 44	295 ± 68	0.010
At2g18680	Expressed protein	36 h	27 ± 6	45 ± 8	0.002
At4g12720	Nucleoside diphosphate-linked moiety	36 h	54 ± 12	91 ± 18	0.001
At5g39670	Calcium-binding EF hand family protein	36 h	35 ± 6	59 ± 11	0.006
At2g30140	UDP-glucosyl transferase family protein	36 h	140 ± 35	226 ± 16	0.007
At1g03220	Extracellular dermal glycoprotein	2 h	76 ± 21	126 ± 58	0.080
At1g27020	Unknown soluble protein	36 h	44 ± 5	82 ± 21	0.003
At4g33050	Calmodulin-binding family protein	36 h	45 ± 10	76 ± 10	0.002
At1g64810	Accumulation of photosystem one	36 h	41 ± 10	68 ± 19	0.026
At3g12740	Unknown	36 h	130 ± 20	219 ± 47	0.018
At4g20860	FAD-binding domain-containing protein	36 h	24 ± 12	45 ± 16	0.024
At5g52750	Heavy-metal-associated domain-containing protein	36 h	68 ± 18	142 ± 42	0.004
At3g52400	Syntaxin	36 h	28 ± 7	54 ± 20	0.036
At3g03470	Cytochrome P450	36 h	80 ± 11	174 ± 56	0.008
At3g52430	Phytoalexin *PAD4*	36 h	30 ± 12	69 ± 22	0.003
At4g13180	Short-chain dehydrogenase/reductase (*SDR*) family	36 h	27 ± 9	67 ± 37	0.022
At2g18690	Expressed protein	36 h	35 ± 9	80 ± 29	0.013
At5g63790	No apical meristem (*NAM*) family protein	36 h	51 ± 5	124 ± 67	0.043
At4g02520	Glutathione transferase *GST2*	36 h	638 ± 273	1630 ± 693	0.017
At5g20230	Blue copper-binding protein	36 h	61 ± 17	150 ± 54	0.021
At2g02930	Glutathione transferase *GST16*	36 h	95 ± 28	274 ± 128	0.017
At3g16530	Legume lectin family protein	36 h	147 ± 47	558 ± 253	0.008
At1g02930	Glutathione transferase *GST1*	36 h	99 ± 49	390 ± 232	0.018

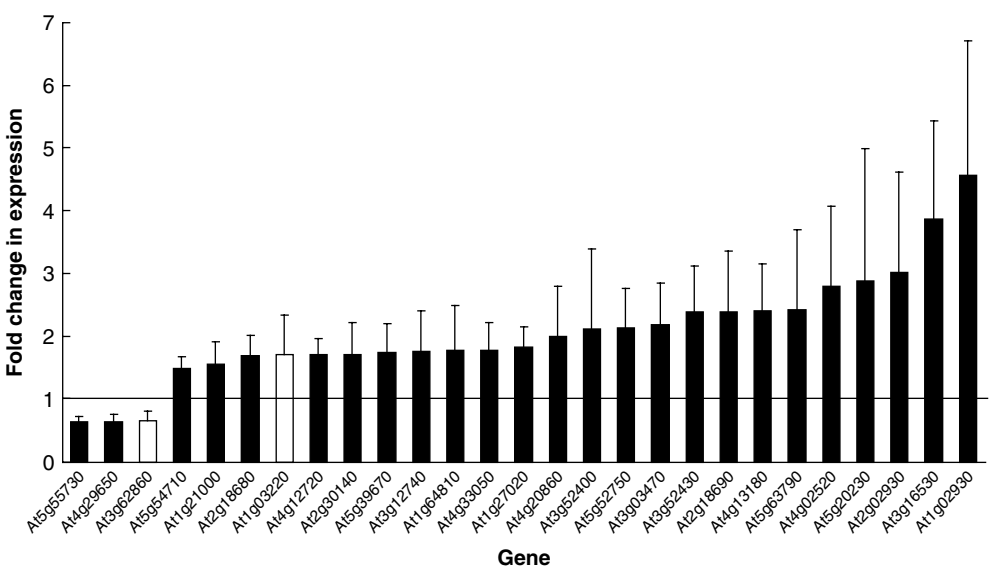

Fig. 1. Fold change in *Arabidopsis* genes showing significant changes in expression following 2 h (open bars) or 36 hours (closed bars) infestation of rosette leaves by 4th instar nymphs of *Myzus persicae*. Each leaf was infested with ten aphids; two leaves per plant were infested and mRNA was extracted from infested leaves only. Significant changes in expression were determined by Genespring analysis of the six independent replicates. Bars represent average ± SD of fold change in expression of the six, paired, independent biological replicates. The horizontal line represents no change in expression.

https://doi.org/10.1017/S0007485307005160 Published online by Cambridge University Press

at 36 h fit into this category. The first, a FAD-Binding domain-containing protein similar to a reticuline oxidase precursor, is a documented oxidative stress-responsive gene with a role in oxidoreductase activity and possible antimicrobial activity (Custers *et al*., 2004; Kim *et al*., 2005). A blue-copper binding protein, also up-regulated after 36 h, is known to be locally induced in response to wounding as a likely mechanism of increased resistance to oxidative stress (Yang *et al*., 2002). The third gene was an ankyrin repeat family protein. Ankyrin proteins have a documented role in plant disease resistance and regulation of antioxidation metabolism (Yan *et al*., 2002; Dong, 2004). Antioxidant enzymes are likely to be important in sequestering free radicals produced by physical damage (Dillwith *et al*., 1991). Spontaneous DNA mutations may occur as a potential knock-on effect of free radical production and, under such conditions, the up-regulation of DNA repair enzymes can be observed (Filkowski *et al*., 2004). This may explain the observed increase in expression of a nucleoside diphosphate-linked moiety transcript annotated with a function involved in DNA repair.

Likely elicitors of oxidative stress produced by aphids include cell wall fragments and oligosaccharides that are the products of physical damage caused during stylet penetration activity (Moran *et al*., 2002). Aphid saliva may also be an elicitor involved in the alteration of plant oxidative conditions (Dillwith *et al*., 1991; Miles & Oertli, 1993; Miles, 1999; Walling, 2000). Therefore, induction of some of these genes suggests that the aphid may be altering the oxidative status of the plant.

Defence during pathway phase

Glutathione transferases (GSTs) are a class of well-described enzymes involved in xenobiotic detoxification, mediating specific conjugation and peroxidase reactions with glutathione (Wagner *et al*., 2002; Dixon *et al*., 2005; Edwards *et al*., 2005). Aphid feeding after 36 h induced significant up-regulation of three glutathione transferases, *GST1*, *GST2* and *GST16*. *GST1* was the most strongly induced, with 4.6-fold increased mean expression in the infested plants compared with the control. Strong induction in response to wounding, low temperature, high salt and aluminium treatments has previously been demonstrated (Yang *et al*., 1998; Ezaki *et al*., 2004). *GST2* induction in response to a range of biotic and abiotic stresses is also well documented, including following exposure to copper and hydrogen peroxide (Lieberherr *et al*., 2003; Smith *et al*., 2004). Regulation is known to occur via ethylene and salicylic acid signalling pathways (Zhou & Goldsbrough, 1993; Lieberherr *et al*., 2003; Smith *et al*., 2003; Mang *et al*., 2004). Eliciting factors from the aphid that may induce such genes are likely to include a range of glycoproteins, oligosaccharides and enzymes secreted as constituents of the gelling saliva (Miles, 1999; Cherqui & Tjallingii, 2000). These genes could also be included in the oxidative stress category, as reduced glutathione has redox buffering power, which may confer cellular protection from this type of stress (Ogawa, 2005).

Induction of a *cytochrome P450* gene is also consistent with plant defence in response to aphid stylet penetration activity. While there are two broad classes of these enzymes, both have a defensive role, being involved in either detoxification of xenobiotics or biosynthesis of a range of molecules associated with defence and signalling (Li *et al*., 2002). A gene coding for an esterase family protein was up-regulated 2 h post-infestation. Classically, esterases play a role in detoxification; significant increases in esterase activity have previously been demonstrated in barley (*Hordeum vulgare* L.) in response to *Diuraphis noxia* (Kurdj.) feeding and hypothesised to be related to toxic and oxidative stress imposed by the aphid (Ni & Quisenberry, 2003).

Cell wall modification

Two genes with annotations associated with cell wall modification showed significant differential expression at 2 and 36 h. These genes are likely to be associated with the physical damage to cell walls caused by the intercellular passage of the stylets and the profuse number of cell punctures during the pathway phase. The first of these genes codes for an extracellular dermal glycoprotein, known to be an inhibitor of endo-glucanases (York *et al*., 2004). This function may confer a plant defence response by blocking cell wall degrading enzymes, such as pectinase, present in the watery saliva (Cherqui & Tjallingii, 2000). Early up-regulation at 2 h is consistent with EPG data, showing that most pathway (and, thus, physical damage) occurs during this time. A homologue in carrot has been shown to play an important role in plant defence and signal transduction during wounding (Satoh *et al*., 1992; Shang *et al*., 2004).

A gene for a fasciclin-like arabinogalactan-protein was down-regulated at 36 h. Arabinogalactan-proteins are a family of hyperglycosylated, hydroxyproline-rich proteins found at the surface of plant cells (Borner *et al*., 2003) Up-regulation in response to salt stress has previously been documented (Lamport & Kieliszewski, 2005). The reversed expression observed in this study may be consistent with the type of stress imposed. Arabinogalactan-proteins may have a role in reducing the number of pectin cross-linkages in the cell wall with the result of increasing porosity (Lamport & Kieliszewski, 2005). This would facilitate cell wall penetration by the aphid, and it can be speculated that down-regulation of this gene represents active facilitation of feeding by aphids.

Potential phloem defence

Plant defence responses may also be specifically targeted at mechanical damage in the sieve element. A gene for a UDP-glucosyl transferase family protein involved in the glycosylation of aglycones (xenobiotics, hormones and defence-related metabolites) (Li *et al*., 2002) was up-regulated at 36 h. Some UDP-glucosyl transferases have been shown to be involved in callose synthesis (Hong *et al*., 2001). Callose deposition at the sieve plate is a common defence response to sieve element disruption and may be linked with sieve element damage caused by the aphid stylets (Will & van Bel, 2006).

A legume lectin family protein gene was up-regulated 3.9-fold after 36 h. While lectins (carbohydrate-binding proteins), are known to play a role in defence responses against a range of bacteria, fungi, viruses and other plant antagonists, there is evidence that some lectins are phloem-specific (Vijayan & Chandra, 1999; Dinant *et al*., 2003). Aphid growth and fecundity is known to be affected by jackbean (*Canavalia gladiata* Jacq) and snowdrop (*Galanthus nivalis* L.) lectins (Stoger *et al*., 1999; Sauvion *et al*., 2004; Vasconcelos & Oliveira, 2004).

https://doi.org/10.1017/S0007485307005160 Published online by Cambridge University Press

Also up-regulated at 36 h was a gene for a syntaxin protein (*AtSyp122*) involved in vesicle trafficking of the defence-related molecules, SNAREs (soluble N-ethylmaleimide-sensitive factor adaptor protein receptor) (Sanderfoot *et al*., 2000). The modified plasmodesmata, which connect sieve elements to companion cells, are thought to be one of the major pathways by which macromolecular signals are transported in the plant (Oparka, 2004). It is possible that syntaxin may be involved in this type of trafficking. However, syntaxin receptors may be localised to various membranes throughout the plant to induce a defence response (Bassham *et al*., 1995). Indeed, a calcium-dependent reaction of the *AtSyp122* gene, elicited by a bacterial flagellin peptide, leads to exocytosis of hypothesised defence-related proteins in cultured *Arabidopsis* cells (Nühse *et al*., 2003).

Calcium-dependent signalling

Calcium is an important message in the transduction of developmental and environmental signals, including biotic and abiotic stresses (Reddy & Reddy, 2004). At 36 h, two genes with functions associated with calcium homeostasis showed significant up-regulation, including a gene for calcium-binding EF hand family protein and a calmodulin-binding family protein (At4g33050). Calmodulin is a calcium-binding protein that can bind to, and regulate, an assembly of different proteins. Calmodulin-related signalling genes in the *TCH* family were up-regulated by aphid infestation after 72 h (Moran *et al*., 2002). A defence role has been implicated in downy mildew infestation (León *et al*., 1998; Bergey & Ryan, 1999; Eulgem *et al*., 2004). Indeed, At4g33050 may be a target of whirly transcription factors that regulate defence gene expression (Desveaux *et al*., 2005).

Signalling

A transcript for the *PAD4* gene was up-regulated at 36 h; *PAD4* may operate to integrate signals from the salicylate and jasmonate defence pathways (Zhou *et al*., 1998; Pegadaraju *et al*., 2005). *PAD4* is implicated in plant defence since a loss-of-function mutant in *Arabidopsis* for the *PAD4* gene supported a higher aphid population (Pegadaraju *et al*., 2005). It is also part of a suite of genes involved in plant disease resistance against *Pseudomonas syringae* (pv) and *Peronospora parasitica* (Pers) (Glazebrook *et al*., 1997; Feys *et al*., 2001). The salicylic acid (SA) signalling pathway may mediate defence induction in *A. thaliana* under feeding pressure by *M. persicae* (Moran *et al*., 2002). This is consistent with observations of *M. persicae* and *M. euphorbiae* on tomato, which induced strong up-regulation of the salicylic pathway *PR* and *P4* genes (Fidantsef *et al*., 1999). However, the role of the SA is not yet resolved, and aphid reproduction was unaffected on mutant *Arabidopsis* plants in which SA signalling was compromised (Pegadaraju *et al*., 2005). In *Arabidopsis* leaves, aphid feeding did not induce any measurable changes in SA levels (De Vos *et al*., 2005).

A short-chain dehydrogenase/reductase family protein was significantly up-regulated at 36 h post-infestation. This protein has a function in cellular differentiation and signalling (Kallberg *et al*., 2002). Its signalling role is likely to be important in the transduction of stress elicitors.

Carbohydrate metabolism

A transcript annotated 'accumulation of photosystem one (*APO1*)' was up-regulated at 36 h. This gene has a role in chloroplast cluster complex accumulation (Amann *et al*., 2004). When the aphid is feeding, it effectively becomes a new solute sink within the plant (Douglas, 2003) so that its up-regulation may be associated with alterations in carbon partitioning, either through aphid-induction to manipulate the plant to produce a more favourable diet, or as a passive response to the aphid becoming more of a sink on the plant.

Transcription

Five percent of the *Arabidopsis* genome is known to be involved in transcriptional regulation coding for more than 1500 transcription factors (Riechmann *et al*., 2000). Several transcripts differentially expressed at 36 h were annotated with functions involved in regulation of gene expression. The first of these genes, coding for a no apical meristem family protein, has similarities with NAC-domain proteins that are known to have transcription factor activity (Ooka *et al*., 2003). A number are reported to be involved in responses to a range of biotic and abiotic stresses, including *Sclerotinia sclerotiorum* (Lib.), flea beetle feeding, wounding, drought and low temperature (Seki *et al*., 2002; Hegedus *et al*., 2003; Delessert *et al*., 2005; Mahalingam *et al*., 2005). Also up-regulated was a gene for a zinc-binding family protein, part of the PLATZ1 class of zinc-dependent DNA-binding proteins which repress transcription of A/T-rich sequence motifs (Nagano *et al*., 2001). Finally, a cytidine deaminase, down-regulated at 36 h, may have a role in RNA turnover, catalysing the hydrolysis of cytidine into uridine and ammonia (Faivre-Nitschke *et al*., 1999).

Unknown

Five transcripts (At1g27020, At2g18680, At2g18690, At3g12740 and At5g52750) were significantly up-regulated at 36 h, but literature and BLAST searches revealed unknown functions. These transcripts could perhaps be of most interest, as they could provide information about previously uncharacterised defence-related processes.

Conclusions

This study has identified candidate plant genes that are strongly implicated in the aphid-plant interaction and provides sound target genes for future projects to study their function in more detail. These targets will inform the development of novel defence strategies (Moran *et al*., 2002). Future work will aim to localise cellular expression of each of these genes and study detailed changes in expression using RT-PCR. Examination of aphid performance on knockout mutants will provide further information about the function of these individual genes. Finally, the small number of genes that we have confidently identified will facilitate focused systems biology studies examining the co-regulation of these genes within gene networks.

Acknowledgements

The authors thank the Biotechnology and Biological Sciences Research Council for funding the studentship for

Clare Couldridge and Syngenta for both funding and technical support with the microarrays. We acknowledge the helpful comments of two anonymous referees.

References

Abate, T., van Huis, A. & Ampofo, J.K.O. (2000) Pest management strategies in traditional agriculture: An African perspective. *Annual Review of Entomology* **45**, 631–659.

Amann, K., Lezhneva, L., Wanner, G., Herrmann, R.G. & Meurer, J. (2004) Accumulation of photosystem one1, a member of a novel gene family, is required for accumulation of cluster-containing chloroplast complexes and antenna proteins. *Plant Cell* **16**, 3084–3097.

Barnes, A., Bale, J.S., Constantinidou, C., Ashton, P., Jones, A. & Pritchard, J. (2004) Determining protein identity from sieve element sap in *Ricinus communis* l. by quadrupole time of flight (Q-TOF) mass spectrometry. *Journal of Experimental Botany* **55**, 1473–1481.

Bassham, D.C., Gal, D., Conceicao, A.D. & Raikhel, N.V. (1995) An Arabidopsis syntaxin homologue isolated by functional complementation of a yeast pep12 mutant. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 7262–7266.

Benjamini, V. & Hochberg, V. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B* **57**, 289–300.

Bergey, D.R. & Ryan, C.A. (1999) Wound- and systemin-inducible calmodulin gene expression in tomato leaves. *Plant Molecular Biology* **40**, 815–823.

Borner, G.H.H., Lilley, K.S., Stevens, T.J. & Dupree, P. (2003) Identification of glycosylphosphatidylinositol-anchored proteins in Arabidopsis. a proteomic and genomic analysis. *Plant Physiology* **132**, 568–577.

Boyes, D.C., Zayed, A.M., Ascenzi, R., McCaskill, A.J., Hoffman, N.E., Davis, K.R. & Gorlach, J. (2001) Growth stage-based phenotypic analysis of Arabidopsis: a model for high throughput functional genomics in plants. *Plant Cell* **13**, 1499–1510.

Cherqui, A. & Tjallingii, W.F. (2000) Salivary proteins of aphids, a pilot study on identification, separation and immunolocalisation. *Journal of Insect Physiology* **46**, 1177–1186.

Custers, J.H.H.V., Harrison, S.J., Sela-Buurlage, M.B., Van Deventer, E., Lageweg, W., Howe, P.W., Van Der Meijs, P.J., Ponstein, A.S., Simons, B.H., Melchers, L.S. & Stuiver, M.H. (2004) Isolation and characterisation of a class of carbohydrate oxidases from higher plants, with a role in active defence. *Plant Journal* **39**, 147–160.

De Ilarduya, O.M., Xie, Q.G. & Kaloshian, I. (2003) Aphid-induced defense responses in Mi-1-mediated compatible and incompatible tomato interactions. *Molecular Plant-Microbe Interactions* **16**, 699–708.

Delessert, C., Kazan, K., Wilson, I.W., Van Der Straeten, D., Manners, J., Dennis, E.S. & Dolferus, R. (2005) The transcription factor ataf2 represses the expression of pathogenesis-related genes in Arabidopsis. *Plant Journal* **43**, 745–757.

Desveaux, D., Marechal, A. & Brisson, N. (2005) Whirly transcription factors: Defense gene regulation and beyond. *Trends in Plant Science* **10**, 95–102.

De Vos, M., van Oosten, V.R., van Poecke, R.M.P., van Pelt, J.A., Pozo, M.J., Mueller, M.J., Buchala, A.J., Metraux, J.P., van Loon, L.C., Dicke, M. & Pieterse, C.M.J. (2005) Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. *Molecular Plant-Microbe Interactions* **18**, 923–937.

Dillwith, J.W., Berberet, R.C., Bergman, D.K., Neese, P.A., Edwards, R.M. & McNew, R.W. (1991) Plant biochemistry and aphid populations – Studies on the Spotted Alfalfa Aphid, *Therioaphis maculata*. *Archives of Insect Biochemistry and Physiology* **17**, 235–251.

Dinant, S., Lark, A.M., Zhu, Y.M., Vilaine, F., Palauqui, J.C., Kusiak, C. & Thompson, G.A. (2003) Diversity of the superfamily of phloem lectins (phloem protein 2) in angiosperms. *Plant Physiology* **131**, 114–128.

Divol, F., Vilaine, F., Thibivilliers, S., Amselem, J., Palauqui, J.C., Kusiak, C. & Dinant, S. (2005) Systemic response to aphid infestation by *Myzus persicae* in the phloem of *Apium graveolens*. *Plant Molecular Biology* **57**, 517–540.

Dixon, D.P., Skipsey, M., Grundy, N.M. & Edwards, R. (2005) Stress-induced protein S-glutathionylation in Arabidopsis. *Plant Physiology* **138**, 2233–2244.

Doering-Saad, C., Newbury, H.J., Bale, J.S. & Pritchard, J. (2002) Use of aphid stylectomy and RT-PCR for the detection of transporter mRNAs in sieve elements. *Journal of Experimental Botany* **53**, 631–637.

Doering-Saad, C., Newbury, H.J., Couldridge, C.E., Bale, J.S. & Pritchard, J. (2006) A phloem-enriched cDNA library from *Ricinus*: insights into phloem function. *Journal of Experimental Botany* **57**, 3183–3193.

Dong, X. (2004) The role of membrane-bound ankyrin-repeat protein ACD6 in programmed cell death and plant defense. *Science's STKE* **221**, 6.

Douglas, A.E. (2003) The nutritional physiology of aphids. *Advances in Insect Physiology* **31**, 73–140.

Edwards, R., Brazier-Hicks, M., Dixon, D.P. & Cummins, I. (2005) Chemical manipulation of antioxidant defences in plants. *Advances in Botanical Research Incorporating Advances in Plant Pathology* **42**, 1–32.

Eulgem, T., Weigman, V.J., Chang, H.S., McDowell, J.M., Holub, E.B., Glazebrook, J., Zhu, T. & Dangl, J.L. (2004) Gene expression signatures from three genetically separable resistance gene signalling pathways for downy mildew resistance. *Plant Physiology* **135**, 1129–1144.

Ezaki, B., Suzuki, M., Motoda, H., Kawamura, M., Nakashima, S. & Matsumoto, H. (2004) Mechanism of gene expression of Arabidopsis glutathione S-transferase, atgst1, and atgst11 in response to aluminum stress. *Plant Physiology* **134**, 1672–1682.

Faivre-Nitschke, S.E., Grienenberger, J.M. & Gualberto, J.M. (1999) A prokaryotic-type cytidine deaminase from *Arabidopsis thaliana* – gene expression and functional characterization. *European Journal of Biochemistry* **263**, 896–903.

Feys, B.J., Moisan, L.J., Newman, M.A. & Parker, J.E. (2001) Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. *EMBO Journal* **20**, 5400–5411.

Fidantsef, A.L., Stout, M.J., Thaler, J.S., Duffey, S.S. & Bostock, R.M. (1999) Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor ii, and pathogenesis-related protein p4 in the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology* **54**, 97–114.

Filkowski, J., Kovalchuk, O. & Kovalchuk, I. (2004) Genome stability of vtc1, tt4, and tt5 *Arabidopsis thaliana* mutants

impaired in protection against oxidative stress. *Plant Journal* **38**, 60–69.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E.E., Crute, I.R., Holub, E.B., Hammerschmidt, R. & Ausubel, F.M. (1997) Phytoalexin-deficient mutants of Arabidopsis reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. *Genetics* **146**, 381–392.

Hale, B.K., Bale, J.S., Pritchard, J., Masters, G.I. & Brown, V.K. (2003) Effects of host plant drought stress on the performance of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.): A mechanistic analysis. *Ecological Entomology* **28**, 666–677.

Hegedus, D., Yu, M., Baldwin, D., Gruber, M., Sharpe, A., Parkin, I., Whitwill, S. & Lydiate, D. (2003) Molecular characterization of *Brassica napus* NAC domain transcriptional activators induced in response to biotic and abiotic stress. *Plant Molecular Biology* **53**, 383–397.

Hong, Z.L., Zhang, Z.M., Olson, J.M. & Verma, D.P.S. (2001) A novel udp-glucose transferase is part of the callose synthase complex and interacts with phragmoplastin at the forming cell plate. *Plant Cell* **13**, 769–779.

Hunt, E.J., Pritchard, J., Bennett, M.J., Zhu, X., Barrett, D.A., Allen, T., Bale, J.S. & Newbury, H.J. (2006) The *Arabidopsis thaliana*/*Myzus persicae* model system demonstrates that a single gene can influence the interaction between a plant and a sap-feeding insect. *Molecular Ecology* **15**, 4203–4213.

Jammes, F., Lecompte, P., de Almida-Engler, J., Bitton, F., Martin-Magniette, M-L., Renou, J.P., Abad, P. & Favery, B. (2005) Genome-wide expression profiling of the host response to root-knot nematode infection in Arabidopsis. *The Plant Journal* **44**, 447–458.

Kallberg, Y., Oppermann, U., Jornvall, H. & Persson, B. (2002) Short-chain dehydrogenase/reductase (SDR) relationships: a large family with eight clusters common to human, animal, and plant genomes. *Protein Science* **11**, 636–641.

Kempema, L.A., Cui, X., Holzer, F.M. & Walling, L.L. (2007) Arabidopsis transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology*, in press.

Kim, H.G., Kim, B.C., Park, E.H. & Lim, C.J. (2005) Stress-dependent regulation of a monothiol glutaredoxin gene from *Schizosaccharomyces pombe*. *Canadian Journal of Microbiology* **51**, 613–620.

Lamport, D.T.A. & Kieliszewski, M.J. (2005) Stress upregulates periplasmic arabinogalactan-proteins. *Plant Biosystems* **139**, 60–64.

Lee, M.T., Kuo, F.C., Whitmore, G.A. & Sklar, J. (2000) Importance of replication in microarray gene expression studies: Statistical methods and evidence from repetitive cDNA hybridizations. *Proceedings of the National Academy of Sciences* **97**, 9834–9839.

León, J., Rojo, E., Titarenko, E. & Sanchez-Serrano, J.J. (1998) Jasmonic acid-dependent and -independent wound signal transduction pathways are differentially regulated by Ca^{2+} calmodulin in *Arabidopsis thaliana*. *Molecular and General Genetics* **258**, 412–419.

Li, X.C., Schuler, M.A. & Berenbaum, M.R. (2002) Jasmonate and salicylate induce expression of herbivore cytochrome p450 genes. *Nature* **419**, 712–715.

Lieberherr, D., Wagner, U., Dubuis, P.H., Metraux, J.P. & Mauch, F. (2003) The rapid induction of glutathione S-transferases atgstf2 and atgstf6 by a virulent *Pseudomonas syringae* is the result of combined salicylic acid and ethylene signalling. *Plant and Cell Physiology* **44**, 750–757.

Mahalingam, R., Shah, N., Scrymgeour, A. & Fedoroff, N. (2005) Temporal evolution of the Arabidopsis oxidative stress response. *Plant Molecular Biology* **57**, 709–730.

Mang, H.G., Kang, E.O., Shim, J.H., Kim, S.Y., Park, K.Y., Kim, Y.S., Bahk, Y.Y. & Kim, W.T. (2004) A proteomic analysis identifies glutathione S-transferase isoforms whose abundance is differentially regulated by ethylene during the formation of early root epidermis in Arabidopsis seedlings. *Biochimica et Biophysica Acta-Gene Structure and Expression* **1676**, 231–239.

Miles, P.W. (1999) Aphid saliva. *Biological Reviews* **74**, 41–85.

Miles, P.W. & Oertli, J.J. (1993) The significance of antioxidants in the aphid-plant interaction – the redox hypothesis. *Entomologia Experimentalis et Applicata* **67**, 275–283.

Mittler, T.E. (1958) Studies on the feeding and nutrition of *Tuberolachnus salignus* (Gmelin) ii, the nitrogen and sugar composition of ingested phloem sap and excreted honeydew. *Journal of Experimental Biology* **35**, 74–84.

Moran, P.J., Cheng, Y.F., Cassell, J.L. & Thompson, G.A. (2002) Gene expression profiling of *Arabidopsis thaliana* in compatible plant-aphid interactions. *Archives of Insect Biochemistry and Physiology* **51**, 182–203.

Nagano, Y., Furuhashi, H., Inaba, T. & Sasaki, Y. (2001) A novel class of plant-specific zinc-dependent DNA-binding protein that binds to A/T-rich DNA sequences. *Nucleic Acids Research* **29**, 4097–4105.

Ni, X.Z. & Quisenberry, S.S. (2003) Possible roles of esterase, glutathione s-transferase, and superoxide dismutase activities in understanding aphid-cereal interactions. *Entomologia Experimentalis et Applicata* **108**, 187–195.

Ni, X.Z., Quisenberry, S.S., Heng-Moss, T., Markwell, J., Sarath, G., Klucas, R. & Baxendale, F. (2001) Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. *Journal of Economic Entomology* **94**, 743–751.

Nühse, T.S., Boller, T. & Peck, S.C. (2003) A plasma membrane syntaxin is phosphorylated in response to the bacterial elicitor flagellin. *Journal of Biological Chemistry* **278**, 45248–45254.

Ogawa, K. (2005) Glutathione-associated regulation of plant growth and stress responses. *Antioxidants & Redox Signaling* **7**, 973–981.

Ooka, H., Satoh, K., Doi, K., Nagata, T., Otomo, Y., Murakami, K., Matsubara, K., Osato, N., Kawai, J., Carninci, P., Hayashizaki, Y., Suzuki, K., Kojima, K., Takahara, Y., Yamamoto, K. & Kikuchi, S. (2003) Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Research* **10**, 239–247.

Oparka, K.J. (2004) Getting the message across: how do plant cells exchange macromolecular complexes? *Trends in Plant Science* **9**, 33–41.

Pavlidis, P., Qinghong, L. & Stafford Nobel, W. (2003) The effect of replication on gene expression microarray experiments. *Bioinformatics* **19**, 1620–1627.

Pegadaraju, V., Knepper, C., Reese, J. & Shah, J. (2005) Premature leaf senescence modulated by the Arabidopsis PHYTOALEXIN DEFICIENT4 gene is associated with defense against the phloem-feeding green peach aphid. *Plant Physiology* **139**, 1927–1934.

Ponder, K.L., Pritchard, J., Harrington, R. & Bale, J.S. (2000) Difficulties in location and acceptance of phloem sap combined with reduced concentration of phloem amino acids explain lowered performance of the aphid

Rhopalosihum padi on nitrogen deficient barley (*Hordeum vulgare*) seedlings. *Entomologia Experimentalis et Applicata* **97**, 203–210.

Ponder, K.L., Pritchard, J., Harrington, R. & Bale, J.S. (2001) Feeding behaviour of the aphid *Rhopalosiphum padi* (Hemiptera: Aphididae) on nitrogen and water stressed barley (*Hordeum vulgare*) seedlings. *Bulletin of Entomological Research* **91**, 1–9.

Prado, E. & Tjallingii, W.F. (1997) Effects of previous plant infestation on sieve element acceptance by two aphids. *Entomologia Experimentalis et Applicata* **82**, 189–200.

Pritchard, J., Griffiths, B. & Hunt, E.J. (2007) Can the effect of climate change on the performance of phloem-feeding insect herbivores be predicted? *Global Change Biology*. in press.

Qubbaj, T., Reineke, A. & Zebitz, C.P.W. (2005) Molecular interactions between rosy apple aphids, *Dysaphis plantaginea*, and resistant and susceptible cultivars of its primary host *Malus domestica*. *Entomologia Experimentalis et Applicata* **115**, 145–152.

Reddy, V.S. & Reddy, A.S.N. (2004) Proteomics of calcium-signaling components in plants. *Phytochemistry* **65**, 1745–1776.

Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C.Z., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., Creelman, R., Pilgrim, M., Broun, P., Zhang, J.Z., Ghandehari, D., Sherman, B.K. & Yu, C.L. (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science* **290**, 2105–2110.

Sanderfoot, A.A., Assaad, F.F. & Raikhel, N.V. (2000) The Arabidopsis genome. An abundance of soluble N-ethylmaleimide-sensitive factor adaptor protein receptors. *Plant Physiology* **124**, 1558–1569.

Sandström, J., Telang, A. & Moran, N.A. (2000) Nutritional enhancement of host plants by aphids – a comparison of three aphid species on grasses. *Journal of Insect Physiology* **46**, 33–40.

Satoh, S., Sturm, A., Fujii, T. & Chrispeels, M.J. (1992) cDNA cloning of an extracellular dermal glycoprotein of carrot and its expression in response to wounding. *Planta* **188**, 432–438.

Sauvion, N., Charles, H., Febvay, G. & Rahbe, Y. (2004) Effects of jackbean lectin (conA) on the feeding behaviour and kinetics of intoxication of the pea aphid, *Acyrthosiphon pisum*. *Entomologia Experimentalis et Applicata* **110**, 31–44.

Seki, M., Narusaka, M., Ishida, J., Nanjo, T., Fujita, M., Oono, Y., Kamiya, A., Nakajima, M., Enju, A., Sakurai, T., Satou, M., Akiyama, K., Taji, T., Yamaguchi-Shinozaki, K., Carninci, P., Kawai, J., Hayashizaki, Y. & Shinozaki, K. (2002) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant Journal* **31**, 279–292.

Shang, C.W., Shibahara, T., Hanada, K., Iwafune, Y. & Hirano, H. (2004) Mass spectrometric analysis of posttranslational modifications of a carrot extracellular glycoprotein. *Biochemistry* **43**, 6281–6292.

Sjölund, R.D. (1997) The phloem sieve element: a river runs through it. *Plant Cell* **9**, 1137–1146.

Slosser, J.E. (1993) Influence of planting date and insecticide treatment on insect pest abundance and damage in dryland cotton. *Journal of Economic Entomology* **86**, 1213–1222.

Smith, A.P., Nurizadeh, S.D., Peer, W.A., Xu, J.H., Bandyopadhyay, A., Murphy, A.S. & Goldsbrough, P.B. (2003) Arabidopsis AtGSTF2 is regulated by ethylene and auxin, and encodes a glutathione S-transferase that interacts with flavonoids. *Plant Journal* **36**, 433–442.

Smith, A.P., Deridder, B.P., Guo, W.J., Seeley, E.H., Regnier, F.E. & Goldsbrough, P.B. (2004) Proteomic analysis of Arabidopsis glutathione S-transferases from benoxacor- and copper-treated seedlings. *Journal of Biological Chemistry* **279**, 26098–26104.

Stoger, R., Williams, S., Christou, P., Down, R.E. & Gatehouse, J.A. (1999) Expression of the insecticidal lectin from snowdrop (*Galanthus nivalis* agglutinin; GNA) in transgenic wheat plants: effects on predation by the grain aphid *Sitobion avenae*. *Molecular Breeding* **5**, 65–73.

Thompson, G.A. & Goggin, F.L. (2006) Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. *Journal of Experimental Botany* **57**, 755–766.

Tjallingii, W.F. (2006) Salivary secretions by aphids interacting with proteins of phloem wound responses. *Journal of Experimental Botany* **57**, 139–745.

Tjallingii, W.F. & Hogen Esch, T. (1993) Fine-structure of aphid stylet routes in plant-tissues in correlation with EPG signals. *Physiological Entomology* **18**, 317–328.

Urbanska, A., Tjallingii, W.F., Dixon, A.F.G. & Leszczynski, B. (1998) Phenol oxidising enzymes in the grain aphid's saliva. *Entomologia Experimentalis et Applicata* **86**, 197–203.

Van Bel, A.J.E. (2003) The phloem, a miracle of ingenuity. *Plant Cell and Environment* **26**, 125–149.

Vasconcelos, I.M. & Oliveira, J.T.A. (2004) Antinutritional properties of plant lectins. *Toxicon* **44**, 385–403.

Vijayan, M. & Chandra, N. (1999) Lectins. *Current Opinion in Structural Biology* **9**, 707–714.

Voelckel, C., Weisser, W.W. & Baldwin, I.T. (2004) An analysis of plant-aphid interactions by different microarray hybridization strategies. *Molecular Ecology* **13**, 3187–3195.

Wagner, U., Edwards, R., Dixon, D.P. & Mauch, F. (2002) Probing the diversity of the Arabidopsis glutathione S-transferase gene family. *Plant Molecular Biology* **49**, 515–532.

Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* **19**, 195–216.

Will, T. & van Bel, A.J.E. (2006) Physical and chemical interactions between aphids and plants. *Journal of Experimental Botany* **57**, 729–737.

Yan, J., Wang, J. & Zhang, H. (2002) An ankyrin repeat-containing protein plays a role in both disease resistance and antioxidation metabolism. *The Plant Journal* **29**, 193–202.

Yang, K.Y., Kim, E.Y., Kim, C.S., Guh, J.O., Kim, K.C. & Cho, B.H. (1998) Characterization of a glutathione S-transferase gene AtGST1 in *Arabidopsis thaliana*. *Plant Cell Reports* **17**, 700–704.

Yang, K.Y., Im, Y.J., Chung, G.C. & Cho, B.H. (2002) Activity of the Arabidopsis blue copper-binding protein gene promoter in transgenic tobacco plants upon wounding. *Plant Cell Reports* **20**, 987–991.

York, W.S., Qin, Q.A. & Rose, J.K.C. (2004) Proteinaceous inhibitors of endo-beta-glucanases. *Biochimica et Biophysica Acta-Proteins and Proteomics* **1696**, 223–233.

Zhou, J.M. & Goldsbrough, P.B. (1993) An Arabidopsis gene with homology to glutathione S-transferases is regulated by ethylene. *Plant Molecular Biology* **22**, 517–523.

Zhou, N., Tootle, T.L., Tsui, F., Klessig, D.F. & Glazebrook, J. (1998) PAD4 functions upstream from salicylic acid to

control defense responses in Arabidopsis. *Plant Cell* **10**, 1021–1030.

Zhu, T. (2003) Global analysis of gene expression using GeneChip microarrays. *Current Opinion in Plant Biology* **6**, 418–425.

Zhu, T., Budworth, P., Han, B., Brown, D., Chang, H-S., Zou, G. & Wang, X. (2001) Toward elucidating the global gene expression patterns of developing *Arabidopsis*: Parallel analysis of 8300 genes by a high-density oligonucleotide probe array. *Plant Physiology and Biochemistry* **39**, 221–242.

Zhu-Salzman, K., Salzman, R.A., Ahn, J.E. & Koiwa, H. (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. *Plant Physiology* **134**, 420–431.

(Accepted 30 January 2007)