Biotic stress globally downregulates photosynthesis genes

DAMLA D. BILGIN[1], JORGE A. ZAVALA[1,5], JIN ZHU[2], STEVEN J. CLOUGH[2,3], DONALD R. ORT[1–4] & EVAN H. DeLUCIA[1,4]

[1]*Institute for Genomic Biology and* [2]*Department of Crop Sciences, University of Illinois at Urbana-Champaign,* [3]*USDA-ARS and* [4]*Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA and* [5]*CONICET-Universidad Católica Argentina – Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires, Argentina*

ABSTRACT

To determine if damage to foliage by biotic agents, including arthropods, fungi, bacteria and viral pathogens, universally downregulates the expression of genes involved in photosynthesis, we compared transcriptome data from microarray experiments after twenty two different forms of biotic damage on eight different plant species. Transcript levels of photosynthesis light reaction, carbon reduction cycle and pigment synthesis genes decreased regardless of the type of biotic attack. The corresponding upregulation of genes coding for the synthesis of jasmonic acid and those involved in the responses to salicylic acid and ethylene suggest that the downregulation of photosynthesis-related genes was part of a defence response. Analysis of the sub-cellular targeting of co-expressed gene clusters revealed that the transcript levels of 84% of the genes that carry a chloroplast targeting peptide sequence decreased. The majority of these downregulated genes shared common regulatory elements, such as G-box (CACGTG), T-box (ACTTTG) and SORLIP (GCCAC) motifs. Strong convergence in the response of transcription suggests that the universal downregulation of photosynthesis-related gene expression is an adaptive response to biotic attack. We hypothesize that slow turnover of many photosynthetic proteins allows plants to invest resources in immediate defence needs without debilitating near term losses in photosynthetic capacity.

Key-words: chloroplast; *cis*-regulatory elements; defence; gene expression; microarray.

INTRODUCTION

Plants are under constant assault by biotic agents, including viral, bacterial and fungal pathogens, parasitic plants and insect herbivores, with enormous economic and ecological impact (Pimentel 1991, 2002). Plants are locked in an evolutionary arms race with their attackers, and faced with this onslaught have evolved myriad defences. Once an attack is

Correspondence: Evan H. DeLucia. E-mail: delucia@life.illinois.edu

perceived, plant metabolism must balance potentially competing demands for resources to support defence versus requirements for cellular maintenance, growth and reproduction (Herms & Mattson 1992; Zangerl & Berenbaum 1997, 2003; Berger, Sinha & Roitsch 2007a). Upon introduction of various elicitors, such as pathogen-associated molecular patterns (PAMPs), viral coat proteins or fatty acid conjugates in the oral secretions of insect saliva, a massive reprogramming of plant gene expression, hormonal and chemical defence responses are initiated, a process that can be costly in terms of plant growth and fitness (Tian *et al.* 2003; Zavala & Baldwin 2004). In addition to triggering defences to dissuade pathogen and herbivore attack by allocating resources from growth to defence, a reduction of photosynthetic capacity in remaining leaf tissues may represent a 'hidden cost' of defence (Zangerl *et al.* 2002; Aldea *et al.* 2006; Berger *et al.* 2007b; Bilgin *et al.* 2008; Nabity, Zavala & DeLucia 2009).

Although there are examples of compensatory stimulations of photosynthesis (Trumble, Kolondy-Hirsch & Ting 1993), a decline in photosynthetic rate following attack by insects or pathogens is well documented (Welter 1989; Schenk *et al.* 2000; Sasaki *et al.* 2001; Macedo *et al.* 2003; Sasaki-Sekimoto *et al.* 2005; Uppalapati *et al.* 2005; Zou *et al.* 2005; Aldea *et al.* 2006; Major & Constabel 2006; Shimizu *et al.* 2007; Vogel, Kroymann & Mitchell-Olds 2007; Yang *et al.* 2007; Bozso *et al.* 2009; Nabity *et al.* 2009). Beyond the actual rates of CO_2 assimilation, early examinations of the plant transcriptome revealed that many photosynthetic genes are downregulated following biotic attack (Zou *et al.* 2005; Berger *et al.* 2007b). The linkage between photosynthesis and defence is further illustrated by the observation that silencing of gene expression for two central photosynthetic proteins, ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) and Rubisco activase, affected herbivore resistance in *Nicotiana attenuata* (Giri *et al.* 2006; Mitra & Baldwin 2008). Assault by insects or pathogens reduces the mRNA levels coding for Rubisco small subunit, as well as genes coding for the components of the antenna complexes in both photosystems (Logemann *et al.* 1995; Ehness *et al.* 1997; Hermsmeier, Schittko & Baldwin 2001; Montesano *et al.* 2004; Zou *et al.* 2005). With

the public availability of data derived from whole-genome microarray experiments (Galbraith 2006), we were able to examine, across a broad spectrum of biotic agents and plant species, if attack by insects and infection by pathogens causes a global downregulation of genes coding for photosynthetic proteins as part of basal defence response, and if the transcriptional response of plants is conserved when exposed to different types of biotic assault.

Building the observed responses of specific plant species to specific types of biotic assault mentioned earlier, we asked whether damage to foliage by biotic agents, including arthropods, fungi, bacteria and viral pathogens, causes a universal downregulation of genes involved in photosynthesis. This regulatory set includes genes coding for pigments and proteins involved in electron transport and genes coding for related aspects of carbon metabolism. We used a meta-genomic approach by comparing the results from published experiments that examined genome-wide responses to various biotic agents. To explore the possible significance of this putative downregulation of photosynthetic genes, the response of genes involved in defence signalling and the reactive oxygen species (ROS) scavenging network specifically were examined. If reallocating resources made available by downregulation of photosynthesis gene expression supports a reorientation of metabolism towards defence, a corresponding upregulation of genes involved in defence signalling is expected. Moreover, if these biotic agents induce ROS formation, a corresponding upregulation of genes involved in the detoxification of ROS is anticipated. Although these predictions are not mutually exclusive they may provide insight on the adaptive consequences of reducing plant energy supply when confronted with biotic attack.

MATERIALS AND METHODS

Data sources and analysis

To examine the response to biotic attack, gene lists for photosynthesis light reaction and carbon fixation, starch and sucrose metabolism, and flavonoid biosynthesis were assembled from metabolic and signalling pathways illustrated by the KEGG PATHWAY Database (http://www.genome.ad.jp/kegg/pathway.html), the Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org/) and the Signal Transduction Knowledge Environment database (STKE; http://stke.sciencemag.org/). At the same time, to observe changes in other signalling and biosynthetic pathways, gene lists of different processes such as, photorespiration, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET)-related defence response, reactive oxygen scavenging network were formed.

We conducted a search of *Science Citation Index Expanded* database from *ISI Web of Knowledge, Web of Science*, with 'microarray', 'herbivory', 'pathogen' and 'biotic stress' as keywords, with no restrictions on date of publication. Additional papers were identified from the literature cited sections of papers from the electronic search. Only papers presenting genome-wide responses of leaf tissue following damage were incorporated in our analysis; these papers utilized cDNA or oligonucleotide (Affymetrix©, NimbleGen©) microarrays. When not given in the published article, authors were asked to provide the identification number, fold-change (log_2) relative to control and the '*P*' value for each gene. Additional data sets were obtained from a public repository of microarray data (Genevestigator; https://www.genevestigator.ethz.ch/). Transgenic and mutant plants and resistant biotypes were excluded from the analysis.

Groups of genes corresponding to photosynthesis and other metabolic and signalling pathways were extracted from these original data sets to determine the changes in transcript levels. The data sets were log transformed and significant genes were selected according to $P < 0.05$. Where data for multiple time points were available, the earliest time point representing the maximum genomic response (absolute value of the sum of up- and downregulated genes) was selected. Values obtained from Genevestigator database were an averaged single value for each gene that represented the overall transcript change across time points.

Gene functions were based on annotation of The Arabidopsis Information Resource database (Rhee *et al.* 2003; TAIR; http://www.arabidopsis.org). The *Arabidopsis* top match were used from the original studies and where necessary (e.g. Halitschke *et al.* 2003; Izaguirre *et al.* 2003; Voelckel & Baldwin 2004; Schmidt *et al.* 2005; Coram & Pang 2006; Casteel *et al.* 2008), gene sequences were compared to *Arabidopsis* with the Basic Local Alignment Search Tool (BLAST) from TAIR and sequence homologs were determined with a 10^{-10} *e*-value cutoff (Supporting Information Table S3). It should be noted that because of the absence of full sequence data for host species in this study other than *Arabidopsis*, the gene identifiers should be treated as tentative. The genes that were not on the array or did not show significant sequence homology during BLAST search because they did not meet the cutoff value were scored zero for the purpose of running the clustering algorithm (Eisen *et al.* 1998; Alizadeh *et al.* 2000). The missing log_2 transformed gene expressions that were replaced with zero were marked with grey colour in the Figs 1–4, Supporting Information Figs S1–3 and Table S2.

In some cases multiple transcripts from the host tissue aligned to the same *Arabidopsis* gene following the BLAST search. It was assumed that different host transcripts with sufficiently high sequence homology to *Arabidopsis*, as indicated by the stringent *e*-value cutoff, represented variants of the same gene and the fold-change values for these multiple transcripts were averaged. Alternatively, the single host transcript with the smallest *e*-value could have been selected. In most cases, transcripts with high sequence homology responded similarly to a specific biotic stress (Supporting Information Table S3), so averaging the fold-change data across similar genes was not likely to influence the results.

Data sets were subjected to hierarchical average-linkage clustering with Cluster software (Eisen *et al.* 1998) and displayed with TreeView version 1.60 (http://rana.lbl.gov/

EisenSoftware.htm). The output from the clustering algorithm provided a graphical display of the similarity of expression data as well as similarity among the response of different forms of biotic stress. Shades of green and magenta represent genes that were downregulated and upregulated, respectively, and the intensity of colour represents the magnitude of the fold-change relative to controls. No 'mask' was applied to the graphical output, so all significant genes were displayed regardless of the magnitude of the response; the same 'image contrast' was applied to each analysis, so the fold-change values represented by the colour intensity were consistent across figures. The black colour represented the genes with no significant transcript change for the studies performed with *Arabidopsis*. For species other than *Arabidopsis*, dark grey represented the absence of the gene on the microarray or the inability to identify sequence homologs to *Arabidopsis* (Figs 1–4, Supporting Information Table S2).

Determining subcellular localization and potential *cis*-regulating elements

The cluster analysis of photosynthesis light reaction, carbon fixation, photorespiration, ROS scavenging network and starch and sucrose metabolism genes showed co-expression patterns. The protein sequences of these co-expressed genes were obtained from TAIR and subcellular localization of each gene was determined by ChloroP 1.1 (Emanuelsson, Nielsen & von Heijne 1999; http://www.cbs.dtu.dk/services/ChloroP/). Within co-expressed clusters the genes were grouped according to their subcellular localization as chloroplast targeted or not.

To determine the enriched DNA motifs that might be co-regulating elements in co-expressed gene clusters, the 2 kb upstream sequences of co-expressed and chloroplast targeted and non-targeted genes were obtained from Matt Hudson Lab Bioinformatics and Plant Genomics database (Hudson & Quail 2003; http://stan.cropsci.uiuc.edu/index.php). The enriched DNA motifs in these upstream sequences were determined with the same database. The number of occurrences of each motif was compared with the frequency of that element in the sequence of the promoters for the whole genome by a version of one-degree-of-freedom chi squared test. The over-represented elements with $P < 0.001$ were analysed a second time to determine the probability of the element being present in the promoters of the query set and the known elements were selected according to statistical significance e-value $\leq 10^{-3}$ (for details see Hudson & Quail 2003).

RESULTS AND DISCUSSION

The downregulation of genes coding for photosynthetic proteins frequently has been observed for individual pairs of plants and biotic agents. The microarray studies used in this study individually have analysed the changes of the transcripts of the whole genome and provided the list of genes that change significantly in response to a particular

biotic stress. Among these differentially regulated genes, we focused our meta-analysis of photosynthesis-related genes and the pathways that may interact directly or indirectly with nuclear encoded photosynthesis genes.

Data included in this study originated from 20 sources that examined basal defence response against pathogen and herbivore attack, including publications and web-based archives (Table 1). In addition to the application of insect regurgitant and various phytohormones and their precursors (e.g. JA, SA, ET and aminocyclopropane carboxylic acid; ACC), eight different types of biotic damage were examined (Table 1). The largest portion of data pertains to *Arabidopsis thaliana*, but microarray data from five other herbaceous species and two tree species were included in our analysis.

The datasets relied on oligonucleotide and custom cDNA array platforms that exclusively represented nuclear encoded genes. Even though photosynthetic genes encoded in the chloroplast genome were not included in the analysis, many nuclear-encoded chloroplast proteins are regulated by nuclear transcription and control chloroplast function and proteome composition (Kleffmann *et al*. 2004; Woodson & Chory, 2008). Additionally, the regulation of gene expression in the chloroplast is coordinated with the nucleus, and although most of this regulation occurs through post-transcriptional mechanisms, transcriptional regulation also is evident (Jarvis & Soll 2001; Jarvis 2001; Woodson & Chory 2008).

Photosynthesis genes respond to biotic damage

In spite of wide variation in the type of damage, host plant and sampling time, biotic damage to foliage caused a near global downregulation of genes involved in photosynthesis. This response was particularly evident for genes involved in pigment synthesis and electron transport (Fig. 1). Genes coding for proteins in photosystem I (PSI) and photosystem II (PSII) reaction centres, ATP synthase and several elements of the light-harvesting complex (LHCII) associated with PSII were downregulated by biotic damage.

Reduced gene expression does not, however, necessarily translate to loss of function. The temporal relationship between the expression of 'light reaction' genes and the function of electron transport from water splitting to the reduction of $NADP^+$ is not immediate because of the long functional lifetime of these proteins. While some elements of PSII reaction centre are highly labile, most notably the chloroplast-encoded D1 protein, and require rapid synthesis, the production and reassembly of function PSII is not proximally regulated by transcription. Under chilling stress, for example, the dramatic decline in D1 protein synthesis occurred with constant steady-state levels of *psbA* mRNA and the decline in D1 was attributed to interference with translation (Grennan & Ort 2007).

A notable exception to the downregulations of pigment and light-reaction genes in response to biotic attack was the genes coding for ferredoxin (Fd) and ferredoxin NADPH

Table 1. Studies that examined the effect of damage by different biotic agents on the transcriptome of leaf tissue. 'Time' represents hours (h), or days (d) in one study, post-damage; values with an asterisk represent studies that included multiple time points, typically between 0.5 and 72 hours. 'Rep' is the number of independent biological replicates used in the statistical analysis. The transciptome was assayed with commercial oligonucleotide (Affymetrix, NimbleGen) or custome cDNA micro- (macro) arrays; the values in parentheses following the platform represent the number genes on the array

Damage type/biotic agent	Host	Time	Rep	Micoarray platform	Ref[a]
Virus					
Soybean mosaic virus	Glycine max	8 h[a]	4	Oligonucleotide (Affy)	12
Bacteria/pathogen					
Pseudomonas syringae	Arabidopsis thaliana	12 h[a]	4	Oligonucleotide (Affy)	1
Pseudomonas syringae	Arabidopsis thaliana	12 h[a]	3	Oligonucleotide (Affy)	14
Pseudomonas syringae	Arabidopsis thaliana	Avg	3	Oligonucleotide (Affy)	8
Pseudomonas syringae	Glycine max	8 h[a]	2	cDNA (22 000)	11
Agrobacterium tumefaciens	Arabidopsis thaliana	Avg	2	Oligonucleotide (Affy)	8
Fungus/pathogen					
Alternaria brassiciciola	Arabidopsis thaliana	24 h[a]	4	Oligonucleotide (Affy)	1
Ascochyta rabiei	Cicer arietinum	48 h[a]	3	cDNA (715)	10
Botrytis cinerea	Arabidopsis thaliana	48 h[a]	2	Oligonucleotide (Affy)	7
Botrytis cinerea	Arabidopsis thaliana	Avg	3	Oligonucleotide (Affy)	8
Erysiphe orontii	Arabidopsis thaliana	Avg	3	Oligonucleotide (Affy)	8
Erysiphe cichoracearum	Arabidopsis thaliana	Avg	4	Oligonucleotide (Affy)	8
Melampsora larici-populina	Populus hybrid	24 h[a]	3	Oligonucleotide (NimbleGen)	17
Phytophtora infestans	Arabidopsis thaliana	Avg	3	Oligonucleotide (Affy)	8
Insect/chewing					
Choristoneura occidentalis	Picea sitchensis	52 h	5	cDNA (9 720)	4
Malacosoma disstria	Populus hybrid	24 h	5	cDNA (15 496)	3
Manduca sexta	Nicotiana attenuata	24 h	3	cDNA (TIGR potato 10 K v1)	18
Manduca sexta	Nicotiana longiflora	24 h	3	cDNA	19
Manduca sexta	Solanum nigrum	24 h	3	cDNA (TIGR potato 10 K v1)	18[a]
Pieris rapae	Arabidopsis thaliana	24 h[a]	4	Oligonucleotide (Affy)	1
Pieris rapae	Arabidopsis thaliana	24 h	3	cDNA (12 135)	2
Popillia japonica	Glycine max	3 d[a]	4	Oligonucleotide (Affy)	13
Spodoptera littoralis	Arabidopsis thaliana	24 h	3	cDNA (12 135)	2
Puncture/thrip scraping					
Frankliniella occidentalis	Arabidopsis thaliana	24 h[a]	4	Oligonucleotide (Affy)	1
Penetration/stylet					
Bemisia tabaci	Arabidopsis thaliana	21 d	2	Oligonucleotide (Affy)	5
Myzus persicae	Arabidopsis thaliana	48 h[a]	4	Oligonucleotide (Affy)	1
Myzus persicae	Arabidopsis thaliana	Avg	3	Oligonucleotide (Affy)	8
Tupiocoris notatus	Nicotiana attenuata	24 h	4	Oligonucleotide (790)	16
Oviposition					
Pieris brassicae	Arabidopsis thaliana	72 h[a]	6	cDNA (22 072)	6
Pieris rapae	Arabidopsis thaliana	72 h[a]	6	cDNA (22 072)	6
Caterpillar regurgitant					
Manduca quinquemaculata	Nicotiana attenuata	10 h	1	cDNA	20
Manduca sexta	Nicotiana attenuata	10 h	1	cDNA	20
Hormone/other					
Methyl jasmonate (MeJA)	Arabidopsis thaliana	6 h	4	Oligonucleotide (Affy)	1
MeJA	Arabidopsis thaliana	6 h	3	cDNA (12 135)	2
MeJA	Cicer arietinum	27 h	3	cDNA macroarray (559)	9
MeJA	Arabidopsis thaliana	6 h[a]	3	Oligonucleotide (Affy)	15
SA	Cicer arietinum	27 h	3	cDNA macroarray (559)	9
ACC	Cicer arietinum	27 h	3	cDNA macroarray (559)	9
Mechanical wounding					
	Arabidopsis thaliana	5 h	3	cDNA (12 135)	2
	Arabidopsis thaliana	6 h[a]	2	Oligonucleotide (Affy)	15
	Arabidopsis thaliana	Avg	2	Oligonucleotide (Affy)	8
	Picea sitchensis	24 h	5	cDNA (9 720)	4

[a]1, De Vos et al. (2005); 2, Reymond et al. (2004); 3, Ralph et al. (2006a); 4, Ralph et al. (2006b); 5, Kempema et al. (2007); 6, Little et al. (2007); 7, Ferrari et al. (2007); 8, Genevestigator, Zimmermann et al. (2005); 9, Coram & Pang (2007); 10, Coram & Pang (2006); 11, Zou et al. (2005); 12, Bilgin et al. (2008); 13, Casteel et al. (2008); 14, Truman, de Zabala, Grant (2006); 15, Devoto et al. (2005); 16, Voelckel & Baldwin (2004); 17, Rinaldi et al. (2007); 18, Schmidt et al. (2005); 18[a], Schmidt et al. (2005); 19, Izaguirre et al. (2003); 20, Halitschke et al. (2003).
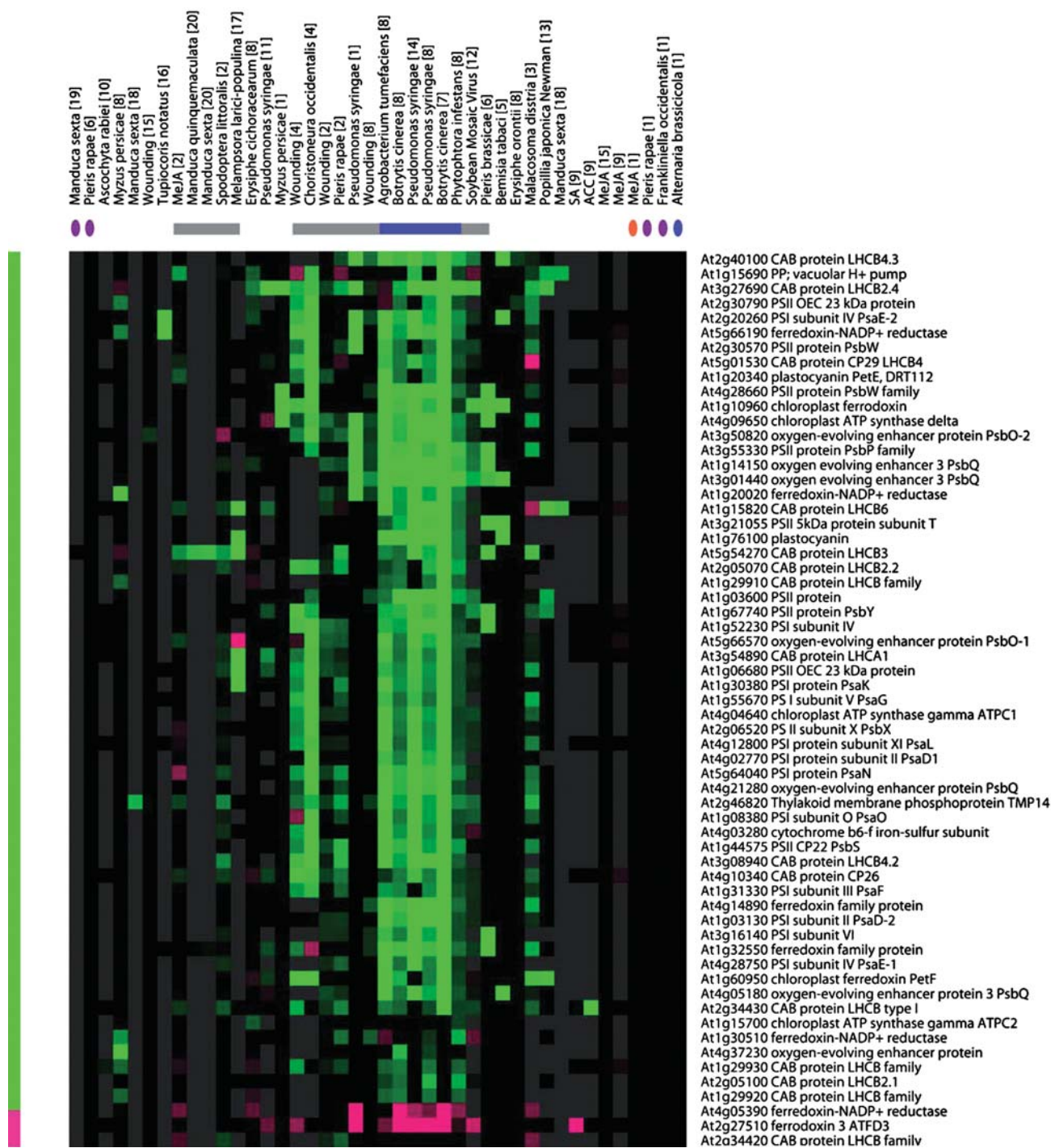
Figure 1. Hierarchical cluster analysis of the expression of genes involved in the synthesis of photosynthetic pigments and the 'light reactions' of photosynthesis following damage by different biotic agents. Genes are listed to the right and biotic agent and their references corresponding to Table 1 in parentheses are listed across the top of the 'heat map'. Genes that were upregulated by the treatments are illustrated in magenta and those that were downregulated are illustrated in green, and brightness is proportional to the strength of the effect. Genes that were not significantly affected by the treatment are represented by black and those that were not represented on the microarray or where a sequence homolog could not be determined are displayed in dark grey. The vertical bars represent the groups derived from the gene tree of hierarchical clustering. The green bar is used for downregulated and magenta bar is for upregulated gene groups. The horizontal bars represent the groups derived from the array tree of hierarchical clustering. Various colours were used to represent different treatments, blue: pathogen infection, purple: insect infestation, orange: phytohormone treatment, grey: combination of treatments. The filled circles represent treatment types that did not cluster and originated from the base of tree. The numbers in parentheses following the treatment names refer to the publications listed in Table 1.
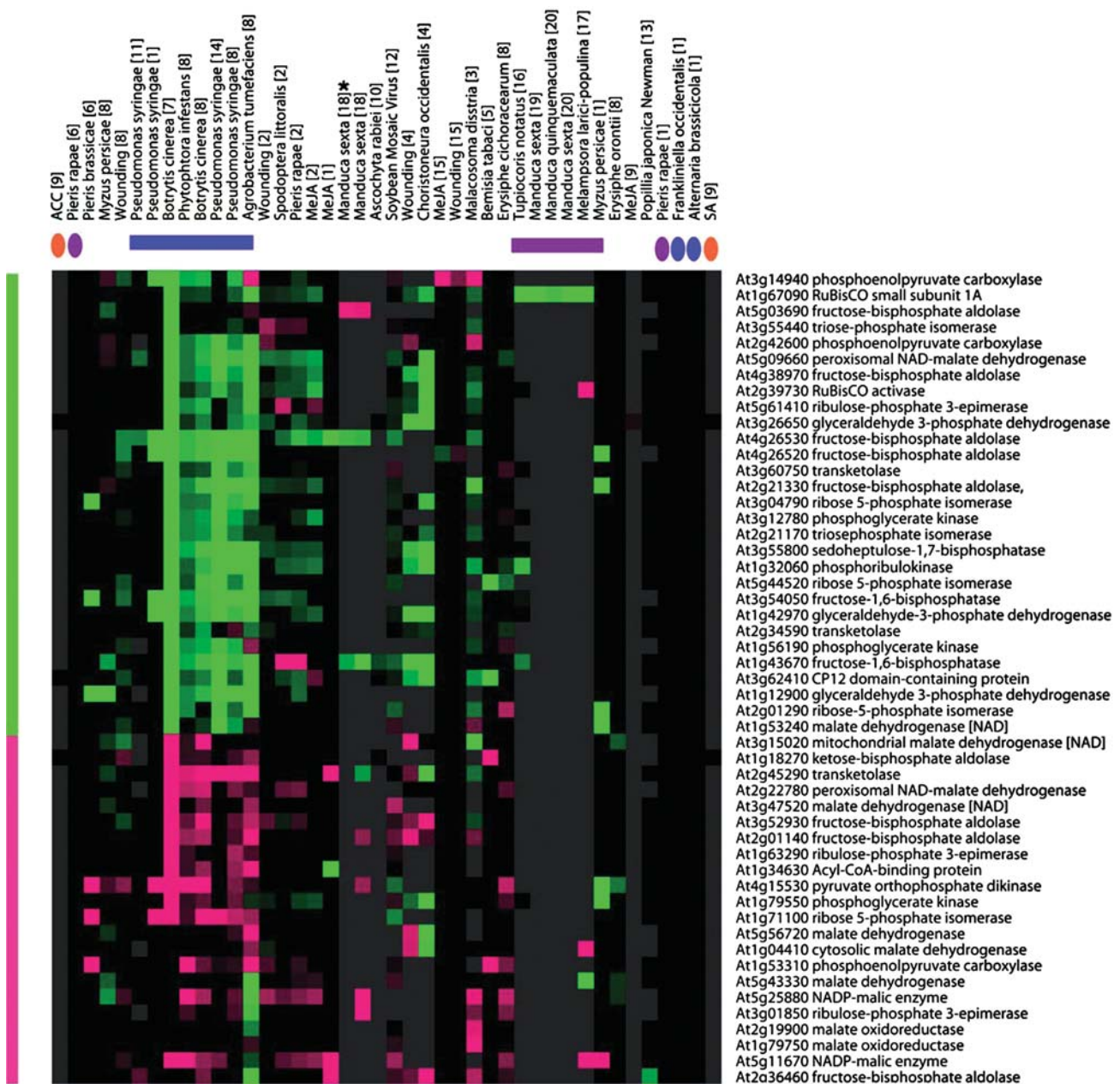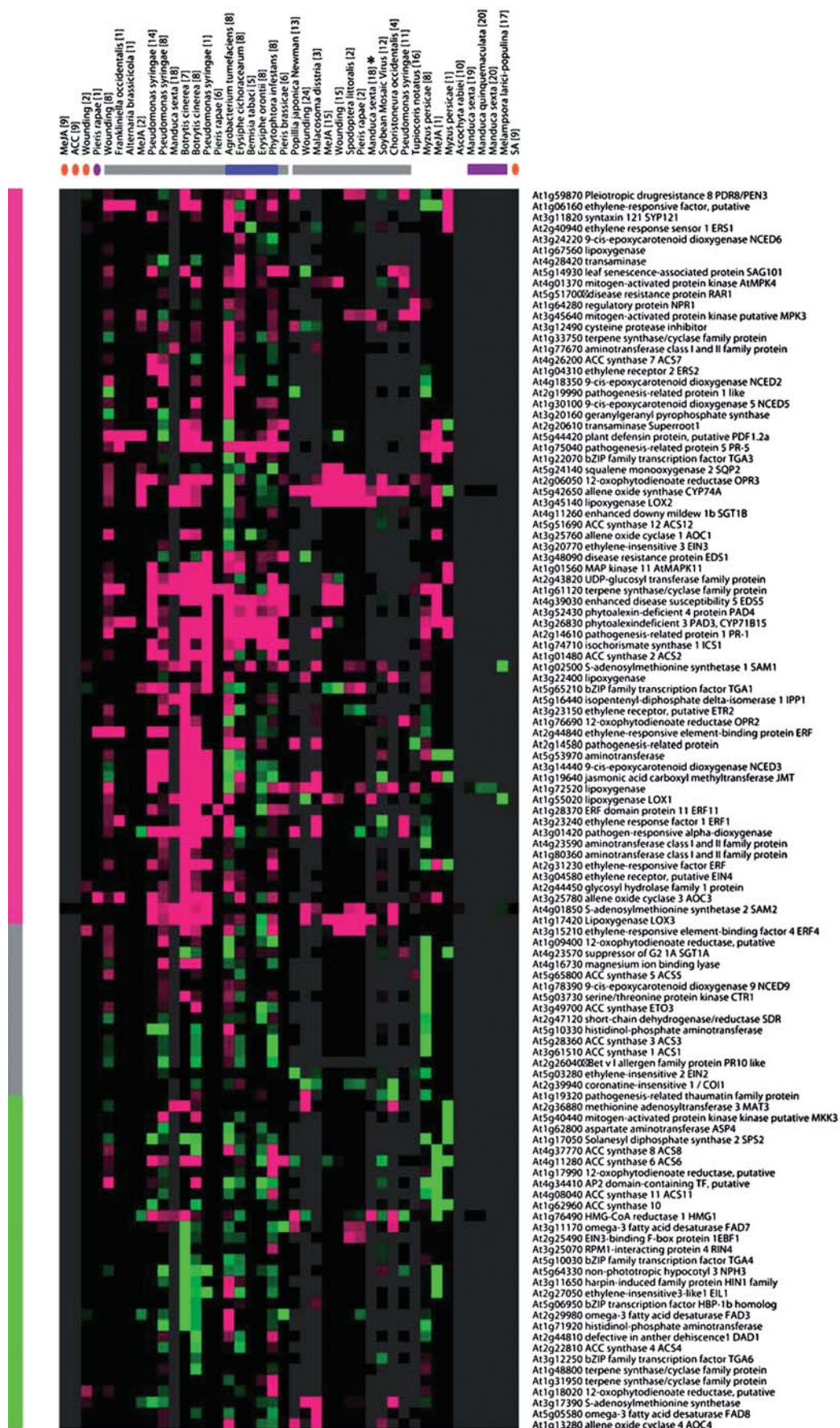
Figure 2. Cluster analysis of the expression of genes following biotic assault involved in the Calvin cycle. For detailed description of colour coding please see the legend of Fig. 1.

oxidoreductase (FNR; Fig. 1). Particularly following pathogen infection, these genes were strongly upregulated. In photosynthesis, Fd accepts electrons from PSI and reduces $NADP^+$ via FNR. However, Fd also participates in other reactions in the chloroplast, including nitrogen and sulfur assimilation, amino acid and fatty acid synthesis, and redox regulation (Knaff & Hirasawa 1991), and different isoforms are present in photosynthetic and non-photosynthetic tissues (Green *et al.* 1991; Hanke *et al.* 2004). The transcriptional upregulation of Fd may reflect its direct participation in pathogen defence. Dayakar *et al.* (2003) observed a synergy between a ferredoxin-like protein and harpin, an

elicitor from *Pseudomonas syringae*; possibly by altering cellular redox state, the ferredoxin-like protein enhanced the ability of harpin to induce production of active oxygen species to mount a hypersensitive response. Recently, it was observed that over-expression of ferredoxin in tobacco conferred resistance to *P. syringae* and *Erwinia carotovora* (Huang *et al.* 2007). The upregulation of Fd and FNR gene expression following biotic assault (Fig. 1) may be related to the role of these proteins in defence rather than a response of photosynthesis per se.

Unlike genes coding for the major elements of photosynthetic electron transport and thylakoid pigments, only

Figure 3. Cluster analysis of the expression of genes following biotic assault involved in the biosynthesis and response to jasmonic acid (JA) and ethylene (ET), and those involved in the response to salicylic acid (SA). For detailed description of colour coding see the legend of Fig. 1.
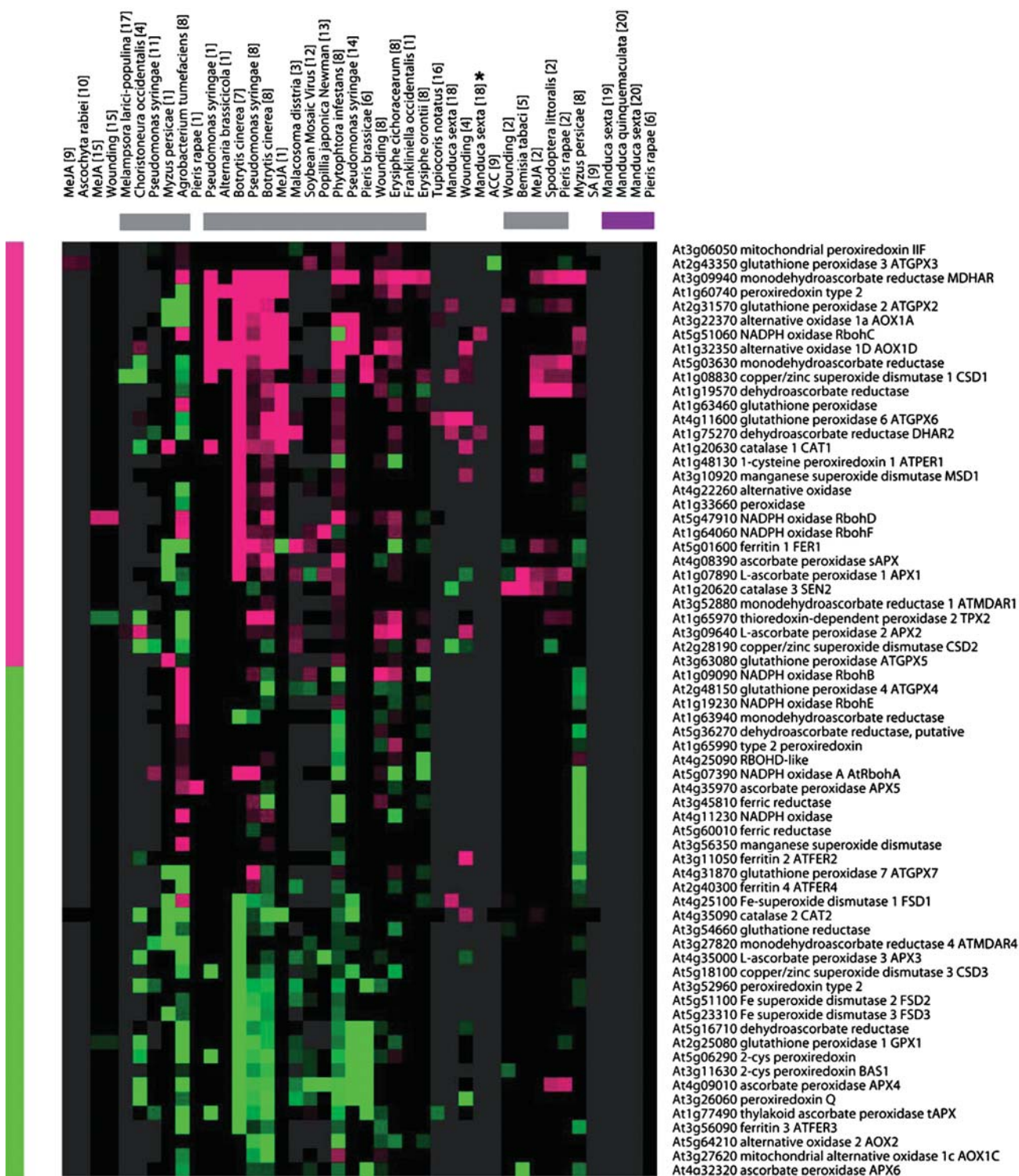
Figure 4. Cluster analysis of the expression of genes involved in the production and detoxification of reactive oxygen species after biotic stress. For detailed description of colour coding see the legend of Fig. 1.

slightly more than half of those coding for enzymes in the Calvin cycle were downregulated following biotic damage (Fig. 2). This response may stem from the dual role of the enzymes coded by genes represented on the microarrays. The Calvin cycle is comprised of 11 different enzymes,

which catalyse 13 different reactions (Gontero, Avilan & Lebreton 2006); however, several Calvin cycle enzymes and metabolites also participate in the oxidative pentose phosphate pathway (OPPP) – a source of reducing power in the form of NADPH. *Arabidopsis*, for example, contains genes

in the chloroplast and nuclear genomes for ribulose-5-isomerase and ribulose-5-epimerase, and transketolase and transaldolase that function both in the Calvin Cycle and OPPP (Kopriva, Koprivova & Suss 2000; Kruger & von Schaewen 2003). Overlapping Calvin cycle and OPPP enzymes are differentially regulated by the ferrodoxin-thioredoxin system to prevent a futile cycle that would consume NADPH and ATP (Gontero *et al.* 2006). Following biotic stress, the opposite responses of different genes coding for fructose-bisphophate aldolase, ribose-5-phophate isomerase and ribulose-phophate 3-epimerase, among others, is very likely a consequence of the dual function of these enzymes (Fig. 2).

As was the case for the 'light reaction' genes, a deeper examination of the responses of 'dark reaction' genes revealed that those genes uniquely associated with the Calvin cycle also were uniformly downregulated following biotic damage (Fig. 2). The nuclear encoded gene for the small subunit of Rubisco was downregulated by biotic stress. Rubisco activity is precisely regulated *in vivo*, and its light-dependent activation is controlled by Rubisco activase (RCA; Portis *et al.* 2008). The gene coding RCA also was downregulated by biotic stress.

In addition to Rubisco and RCA, the other genes unique to the Calvin cycle downregulated following biotic damage were those coding for phosphoribulose kinase (PRK), CP12 and sedoheptulose-1,7-bisphosphatase (SBPase). PRK catalyses the phophorylation of ribulose-5-phosphate to regenerate ribulose bisphosphate. In the stroma, PRK forms a supramolecular complex with glyceraldehyde-3-phophate dehydrogenase (GAPDH) and CP12; the expression of the genes for these proteins is tightly coordinated and regulated by light intensity and sucrose concentration (Marri *et al.* 2005; Howard *et al.* 2008). SBPase functions in the 'regenerative phase' of the Calvin cycle where it catalyses the dephosphorylation of sedaheptulose-1,7-bisphosphate on route to regenerating ribulose bisphosphate. It is an important regulator of the Calvin cycle, directly affecting the rate of photosynthesis (Lefebvre *et al.* 2005; Raines & Paul 2006; Tamoi *et al.* 2006), and expression of the gene coding for SBPase is affected by irradiance and sugar levels (Raines, Lloyd & Dyer 1999).

Genes coding for the major elements of the light reactions and those unique to the Calvin cycle were uniformly downregulated by biotic stress and this downregulation appeared to be coordinated. Regulation of energy distribution between reaction centres, and precise coordination between the production of reductant and ATP from the light reactions and its consumption and subsequent reduction of CO_2 in the Calvin cycle (Wullschleger 1993; Walters 2004) ensure homeostasis of photosynthetic processes when faced with changing environmental conditions. Similar responses for light reaction and Calvin cycle gene expression suggest that the transcriptional responses of these processes to biotic stress also are coordinated.

Although most genes coding for proteins involved in photosynthetic light reactions were downregulated following biotic stress, not all were downregulated to the same extent.

Exposure to methyl jasmonate (MeJA), for example caused only a small response (De Vos *et al.* 2005; Devoto *et al.* 2005; Coram & Pang 2007). Two of three MeJA experiments in this analysis were performed with Affymetrix ATH1 full-genome GeneChips that represent approximately 23 750 *Arabidopsis* genes. The selected genes of all the pathways were present on the array and no elimination due to BLAST search was involved. Even though MeJA treatment altered the transcript levels of various defence and ROS scavenging network genes, it did not have a strong effect on photosynthesis-related genes. This expression pattern may suggest that the involvement of MeJA might be more active and direct in defence signalling than photosynthesis-related reactions and ROS scavenging network.

Phytohormone treatments (MeJA, SA and ACC) of *Cicer arietinum* did not cause a robust change in the transcript levels of tested genes (Figs 1–4; Coram & Pang 2007). The *Cicer arietinum* cDNA macroarray used in this study contained a relatively low number of genes (559 cDNAs) and only 39% of these could be assigned a function, based on a stringent comparison with *Arabidopsis* (top hit with an e-value $\leq 10^{-10}$). However, eliminating this study did not cause a major change in the cluster pattern (Supporting Information Fig. S4). For probably similar reasons, subjecting *Nicotiana attenuata* to herbivory by *Manduca sexta* or *Manduca quinquemaculata* did not cause strong changes in expression levels of photosynthesis or signalling genes (Figs 1–4).

Two independent studies of *P. rapae* oviposition and infestation did not alter the transcript levels of the genes in all four pathways (Figs 1–4). In general, insect treatments did not alter transcript levels as strongly as pathogen treatments; however, *P. rapae* regurgitant treatment and infestation were among the weakest responses. In the case of chewing insects, even if the insect saliva induces a response in the host that particular tissue is consumed by the insect and the response could not be detected. Missing data, either because a gene was not present on the array or because it could not be assigned a function, was concentrated in insect experiments conducted with plant species other than *Arabidopsis*. Therefore, conclusions about the relative strength of the transcriptional response to insect damage should be treated with caution.

Signalling genes and defence against biotic assault

The theoretical foundation for the notion that the response of plants to biotic assault marks a transition from a growth and reproductive posture to a defensive posture is captured in the growth-differentiation hypothesis (Herms & Mattson 1992). The upregulation of genes coding for defence and the downregulation of genes coding for photosynthesis proteins (Hermsmeier *et al.* 2001) may represent a genomic manifestation of this hypothesis.

Because of the bewildering number of defensive compounds induced following biotic assault, the species dependent variation in the compounds produced and the large

number of metabolic pathways involved in their production, examining the genomic response of each defence pathway as it relates to the expression of photosynthetic genes was impractical. Instead, the expression of genes involved in SA, JA and ET hormonal biosynthesis and signalling following the attack were examined on the basis that upregulation of 'defence hormones' signals the transition from a growth to a defensive posture and ultimately induces species-specific defence responses (Fig. 3).

Plant defence response to pathogens and insects involves the activation of receptors that recognize pathogen-derived proteins or insect-modified proteins and this recognition then induces the production and transport of three major defence hormones, SA, JA and ET (Howe & Jander 2008; Lopez, Bannenberg & Castresana 2008; Spoel & Dong 2008). While many other primary and secondary signals participate in defence signalling such as calcium, these three hormones form the core of a coordinated defence and their relative contributions vary with the nature of the assault. Generally, JA and ET mediate defence responses to necrotrophic pathogens and chewing insects, while SA mediates responses to biotrophic pathogens and piercing and sucking insects (Felton *et al.* 1999a,b; Koornneef & Pieterse 2008). There are, however, many exceptions to this generalization and JA/ET and SA pathways can interact antagonistically or synergistically depending on the nature of the attack (De Vos *et al.* 2005; Halim *et al.* 2006; Berger *et al.* 2007b).

While there was considerably more variation in the defence-related transcriptome to biotic damage than genes involved in photosynthesis, approximately two thirds of the genes selected to represent hormonal signalling were upregulated following biotic assault (Fig. 3). In this cluster the majority of the genes were ET and SA mediated signalling genes including ET response factor 1 (*ERF1*), Ethylene Insensitive 3 (*EIN3*), Pathogenesis-related protein 1 and 5 (*PR1*, *PR5*). Paradoxically, the majority of genes involved in regulating synthesis of ET and SA were in the downregulated gene cluster.

In contrast to ET and SA, JA biosynthesis genes, such as lipoxygenases (*LOX*), allene oxide cyclase (*AOC*) and 12-oxophytodienoate reductase (*OPR3*), were upregulated, indicating a positive feedback in the regulation of JA biosynthesis. JA biosynthetic genes are upregulated following treatment with JA and *AOC* expression is downregulated in JA-deficient *opr3* mutants (Stenzel *et al.* 2003). The upregulation of *LOX* and *AOC* genes suggests an increase in JA levels in response to pathogen and herbivore attack. Also, the transcript level of *SGT1b* which is a pleitrophic effector functioning in JA, auxin and pathogen signalling increases, and *SGT1A* suppressor transcript levels decrease in response to biotic stress (Lorenzo & Solano 2005). Even though the number of genes differentially regulated by pathogen infections were higher compared with herbivore and wounding effects, *LOX* genes were most strongly upregulated in response to herbivores.

JA, often interacting with ET, plays a key role orchestrating the defence response against arthropods, and is strongly

associated with downregulation of photosynthesis genes (Weidhase *et al.* 1987; Giri *et al.* 2006; Wasternack 2007; Howe & Jander 2008). JA originates from α-linolenic acid released from chloroplast membranes and enzymes early in the octedecanoid pathway producing JA, *LOX* and *AOS*, are localized in the chloroplast (Wasternack 2007). The co-location of photosynthesis and octedecanoid metabolism suggests a functional relationship between these processes. Even before the downregulation of transcription, Roloff, Parthier & Wasternack (1994) observed that barley exposed to MeJA no longer translated mRNAs for Rubisco small subunit, chlorophyll a/b binding protein and proteins in photosystem II. Also, it is suggested that phytochrome regulated selective desensitization to JA plays a role in the allocation of resources between plant growth and anti-herbivore defence (Ballare 2009). The decrease in photosynthetic capacity of *N. attenuata* by silencing of ribulose-1,5-bisphosphate carboxylase/oxygenase activase reduced plant's ability to elicit some of the herbivore resistance traits due to limitations in the production of jasmonate-isoleucine (Mitra & Baldwin 2008). In crowded habitats, phytochrome inactivation by far-red radiation increased the susceptibility of *Arabidopsis* to *Spodoptera frugiperda* caterpillar (Moreno *et al.* 2009). These examples point to a possible connection between JA induced defence response and photosynthesis.

The gaseous hormone ET is a potent regulator of plant growth and development, and often acts synergistically with JA to trigger the expression of defence proteins and other aspects of defence-related secondary metabolism. The maximum production of JA often requires ET (Wang, Li & Ecker 2002; Li & Guo 2007; Howe & Jander 2008). Biotic assault strongly affected the expression of genes related to ET, but appeared to affect genes involved in ET biosynthesis and ET responsive genes differently (Fig. 3). Biosynthesis of ET involves ACC synthase (*ACS*) and ACC oxidase (*ACO*; Kende 1993; Wang *et al.* 2002; von Dahl *et al.* 2007), both of which are encoded by multi-gene families (Argueso, Hansen & Kieber 2007; von Dahl *et al.* 2007). The perception of ET is regulated by a family of five receptor proteins in *Arabidopsis* (ETR1, ERS1, ETR2, EIN4 and ERS2; Wang *et al.* 2002). Biotic assault downregulated many of the genes coding for ACC synthase (*ACS*), while simultaneously increasing the expression of many ET responsive genes (Fig. 3). In the absence of ET, the receptors suppress ET responses by activating CTR1 (constitutive triple response 1) suppressor protein. The transcript level of *CTR1* was downregulated by pathogen and herbivore attack. The downstream signalling genes *EIN3*, *EIN4*, *ERF1* and *ERF4* were clustered in the upregulated gene cluster. The differential expression of ET synthesis and ET responsive genes may reflect the kinetics of the response to wounding, as wound-induced ET biosynthesis negatively regulates the activity of *ACS* and *ACO* (ACC oxidase; Wang *et al.* 2002; von Dahl *et al.* 2007).

Plant resistance to biotrophic pathogens is mediated by SA, which, following infection, induces the production of 'pathogen-related' proteins (PR; Loake & Grant 2007). Accumulation of SA following infections requires *de novo*

synthesis via the isochorismate pathway in *Arabidopsis* or the phenylpropanoid pathway in potato (Halim *et al.* 2006). Even though our focus was primarily on SA responsive genes rather than biosynthetic genes because of species-specific differences in the choice of these pathways (isochorismate pathway versus phenylpropanoid pathway), the phenylalanine-ammonia lyase 1 (*PAL1*; in flavonoid pathway; Supporting Information Fig. S1) transcript levels increased in response to pathogen and herbivore attacks. However, isochorismate synthase 1 (*ICS1*) transcripts were upregulated only in response to attack by pathogens, the sucking insect *M. persicae* and *P. brassicae* oviposition, but not by other herbivores. Similar to ET and JA, genes responsive to SA largely were induced by biotic assault (Fig. 3). Several *PR* genes and the genes coding for NPR1 protein (non expressor of *PR* genes) and *EDS1* and *EDS5* (enhanced disease susceptibility 1 and 5) were upregulated by biotic assault. NPR1 mediates protein-protein interactions and appears to play a pivotal role in signal transduction leading to systemic acquired resistance and induced acquired resistance (Bostock 2005). Additionally, NPR1 may mediate antagonism between JA and SA signalling pathways (Bostock 2005; Zhao & Qi 2008). While this antagonism is not generally apparent in this data set, possibly because data were averaged across different time points, individual examples are evident. For example, infection of *Arabidopsis* by the pathogen *Agrobacterium tumefaciens* strongly induced SA biosynthesis and signalling genes (*ICS1*, *EDS1*, *PAD4*, *NPR1 and PR-1*) while suppressing genes coding for JA biosynthesis and signalling (*AOS*, *CYP74A*; allene oxide synthase; *AOC*, allene oxide cyclase, *COI1*, and *JMT*; Fig. 3).

Among different types of biotic attack, pathogen treatments clustered together and plants responded to pathogen infection more vigorously by differentially regulating the transcription of a high number of defence-related genes. Plant defence response to the penetrating insect *Bemisia tabaci* and deposition of egg batches by *Pieris brassicae* changed the transcript levels of defence genes similarly to pathogen infection (Fig. 3). Even though *Myzus persicae* treatments were clustered separately, SA biosynthesis and signalling-related genes, such as *ICS1*, *EDS1*, *PR-1*, were upregulated and ET biosynthesis genes were downregulated in a similar mode to pathogen infection at the early time point (8 h post infection).

The strong and uniform upregulation of genes coding for proteins involved in biosynthesis of JA as well as those involved in ET or SA signalling suggests that attack by widely divergent biotic agents induced a defence response while at the same time causing a global decrease in photosynthesis genes transcripts (Figs 1 and 3).

Biotic attack and genes coding for the synthesis or detoxification of ROS

ROS are produced as byproducts of metabolism and as part of defence responses against biotic stresses (Inze & Van Montagu, 2002). They are, however, hazardous to DNA and proteins and are detoxified by enzymatic and non-enzymatic scavengers. The expression of genes coding for these detoxification enzymes are differentially regulated by bacterial, fungal, viral infections and herbivore infestations (Fig. 4). The accumulation of ascorbate, glutathione and cysteine, and the activity of dehydroascorbate reductase, which are important defence components against oxidative stress were shown to be induced by JA (Sasaki-Sekimoto *et al.* 2005). Following biotic assault, genes coding for ROS scavenging enzymes clustered in two groups; 30 genes were upregulated and 35 genes were downregulated (Fig. 4).

Under ideal conditions, ROS are produced and detoxified in an orderly fashion and are used as signalling agents to initiate developmental and regulatory processes (Foyer & Noctor 2005; Carol & Dolan 2006; Wang & Song 2008). Exposure to biotic stress induces ROS production, which can cause irreversible oxidative damage to cells. Varying intensity and duration of oxidative burst has been observed in response to pathogen invasion. Plasmalemma-bound NADPH oxidases are important source of ROS following pathogen infection (Torres & Dangl 2005), and especially H_2O_2 that can serve as a diffusible signalling molecule in response to pathogen infection (Foyer & Harbinson 1997). Typically, for compatible interactions an early, mild oxidative burst is observed, on the other hand, long-lasting bi- or triphasic ROS production occurs early in incompatible and non-host plant-pathogen interactions (Baker & Orlandi 1995; Huckelhoven & Kogel 2003). To increase the ROS concentrations within the cytoplasm, the levels and activity of the ROS detoxifying enzymes such as CAT and APX are suppressed (Clark *et al.* 2000). Upon biotic attack both the amount of ROS and their localization are important aspects of the defence response.

Genes representing members of the same family of ROS scavenging enzyme coding genes were clustered in up- and downregulated groups (Fig. 4). There was no distinction according to the reaction they catalysed but ~55% of the downregulated genes carried a putative-chloroplast targeting sequence (cTP) determined by ChloroP subcellular localization prediction program (Emanuelsson *et al.* 1999) even though only ~17% of the upregulated genes were potentially targeted to chloroplast. Increases in ROS concentration act as an antimicrobial agent, and to enhance ROS production in response to biotic attack, plant cells may downregulate chloroplast-targeted ROS scavenging enzymes as this organelle is a main source of ROS (Apel & Hirt 2004). Faced with the downregulation of photosynthetic genes, and a moderate reduction in the rate of photosynthesis and subsequent capacity to generate ROS, the reduction in chloroplast-targeted ROS savaging genes may ensure sufficient concentrations of ROS to mount an effective defence. However, the toxic effect of ROS to a pathogen depends on the pathogen sensitivity to the ROS concentration (Levine *et al.* 1994; Shetty *et al.* 2008).

In addition to acting as a direct defence against pathogens, ROS are involved in induction of defence signalling pathways such as triggering phytoalexin accumulation, increasing cytosolic calcium concentration and inducing

defence-related gene expression (Price *et al.* 1994, 1996; Mou, Fan & Dong 2003; Mittler *et al.* 2004; Tada *et al.* 2008). ROS can also function as retrograde signals to communicate stressful conditions to the nucleus (Mayfield & Taylor 1987; Lee *et al.* 2007; Woodson & Chory 2008; Foyer & Noctor 2009). Variation in the response of genes involved in ROS production and detoxification following biotic assault may stem from the multiple roles of this family of compounds.

Cellular localization and potential cis-regulatory elements

The global downregulation of genes involved photosynthesis across many biotic agents suggests that this response was coordinated and adaptive. To look for putative mechanisms supporting this coordination, the promoter regions of these genes were examined for common regulatory elements. For this purpose, genes involved in photosynthesis light reactions (Fig. 1), carbon fixation (Fig. 2), ROS scavenging network (Fig. 4), photorespiration (Supporting Information Fig. S2) and starch and sucrose metabolism (Supporting Information Fig. S3) were grouped as 'photosynthesis-related pathways' and classified as up- or downregulated.

A search of the ChloroP data base revealed that in the photosynthesis-related pathways, 85 (<61%) out of 139 downregulated genes had chloroplast transit peptides (cTP) but only 16 (<17%) of 92 upregulated genes had cTP, indicating their gene products were targeted to the chloroplast. This chloroplast transit peptide sequence that is commonly present in the majority of the downregulated genes may be a source of transcriptional regulation by micro-RNA (miRNA; Jones 2002). However, a search for potential miRNA binding sequences in targeted genes (http://asrp.cgrb.oregonstate.edu/db/) was negative. Alternatively, transcription may have been coordinated by *cis*-regulatory elements in the promoter region of responsive genes.

Computational analysis as in Hudson & Quail (2003) of base pairs upstream of transcribed regions for genes in the photosynthesis-related pathways that were significantly affected by biotic attack revealed several common regulatory elements (Supporting Information Table S1). Of the downregulated, chloroplast-targeted genes, G-box, I-box and T-box regulatory elements were over represented relative to the entire genome by 42, 75 and 87%, respectively, and ≥86% of all downregulated genes in photosynthesis related pathways, regardless of subcellular localization, had over representation of SORLIP1 in their promoters. SORLIP1 was found to be present in phyA-induced promoters (Hudson & Quail, 2003), however there is no previous information regarding its involvement in gene expression regulation in response to biotic stress. The G box (Giuliano *et al.* 1988; Menkens, Schindler & Cashmore 1995; Chattopadhyay *et al.* 1998; Martinez-Garcia, Huq & Quail 2000), I box (Donald & Cashmore 1990) and T box motifs (Chan, Guo & Shih 2001) appear to be light regulation elements and it may therefore be coincidental that genes responsive to biotic attack contain similar regulatory elements, as many photosynthetic genes are light regulated (Tyagi & Gaur 2003). However, a substantial number of ROS scavenging and starch and sucrose mechanism genes that responded to biotic attack also had these motifs enriched in their promoter sequences. While the mechanism is not resolved, the regulatory elements among genes in the photosynthesis-related pathways have sufficient similarity to justify the hypothesis that they are regulated at the transcriptional level following biotic attack by common regulatory elements.

CONCLUSIONS

The defence responses to biotic assault are extremely variable, in part because of the unique co-evolutionary relationships between specific plant species and the specific agents of damage. Faced with this diversity, it is remarkable that biotic attack triggers a uniform and apparently regulated reduction in transcription of nuclear genes coding for the major components of photosynthesis, regardless of the plant host or damage vector. A uniform downregulation of photosynthetic gene transcription when faced with stress is not unprecedented, as environmental stresses, including drought, salinity and low temperatures, elicit a similar response (Saibo, Lourenco & Oliveira 2009).

The observed downregulation of photosynthesis genes by biotic attack raises important questions: is this response adaptive and how is it regulated? It has been hypothesized that a reduced investment in photosynthetic proteins following herbivory or pathogen infection is necessary to support the induction of a defence response. A high proportion of leaf N is invested in photosynthetic proteins, primarily Rubisco (Evans 1989); faced with N limitations, supporting the induction of defensive compounds may necessitate a lower N investment or even withdrawing N from Rubisco (Stitt & Schulze 1994; Paul & Foyer 2001) and a corresponding rebalancing of protein levels, beginning with the regulation of transcription. Common regulatory elements in the promoter region of photosynthetic genes and genes involved in sugar metabolism and ROS detoxification suggest that the transcriptional response to biotic stress is highly coordinated. Because of the long turnover time of many photosynthetic enzymes, the downregulation of transcription of photosynthetic genes may permit reallocation of nitrogen to the defence response while causing only moderate losses in the rate of carbon assimilation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cluster analysis of the expression of genes involved in the flavonoid biosynthesis following biotic stress. For detailed description of colour coding please see the legend of Fig. 1.

Figure S2. Cluster analysis of the expression of genes involved in the photorespiration following biotic stress. For detailed description of colour coding please see the legend of Fig. 1.

Figure S3. Cluster analysis of the expression of genes involved in the starch and sucrose metabolism following biotic stress. For detailed description of colour coding please see the legend of Fig. 1.

Figure S4. Hierarchical cluster analysis of the expression of genes involved in the synthesis of photosynthetic pigments and the 'light reactions' of photosynthesis following damage by different biotic agents. The elimination of phytohormone treatments (MeJA, SA and ACC) of *Cicer arietinum* did not cause a major change in the cluster pattern. For detailed description of colour coding please see the legend of Fig. 1.

Table S1. The percentage of the genes that were significantly over-represented *cis*-elements. The number of occurrences of each motif was compared with an expected value derived from the frequency of that element in the sequence of the promoters for the whole genome via one-degree-of-freedom chi squared test ($P < 0.001$). The photosynthesis dark reaction, carbon fixation, photorespiration, ROS scavenging genes and starch and sucrose metabolism genes were pooled and grouped as chloroplast localization and transcript down- or upregulation. Defence-related genes were clustered according to increase or decrease in the transcript level, subcellular localization was not included.

Table S2. The list of *Arabidopsis* genes used in hierarchical cluster analysis. The genes were grouped according to

signalling and biosynthesis pathways. The first vertical column contains the AGI gene codes together with TAIR nomenclature. The biotic agents and their references corresponding to Table 1 in parentheses are listed on the first row. The transcript fold changes were given in $\log_2$. The grey cells point the missing values due to lack of significant homology to *Arabidopsis* or the genes that were not on the array because of incomplete genome sequence.

Table S3. List of clones from soybean, chickpea, poplar, spruce, potato and *N. attenuata* plant arrays used in this study. Their corresponding *Arabidopsis* top hits were determined by sequence homology search, a stringent cut off was applied (*e*-value ≤ 1e-10). Data provided include: clone ID, AGI gene codes and *e*-value.

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