Journal of Experimental Botany, Vol. 57, No. 11, pp. 2847–2865, 2006
doi:10.1093/jxb/erl043 Advance Access publication 25 July, 2006
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany

RESEARCH PAPER

Transcriptional analysis of calcium-dependent and calcium-independent signalling pathways induced by oligogalacturonides

Roberto Moscatiello[1], Paola Mariani[1], Dale Sanders[2] and Frans J. M. Maathuis[2,]*

[1] *Department of Biology, University of Padua, Via Ugo Bassi 58b, I-35131 Padova, Italy*

[2] *Department of Biology (Area 9), University of York, PO Box 373, York YO10 5YW, UK*

Received 5 March 2006; Accepted 9 May 2006

Abstract

α-1,4-linked oligogalacturonides (OGs) are pectic fragments of plant cell walls that are able to induce defence and developmental responses. To understand plant responses to OGs at the transcriptional level, changes in gene expression were examined using oligonucleotide-based microarrays that cover almost the entire *Arabidopsis* transcriptome. In suspension-cultured *Arabidopsis thaliana* (L.) Columbia hypocotyl cells, approximately 4% of the total transcriptome exhibited significant change in abundance in response to treatment with OGs for 2 h. Steady-state changes in the abundance of transcripts encoding stress- and disease-related proteins, signalling components, and transcription factors were particularly noteworthy. As in other plant cell types, OGs elicit a rapid, but transient, elevation in cytosolic free Ca^{2+}. The Ca^{2+} transient can be abolished by the protein kinase inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) and by the Ca^{2+} channel inhibitor La^{3+}, thereby facilitating a distinction between Ca^{2+}-dependent and -independent transcriptional responses. Among the 244 transcripts that were up-regulated by OGs, the response of 93 (38%) was selectively sensitive to abolition of the Ca^{2+} transient. These OG-up-regulated, Ca^{2+}-dependent transcripts included two noteworthy classes, the first comprising genes involved in cell wall modification following pathogen attack, and the second consisting of genes involved in the biosynthesis of jasmonate and C6 volatile compounds. These results support the notion of an important role for cytosolic Ca^{2+} signalling in jasmonate biosynthesis following OG perception. Promoter analysis of OG-induced, inhibitor-sensitive and -insensitive genes identified several putative *cis*-elements that might be involved specifically in Ca^{2+}-dependent transcriptional regulation.

Key words: *Arabidopsis*, calcium, oligogalacturonides, signalling, transcriptomics.

Introduction

During plant–pathogen interactions oligosaccharide fragments, generated by the depolymerization of the parietal polysaccharides (Côté and Hahn, 1994; John *et al.*, 1997), can elicit defence responses such as cell wall fortification through oxidative cross-linking of cell wall polymers, the generation and accumulation of reactive oxygen species (ROS) (Lamb and Dixon, 1997; Cessna and Low, 2001), the production of antimicrobial secondary metabolites such as phytoalexins, and the synthesis of pathogen-related proteins. Among the pectic breakdown fragments, α-1,4-linked oligogalacturonides (OGs) have been shown to be especially potent defence response elicitors (Côté and Hahn, 1994; Ridley *et al.*, 2001). At concentrations lower than those pertaining in defence response signalling, OGs also have profound effects on plant development through interference with auxin-induced cell elongation, flower development, and root organogenesis (Côté and Hahn, 1994; Bellincampi *et al.*, 1996). In addition, OGs have been shown to stimulate stomatal and pericycle cell differentiation (Altamura *et al.*, 1998). Apart from pathogen attack, the action of herbivores may also lead to the production of OGs, either through mechanical tissue damage or through OG release from cell wall pectin by the introduction of polygalacturonase-containing saliva into the wounding site (Miles, 1999).

* To whom correspondence should be addressed. E-mail: fjm3@york.ac.uk

© 2006 The Author(s).
This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/2.0/uk/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Not all OGs are equally capable of generating cellular responses. Oligomers with a degree of polymerization (DP) between 10 and 15 have been shown to be the most potent inducers of defence responses (Darvill *et al*., 1992; Van Cutsem and Messiaen, 1994), a property that has generally been attributed to their ability to form hetero-oligomeric complexes with Ca^{2+} (Liners *et al*., 1992). Nevertheless, smaller oligomers have also been shown to generate defence responses in plants, for example, in potato where OGs with a DP of 2–4 induce resistance against *Erwinia carotorova* (Wegener *et al*., 1996). OGs with a DP less than 8 can trigger plant cell death during tissue decay induced by *E. carotovora* in potato (Weber *et al*., 1996), ethylene production (Simpson *et al*., 1998), induction of genes involved in metabolism and/or synthesis of jasmonic acid (Norman *et al*., 1999), and the accumulation of protease inhibitors (Moloshok *et al*., 1992).

Currently, very little is understood regarding the intermediate processes that couple the perception of OGs to cellular responses such as the induction of defence mechanisms against pathogens. Coupling of primary stimuli, such as OGs, to cellular targets often involves intracellular messengers of which Ca^{2+} is considered one of the most versatile. The capacity of Ca^{2+} to couple a wide range of extracellular signals to meaningful responses relies on generating Ca^{2+} transients with unique stimulus-specific kinetics (calcium signatures: Sanders *et al*., 2002). Previous work from this laboratory (Navazio *et al*., 2002) and from others (Van Cutsem and Messiaen, 1994; Chandra *et al*., 1997) has shown that Ca^{2+} signalling is involved in OG-induced signal transduction. In soybean cells exposure to OGs invoked a rapid and transient increase of cytosolic Ca^{2+} that appeared to precede both alkalinization of the extracellular medium (Felix *et al*., 1993) and H_2O_2 production (Chandra *et al*., 1997; Cessna and Low, 2001; Navazio *et al*., 2002). Both extra- and intracellular stores are likely to contribute to the Ca^{2+} signal. Pretreatment of cells with the Ca^{2+} channel blocker La^{3+} or the protein kinase inhibitor 4,5,6,7-tetrabromobenzotriazole (TBB) completely abolished the Ca^{2+} transient induced by OGs. The effect of TBB suggests an upstream phosphorylation event is essential for the generation of the Ca^{2+} signal. Exposure to TBB also abolished the emergence of extracellular H_2O_2.

Although our knowledge of the initial stages of OG-induced signalling is fragmentary, the pathways by which early events of the signal cascade lead to meaningful cellular responses is even less clear. A productive strategy to identify downstream targets of OG-based stimuli is to query the transcriptome for changes after exposure to OGs. Some studies into the regulation of transcripts in response to pathogens and wounding have been reported (Cheong *et al*., 2002). However, none of these has specifically focused on the role of OGs in such processes, nor were such studies carried out genome-wide. Thus, a microarray approach was used, offering comprehensive coverage of the *Arabidopsis* transcriptome to identify transcripts that are rapidly modulated after exposure of mesophyll suspension cultures to OGs. Moreover, selective abolition of the OG-induced Ca^{2+} transient by TBB and La^{3+} made it possible to distinguish between Ca^{2+}-dependent and Ca^{2+}-independent pathways that exert control over gene transcription downstream of the OG stimulus.

Materials and methods

Plant material

Arabidopsis thaliana (L.) Columbia hypocotyl-derived cell-suspension cultures were maintained at 24 °C on a rotary shaker at 80 rpm under an 18 h photoperiod at 80–100 μmol m^{-2} s^{-1} light intensity. Cells were subcultured every week with a 10% (v/v) inoculum in Murashige and Skoog (MS) liquid medium supplemented with 3% (w/v) sucrose, 0.5 μg ml^{-1} 2,4-dichlorophenoxyacetic acid, and 0.25 μg ml^{-1} 6-benzylaminopurine.

Production and isolation of oligogalacturonic acids

OGs were obtained according to the method described by Simpson *et al*. (1998). Briefly, 5 g of polygalacturonic acid (PGA) from orange pectin (Sigma), dissolved in 0.5% ammonium oxalate (5 mg ml^{-1}), were repeatedly dialysed against dH_2O and concentrated by vacuum evaporation, then de-esterified with cold alkali and freeze-dried. De-esterified PGA was dissolved in dH_2O (5 mg ml^{-1}) and heated to 37 °C, after which 0.03 mU mg^{-1} of *Aspergillus niger* polygalacturonase (Sigma) was added. After incubation for 1 h, digestion was stopped by heating to 100 °C. To isolate oligomers with a DP of 5–15 the digested PGA was selectively precipitated with ethanol and sodium acetate (Spiro *et al*., 1993). The precipitate was redissolved in dH_2O and separated by anion exchange chromatography on a QAE-Sephadex A-25 matrix (Pharmacia, 2.5 cm×60 cm) equilibrated with 50 mM ammonium formate pH 9. OGs were eluted using a linear gradient running from 250 mM to 1000 mM ammonium formate pH 9 at a flow rate of 2 ml min^{-1} (total volume 4.0 l). Fractions (8 ml) were assayed for their uronic acid content by the *m*-hydroxydiphenyl method (Van den Hoogen, 1998) using galacturonic acid as a standard. Individual peaks were pooled, diluted 1:1 with dH_2O, and freeze-dried several times to remove the ammonium formate. The size and purity of the OG oligomers eluted in each peak was determined by MALDI mass spectrometry: samples (125 pmol) were mixed on the target plate with 2,5-dihydroxybenzoic acid and allowed to dry. The target spots were then recrystallized in 0.5 ml ethanol (Harvey, 1993). Positive ion MALDI mass spectra were recorded with a PerSeptive Biosystems Voyager Elite time-of-flight mass spectrometer (nitrogen laser, 337 nm) operating in the reflectron mode. The delayed-extraction ion source was operated with a 75 ns delay, the extraction voltage was 20 kV and the grid voltage was set at 65%.

Finally, individual peaks in the range of DP 10–15 were pooled and de-salted on a 500 ml column of Sephadex G-25 matrix (Pharmacia), equilibrated and eluted with dH_2O.

Reconstitution of aequorin

Aequorin reconstitution was done as previously described (Navazio *et al*., 2002) for soybean cells and entailed incubation of 10-d-old transgenic *Arabidopsis* cells with 5 μM coelenterazine added to the cell culture medium, overnight in darkness. Cells were then washed three times with 10 vols of fresh hormone-free culture medium and used after 30 min.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Aequorin luminescence measurement and Ca^{2+} calibration

Suspension-cultured cells were transferred to a purpose-built chamber placed in close proximity to a low-noise photomultiplier, with a built-in amplifier discriminator (Navazio *et al.*, 2002). All measurements were performed at room temperature in a final volume of 50 μl containing approximately 3 mg (fresh weight) of reconstituted cell-suspension culture. Treatment with OGs was carried out by injecting an equal volume of 2-fold-concentrated stock solutions (dissolved in the basal cell-culture medium) through the luminometer port into the cell-suspension culture, using a light-tight syringe. All experiments were terminated by discharging the remaining aequorin pool with 0.33 M $CaCl_2$ in 10% (v/v) ethanol. The output of the discriminator was captured by a Thorn-EMI photoncounting board and stored in an IBM-compatible computer for further analyses. The aequorin luminescence data were calibrated off-line into $[Ca^{2+}]$ values, using a computer algorithm based on the Ca^{2+} response curve of aequorin, as described by Brini *et al*. (1995).

Microarray hybridization and analysis

10-d-old *Arabidopsis* cells were treated with either (i) oligogalacturonides (200 μg ml^{-1}, 2 h), (ii) oligogalacturonides plus TBB (50 μM, 10 min prior to addtion of OGs), (iii) TBB only, or (iv) oligogalacturonides plus La^{3+} (3 mM, 10 min prior to addtion of OGs), and total RNA was extracted using RNeasy columns (Qiagen, UK) from treated and control cells. Total RNA of three independent growth cultures was pooled for each treatment and this procedure was repeated three times, i.e. a total of 12 (three for each treatment) microarrays was hybridized. For each hybridization, approximately 100 μg of total RNA was primed with Random 15-primer (0.5 μg $μl^{-1}$; Operon) and reverse-transcribed with Superscript II (Invitrogen). Fluorescent labelling was achieved by replacing dCTP in the dNTP mix (Sigma) with Cy3-dCTP and Cy5-dCTP (Amersham, UK). Labelled cDNA was cleaned on a QIAquick spin column (Qiagen, UK). Arabidopsis Oligonucleotide Microarrays (http//ag.arizona.edu/microarray), using the *Arabidopsis* Qiagen-Operon Genome Oligo Set that represents around 26 000 coding sequences, were used for hybridization. Array cross-linking, hybridization, and post-hybridization washes were carried out as described by the manufacturer (http//ag.arizona.edu/microarray).

Arrays were scanned using an Axon (Axon Instruments, Braintree, UK) scanner and initial array analysis was carried out with ScanAlyze2 software (http://rana.lbl.gov/EisenSoftware.htm). Background subtraction, global normalization of fluorescence signals and lowess signal correction were performed using SNOMAD software available at http://pevsnerlab.kennedykrieger.org/snomadinput.html. Signals were designated as 'present' when a signal background ratio of >1.5 was found in at least one channel. Global mean normalization was carried out across microarray surfaces and local mean normalization across element signal intensity. After normalization and log_2-transformation, signal averages and the standard deviations for signal ratios of the three replica experiments were calculated. Transcripts were included for analysis and annotated as significantly regulated when the following criteria were met: (i) a 'present' signal on all three replicas, (ii) a signal ratio average of four ($log_2 2$) or more between treated and control transcripts, and (iii) a ratio between the ratio average and the standard deviation greater than 1+0.5×standard deviation. The fold-change cut off criterion (four) was based on the distribution of fold-changes observed in control data such that the number of treatment-induced false positives is 5% or lower.

RT-PCR analysis

A proportion of the RNA obtained for microarray studies was used for RT-PCR. After DNase I treatment (Ambion Ltd., UK), 5 μg of total RNA was primed with Random Decamers (Ambion), reverse-transcribed with PowerScript Reverse Transcriptase (Clontech, USA) and diluted 1:5. Relative-quantitative RT-PCR was performed with 5 μl diluted first-strand cDNA, using 18S rRNA as an internal standard (QuantumRNA Universal 18S Internal Standards Kit, Ambion Ltd., UK). The 18S Primers:Competimers ratio was established as 1:9. The primers used to obtain gene amplicons (~200 bp) were: *AOS* (At5g42650) 5′-ACGCTCCGGGTTTGATCACTAAATG-3′, 5′-CCCAATTTATCGGCTTCAACGAGAA-3′; *LOX2* (At1g72520) 5′-GAGTCGTGCTTCACTGCTGGTCAAT-3′, 5′-ATAAGAGAC-CGTCGTTGGCGTATGG-3′; *ACS* (At4g11280) 5′-TGGTGGCT-TTTGCAACAGAGAAGAA-3′, 5′-ACGCATCAAATCTCCACA-AAGCTGA-3′; *ACO* (At1g06650) 5′-AGTTCCACGCATCTTT-CATCATCCA-3′, 5′-TGATCACCTGGAAGAAACCCCACTT-3′; *MAPK3* (At3g45640) 5′-ATGCGAAAAGATACATCCGGCA-ACT-3′, 5′-TCATCATTCGGGTCGTGCAATTTAG-3′; *Disease Resistance* (At5g41750) 5′-TCGGTAGGTAAGGGGGCTTTTGA-AG-3′, 5′-AATTTTGACGAGATGTTCCGGGTTG-3′; *MAPKKK5* (At5g66850) 5′-CTGATTTCGGCATGGCTAAACACCT-3′, 5′-CAAGGAGGCTTCCCAGTGAACATCT-3′; *Multi drug resistance* (At4g25960) 5′-AAGGCTGGTGAGATTGCAGAAGAGG-3′, 5′-ACGAGCAAGGCCCAAGATAGAAACA-3′. The thermocycler was programmed with the following parameters: 20 s at 94 °C, 30 s at 68 °C, and Advantage 2 Polymerase Mix (Clontech) was used as *Taq* Polymerase. Densitometric analysis of ethidium bromide-stained agarose gels (0.5 μg ml^{-1}) was performed using Quantity One software (Bio-Rad).

Promoter cis-element analysis

For the detection of putative regulatory *cis* elements in the promoter regions of coregulated transcripts, 5′ upstream sequences of up to 800 bp (avoiding overlap with preceding coding sequences) were uploaded at the 'Regulatory Sequences Analysis Tools' service at 'http://rsat.ulb.ac.be/rsat/'. Sequences were queried using algorithms (van Helden *et al*., 1998) to detect over-represented strings of 4–8 nucleotides searching both DNA strands. The *P*-value represents the probability for the number of detected motifs to occur relative to the expected number of occurrences based on the motif distribution in the background dataset which contains all *Arabidopsis* 5′ upstream sequences. A significance cut-off of $P < 10^{-5}$ was used in all analyses. Identified putative promoter elements were used to query *Arabidopsis cis*-element databases such as PlantCARE (http://oberon.fvms.ugent.be:8080/PlantCARE/), Agris (http://arabidopsis.med.ohio-state.edu/AtcisDB/), and Atprobe (http://exon.cshl.org/cgi-bin/atprobe/atprobe.pl) for known functions.

Results

Arabidopsis cells show a large OG-induced Ca^{2+} transient

It has previously been shown that a cytosolic Ca^{2+} transient is rapidly generated in soybean cells after exposure to OGs (Navazio *et al*., 2002). Figure 1 shows that in *Arabidopsis* mesophyll suspension culture cells a similar Ca^{2+} signal occurs after the addition of 10 μg ml^{-1} OGs (DP 10–15) to the medium. Furthermore, as was observed for soybean cells, pretreatment with 50 μM TBB completely abolished the Ca^{2+} transient. This observation suggests that, in *Arabidopsis*, too, protein kinase-dependent phosphorylation might be involved in the early stages of OG signalling.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

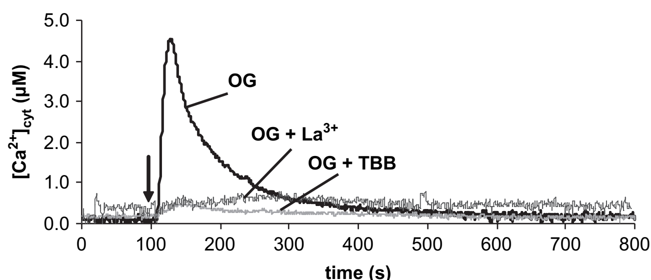

Fig. 1. Effect of the inhibitors TBB and La^{3+} on the transient Ca^{2+} increase induced by OGs. Ten min prior to the addition of OGs (arrow, 10 μg ml^{-1} OGs), cytosolic aequorin-expressing cells were treated with 50 μM TBB or 3 mM La^{3+} which both led to a virtually complete abolition of the OG-induced changes in $[Ca^{2+}]_{cyt}$. Control cells were treated with 0.5% (v/v) DMSO 10 min prior to addition of OGs.

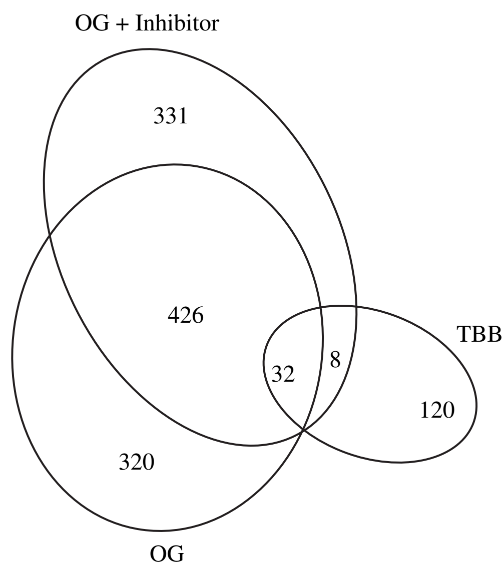

Fig. 2. Venn diagram of genes regulated by 'OGs', 'OGs plus inhibitors', and by 'TBB alone'. Numbers are based on oligonucleotide probes and relate to more than 4-fold changes of signal after 2 h treatments: a total of 746 transcripts was regulated in response to 'OGs'; 757 in response to 'OGs plus inhibitor'; and 160 in response to 'TBB'. Transcripts found in the area corresponding to 'OG only' and the intersection between 'OG' and 'OG plus inhibitor', were used to construct Table 2. Listings for the other transcript groups can be found in the supplementary data at JXB online.

Functional dissection of transcriptional responses to OGs

To study gene expression in response to treatment with OGs, oligonucleotide-based microarrays were used that fully cover the *Arabidopsis* transcriptome. The arrays were probed with cDNAs derived from four different treatments to enable comparison on individual microarrays between (i) 'control' and 'OG-treated' cells, (ii) 'control' and 'OG plus TBB-treated' cells, (iii) 'control' and 'TBB-treated' cells, and (iv) 'control' and 'OG plus La^{3+}-treated, cells. The first condition (i) allows determination of how the presence of OGs impacts on the entire transcriptome. In addition, it is possible to distinguish within the results those changes in transcript level that are due to Ca^{2+}-dependent and Ca^{2+}-independent pathways by comparing the outcome of condition (i) with conditions where the initial Ca^{2+} transient is inhibited. TBB has been shown previously to remove the Ca^{2+} transient totally and thus condition (ii) should provide insight into OG-induced Ca^{2+}-dependent and Ca^{2+}-independent processes. Non-specific effects of the protein kinase inhibitor were accounted for and subsequently eliminated from analysis by including condition (iii). However, it can not be ruled out that TBB may have specific effects during OG-induced transcriptional regulation that do not involve the inhibition of the early Ca^{2+} signal. A second treatment (condition iv), which has also been shown to eliminate the early Ca^{2+} signal (Navazio *et al.*, 2002), was therefore included. Only those transcripts that showed sensitivity to both condition (ii) and (iv) were considered for further analysis. For each treatment, RNA was isolated after 2 h and all data represent three independent experiments for each treatment.

Figure 2 shows a Venn diagram representing the total number of transcripts, 1237, that was changed by the treatments. For clarity, conditions (ii) and (iv), which both act to eliminate the Ca^{2+} transient and largely overlapped, are represented as one 'OG plus inhibitor' dataset. To ensure that analysis was restricted only to significantly changed transcripts, a robust threshold criterion of 4-fold (i.e. 2 on a log_2 base) was applied to qualify for description as a change in transcript abundance. Overlapping regions only contain transcripts that were significantly affected in the same direction (i.e. up or down). Of the 1237 transcripts that exceeded this threshold, 320 transcripts (26%) responded specifically to 'OG' treatment, 424 (34%) were responsive to both 'OG' and 'OG plus inhibitor' treatments, whereas 330 transcripts responded solely to the 'OG plus inhibitor' treatment. Condition (iii), 'TBB' treatment, showed that 158 transcripts responded to TBB in the absence of OGs of which 38 overlapped with the 'OG plus inhibitor' data. These false positives were removed from the 'OG plus inhibitor' dataset in all subsequent analyses. Conditions (ii) and (iv) were compared to identify transcripts that responded to OGs (condition i) but not to 'OG plus TBB' (condition ii) and 'OG plus La^{3+}' (condition iv) and only transcripts that were sensitive to both TBB and La^{3+} were annotated as inhibitor-sensitive and included in the subsequent analyses. Accession numbers and numerical data for all transcripts can be found in the supplementary data files at JXB online.

Independent confirmation of array data using RT-PCR (Table 1) for a number of key transcripts (see below) shows an overall agreement between the two methods although transcript changes were generally found to be less pronounced when assessed by RT-PCR.

The OG-responsive, inhibitor-sensitive fraction and the OG-responsive, inhibitor-insensitive fraction totalled 746

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 1. *Comparison of results obtained with microarrays and RT-PCR*

OG-induced transcript regulation was assessed for a number of key genes using either a microarray approach or RT-PCR. Both types of analysis were carried out in triplicate.

Gene name	ID	RT-PCR	SD	Microarray	SD
AOS	At5g42650	3.03	0.80	4.23	2.07
*LOX*2	At1g72520	2.57	0.64	3.51	1.58
*ACS*6	At4g11280	2.52	1.13	3.82	2.59
ACO	At1g06650	1.28	0.09	3.09	0.87
MAPK 3	At3g45640	2.03	0.68	8.75	2.70
Disease resistance protein (TIR-NBS-LRR class)	At5g41750	1.67	0.07	7.98	2.03
MAPKKK 5	At5g66850	0.37	0.06	−4.97	0.63
Multidrug resistance P-glycoprotein	At4g25960	0.45	0.16	−3.16	1.48

transcripts. Of these, a total of 413 could be classified into specific functional categories. The predominant categories are listed in Table 2 and represent transcripts encoding proteins involved in signal transduction (e.g. protein kinases, phosphatases, calcium binding proteins, G-proteins, ethylene and jasmonate signalling pathways), gene transcription (e.g. ethylene binding factors, bHLH, bZIP, MADS, Myb, Myc, NAC/NAM, Zinc finger, Heat shock, WRKY), stress and disease (e.g. cytochrome P450, disease resistance proteins, chitinases), and cell wall modification (e.g. glycosyl hydrolases, polygalacturonases).

It is noteworthy that treatment with OGs increased the transcript levels of many typical pathogen-induced genes, including plant disease resistance genes (R genes) involved in the detection of pathogens. Many of the up-regulated R genes encode proteins containing nucleotide-binding sites (NBS), toll/interleukin receptor (TIR) domains, and leucine-rich repeat (LRR) domains. Among the up-regulated TIR-NBS and TIR-NBS-LRR type disease resistance transcripts are RPP1-WsA-C and RPP1-WsB-like gene products, but also many that have not been characterized.

Post-translational regulation through phosphorylation during the OG response appears to be prevalent judging by the many phosphatase and kinase encoding transcripts that are affected by OG treatment. These included mitogen-activated protein (MAP) kinases, plant receptor kinases (PRKs), or receptor-like kinases (RLKs), and serine/threonine kinases. MAP kinase cascades have been shown to be involved in pathogen responses, for example, in tobacco cells after OG treatment (Lebrun-Garcia *et al.*, 1998), whereas various transcription factors can also be MAPK targets (Tena *et al.*, 2001). The present data show particularly that *MAPK3* and a putative *MAPK*, which is very similar to *MAPK4* that was previously reported to be induced by wounding (Cheong *et al.*, 2002), were significantly up-regulated after OG treatment. Activation of MAPK3 by fungal elicitors has been observed in alfalfa cells (Cardinale *et al.*, 2000). Further up-regulated *MAPK*s included *MAPKK9*, *MAPKKK19*, and *MAPKKK8* (*MEKK1*). Down-regulated *MAPK*s included *MAPK8*, a *MAPKKK5*, and a *MAPKK1*.

The transcript level of one protein phosphatase 2C (*PP2C*) isoform was greatly increased by OG treatment. PP2C is considered to regulate various signalling pathways (Rodriguez, 1998) and is a specific MAPK inactivator (Tena *et al.*, 2001). Up-regulation of *PP2C* could therefore lead to post-translational reduction in activity of selective MAPK cascades, in addition to possible effects of reduced transcript levels of MAPK-type kinases such as *MAPK8, MEK*, and *MAPKKK5*.

Exposure to OGs has been demonstrated to activate jasmonate (Doares *et al.*, 1995) and ethylene (Simpson *et al.*, 1998) signalling pathways and the interaction between these hormones determines the type of response to pathogen attack or wounding, including the expression of particular defence proteins such as PR1b, PR5 (osmotin) and PDF2.1 (Table 2; Xu *et al.*, 1994; Penninckx *et al.*, 1998). Transcripts involved in the biosynthesis of both hormones were also increased after exposure to OGs. These included genes encoding aminocyclopropane 1-carboxylic acid synthase (ACS-6) and aminocyclopropane 1-carboxylic acid oxidase (ACC oxidase), two enzymes required for ethylene biosynthesis, and for genes encoding lipoxygenase (LOX) and allene oxide synthase (AOS), two enzymes required for jasmonate biosynthesis.

Functional categories of genes show differential inhibitor sensitivity

For many transcripts that were significantly regulated by OG treatment, this regulation was prevented by the inclusion of a Ca^{2+} signal abolishing inhibitor. Although our discrimination criteria were based on one specific cut-off value, Table 2 shows that in most cases there is a large difference between the observed ratio value in the 'OG' condition and the 'OG plus inhibitor' condition, giving extra confidence to our classification of inhibitor-sensitive and inhibitor-insensitive transcripts.

The relative proportions of inhibitor-sensitive and -insensitive transcripts varied greatly across functional categories of OG-responsive genes, (Table 2). To identify which functional categories are likely to require an early Ca^{2+} signalling event for transcriptional regulation, data were therefore analysed for all 413 functionally annotated genes with respect to inhibitor sensitivity. In the complete dataset of 244 up-regulated genes, 97 were found to be inhibitor-sensitive and 147 are inhibitor-insensitive. Among down-regulated genes, 65 were inhibitor-sensitive out of a total of 169, whereas 104 genes were insensitive.

Data from each functional category were analysed to test whether the binomial distribution (see http://fonsg3.let.uva.nl/Service/Statistics/Binomial_proportions.html) of

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 2. *Genes with significantly altered transcript levels after exposure of* Arabidopsis *cells to oligogalacturonides for a period of 2 h*

Genes were listed on the basis of significance criteria (see Materials and methods) and their known classification to functional categories. Experiments were carried out in triplicate. Transcripts that significantly responded to 'OG' treatment but not to 'OG plus inhibitor' treatment were classified as 'inhibitor-insensitive' and show the ratio obtained in the 'OG plus inhibitor' condition in the last column. Positive and negative log ratios indicate increased or decreased levels of transcript in the treated cells, respectively.

ID	Gene family	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG+Inhibitor' (log_2)
	Stress and disease related genes			
At5g41750	Disease resistance protein (TIR-NBS-LRR class), putative	7.98	2.03	
At5g10100	Trehalose-6-phosphate phosphatase-like protein	7.88	2.25	
At1g61340	Late embryogenesis abundant protein, putative	7.76	3.35	
At2g32140	Disease resistance protein (TIR class), putative	6.27	1.54	
At2g17850	Senescence-associated protein	6.13	2.94	
At5g06320	Harpin-induced protein-like	5.68	1.70	
At2g40000	Putative nematode-resistance protein	5.67	0.67	
At1g72910	Disease resistance protein (TIR-NBS class), putative	4.84	0.72	0.48
At1g66090	Disease resistance protein (TIR-NBS class), putative	4.71	0.98	
At1g72900	Disease resistance protein (TIR-NBS class), putative	4.34	1.25	
At1g67360	Stress related protein, putative	4.19	3.25	
At5g46470	Disease resistance protein (TIR-NBS-LRR class)	3.97	0.57	
At1g78040	Allergen, putative	3.96	1.98	
At4g12400	Stress-induced protein sti1-like protein	3.56	0.92	
At5g46480	Disease resistance protein (TIR class), putative	3.51	1.19	
At4g18340	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	3.18	1.90	
At1g72940	Disease resistance protein (TIR-NBS class), putative	3.06	2.28	1.47
At3g44630	Disease resistance protein RPP1-wsb-like (TIR-NBS-LRR class), putative	2.89	1.76	0.56
At4g13600	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	2.77	1.52	
At4g02200	Drought-induced-19-like 1	2.70	0.80	1.13
At1g64760	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	2.58	1.30	
At5g44510	Disease resistance protein (TIR-NBS-LRR class), putative	2.45	1.36	
At4g21980	Symbiosis-related like protein	2.44	1.50	
At4g29360	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	2.43	1.22	
At5g42500	Disease resistance response protein-related	2.34	1.11	
At2g02120	Plant defensin protein, putative (PDF2.1)	2.27	1.41	
At5g43730	Disease resistance protein (CC-NBS-LRR class), putative	2.24	1.04	0.83
At2g19990	Pathogenesis-related protein (PR-1)	2.22	1.00	
At1g63750	Disease resistance protein (TIR-NBS-LRR class), putative	2.18	0.61	
At5g44870	Disease resistance protein (TIR-NBS-LRR class), putative	2.08	1.01	0.41
At3g50790	Putative LEA protein	2.07	0.84	0.70
At3g28250	Glycosyl hydrolase family 17 (endo β-1,3 glucanase)	2.07	1.03	0.48
At1g18250	Pathogenesis-related group 5 protein, putative	2.07	0.83	
At3g44670	Disease resistance protein RPP1-Ws(A,C)-like (TIR-NBS-LRR class), putative	2.01	0.86	
	Protein phosphorylation			
At3g45640	Mitogen-activated protein kinase 3 (MAPK 3)	8.75	2.70	
At1g73500	MAP kinase, putative (MAPKK 9)	8.31	3.85	
At4g28400	Protein phosphatase 2C (PP2C)	7.92	4.08	
At1g01560	MAP kinase, putative (MAPK 4)	4.54	1.03	
At4g23570	Phosphatase-like protein	4.51	3.53	1.82
At1g67580	Putative protein kinase	4.01	1.37	
At2g33580	Putative protein kinase	3.92	0.99	
At5g67080	Protein kinase-like protein (MAPKKK 19)	3.42	2.56	
At3g63260	ATMRK1	3.20	1.30	1.13
At5g58300	Leucine-rich repeat transmembrane protein kinase, putative	3.13	2.04	
At5g40030	Protein kinase-like protein	3.02	0.29	1.20
At4g32710	Putative protein kinase	3.02	2.28	1.73
At4g38470	Protein kinase-like protein	2.83	1.74	
At3g56050	Putative protein kinase	2.74	2.22	0.43
At5g02760	Protein phosphatase-like protein (PP2C)	2.74	0.16	
At2g02800	Putative protein kinase (PK2B)	2.68	0.90	0.20
At2g40270	Putative protein kinase	2.63	1.50	
At3g62220	Serine/threonine protein kinase-like protein	2.61	1.62	0.84
At2g46070	Putative mitogen-activated protein kinase (MAPK 4)	2.56	2.01	1.19
At5g15080	Serine/threonine specific protein kinase-like	2.35	0.92	0.52
At5g45430	Serine/threonine-protein kinase MAK (male germ cell-associated kinase)-like protein	2.32	0.20	1.04
At4g21380	Receptor-like serine/threonine protein kinase ARK3	2.29	0.40	
At4g08500	MEKK1/MAP kinase kinase kinase (MAPKKK 8)	2.27	1.15	
At3g24550	Protein kinase, putative	2.24	0.85	

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 2. (*Continued*)

ID	Gene family	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG+Inhibitor' (log_2)
At5g08160	Serine/threonine protein kinase (ATPK3)	2.22	1.08	0.70
At5g47070	Protein serine threonine kinase-like	2.13	0.83	1.74
At1g06390	Shaggy-like kinase, putative	2.07	0.67	
At1g70740	Putative protein kinase	2.04	1.07	1.42
	Oxidative burst			
At4g21090	Adrenodoxin-like protein (mitochondrial ferridoxin)	5.88	0.62	
At2g29490	Glutathione transferase, putative	3.55	2.13	
At2g29480	Glutathione transferase, putative	3.46	2.78	1.18
At2g48150	Glutathione peroxidase, putative	2.86	2.26	
At1g59670	Glutathione transferase, putative	2.70	1.23	
At3g50820	Photosystem II oxygen-evolving complex 33 (oec33)	2.49	1.68	0.73
At3g63080	Glutathione peroxidase, putative	2.33	0.77	1.02
At4g25100	Iron superoxide dismutase (fsd1)	2.30	0.49	
	CYT P450			
At5g45340	Cytochrome P450 family (CYP707A3)	9.70	0.79	
At4g39510	Cytochrome P450 family (CYP96A12)	3.85	2.48	
At4g19230	Cytochrome P450 family (CYP707A1)	3.78	2.91	
At3g26290	Cytochrome P450 family (CYP71B26)	3.03	0.61	
At5g06350	Cytochrome P450, putative	2.55	0.11	0.72
At4g00360	Cytochrome P450, putative (CYP86A2)	2.25	1.12	0.80
	Ethylene synthesis and signalling			
At4g11280	ACC synthase (ATACS-6)	3.82	2.59	
At1g06650	Oxidoreductase, putative (ACC oxidase e-122)	3.09	0.87	
At2g25450	Putative dioxygenase	2.38	0.99	0.45
At5g47230	Ethylene-responsive element binding factor 5 (AtERF5)	7.67	3.81	
At4g17500	Ethylene-responsive element binding factor 1	6.85	1.62	
At1g21910	TINY-like protein	6.03	0.54	
At2g38340	DREB-like AP2 domain transcription factor	4.99	2.26	
At5g21960	Similar to AP2 domain transcription factor, putative	4.73	1.35	
At3g15210	Ethylene-responsive element binding factor 4 (AtERF4)	3.43	1.66	
At5g05410	Putative DREB2A protein	2.80	1.32	1.36
At3g14230	AP2 domain protein RAP2.2	2.55	0.78	
	Jasmonic acid synthesis			
At5g42650	Allene oxide synthase (CYP74A)	4.23	2.07	1.64
At1g72520	Putative lipoxygenase (LOX 2)	3.51	1.58	1.78
At4g15440	Hydroperoxide lyase (HPOL) like protein (CYP74B2)	3.21	1.45	
At1g17420	Lipoxygenase (LOX 2)	2.23	0.44	0.39
	Calcium/calmodulin			
At5g39670	Calcium-binding-like protein	6.64	2.78	
At1g66400	Calmodulin-related protein	5.00	1.14	
At5g09410	Calmodulin-binding protein	4.34	1.58	
At1g27770	Calcium-atpase 1, plasma membrane-type (Ca^{2+}-ATPase, isoform 1)	3.85	2.10	
At5g62570	Calmodulin-binding protein	3.54	0.98	
At5g66210	Calcium-dependent protein kinase	3.44	0.83	
At3g43810	Calmodulin 7	2.90	0.10	
At5g37710	Putative calmodulin-binding heat-shock protein	2.47	1.92	0.97
At5g44460	Calmodulin-like protein	2.35	0.95	
	Wall modification			
At4g30280	Xyloglucan endotransglycosylase, putative	7.14	2.52	
At4g30290	Xyloglucan endo-1,4-β-D-glucanase-like protein	6.14	1.46	
At3g14310	Putative pectin methylesterase	5.59	4.56	
At4g30270	Xyloglucan endotransglycosylase (meri5B)	5.19	1.86	
At3g05620	Pectin esterase family	4.07	1.92	
At5g53330	Proline-rich cell wall protein-like	3.91	2.98	
At3g62720	α-galactosyltransferase-like protein	3.89	1.36	1.36
At5g63810	Glycosyl hydrolase family 35 (β-galactosidase)	3.66	0.16	
At5g08370	Glycosyl hydrolase family 27 (α-galactosidase/melibiase)	3.44	2.52	1.07
At5g61540	Glycosylasparaginase-like protein	2.77	2.11	0.99
At5g66460	Glycosyl hydrolase family 5/cellulase ((1-4)-β-mannan endohydrolase)	2.69	0.33	1.39
At3g50760	Glycosyltransferase family	2.60	1.86	1.56
At2g26620	Polygalacturonase, putative	2.56	0.99	1.57
At2g40310	Polygalacturonase, putative	2.54	1.61	
At5g08380	Glycosyl hydrolase family 27 (α-galactosidase/melibiase)	2.52	0.94	0.43
At4g02130	Predicted glycosyl transferase	2.11	0.89	1.65
At5g64860	Glycosyl hydrolase family 77 (4-α-glucanotransferase)	2.05	1.44	

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 2. (*Continued*)

ID	Gene family	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG+Inhibitor' (log_2)
	NAC/NAM			
At3g49530	NAC2-like protein	6.76	3.86	
At1g01720	NAC domain protein, putative	4.84	1.20	
At4g01520	Putative NAM-like protein	3.75	0.15	0.87
At1g28470	NAM protein, putative	2.57	0.76	−0.45
At2g27300	NAM (no apical meristem)-like protein	2.13	1.38	0.49
At1g52890	NAM-like protein	2.06	0.91	
At4g01550	Putative NAM-like protein	2.02	0.84	0.91
	Zinc finger			
At1g27730	Salt-tolerance zinc finger protein	7.52	1.41	
At2g28200	Putative zinc-finger protein	4.92	0.85	
At5g43170	Cys2/his2-type zinc finger protein 3 (dbj|BAA85109.1)	4.84	2.18	
At3g54810	GATA zinc finger protein	4.58	0.99	
At5g22480	Zinc finger protein-like	4.55	1.66	
At2g40140	Putative ccch-type zinc finger protein	3.48	0.26	
At3g19580	Zinc finger protein, putative	3.21	0.58	
At1g74410	Putative ring zinc finger protein	3.10	0.84	1.09
At4g09690	CHP-rich zinc finger protein, putative	2.94	1.29	
At1g08930	Zinc finger protein ATZF1, putative	2.82	0.74	0.89
At2g37430	Putative C2H2-type zinc finger protein	2.28	1.44	
At1g20823	Similar to putative ring zinc finger protein	2.27	0.66	0.71
At5g63750	ARi-like RING zinc finger protein-like	2.24	0.82	0.88
At4g00070	Putative ring-finger protein	2.14	1.14	0.16
At2g39100	Putative RING zinc finger protein	2.10	0.69	0.49
At2g34900	Putative RING3 protein	2.07	0.36	0.75
At5g18550	Zinc finger -like protein	2.03	0.47	1.14
	WRKY			
At2g30250	WRKY family transcription factor (WRKY 25)	3.99	1.75	
At5g49520	WRKY family transcription factor (WRKY 48)	3.40	2.07	1.41
At1g80840	WRKY family transcription factor (WRKY 40)	3.29	1.89	1.91
At4g11070	WRKY family transcription factor (WRKY 41)	2.27	0.47	
	Stress and disease related genes			
At1g22900	Disease resistance response protein-related	−2.04	0.85	−0.83
At3g05360	Disease resistance protein family (LRR)	−2.15	1.05	−1.14
At3g28860	Multidrug resistance P-glycoprotein, putative	−2.18	1.44	
At5g40170	Disease resistance protein family1	−2.21	0.51	
At1g29380	β-1,3 glucanase, putative	−2.23	0.49	−1.19
At3g20590	Non-race specific disease resistance protein, putative	−2.24	1.25	−1.71
At1g07390	Disease-resistance protein, putative	−2.26	0.51	
At2g24160	Leucine rich repeat protein family	−2.39	0.82	
At1g61100	Disease-resistance protein (TIR class), putative	−2.48	1.00	−1.47
At1g02520	Multidrug resistance P-glycoprotein, putative	−2.54	1.12	
At1g63730	Disease resistance protein (TIR-NBS-LRR class), putative	−2.60	0.81	
At1g52900	Disease resistance protein (TIR class), putative	−2.67	0.77	0.22
At1g06410	Trehalose-6-phosphate synthase, putative	−2.73	1.10	
At1g16980	Trehalose-6-phosphate synthase, putative	−2.97	1.92	−1.59
At2g36910	Multidrug resistance P-glycoprotein (pgp1)	−2.98	0.86	−0.74
At1g15890	Disease resistance protein (CC-NBS-LRR class), putative	−3.04	0.89	
At1g22210	Trehalose-6-phosphate phosphatase, putative	−3.15	0.41	−0.56
At4g25960	Multidrug resistance P-glycoprotein, putative	−3.16	1.48	
At2g26380	Disease resistance protien-related (LRR)	−3.18	0.77	
At4g22590	Trehalose-6-phosphate phosphatase-like protein	−3.82	1.50	
At3g13650	Disease resistance response protein-related/dirigent protein-related	−3.83	1.49	
At3g50970	Dehydrin Xero2	−3.89	1.69	
At5g38340	Disease resistance protein (TIR-NBS-LRR class), putative	−4.08	2.58	
At1g12220	Disease resistance protein RPS5 (resistance to *Pseudomonas syringae* protein 5) (CC-NBS-LRR class)	−4.51	2.59	
At2g34930	Disease resistance protein family	−4.57	2.54	
At1g33590	Disease resistance protein-related (LRR)	−5.01	1.84	
At1g33670	Leucine rich repeat protein family	−5.41	1.68	
	Protein phosphorylation			
At1g48120	Serine/threonine phosphatase PP7, putative	−2.00	1.10	
At5g09890	Protein kinase	−2.02	1.11	−1.43
At3g04810	Putative kinase	−2.03	1.16	−0.99
At4g22730	Leucine-rich repeat transmembrane protein kinase, putative	−2.03	0.36	−0.93
At4g31220	Protein kinase-like protein	−2.08	1.03	−1.43

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 2. (*Continued*)

ID	Gene family	Ratio 'OG' ($\log_2$)	Standard deviation	Ratio 'OG+Inhibitor' ($\log_2$)
At5g13160	Protein kinase-like	−2.11	0.92	
At2g28990	Putative receptor-like protein kinase	−2.14	0.55	−0.16
At1g49100	Light-repressible receptor protein kinase, putative	−2.15	0.90	−0.30
At5g03700	S-receptor kinase-like protein	−2.19	1.04	−0.74
At3g45860	Protein kinase-like (RLK4)	−2.20	1.23	
At5g63650	Serine/threonine-protein kinase	−2.20	0.73	−0.21
At4g32300	S-receptor kinase-like protein (ARK3)	−2.26	0.73	−0.84
At1g18150	Mitogen-activated protein kinase, putative (MAPK 8)	−2.32	1.62	
At1g50990	Protein kinase, putative	−2.38	1.57	−0.59
At1g16270	Putative Ser/Thr protein kinase	−2.40	1.24	−1.15
At1g16900	Ser/Thr protein kinase, putative	−2.41	0.83	
At1g53430	Receptor-like serine/threonine kinase, putative	−2.43	1.47	
At1g53730	Leucine-rich repeat transmembrane protein kinase 1, putative	−2.62	1.04	
At2g24360	Putative protein kinase	−2.75	1.44	
At2g26290	Putative protein kinase	−2.76	0.01	
At1g66150	Receptor protein kinase (TMK1), putative	−2.86	1.43	
At5g53320	Leucine-rich repeat transmembrane protein kinase	−2.95	1.11	
At1g09970	Leucine-rich repeat transmembrane protein kinase, putative	−3.05	1.42	
At4g26070	Mitogen activated protein kinase kinase (nmapkk) (MEK 1)	−3.43	1.08	
At1g70520	Putative protein kinase	−3.78	0.81	
At1g07430	Protein phosphatase 2C (PP2C), putative	−3.80	0.18	
At4g23270	Serine/threonine kinase	−4.21	1.63	
At5g66850	MAP protein kinase (MAPKKK 5)	−4.97	0.63	
At2g39660	Putative protein kinase	−5.31	1.81	
	Oxidative burst			
At1g17180	Glutathione transferase, putative	−2.03	0.53	−1.26
At1g12520	Copper/zinc superoxide dismutase copper chaperone, putative	−2.31	0.30	−1.53
At2g30870	Glutathione transferase, putative	−2.32	1.55	−1.31
At1g52820	Putative oxidoreductase	−2.41	0.89	−1.83
At5g27380	Glutathione synthetase (GSH2)	−2.49	1.19	−1.12
At5g58390	Peroxidase, putative	−2.91	1.40	
At4g38540	Monooxygenase 2 (MO2)	−2.91	0.91	−0.79
At1g19230	Respiratory burst oxidase protein, putative	−3.18	0.42	−1.17
At1g52810	Putative oxidoreductase	−3.22	0.91	
At5g39580	Peroxidase, putative	−3.22	0.70	
At1g30510	Ferrodoxin NADP oxidoreductase, putative	−4.32	0.67	
At3g49120	Peroxidase, putative	−4.66	2.09	
At4g08770	Peroxidase, putative	−5.25	2.23	
At4g08780	Peroxidase, putative	−5.96	1.64	
	CYT P450			
At1g01280	Cytochrome P450 family (CYP703A2)	−2.12	0.68	
At1g28430	Cytochrome P450 family (CYP705A24)	−2.40	0.67	−0.62
At5g04660	Cytochrome P450, putative (CYP77A4)	−2.74	0.47	
At1g01600	Cytochrome P450, putative (CYP86A4)	−2.75	1.73	
At4g37430	Cytochrome P450 family CYP81F1, CYP91A2, CYT P450 MONOOXYGENASE 91A2	−3.10	0.40	
At2g40890	Cytochrome P450, putative (CYP98A3) coumarate 3-hydroxylase (C3H)	−4.22	0.60	
	Ethylene synthesis and signalling			
At1g01480	1-aminocyclopropane-1-carboxylate synthase (AtACS-2)	−2.00	0.41	−0.57
At4g36920	Apetala2 protein protein	−4.20	1.60	
	Jasmonic acid synthesis and signalling			
At1g52070	Jasmonate inducible protein, putative	−2.91	0.60	
At1g76680	12-oxophytodienoate reductase (OPR1)	−3.06	1.13	
At2g03980	Putative GDSL-motif lipase/hydrolase	−3.07	1.15	
At5g55050	Putative GDSL-motif lipase/hydrolase	−6.48	2.21	
	Calcium/calmodulin			
At1g74740	calcium-dependent protein kinase, putative	−2.36	0.68	
At3g57330	potential calcium-transporting ATPase 11, plasma membrane-type (Ca^{2+}-ATPase, isoform 11)	−2.46	1.12	
At4g04740	putative calcium-dependent protein kinase	−3.26	1.76	
At4g04695	similar to calcium-dependent protein kinase-like protein	−4.25	2.06	
At4g04700	putative calcium-dependent protein kinase	−5.50	3.28	
	Wall modification			
At3g57520	Glycosyl hydrolase family 36	−2.01	0.45	
At4g27820	Glycosyl hydrolase family 1	−2.06	1.19	−1.39
At4g19810	Glycosyl hydrolase family 18	−2.08	0.45	−0.37
At1g45130	Glycosyl hydrolase family 35 (β-galactosidase)	−2.10	0.83	−0.71

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 2. (*Continued*)

ID	Gene family	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG+Inhibitor' (log_2)
At1g41830	Pectin esterase (pectin methylesterase), putative	−2.24	1.26	
At2g44450	Glycosyl hydrolase family 1	−2.25	0.71	
At1g12560	Expansin, putative	−2.29	0.28	
At1g64390	Glycosyl hydrolase family 9 (endo-1,4-β-glucanase)	−2.31	0.96	
At1g55850	Cellulose synthase catalytic subunit, putative	−2.31	0.44	
At3g13750	Glycosyl hydrolase family 35 (β-galactosidase)	−2.39	1.71	
At5g35190	Extensin-like protein	−2.42	1.02	
At1g52400	Glycosyl hydrolase family 1, β-glucosidase (BG1)	−2.64	1.62	0.72
At3g62740	Glycosyl hydrolase family 1	−2.78	0.70	−0.54
At2g39700	Expansin, putative	−2.95	0.40	0.03
At1g48930	Glycosyl hydrolase family 9 (endo-1,4-β-glucanase)	−3.09	1.11	
At1g76160	Pectin esterase (pectin methylesterase), putative	−3.10	1.05	
At1g55120	Glycosyl hydrolase family 32	−4.00	0.95	
At5g11920	Glycosyl hydrolase family 32	−4.44	2.33	
At3g13790	Glycosyl hydrolase family 32	−6.31	1.57	
	NAC/NAM			
At1g56010	NAC1	−2.12	0.82	0.26
At1g71930	NAM-like protein	−2.26	0.36	−1.22
At3g03200	NAM-like protein (no apical meristem)	−2.33	0.68	
At2g33480	putative NAM (no apical meristem)-like protein	−2.46	0.86	−1.95
	Zinc finger			
At1g55410	CHP-rich zinc finger protein, putative	−2.03	0.69	
At1g53010	Zinc finger protein, putative	−2.03	1.38	−1.32
At1g61710	CHP-rich zinc finger protein, putative	−2.09	1.36	
At1g29570	Zinc finger protein, putative	−2.13	1.56	−0.14
At4g01930	CHP-rich zinc finger protein, putative	−2.14	1.09	
At1g55110	Zinc finger protein, putative	−2.16	0.15	−0.86
At1g69570	Dof zinc finger protein	−2.28	0.62	
At4g01910	CHP-rich zinc finger protein, putative	−2.33	0.39	
At3g27500	CHP-rich zinc finger protein, putative	−2.85	0.43	
At2g17450	Putative RING zinc finger protein	−3.38	0.52	
At3g26240	CHP-rich zinc finger protein, putative	−3.55	0.79	
At5g03360	CHP-rich zinc finger protein, putative	−3.56	1.42	0.28
At1g74620	Putative RING zinc finger protein	−3.79	0.40	
At3g45530	CHP-rich zinc finger protein, putative	−3.84	1.50	
At3g27095	Pseudogene, putative zinc finger protein	−4.61	1.19	
	WRKY			
At3g04670	WRKY family transcription factor	−2.68	0.23	

inhibitor-sensitive and -insensitive genes in that particular category deviated significantly from the distribution in the reference dataset consisting of 1117 transcripts of which 320 were inhibitor-sensitive. Table 3 reports those categories that showed a significant change from the null hypothesis $P_1=P_2$ (i.e. binomial distribution of category is not significantly different from the binomial distribution of the background). Among categories of genes that were up-regulated by OGs, those involved in cell wall modification were significantly inhibitor-sensitive (Table 3; 9 out of 17 genes). It is noteworthy that this was the case only for the group of cell wall modification genes that was transcriptionally up-regulated by OGs and did not apply to down-regulated genes. Conversely, Nac/Nam type transcription factors were found to be significantly inhibitor-sensitive, but only for the subset that was *down-regulated* in response to OGs. Of particular interest is the finding that the jasmonate synthesis pathway is disproportionately inhibitor-sensitive, with three out of four OG-up-regulated jasmonate production transcripts showing inhibitor sensitivity. However, the small number of transcripts in this group only yielded a significant P value (0.041) at the 5% level. In all other categories, no evidence for significant deviation from the background distribution of inhibitor sensitivity was obtained, including disproportionate inhibitor insensitivity. Thus, our analysis suggests that two of the processes that contribute to rapid OG responses,

Table 3. *Functional categories that show a significant deviation from the background binomial distribution for inhibitor sensitivity*

As background distribution, the total complement of inhibitor-sensitive transcripts (320 out of a total of 1117) was used.

Calcium-dependent categories up-regulated	P ($P_1=P_2$)
Jasmonic acid pathway	$\leq$0.041
Wall modification	$\leq$0.00842
Calcium-dependent categories down-regulated	P ($P_1=P_2$)
Nac/Nam transcription factors	$\leq$0.00201

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

modification of the cell wall and the induction of jasmonate synthesis, may require an upstream Ca^{2+} signal. By contrast, ethylene synthesis, the oxidative burst, phosphorylation, and the transcriptional regulation of stress-related genes and induction of many transcription factors themselves rely on both Ca^{2+}-dependent and Ca^{2+}-independent upstream events.

To analyse further which OG-induced signalling components require upstream Ca^{2+} transients, the data were reordered (Table 4) into three major classes of 'wounding', 'jasmonate', and 'ethylene'-related genes, since several reports have described a close causal link between wounding, the production of OGs and the induction of jasmonate and ethylene signalling pathways (Schenk *et al.*, 2000; Cheong *et al.*, 2002; Van Zhong *et al.*, 2003; DRASTIC Data Base: http://www.drastic.org.uk/). For example, the dataset of jasmonate signalling-related genes contains, in addition to those directly involved in jasmonic acid synthesis, genes encoding kinases, glycosyl hydrolases, and transcription factors that are known to play a role in jasmonate signalling or known to be transcriptionally regulated by jasmonate treatment. Among transcripts that were significantly regulated by OGs, 101 genes were recognized as associated with wounding, 56 with jasmonate signalling, and 46 with ethylene signalling. To test whether Ca^{2+} dependence was restricted to jasmonate synthesis *per se* or whether it pertains to a broader range of targets in either the jasmonate-associated gene group or the other groups, the same binomial analysis as described above was applied. For all wounding-associated genes the distribution of inhibitor-sensitive and -insensitive genes did not deviate significantly from the distribution found in the background set. A similar finding was made regarding ethylene synthesis and ethylene signalling-related genes. However, both categories of up-regulated and down-regulated jasmonate-associated genes exhibited a highly significant inhibitor sensitivity (Table 4) with an overall significance score for all jasmonate-associated genes of $P \leqslant 1.2e^{-5}$.

These results suggest that, in addition to the gene products responsible for jasmonic acid metabolism (Tables 2, 3) the jasmonate signalling network itself is Ca^{2+}-dependent. By contrast, the wounding response and both ethylene synthesis and the induction of many ethylene signalling associated genes appear to require both Ca^{2+}-dependent and Ca^{2+}-independent components.

To confirm our findings, an alternative analysis was carried out on the OG responsive transcripts that is not based on fold-change criteria but calculates a 'Rank Product' for each transcript (Breitling *et al.*, 2004). Data in the supplementary file 'RankProduct_SupplData.txt' at JXB online not only show a high ranking for the relevant transcripts involved in ethylene and jasmonic acid biosynthesis but also a very low probability of being false positives.

Identification of putative promoter cis*-elements*

The rapid changes in the level of many transcripts that follows exposure to OGs implies that transcriptional regulation of some or many of these genes might rely on common regulatory motifs in their promoters. To determine whether such common motifs or *cis*-elements are present, the 5′ upstream regions of OG-responsive genes were queried for overrepresented motifs. Analyses were carried out for total data sets (i.e. all up-regulated or down-regulated genes), respective functional categories, and across inhibitor-sensitive and -insensitive subcategories. Our particular interest was to identify putative motifs that were associated with either Ca^{2+}-dependent or Ca^{2+}-independent categories.

Table 5 lists for the various data sets putative motifs with a P score $<10^{-5}$. Most of the identified motifs are previously unrecognized and their significance has yet to be established. A few putative motifs, for example, 5′-ACCACCGT-3′, 5′-AGTTTTAT-3′, and 5′-GGATAA-CA-3′, occur only in inhibitor-sensitive categories and may therefore involve Ca^{2+} signalling for the activation of their associated transcription factors.

Of the several motifs that were found and were previously described, the 'Dof core' motif from maize was the most prevalent. Dof proteins are plant transcription factors that contain conserved single Zn-finger motifs. A large number of Zn-finger type transcription factors was found to be up-regulated by OGs (Table 2) and Dof proteins have previously been shown to be elicitor responsive, for example, ERDP from *Pisum sativum* (Lijavetzky *et al.*, 2003). Further known elements included: 'Pollen lelat', one of two co-dependent regulatory elements responsible for pollen-specific activation of the tomato *lat52* gene involved in pollen development; 'CAAT-boxes', common promoter elements that act as transcription enhancers; an 'I-box' that is believed to play a role in light response; and 'ABRElaterd1', an ABA-responsive element-like sequence required for expression of erd1 (early responsive to dehydration).

Discussion

Formation of cell wall degradation products in the form of OGs provides a potent cue for plant cells to respond to attack by pathogenic micro-organisms or herbivores. Processing of this response includes OG perception, subsequent signal transduction, and the activation of cellular targets by transcriptional regulation. Very little is known regarding the details of the signal transduction events between OG perception and downstream targets, but several reports have shown the involvement of Ca^{2+} as a potential second messenger (Messiaen and Van Cutsem, 1994; Chandra *et al.*, 1997; Navazio *et al.*, 2002). Yet the significance of the intermediate Ca^{2+} signal and its downstream targets remain largely unknown.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 4. *Genes with significantly altered transcript levels after exposure of* Arabidopsis *cells to oligogalacturonides for a period of 2 h*

Genes were grouped according to previously desrcibed involvement in either the wounding response, jasmonic acid synthesis and signalling, or ethylene synthesis and signalling. Transcripts that significantly responded to 'OG' treatment but not to 'OG plus inhibitor' treatment were classified as 'inhibitor-insensitive' and show the ratio obtained in the 'OG plus inhibitor' condition in the last column. A binomial distribution test was carried out comparing the inhibitor sensitivity of the background dataset with that of the respective categories. Positive and negative log ratios indicate increased or decreased levels of transcript in the treated cells respectively.

ID	Wounding up-regulated genes Binomial distribution of inhibitor sensitivity relative to background ($P \leqslant 0.339$)	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG plus Inhibitor' (log_2)
At5g41750	Disease resistance protein (TIR-NBS-LRR class), putative	7.98	2.03	
At5g10100	Trehalose-6-phosphate phosphatase-like protein	7.88	2.25	
At1g61340	Late embryogenesis abundant protein, putative	7.76	3.35	
At2g17850	Senescence-associated protein	6.13	2.94	
At4g18340	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	3.18	1.90	
At3g44630	Disease resistance protein RPP1-WsB-like (TIR-NBS-LRR class), putative	2.89	1.76	0.56
At4g13600	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	2.77	1.52	
At1g64760	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	2.58	1.30	
At4g29360	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	2.43	1.22	
At2g02120	Plant defensin protein, putative (PDF2.1)	2.27	1.41	
At3g50790	Putative LEA protein	2.07	0.84	0.70
At3g28250	Glycosyl hydrolase family 17; (endo β-1,3 glucanase)	2.07	1.03	0.48
At3g44670	Disease resistance protein RPP1-Ws(A,C)-like (TIR-NBS-LRR class)	2.01	0.86	
At3g45640	Mitogen-activated protein kinase 3 (MAPK 3)	8.75	2.70	
At4g28400	Protein phosphatase 2C (PP2C)	7.92	4.08	
At1g01560	MAP kinase, putative (MAPK 4)	4.54	1.03	
At5g02760	Protein phosphatase-like protein; (PP2C)	2.74	0.16	
At2g46070	Putative mitogen-activated protein kinase; (MAPK 4)	2.56	2.01	1.19
At5g15080	Serine/threonine specific protein kinase-like	2.35	0.92	0.53
At4g21380	Receptor-like serine/threonine protein kinase ARK3	2.29	0.40	
At5g47070	Protein serine/threonine kinase-like	2.13	0.83	1.74
At2g29490	Glutathione transferase, putative	3.55	2.13	
At2g29480	Glutathione transferase, putative	3.46	2.78	1.18
At2g48150	Glutathione peroxidase, putative	2.86	2.26	
At1g59670	Glutathione transferase, putative	2.70	1.23	
At3g63080	Glutathione peroxidase, putative	2.33	0.77	1.02
At4g11280	ACC synthase (AtACS-6)	3.82	2.59	
At5g42650	Allene oxide synthase (CYP74A)	4.23	2.07	1.64
At1g72520	Putative lipoxygenase; (LOX 2)	3.51	1.58	1.78
At4g15440	Hydroperoxide lyase (HPOL) like protein; (CYP74B2)	3.21	1.45	
At1g17420	Lipoxygenase; (LOX 2)	2.23	0.44	0.39
At5g14700	Cinnamoyl CoA reductase-like protein	5.47	2.09	
At5g22630	Chorismate mutase/prephenate dehydratase-like protein	3.55	1.10	
At2g33600	Putative cinnamoyl-CoA reductase	3.03	1.65	
At1g77530	*O*-methyltransferase, family 2 family	2.21	1.49	0.22
At4g30470	Cinnamoyl-CoA reductase-like protein	2.02	0.31	1.26
At4g30280	Xyloglucan endotransglycosylase, putative	7.14	2.52	
At3g14310	Putative pectin methylesterase	5.59	4.56	
At4g30270	Xyloglucan endotransglycosylase (meri5B)	5.19	1.86	
At3g05620	Pectinesterase family	4.07	1.92	
At3g50760	Glycosyltransferase family	2.60	1.86	1.56
At1g59860	Heat shock protein, putative	7.41	1.84	
At1g54050	Heat-shock protein, putative	6.37	3.10	
At5g01390	Heat shock protein 40-like	2.32	0.71	
At5g47230	Ethylene responsive element binding factor 5 (AtERF5)	7.67	3.81	
At4g17500	Ethylene responsive element binding factor 1	6.85	1.62	
At2g38340	DREB-like AP2 domain transcription factor	4.99	2.26	
At5g21960	Similar to AP2 domain transcription factor, putative	4.73	1.35	
At3g15210	Ethylene responsive element binding factor 4 (AtERF4)	3.43	1.66	
At5g05410	Putative DREB2A protein	2.80	1.32	1.36
At4g21440	Myb family protein; (Myb related preotein 4) (MYB102)	3.06	0.41	
At5g62470	MYB96 transcription factor-like protein	2.86	2.34	1.17
At3g49530	NAC2-like protein	6.76	3.86	
At1g01720	NAC domain protein, putative	4.84	1.20	
At4g01520	Putative NAM-like protein	3.75	0.15	0.87
At1g28470	NAM protein, putative	2.57	0.76	−0.45
At2g27300	NAM (no apical meristem)-like protein	2.13	1.38	0.49
At1g52890	NAM-like protein	2.06	0.91	
At4g01550	Putative NAM-like protein	2.02	0.84	0.91

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 4. (*Continued*)

	Wounding up-regulated genes			
ID	Binomial distribution of inhibitor sensitivity relative to background ($P \leqslant 0.339$)	Ratio 'OG' (log$_2$)	Sandard deviation	Ratio 'OG plus Inhibitor' (log$_2$)
At1g27730	Salt-tolerance zinc finger protein	7.52	1.41	
At3g54810	GATA zinc finger protein	4.58	0.99	
At2g40140	Putative CCCH-type zinc finger protein	3.48	0.26	
At3g51910	Putative heat shock transcription factor	4.60	2.02	
At2g29500	Putative small heat shock protein	3.48	1.03	
At5g62020	Heat shock factor 6	2.99	1.76	
At3g02990	Putative heat shock transcription factor	2.37	1.23	
At1g80840	WRKY family transcription factor	3.29	1.89	1.91
At3g13310	DnaJ protein, putative	2.65	1.69	0.52
At4g13830	DnaJ-like protein	2.28	1.52	0.89

	Wounding down-regulated genes			
ID	Binomial distribution of inhibitor sensitivity relative to backgroun ($P \leqslant 0.320$)	Ratio 'OG' (log$_2$)	Standard deviation	Ratio 'OG plus inhibitor' (log$_2$)
At1g29380	β-1,3 glucanase, putative	−2.23	0.49	−1.19
At1g06410	Trehalose-6-phosphate synthase, putative	−2.73	1.10	
At1g16980	Trehalose-6-phosphate synthase, putative	−2.97	1.92	−1.59
At1g22210	Trehalose-6-phosphate phosphatase, putative	−3.15	0.41	−0.56
At4g22590	Trehalose-6-phosphate phosphatase-like protein	−3.82	1.50	
At4g32300	S-receptor kinase-like protein (ARK3)	−2.26	0.73	−0.84
At1g16900	Ser/Thr protein kinase, putative	−2.41	0.83	
At1g09970	Leucine-rich repeat transmembrane protein kinase, putative	−3.05	1.42	
At1g07430	Protein phosphatase 2c (pp2c), putative	−3.80	0.18	
At1g17180	Glutathione transferase, putative	−2.03	0.53	−1.26
At2g30870	Glutathione transferase, putative	−2.32	1.55	−1.31
At5g39580	Peroxidase, putative	−3.22	0.70	
At3g49120	Peroxidase, putative	−4.66	2.09	
At4g08770	Peroxidase, putative	−5.25	2.23	
At4g08780	Peroxidase, putative	−5.96	1.64	
At1g52070	Jasmonate inducible protein, putative	−2.91	0.60	
At1g76680	12-oxophytodienoate reductase (OPR1)	−3.06	1.13	
At3g29200	Chorismate mutase	−2.11	0.52	
At5g19440	Cinnamyl-alcohol dehydrogenase-like protein	−2.69	1.12	
At1g80820	Cinnamoyl CoA reductase, putative	−3.22	1.35	
At1g12560	Expansin, putative	−2.29	0.28	
At5g35190	Extensin-like protein	−2.42	1.02	
At2g39700	Expansin, putative	−2.95	0.40	0.03
At1g79920	Putative heat-shock protein	−2.45	1.28	−1.23
At4g37390	Auxin-responsive GH3-like protein	−4.82	1.26	
At1g59500	Auxin-regulated protein GH3, putative	−5.51	1.66	
At3g18820	GTP-binding protein, putative	−2.37	0.96	−0.45
At1g56010	NAC1	−2.12	0.82	0.26
At1g71930	NAM-like protein	−2.26	0.36	−1.22
At3g03200	NAM-like protein (no apical meristem)	−2.33	0.68	
At2g33480	Putative NAM (no apical meristem)-like protein	−2.46	0.86	−1.95
At1g69570	Dof zinc finger protein	−2.28	0.62	

	Jasmonate up-regulated genes			
ID	Binomial distribution of inhibitor sensitivity relative to background ($P \leqslant 0.0021$)	Ratio 'OG' (log$_2$)	Standard deviation	Ratio 'OG plus inhibitor' (log$_2$)
At4g18340	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	3.18	1.90	
At4g13600	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	2.77	1.52	
At1g64760	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	2.58	1.30	
At4g29360	Glycosyl hydrolase family 17 (chitinase/β-1,3 glucanase)	2.43	1.22	
At2g19990	Pathogenesis-related protein (PR-1)	2.22	1.00	
At3g28250	Glycosyl hydrolase family 17 (endo β-1,3 glucanase)	2.07	1.03	0.48
At1g18250	Pathogenesis-related group 5 protein, putative	2.07	0.83	
At3g45640	Mitogen-activated protein kinase 3 (MAPK 3)	8.75	2.70	
At4g08500	MEKK1/MAP kinase kinase kinase (MAPKKK 8)	2.27	1.15	
At2g48150	Glutathione peroxidase, putative	2.86	2.26	
At3g63080	Glutathione peroxidase, putative	2.33	0.77	1.02
At1g06650	Oxidoreductase, putative (ACC oxidase e-122)	3.09	0.87	

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 4. (*Continued*)

ID	Jasmonate up-regulated genes Binomial distribution of inhibitor sensitivity relative to background ($P \leq 0.0021$)	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG plus inhibitor' (log_2)
At2g25450	Putative dioxygenase	2.38	0.99	
At5g42650	Allene oxide synthase (CYP74A)	4.23	2.07	1.64
At1g72520	Putative lipoxygenase (LOX 2)	3.51	1.58	1.78
At4g15440	Hydroperoxide lyase (HPOL) like protein (CYP74B2)	3.21	1.45	
At1g17420	Lipoxygenase (LOX 2)	2.23	0.44	0.35
At1g77530	*O*-methyltransferase, family 2 family	2.21	1.49	0.22
At4g30280	Xyloglucan endotransglycosylase, putative	7.14	2.52	
At4g30270	Xyloglucan endotransglycosylase (meri5B)	5.19	1.86	
At5g53330	Proline-rich cell wall protein-like	3.91	2.98	
At3g50760	Glycosyltransferase family	2.60	1.86	1.56
At4g02130	Predicted glycosyl transferase	2.11	0.89	1.65
At1g64230	E2, ubiquitin-conjugating enzyme, putative	2.69	1.36	1.21
At5g51210	Oleosin	2.21	0.72	0.97
At5g07600	Oleosin	2.05	0.78	0.23
At4g17500	Ethylene responsive element binding factor 1	6.85	1.62	
At2g31370	bZIP transcription factor (POSF21) (bZIP59)	2.29	1.09	1.80
At3g49530	NAC2-like protein	6.76	3.86	
At1g01720	NAC domain protein, putative	4.84	1.20	
At4g01520	Putative NAM-like protein	3.75	0.15	0.87
At1g28470	NAM protein, putative	2.57	0.76	−0.45
At2g27300	NAM (no apical meristem)-like protein	2.13	1.38	0.49
At1g52890	NAM-like protein	2.06	0.91	
At4g01550	Putative NAM-like protein	2.02	0.84	0.91
At3g02060	DEAD/DEAH box helicase protein, putative	2.53	0.61	
At3g13310	DnaJ protein, putative	2.65	1.69	0.52
At4g13830	DnaJ-like protein	2.28	1.52	0.89

ID	Jasmonate down-regulated genes Binomial distribution of inhibitor sensitivity relative to background ($P \leq 0.0035$)	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG plus Inhibitor' (log_2)
At1g29380	β-1,3 glucanase, putative	−2.23	0.49	−1.19
At1g30510	Ferrodoxin nadp oxidoreductase, putative	−4.32	0.67	
At1g52070	Jasmonate-inducible protein, putative	−2.91	0.60	
At1g76680	12-oxophytodienoate reductase (OPR1)	−3.06	1.13	
At4g34850	Chalcone synthase-like protein	−2.05	0.68	−1.10
At1g48850	Chorismate synthase, putative	−3.45	1.54	−1.99
At4g27820	Glycosyl hydrolase family 1	−2.06	1.19	−1.39
At2g44450	Glycosyl hydrolase family 1	−2.25	0.71	
At5g35190	Extensin-like protein	−2.42	1.02	
At1g52400	Glycosyl hydrolase family 1, β-glucosidase (BG1)	−2.64	1.62	0.72
At3g62740	Glycosyl hydrolase family 1	−2.78	0.70	−0.54
At1g48930	Glycosyl hydrolase family 9 (endo-1,4-β-glucanase)	−3.09	1.11	
At4g02890	Polyubiquitin (UBQ14)	−2.54	0.51	−0.95
At1g56010	NAC1	−2.12	0.82	0.26
At1g71930	NAM -like protein	−2.26	0.36	−1.22
At3g03200	NAM -like protein (no apical meristem)	−2.33	0.68	
At2g33480	Putative NAM (no apical meristem)-like protein	−2.46	0.86	−1.95
At1g71280	DEAD/DEAH box rna helicase protein, putative	−2.44	0.88	−1.62

ID	Ethylene up-regulated genes Binomial distribution of inhibitor sensitivity relative to background ($P \leq 0.310$)	Ratio 'OG' (log_2)	Standard deviation	Ratio 'OG plus Inhibitor' (log_2)
At4g18340	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	3.18	1.90	
At4g13600	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	2.77	1.52	
At1g64760	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	2.58	1.30	
At4g29360	Glycosyl hydrolase family 17; (chitinase/β-1,3 glucanase)	2.43	1.22	
At2g02120	Plant defensin protein, putative (pdf2.1)	2.27	1.41	
At3g45640	Mitogen-activated protein kinase 3 (mapk 3)	8.75	2.70	
At4g28400	Protein phosphatase 2C (PP2C)	7.92	4.08	
At1g01560	Map kinase, putative (MAPK 4)	4.54	1.03	
At5g02760	Protein phosphatase-like protein; (PP2C)	2.74	0.16	
At2g29490	Glutathione transferase, putative	3.55	2.13	

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 4. (*Continued*)

ID	Ethylene up-regulated genes Binomial distribution of inhibitor sensitivity relative to background ($P \leq 0.0021$)	Ratio 'OG' ($\log_2$)	Standard deviation	Ratio 'OG plus inhibitor' ($\log_2$)
At2g29480	Glutathione transferase, putative	3.46	2.78	1.18
At1g59670	Glutathione transferase, putative	2.70	1.23	
At4g11280	ACC synthase (ATACS-6)	3.82	2.59	
At1g06650	Oxidoreductase, putative (ACC oxidase e-122)	3.09	0.87	
At1g77530	*O*-methyltransferase, family 2 family	2.21	1.49	0.22
At4g30290	Xyloglucan endo-1,4-β-D-glucanase-like protein	6.14	1.46	
At3g14310	Putative pectin methylesterase	5.59	4.56	
At3g05620	Pectinesterase family	4.07	1.92	
At3g50760	Glycosyltransferase family	2.60	1.86	1.56
At2g26620	Polygalacturonase, putative	2.56	0.99	1.57
At2g40310	Polygalacturonase, putative	2.54	1.61	
At4g02130	Predicted glycosyl transferase	2.11	0.89	
At5g47230	Ethylene responsive element binding factor 5 (AtERF5)	7.67	3.81	
At4g17500	Ethylene responsive element binding factor 1	6.85	1.62	
At2g38340	DREB-like AP2 domain transcription factor	4.99	2.26	
At5g21960	Similar to AP2 domain transcription factor, putative	4.73	1.35	
At3g15210	Ethylene responsive element binding factor 4 (AtERF4)	3.43	1.66	
At3g14230	AP2 domain protein RAP2.2	2.55	0.78	
At1g27730	Salt-tolerance zinc finger protein	7.52	1.41	

ID	Ethylene down-regulated genes Binomial distribution of inhibitor sensitivity relative to background ($P \leq 0.60$)	Ratio 'OG' ($\log_2$)	Standard deviation	Ratio 'OG plus Inhibitor' ($\log_2$)
At2g28990	Putative receptor-like protein kinase	−2.14	0.55	−0.16
At1g49100	Light repressible receptor protein kinase, putative	−2.15	0.90	−0.30
At1g07430	Protein phosphatase 2c (PP2C), putative	−3.80	0.18	
At1g17180	Glutathione transferase, putative	−2.03	0.53	−1.26
At1g30510	Ferrodoxin NADP oxidoreductase, putative	−4.32	0.67	
At 2g03980	Putative GDSL-motif lipase/hydrolase	−3.07	1.15	
At5g55050	Putative GDSL-motif lipase/hydrolase	−6.48	2.21	
At1g48850	Chorismate synthase, putative	−3.45	1.54	
At1g64390	Glycosyl hydrolase family 9 (endo-1,4-β-glucanase)	−2.31	0.96	
At5g35190	Extensin-like protein	−2.42	1.02	
At1g48930	Glycosyl hydrolase family 9 (endo-1,4-β-glucanase)	−3.09	1.11	
At1g55120	Glycosyl hydrolase family 32	−4.00	0.95	
At5g11920	Glycosyl hydrolase family 32	−4.44	2.33	
At3g13790	Glycosyl hydrolase family 32	−6.31	1.57	
At4g02890	Polyubiquitin (UBQ14)	−2.54	0.51	−0.95
At4g37390	Auxin-responsive GH3-like protein	−4.82	1.26	
At1g59500	Auxin-regulated protein GH3, putative	−5.51	1.66	

In a previous study (Navazio *et al.*, 2002), it was shown that, in soybean cells, the OG-induced Ca^{2+} transient can be completely abolished by the administration of the protein kinase inhibitor TBB, suggesting that a phosphorylation event is essential for the generation of the Ca^{2+} signal. The current work demonstrates that in *Arabidopsis* as well, the Ca^{2+} transient induced by OGs is completely abolished by TBB pretreatment (Fig. 1). Therefore, using a microarray-based approach with TBB as a diagnostic tool, it was possible to dissect OG-induced signalling into Ca^{2+}-dependent and Ca^{2+}-independent components and to establish the role of OGs in the transcriptional regulation of targets involved in the pathogen response.

Using our significance criteria (see Materials and methods) around 1080 transcripts, or 4% of the *Arabidopsis* transcriptome, were found to change in abundance within 2 h exposure to OGs. Apart from a large number of unknown and hypothetical proteins, this group of genes predominantly encoded stress- and disease-related proteins, signalling components, and transcription factors. Within these groups many transcript levels changed substantially, even after the relatively short period of OG exposure. Transcripts that changed most markedly in abundance included those related to disease resistance proteins, kinases, and cytochrome P450.

Analysed on the basis of sensitivity to inhibitors that abolish the Ca^{2+} transient, specific functional categories were found that appear to be more Ca^{2+} dependent. Although these data do not provide direct evidence for altered protein activity, they may form an indication that specific biochemical functions do require an initial Ca^{2+} signal. First, many of the genes involved in post-pathogen

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Table 5. *Identification of putative regulatory elements*

5′ Upstream sequences of all different categories were queried for overrepresented sequence motifs of 4–8 bases length using the 'Regulatory Sequences Analysis Tools' service at http://rsat.ulb.ac.be/rsat/. The *P* value represents the probability for the number of detected motifs to occur relative to the expected number of occurrences based on the motif distribution in the background dataset which contains all *Arabidopsis* 5′ upstream sequences. A significance cut-off of $P < 10^{-5}$ was used in all analyses. Total number of trancripts queried for each category is given in brackets.

Category	Motif	Occurrence	Expected occurrence	*P*	Name
OGs up-regulated (244)	CAAAA|TTTTG	2631	2417.3	8.70E-06	
OGs down-regulated (169)	AAAAT|ATTTT	2775	2441.87	1.70E-11	
	GAAAA|TTTTC	1783	1540.12	7.20E-10	
	AAAGA|TCTTT	1605	1414	3.20E-07	Dof core ZM
	AAAGT|ACTTT	1105	948.43	3.60E-07	
	CAAAA|TTTTG	1996	1789.62	7.80E-07	
	AATAA|TTATT	1917	1718.17	1.20E-06	
	AGAAA|TTTCT	1762	1585.68	6.60E-06	Pollen1lelat52
	AAAAG|CTTTT	1576	1409.85	6.80E-06	Dof core ZM
	AAAAAT|ATTTTT	1104	940.09	9.80E-08	
OGs up-regulated inhibitor-sensitive (101)	GAAAAAA|TTTTTTC	207	150.91	8.60E-06	
OGs down-regulated inhibitor-insensitive (100)	AAAAT|ATTTT	1747	1475.24	2.40E-12	
	CAAAA|TTTTG	1079	932.5	1.40E-06	
	AAAGT|ACTTT	686	573.93	2.90E-06	Dof core ZM
	AAAAAT|ATTTTT	715	568.14	1.60E-09	
	CAAAAA|TTTTTG	508	417.52	9.60E-06	
Wounding up-regulated (69)	ACACGT|ACGTGT	63	34.57	9.00E-06	ABRElaterd1
Wounding up-regulated inhibitor-sensitive (25)	AAAAT|ATTTT	428	332.33	2.40E-07	
Wounding up-regulated inhibitor-insensitive (44)	ACAGAGG|CCTCTGT	24	5.58	6.40E-09	
	AAGATGAT|ATCATCTT	21	6.32	3.20E-06	I-box
	AACAGAGG|CCTCTGTT	14	3	3.40E-06	
Wounding down-regulated inhibitor-sensitive (13)	AAAG|CTTT	347	267.99	1.80E-06	Dof core ZM
Wounding down-regulated inhibitor-insensitive (19)	AAATTAAT|ATTAATTT	26	9.39	6.10E-06	
Jasmonate down-regulated (18)	ATCCAAGC|GCTTGGAT	7	0.43	3.80E-07	CAAT-Box
	AGATACAT|ATGTATCT	9	1.21	5.30E-06	
	CTTATAAA|TTTATAAG	13	2.71	5.50E-06	
Jasmonate up-regulated inhibitor-sensitive (22)	ACCACCGT|ACGGTGGT	6	0.46	8.50E-06	
	AGTTTTAT|ATAAAACT	16	4.21	9.20E-06	
Jasmonate down-regulated inhibitor-sensitive (12)	ATCCAAGC|GCTTGGAT	6	0.29	6.10E-07	CAAT-Box
	CTTGGA|TCCAAG	20	6.22	8.50E-06	CAAT-Box
	ACATATA|TATATGT	23	7.44	3.60E-06	
Ethylene down-regulated (17)	ATAA|TTAT	540	418.49	4.90E-09	
	TTAA|TTAA	279	213.39	8.90E-06	
	ATAATAT|ATATTAT	41	16.27	1.90E-07	
	AATAA|TTATT	236	170.14	9.40E-07	
Ethylene up-regulated inhibitor-sensitive (7)	AATAA|TTATT	100	56.75	1.20E-07	
Ethylene down-regulated inhibitor-sensitive (4)	GGATAACA|TGTTATCC	4	0.12	7.40E-06	
Ethylene down-regulated inhibitor-insensitive (13)	ATAATAT|ATATTAT	33	12.96	2.20E-06	
	TAATA|TATTA	140	94.41	6.50E-06	
	ATAAT|ATTAT	155	107.59	9.70E-06	
	ATATTATA|TATAATAT	16	4.18	8.30E-06	
	ATAATA|TATTAT	65	36.03	8.70E-06	

attack cell-wall modification, for example, lignin formation, fail to be induced after inhibitor treatment and therefore seem to require an upstream Ca^{2+} signal. The group of genes involved in the biosynthesis of jasmonate (*AOS* and *LOX*) and of C6 volatile compounds showed sensitivity in three out of four. The latter group is known to be involved in many signal pathways, for example, those occurring after wounding (Leon *et al.*, 2001). By contrast, transcriptional regulation of very few genes involved in ethylene synthesis and signalling (*ACS, ACO*) was affected by TBB. This appears to contradict earlier studies invoking Ca^{2+} as an effector of transcript levels of both *ACS* and

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

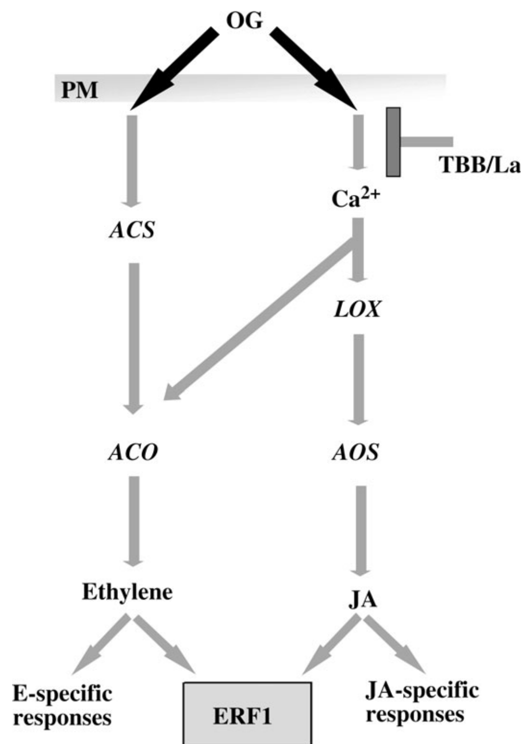

Fig. 3. Diagram depicting transcriptional regulation of genes involved in ethylene and jasmonate pathways during pathogen and wounding response. The formation of OGs triggers a rapid Ca^{2+} transient that can be blocked by the Ca^{2+} channel blocker La^{3+} or the protein kinase inhibitor TBB. Blockage of the Ca^{2+} signal is likely to affect transcriptional regulation of the entire jasmonate biosynthesis pathway and many downstream jasmonate targets, whereas transcriptional regulation of the ethylene pathway only partly relies on the occurrence of an upstream Ca^{2+} transient. Both pathways converge in the transcriptional activation of the ethylene-response factor ERF1. *LOX*: lipoxygenase; *AOS*: allene oxide synthase; JA: jasmonic acid; *ACS*: 1-aminocyclopropane-1-carboxylic acid synthase; *ACO* aminocyclopropane 1-carboxylic acid oxidase.

ACO (Petruzzelli *et al*., 2003). However, the latter study was carried out over a much longer time scale of 8 h and investigated the effect of Ca^{2+} in combination with the presence of ethylene. By contrast, this study points to a minor role of Ca^{2+} during the initial response phase to OGs, during which ethylene is likely to be synthesized.

These results suggest that, in the presence of TBB, OGs are able to bind to their putative receptors and that a substantial part of the OG signal transduction network remains active. However, the absence of a Ca^{2+} signal results in the inactivation of a large part of the ensuing jasmonate-based signalling. The possible role of Ca^{2+} in the induction of jasmonate accumulation in response to OGs was previously highlighted in a study by Hu *et al*. (2003) who, by exposing ginseng cells to $LaCl_3$ and ruthenium red, showed that pretreatment with these Ca^{2+} channel inhibitors largely blocked OG-induced jasmonate biosynthesis.

One of the crucial junctions between the jasmonate and ethylene pathways is formed by transcription factors of the ethylene response factor (ERF) family. At this junction, the jasmonate and ethylene pathways are believed to converge (Fig. 3) and transcriptional activation of ERF1 is a key element in pathogen response signal integration and the regulation of the defence genes (Lorenzo *et al*., 2000). The data show (Table 2) that up-regulation of *ERF1*, and several other *ERF*s, occurs irrespective of Ca^{2+} signal inhibitors. Thus, transcriptional up-regulation of *ERF1 per se* does not appear to require the jasmonate pathway, in contrast to earlier speculation (Lorenzo *et al*., 2003).

TBB is highly specific in its inhibitory action against the casein kinase CK2. Abolition by TBB of the OG-induced Ca^{2+} transient within seconds of the addition of OGs implies that a phosphorylation event forms part of the initial stages of the signalling pathway, possibly soon after binding of OGs with their receptor. Phosphorylation might directly impact on Ca^{2+} signalling, for example, through activation of Ca^{2+} channels. The activity of several animal Ca^{2+} channels has been shown to be increased by phosphorylation and, recently, Kimura and Kubo (2003) reported that the β subunit of a squid plasma membrane Ca^{2+} channel contains a putative CK2 phosphorylation site that leads to channel activation when phosphorylated.

In the 5′ upstream regions of genes that were significantly changed in transcript levels, both known and potentially new promoter *cis*-elements were identified. Several of the identified patterns contain poly-A stretches typical of repetitive, low complexity, sequences and therefore are unlikely to constitute genuine regulatory motifs. Furthermore, none of the previously described motifs could be correlated with TBB-sensitivity and hence with Ca^{2+} dependence. However, putative new elements were identified that only occurred in the TBB-sensitive categories and hence might form transcriptional regulatory domains requiring upstream Ca^{2+} signalling events. At this stage it can only be speculated about the precise role of such motifs, but some of them may be targets of Ca^{2+}-dependent, jasmonate-regulated transcription factors. Direct interaction of transcription factors with calcium/calmodulin comprises another potential mechanism of Ca^{2+}-dependent gene activation.

Supplementary data

Supplementary data can be found at JXB online.

Acknowledgements

Arabidopsis seeds expressing cytoplasmic aequorin were kindly provided by Dr Marc Knight (University of Oxford, UK). We would also like to thank Dr David Ashford (University of York, UK) for his expertise and help in the production of oligogalacturonides, Dr Chiara Romualdi (University of Padua, Italy) for help with statistical analysis of data, and Professor Flavio Meggio for kindly providing the protein kinase inhibitor TBB. Financial support was provided by an award of the Koerber Foundation.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

References

Altamura MM, Zaghi D, Salvi G, De Lorenzo G, Bellincampi D. 1998. Oligogalacturonides stimulate pericycle cell wall thickening and cell divisions leading to stoma formation in tobacco leaf explants. *Planta* **204,** 429–436.

Bellincampi D, Cardarelli M, Zaghi D, Serino G, Salvi G, Gatz C, Cervone F, Altamura MM, Constantino P, De Lorenzo G. 1996. Oligogalacturonides prevent rhizogenesis in rolB transformed tobacco explants by inhibiting auxin-induced expression of the *rolB* gene. *The Plant Cell* **8,** 477–487.

Breitling R, Armengaud P, Amtmann A, Herzyk P. 2004. Rank products: a simple, yet powerful, new method to detect differentially regulated genes in replicated microarray experiments. *FEBS Letters* **573,** 83–92.

Brini M, Marsault R, Bastianuto C, Alvarez J, Pozzan T, Rizzuto R. 1995. Transfected aequorin in the measurament of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$). *Journal of Biological Chemistry* **270,** 9896–9903.

Cardinale F, Jonak C, Ligterink W, Niehaus K, Boller T, Hirt H. 2000. Differential activation of four specific MAPK pathways by distinct elicitors. *Journal of Biological Chemistry* **275,** 36734–36740.

Cessna SG, Low SL. 2001. An apoplastic Ca^{2+} sensor regulates internal Ca^{2+} release in aequorin-transformed tobacco cells. *Journal of Biological Chemistry* **276,** 10655–10662.

Chandra S, Stennis M, Low PS. 1997. Measurement of Ca^{2+} fluxes during elicitation of the oxidative burst in aequorin-transformed tobacco cells. *Journal of Biological Chemistry* **272,** 28274–28280.

Cheong YH, Chang H-S, Gupta R, Wang X, Zhu T, Luan S. 2002. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress and hormonal responses in *Arabidopsis. Plant Physiology* **129,** 661–667.

Côté F, Hahn MG. 1994. Oligosaccharins: structures and signal transduction. *Plant Molecular Biology* **26,** 1379–1411.

Darvill A, Augur C, Bergmann C, *et al.* 1992. Oligosaccharins-oligosaccharides that regulate growth, development and defence responses in plants. *Glycobiology* **2,** 181–198.

Doares SH, Syrovets T, Weiler EW, Ryan C. 1995. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proceedings of the National Academy of Sciences, USA* **92,** 4095–4098.

Felix G, Regenass M, Boller T. 1993. Specific perception of subnanomolar concentrations of chitin fragments by tomato cells: induction of extracellular alkalinization, changes in protein phosphorylation, and establishment of a refractory state. *The Plant Journal* **4,** 307–316.

Harvey DJ. 1993. Quantitative aspects of the matrix-assisted laser-desorption mass-spectrometry of complex oligosaccharides. *Rapid Communications in Mass Spectrometry* **7,** 614–619.

Hu X, Neill S, Cai W, Tang Z. 2003. Hydrogen peroxide and jasmonic acid mediate oligogalacturonic acid-induced saponin accumulation in suspension-cultured cells of *Panax ginseng. Physiologia Plantarum* **118,** 414–421.

John M, Röhring H, Schmidt J, Walden R, Schell J. 1997. Cell signalling by oligosaccharides. *Trends in Plant Science* **2,** 111–115.

Kimura T, Kubo T. 2003. Cloning and functional characterization of squid voltage-dependent Ca^{2+} channel β subunits: involvement of N-terminal sequences in differential modulation of current. *Neuroscience Research* **46,** 105–117.

Lamb C, Dixon RA. 1997. The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology* **48,** 251–275.

Lebrun-Garcia A, Ouked F, Chiltz A, Pugin A. 1998. Activation of MAPK homologues by elicitors in tobacco cells. *The Plant Journal* **15,** 773–781.

Leon J, Rojo E, Sánchez-Serrano JJ. 2001. Wound signalling in plants. *Journal of Experimental Botany* **52,** 1–9.

Lijavetzky D, Carbonero P, Vicente-Carbajosa J. 2003. Genome-wide comparative phylogenetic analysis of the rice and *Arabidopsis* Dof gene families. *BMC Evolutionary Biology* **3,** 17.

Liners F, Thibault J-F, Van Cutsem P. 1992. Influence of the degree of polymerization of oligogalacturonates and of esterification pattern of pectin on their recognition by monoclonal antibodies. *Plant Physiology* **99,** 1099–1104.

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R. 2003. Ethylene response factor1 integrates signals from ethylene and jasmonate pathways in plant defence. *The Plant Cell* **15,** 165–178.

Messian J, Van Cutsem P. 1994. Pectic signal transduction in carrot cells: membrane, cytosolic and nuclear responses induced by oligogalacturonides. *Plant Cell Physiology* **35,** 677–689.

Miles PW. 1999. Aphid saliva. *Biological Reviews* **74,** 41–85.

Moloshok T, Pearce G, Ryan CA. 1992. Oligouronide signaling of proteinase inhibitor genes in plants: structure-activity relationships of di- and trigalacturonic acids and their derivatives. *Archives of Biochemistry and Biophysics* **294,** 731–734.

Navazio L, Moscatiello R, Bellincampi D, Baldan B, Meggio F, Brini M, Bowler C, Mariani P. 2002. The role of calcium in oligogalacturonide-activated signalling in soybean cells. *Planta* **215,** 596–605.

Norman C, Vidal S, Palva ET. 1999. Oligogalacturonide-mediated induction of a gene involved in jasmonic acid synthesis in response to the cell-wall-degrading enzymes of the plant pathogen *Erwinia carotovora. Molecular Plant–Microbe Interactions* **12,** 640–644.

Penninckx IAMA, Thomma BPHJ, Buchala A, Métraux J-P, Broekaert WF. 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis. The Plant Cell* **10,** 2103–2113.

Petruzzelli L, Sturaro M, Mainieri D, Leubner-Metzger D. 2003. Calcium requirement for ethylene-dependent responses involving 1-aminocyclopropane-1-carboxylic acid oxidase in radicle tissues of germinated pea seeds. *Plant, Cell and Environment* **26,** 661–671.

Ridley BL, O'Neill MA, Mohnen D. 2001. Pectins: structure, biosynthesis, and oligogalacturonides-related signaling. *Phytochemistry* **57,** 929–967.

Rodriguez PL. 1998. Protein phosphatase 2C (PP2C) function in higher plants. *Plant Molecular Biology* **38,** 919–927.

Sanders D, Pelloux J, Brownlee C, Harper JF. 2002. Calcium at the crossroads of signalling. *The Plant Cell* **14,** S401–S407.

Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM. 2000. Coordinated plant defence responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Sciences, USA* **97,** 11655–11660.

Simpson SD, Ashford DA, Harvey DJ, Bowles DJ. 1998. Short chain oligogalacturonides induce ethylene production and expression of the gene encoding aminocyclopropane 1-carboxylic acid oxidase in tomato plants. *Glycobiology* **8,** 579–583.

Spiro MD, Kates KA, Koller AL, O'Neill MA, Albersheim P, Darvill A. 1993. Purification and characterization of biologically active 1,4-linked α-D-oligogalacturonic acids after partial digestion of polygalacturonic acid with endopolygalacturonase. *Carbohydrate Research* **247,** 9–20.

Tena G, Asai T, Chiu W-L, Sheen J. 2001. Plant mitogen-activated protein kinase signaling cascades. *Current Opinion in Plant Biology* **4,** 392–400.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018

Van Cutsem P, Messiaen J. 1994. Biological effects of pectic fragments in plant cells. *Acta Botanica Neerlandica* **43,** 231–245.

Van den Hoogen BM, van Weeren PR, Lopes-Cardoso M, van Golde LMG, Barneveld A, van de Lest CHA. 1998. A microtiter plate assay for the determination of uronic acids. *Analytical Biochemistry* **257,** 107–111.

van Helden J, André B, Collado-Vides J. 1998. Extracting regulatory sites from the upstream region of yeast genes by computational analysis of oligonucleotide frequencies. *Journal of Molecular Biology* **281,** 827–842.

Van Zhong G, Burns JK. 2003. Profiling ethylene-regulated gene expression in *Arabidopsis thaliana* by microarray analysis. *Plant Molecular Biology* **53,** 117–131.

Weber J, Olsen O, Wegener C, von Wettstein D. 1996. Digalacturonates from pectin degradation induce tissue responses against potato soft rot. *Physiological and Molecular Plant Pathology* **48,** 389–401.

Wegener C, Bartling S, Olsen O, Weber J, von Wettstein D. 1996. Pectate lyase in transgenic potatoes confers preactivation of defence against *Erwinia carotovora. Physiological and Molecular Plant Pathology* **49,** 359–376.

Xu Y, Chang P-FL, Liu D, Narasimhan ML, Raghothama KG, Hasegawa PM, Bressan RA. 1994. Plant defence genes are synergistically induced by ethylene and methyl jasmonate. *The Plant Cell* **6,** 1077–1085.

Downloaded from https://academic.oup.com/jxb/article-abstract/57/11/2847/680885
by guest
on 28 July 2018