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Oligogalacturonide-induced changes in the nuclear proteome of *Arabidopsis thaliana*

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Abstract

Oligogalacturonides (OGs), derived from the degradation of the plant cell wall homogalacturonan, are elicitors of plant defence responses. A proteomic study of the nuclear compartment was carried out in *Arabidopsis thaliana* seedlings in order to identify proteins whose abundance varied in response to OGs. A nuclear protein map was obtained and 72 spots corresponding to 58 different proteins were identified by MALDI-ToF mass spectrometry. OG-induced changes in protein spot abundance were studied by two-dimensional difference in gel electrophoresis (2D-DIGE). Significant changes in protein abundance were observed for 19 protein spots. Proteins responding to the OG treatment were mainly involved in the protein translation machinery and regulation suggesting a general reprogramming of the plant cell metabolism in response to OGs.

Keywords: Oligogalacturonide; *Arabidopsis thaliana*; DIGE; Proteomics

1. Introduction

Plants are normally exposed to the attack of a large variety of microorganisms, such as fungi, bacteria, and viruses. In order to resist, they have developed perception systems of microbe-derived as well as plant-derived signal molecules produced during the infection. These molecules act as elicitors of plant defence responses [1] and trigger a complex network of intra- and intercellular signalling cascades leading to large changes in gene activity and, subsequently, to massive reprogramming of cell metabolism [2,3].

To fully understand the events that follow elicitor recognition, we need studies of protein expression that take into account relative abundances, sub-cellular localization, profiling of variants generated by mRNA splicing and post-translational modifications. Moreover, the identification of proteins recruited to fulfil specific functions in sub-cellular compartments is expected to give an additional dimension to the proteome analysis. Much of this information can be derived from analysis of sub-cellular structures using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) in combination with mass spectrometric identification.

(1 → 4)-Linked α-D-oligogalacturonides (OGs) are non-specific elicitors that are produced by the enzymatic action of microbial polygalacturonases on the pectic component of the plant cell wall. They are perceived by plants as indicators of the presence of pathogens [4]. Previous studies showed that OGs induce early events including protein phosphorylation and activation of mitogen-activated protein kinases (MAPKs) [5], activation of ion fluxes and membrane depolarization with H^+ influx and K^+ efflux, production of active oxygen species (H_2O_2, and O_2^-) [6,7], synthesis of phytoalexins [8] and transcriptional activation of defense genes [9]. OGs also influence both Ca^{2+} influx and efflux and the activity of a plasma membrane Ca^{2+}-ATPase involved in the oxidative burst [10].

OGs are not only involved in defence but also in plant growth and development. They inhibit auxin-induced stem elongation [11] and rhizogenesis [12], induce the formation of flowers [13], stimulate cell divisions leading to stoma formation [14], and

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inhibit the expression of late auxin-responsive genes [15]. Little is known, however, about the events of the OG signal transfer to the plant cell nucleus or the interaction of nuclear factors with regulatory elements that modulate the transcription rates of response genes.

In this paper we have carried out a DIGE-based proteomic study of the changes occurring in the nuclear proteome of *Arabidopsis thaliana* in response to OGs.

2. Materials and methods

2.1. Plant growth and OG treatment

Arabidopsis seedlings (ecotype Columbia, Col-0) were grown for 10 days in a growth room at 21 °C with a 16/8 light/dark cycle. For treatment with oligogalacturonides (OGs), 10 plates containing 10-day-old seedlings were treated with 100 μg/mL OGs by adding 20 μL of a 5 mg/mL sterile stock solution to the medium. The OGs were prepared according to Bellincampi et al. [12] and have a DP ranging between 9 and 18. Ten control plates containing 10-day-old seedlings were prepared by adding 20 μL of sterile water to the medium. Seedlings were incubated for 1 h and 30 min at 21 °C in constant light, harvested and gently blotted on Whatman paper to remove residual medium. The plant material was frozen in liquid nitrogen and stored at −80 °C.

2.2. Preparation of nuclear proteins

Nuclei were prepared according to the method described by Hamilton et al. [16] with some modifications by Folta and Kaufman [17]. All steps were performed on ice. About 5 g of 10-day-old *Arabidopsis* seedlings were homogenized using a Polytron tissue homogenizer in 3 volumes of extraction buffer (1.0 M sucrose, 10 mM Tris–HCl (pH 7.2), 5 mM $MgCl_2$, 5 mM 2-β-mercaptoethanol, 20 mM PMSF (phenylmethylsulphonylfluoride)) then filtered twice through a double layer of Miracloth. The filtrate was centrifuged at 8800 × *g* for 15 min at 4 °C; the pellet was resuspended in 2.5 mL of extraction buffer containing 0.5% (v/v) Triton X-100 to lyse organellar membranes, then centrifuged at 6400 × *g* for 10 min. Each pellet was resuspended in 1 mL of extraction buffer containing 0.5% (v/v) Triton X-100 then centrifuged at 4000 × *g* for 10 min. Finally the pellet was resuspended in 1 mL of extraction buffer containing 0.5% (v/v) Triton X-100. Crude nuclei were layered onto a discontinuous percoll gradient in 50 mL Falcon tubes containing from the bottom upward 16 mL 80% (v/v) and 16 mL 35% (v/v) percoll A in extraction buffer. Percoll A solution contained 34% (w/v) sucrose, 10 mM Tris–HCl (pH 7.2), 5 mM $MgCl_2$, 5 mM 2-β-mercaptoethanol and Percoll. The gradient was centrifuged by two-step centrifugation (400 × *g* for 5 min and 6500 × *g* for 45 min). The bulk of the nuclei localized at the 35–80% interface. This zone was collected, diluted in 5–10 volumes of extraction buffer and pelleted by centrifugation at 6500 × *g* for 10 min. Nuclear proteins were prepared using TRI Reagent (Sigma, T9424, Tri Reagent) according to the manufacturer's instructions. In order to obtain an amount of proteins suitable for the DIGE analysis, for each sample three independent nuclear protein extracts were pooled.

2.3. Fluorescence microscopy

Nuclei enriched fractions were treated with DAPI (4′,6-diamidino-2-phenylindolecloridrato, 10 μg/mL) and visualized by fluorescence microscopy using a N-400LD Optika microscope.

2.4. Glucose 6-P dehydrogenase activity (G6PDH)

The activity of G6PDH, a cytosolic protein marker, was assayed from the supernatants and the nuclei enriched fractions. Samples were added to 100 mM Gly-NaOH (pH 8.0) containing 0.2 mM $NADP^+$, 1 mM glucose 6-P and 1 mM $MgCl_2$. G6PDH activity was calculated by observing the absorbance at 340 nm for 5 min.

2.5. Protein quantification

Proteins were quantified using the "PlusOne 2D Quant" (GE Healthcare, 80-6485-56) according to the manufacturer's instructions.

2.6. Western blotting

For immunoblotting, proteins were separated by sodium dodecyl sulphate (SDS) poly-acrylamide gel electrophoresis (PAGE) on 12% (v/v) acrylamide gels and then electrotransferred onto a nitrocellulose membrane. The gel was loaded with 10 μg of proteins for samples loaded in lanes 1, 2, 3 and 1 μg in the case of nuclear proteins (line 4).

The membrane was incubated overnight with a 1:500 dilution of primary antibody anti-TATA binding protein [18] (kindly given by Dr. Claus Schwechheimer). The membrane was then washed and incubated with a 1:5000 dilution of secondary antibody, anti-rabbit IgG conjugated to horse radish peroxidase (HRP). Detection of antibody binding was performed by the enhanced chemiluminescent method according to the manufacturer's instructions (ECL Western blotting kit, GE Healthcare).

2.7. Differential in-gel electrophoresis (DIGE)

The DIGE analysis was performed using samples obtained from three independent experiments (two gels for each experiment) according to the scheme in Table 1.

Samples were labelled using the fluorescent cyanine dyes developed for DIGE (GE Healthcare) according to the manufacturer's instructions. The 20 μg of protein were labelled with 200 pmol of amine reactive cyanine dyes, freshly dissolved in anhydrous dimethyl formamide. The labelling reaction was incubated at room temperature in the dark for 30 min and the reaction was terminated by addition of 10 nmol lysine. An equal volume of sample buffer (AUT (2% (v/v) ASB14, 7 M Urea, 2 M Thiourea), DTT 130 mM, 2% (v/v) of IPG buffer pH 4-7) was added to each of the labelled protein samples, and then the three

Table 1
Experimental design of the labelling of samples for the DIGE

	Exp 1 C1T1	Exp 2 C2T2	Exp 3 C3T3	Exp1 C1T1 duplicate	Exp 2 C2T2 duplicate	Exp 3 C3T3 duplicate
Control	Cy3	Cy5	Cy3	Cy5	Cy3	Cy5
OG-treated	Cy5	Cy3	Cy5	Cy3	Cy5	Cy3
IS	Cy2	Cy2	Cy2	Cy2	Cy2	Cy2

IS, internal standard, a pool of all the samples (C1 + C2 + C3 + T1 + T2 + T3).

samples were mixed. Immobilized pH gradient strips (13 cm, pH 4–7 linear) were rehydrated for 12 h with a rehydration solution (AUT, 12 μL/mL DESTREAK, 1% (v/v) IPG buffer pH 4-7 and a pinch of bromophenol blue). IEF was performed for a total of 30,000 voltage hours (Vhr) on the IPGphor system (Amersham Biosciences). Focused strips where then transferred to equilibration buffer (containing 50 mM Tris–HCl pH 8.6, 6 M urea, 30% (v/v) glycerol and 2% (w/v) SDS). IPG strips were placed in the slots of the Immobiline DryStrip Reswelling Tray and equilibrated for 15 min in 2 mL of equilibration buffer containing 40 mg dithioerythritol (DTE), then for 15 min in 2 mL of equilibration buffer containing 100 mg iodoacetamide and a pinch of bromophenol blue. For protein separation in the second dimension, 12% (w/v) SDS poly-acrylamide gels were used. The gels were run at 20 mA for 15 min and then at 40 mA until the bromophenol blue dye front had run off the bottom of the gel. Labelled proteins were visualized using a Typhoon™ 9410 imager (GE Healthcare). The Cy3 images were scanned using a 532 nm laser and a 580 nm band pass (BP) 30 emission filter. Cy5 images were scanned using a 633 nm laser and a 670 nm BP30 emission filter. Cy2 images were scanned using a 488 nm laser and an emission filter of 520 nm BP40. All gels were scanned at 100 mm resolution. The PMT was set to ensure a maximum pixel intensity of between 40,000 and 60,000 pixels. Gel analysis was performed using DeCyder BVA V 6.5 (GE Healthcare), a 2DE analysis software package designed specifically to be used for DIGE, according to manufacturer's instructions. Protein spots showing a significant Student's *t*-test ($p < 0.05$) were considered differentially expressed between control and treated samples.

2.8. Protein identification by MALDI-ToF mass spectrometry

Protein spots were excised from the gel and then in-gel digestion with trypsin was performed using the Montage In-Gel Digest ZipPlate Kit (Millipore, Bedford, MA, USA) according to the manufacturer's instructions. After trypsin digestion, peptides were directly spotted from the ZipPlate onto a MALDI target plate using 1 μL of a 3% solution of matrix (α-ciano-4-hydroxycinnamic acid) in 50% CH_3CN, 0.1% TFA, air dried and analyzed by MALDI-ToF mass spectrometry (Voyager DE-STR Applied Biosystems, MA). The MALDI was operated in positive reflector mode; the spectra were internally calibrated using trypsin autoproteolysis products. Contaminant peaks (peaks from contaminating human keratin or from the trypsin added for digestion of proteins, and peaks present in all mass spectra) were identified and eliminated from the MS spectra using the software PeakErazor (http://www.welcome.to/GPMAW). Protein identification was finally performed entering the mass list into the Mascot PMF database (MASCOT Peptide Mass Fingerprint, http://www.matrixscience.com). Searches were carried out against the MSDB database using the following parameters: one allowed missed cleavage; carbamidomethyl cysteine as fixed modification and oxidation of methionine as variable modification; mass tolerance 10–50 ppm.

3. Results and discussion

Nuclei were isolated from homogenates of *Arabidopsis* seedlings using percoll density gradient centrifugation. The integrity of the isolated nuclei was analyzed by DAPI staining (data not shown). Absence of activity of glucose 6-P dehydrogenase (G6PDH), an abundant cytosolic enzyme often used as a good marker of contamination, indicated no significant contamination of the nuclear preparation by cytosolic components (data not shown). The enrichment of nuclear proteins was evaluated by Western blot analysis using an antibody against an *Arabidopsis* TATA binding protein [18]. This nuclear protein was found in the total extract and in the nuclear fraction, but not in the cytosolic fractions obtained during sequential centrifugation (Fig. 1).

Isolated nuclear proteins were subjected to 2D-PAGE, protein spots were cut out from Coomassie and silver stained gels and analyzed by MALDI-ToF mass spectrometry. In our reference gel a total number of 203 spots were detected. The number of spots resolved is lower than that reported for nuclear proteins from *Arabidopsis* in a previous proteomic study [19]. This difference is probably due to the multiple pH ranges used by Bae et al. [19] for IEF. However, several proteins were identified in both studies, three of which (the precursor of the elongation factor tu, a ribosomal protein and a putative lectin) significantly responded to cold and OG treatment as discussed later.

Seventy-two spots, corresponding to 58 different proteins, were successfully identified (Table S1).

The DIGE approach was used to detect changes on protein abundance after the OG treatment. Changes in the transcriptome of *Arabidopsis* seedlings in response to OGs occur mainly

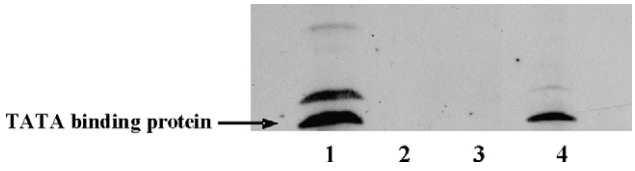

Fig. 1. Western blot analysis using an anti-TATA binding protein antibody. (1) Total protein extract (10 μg); (2 and 3) cytosolic fractions obtained during sequential centrifugation (10 μg); (4) nuclear fraction from percoll density gradient centrifugation (1 μg).

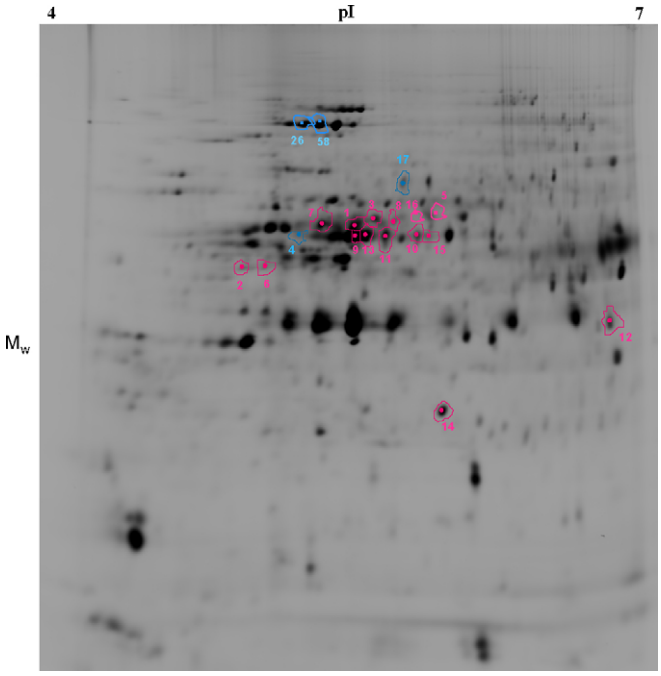

Fig. 2. 2D map of nuclear proteins. Highlighted in red are the repressed proteins, in blue the induced proteins.

after 1 h of treatment [20]. In this study we chose a treatment time of 1.5 h to analyse changes in the nuclear proteins. The analysis was performed using samples obtained from independent experiments (two gels for each experiment) according to the scheme shown in Table 1 (see Section 2). Proteins extracted from OG-treated and control tissues were labelled with Cy5 and Cy3, respectively, in one gel, and with inverted fluorophores in the duplicate gel. After 2D-PAGE, the image of the gel map was visualized and analyzed using DeCyder software (Fig. 2). The analysis was carried out using the BVA module (Biological Variation Analysis), which includes the use of an internal standard consisting of a pool of all samples analyzed, labelled with Cy2. The internal standard was run in each gel together with the Cy3 and Cy5 labelled samples. BVA analysis generates a value indicating protein variation level paired with the *p* value of a *t*-test used to determine statistical significance of protein abundance variation between control and treated samples.

Nineteen spots corresponding to proteins regulated by OGs were detected: 4 were up-regulated, whereas 15 were less abundant upon OG treatment (Table 2). Spots corresponding to differentially expressed proteins were recovered from a preparative gel stained with Coomassie and matched with the DIGE protein map, digested with trypsin and analyzed by MALDI-ToF mass spectrometry. Table 2 shows the sub-cellular localization predicted from the sequence analysis and the experimental evidences of nuclear localization based on data available at the SUBA database [21].

Among the proteins less abundant in OG-treated plants we found the eukaryotic translation initiation factor 4A-1 (eIF4A-1). This is a nuclear protein with a DEAD/DEAH box helicase domain and an ATP-dependent helicase activity. eIF4A participates in ATP-dependent unwinding of the mRNA [22] and was recently reported to be associated with a cyclin-dependent kinase (CDKA) during cell proliferation [23]. Results from Hutchins and co-authors suggest that eIF4A may be part of a protein complex that targets CDKA to the translation machinery, putatively involved in the regulation of translation during plant cell growth and development. In our study the abundance of two different spots (1 and 7), corresponding to eIF4A at two different isoelectric points, significantly decreased after OG treatment (Fig. 3). Since eIF4A is phosphorylated in response to various stimuli [24] and *S*-nytrosilation has been demonstrated for this protein [25], these post-translational modifications might explain eIF4A multispots. Incomplete sequence coverage did not allow to identify post-translationally modified peptides in our spectra. Global transcript analysis shows that eIF4A transcripts do not significantly change in *Arabidopsis* seedling treated with OGs (Table 2).

Changes in abundance of nuclear proteins in response to OGs may arise not only from enhanced or reduced expression but also from post-translational modifications, degradation and/or translocation of proteins to different compartments. For example, degradation of the phosphorylated form of transcription factor SLR1 occurs after GA application [26], and the elicitor-responsive MAP (ERM) kinase is translocated into the nucleus very rapidly within 5 min after elicitor application to parsley cells [27]. Similarly, in *Arabidopsis* plants exposed to ozone, the generation of ROS in the apoplast causes a translocation of AtMPK3 and AtMPK6 to the nucleus 30 min after treatment [28]. In another case the protein NPR1, that regulates transcription of SAR-related genes, is sequestered in the cytoplasm of non-induced cells as an inactive oligomer, and is found in the nucleus as a monomeric form after 24 h [29].

A precursor of the elongation factor tu (EF-tu), which has a predicted chloroplastic localization but is often found in the nuclear proteome [19,30,31], was found to respond to OGs. This factor may be involved in protein synthesis and may promote GTP-dependent binding of aminoacyl-tRNA with the site A of ribosomes. EF-tu was identified in multiple spots, probably corresponding to post-translational modifications of the same gene product. Spots 2, 6, 9, 10, 13 and 15 were all down-regulated upon OG treatment (Fig. 3). Spots 2 and 6 also displayed a lower apparent molecular mass compared to the other spots, possibly due to a truncated form of the peptide. However it was not possible to obtain a total sequence coverage for any of the spots from the mass spectra.

Furthermore, a ribosomal protein S5 involved in protein synthesis, representing one of the constituents of ribosome 30*S* subunit and previously identified among the nuclear proteome of cold-stressed plants [30], is repressed upon OG treatment. Finally, three different isoforms of *S*-adenosilmethionine synthase (SAM) are down-regulated by OGs. These enzymes are involved in DNA methylation and in methylation at 5′ of mRNAs; they are also involved in the biosynthesis of polyamines, lignin and ethylene. These SAM synthases were also found in the *Arabidopsis* nuclear matrix and nucleolar proteome [31,32].

Among the induced proteins we found a putative lectin, which has been identified among the induced proteins in the

Table 2
Protein spots differentially expressed upon OG treatment

Spot no.	Acc. number	Protein name	DIGE data OG 1.5 h		Microarray data OG 1 h		Microarray data OG 3 h		SUBA	
			Ratio	*p*	Fold change	*p*	Fold change	*p*	Sub-cellular localization	Organelle targeting prediction
3	At1g02500	*S*-adenosylmethionine synthetase 1	−1.2	0.0042	1.1	0.07577	1.3	0.00221	Nucleus (mass spec)	Cytosol/peroxisome
16	At1g02500	*S*-adenosylmethionine synthetase 1	−1.33	0.043	1.1	0.07577	1.3	0.00221	Nucleus (mass spec)	Cytosol/peroxisome
12	At2g33800	30*S* ribosomal protein S5	−1.44	0.032	−1.0	0.25403	−1.2	0.00008	Nucleus (mass spec), plastid (mass spec)	Chloroplastm/mitochondrion
1	At3g13920	Eukaryotic translation initiation factor 4A-1 (eIF4A-1)	−1.17	0.00031	1.1	0.0237	1.0	0.35804	Nucleus (mass spec)	Nucleus
7	At3g13920	Eukaryotic translation initiation factor 4A-1 (eIF4A-1)	−1.29	0.01	1.1	0.0237	1.0	0.35804	Nucleus (mass spec)	Nucleus
26	At3g16460	Putative lectin	1.45	0.03	1.0	0.80211	−1.0	0.84906	Nucleus (mass spec), peroxisome (GFP)	Cytosol/chloroplast
58	At3g16460	Putative lectin	1.48	0.0072	1.0	0.80211	−1.0	0.84906	Nucleus (mass spec), Peroxisome (GFP)	Cytosol/chloroplast
11	At3g17390	*S*-adenosylmethionine synthetase, putative similar to S-adenosylmethionine synthetase 2	−1.31	0.022	1.6	1.73E−14	1.3	0.00713	Nucleus (mass spec), Plasma Membrane (mass spec)	Cytosol
14	At3g52150	RNA recognition motif (RRM)-containing protein similar to 33 kDa ribonucleoprotein	−1.46	0.035	−1.0	0.46378	−1.3	4.29639E−06	Plastid (mass spec)	Cytosol/chloroplast/ mitochondrion
5	At4g01850	*S*-adenosylmethionine synthetase 2	−1.2	0.0071	1.1	0.35119	1.2	0.00425	Nucleus (mass spec, GFP)	Cytosol/peroxisome
2	At4g20360	Elongation factor tu, chloroplast precursor (EF-tu)	−1.29	0.0033	−1.0	0.72537	−1.1	0.09477	Nucleus (mass spec), Plastid (mass spec, SwissProt)	Chloroplast/mitochondrion
6	At4g20360	Elongation factor tu, chloroplast precursor (EF-tu)	−1.23	0.0075	−1.0	0.72537	−1.1	0.09477	Nucleus (mass spec), Plastid (mass spec, SwissProt)	Chloroplast/mitochondrion
9	At4g20360	Elongation factor tu, chloroplast precursor (EF-tu)	−1.38	0.016	−1.0	0.72537	−1.1	0.09477	Nucleus (mass spec), Plastid (mass spec, SwissProt)	Chloroplast/mitochondrion
10	At4g20360	Elongation factor tu, chloroplast precursor (EF-tu)	−1.22	0.018	−1.0	0.72537	−1.1	0.09477	Nucleus (mass spec), Plastid (mass spec, SwissProt)	Chloroplast/mitochondrion
13	At4g20360	Elongation factor tu, chloroplast precursor (EF-tu)	−1.41	0.032	−1.0	0.72537	−1.1	0.09477	Nucleus (mass spec), Plastid (mass spec, SwissProt)	Chloroplast/mitochondrion
15	At4g20360	Elongation factor tu, chloroplast precursor (EF-tu)	−1.23	0.036	−1.0	0.72537	−1.1	0.09477	Nucleus (mass spec), Plastid (mass spec, SwissProt)	Chloroplast/mitochondrion
4		Not identified	1.71	0.0067						
8		Not identified	−1.2	0.012						
17		Not identified	1.27	0.043						

Spot no., numbers correspond to spots shown in Fig. 2; acc. number, accession number; protein name, entry name according to the NCBI database; ratio, indicates the standardized protein abundance ratio between corresponding control and OG-treated spots. Positive and negative values are indicated for increases and decreases in expression, respectively; *p*-value: the Student's *t*-test *p*-value represents the probability of obtaining the observed ratio if control and OG-treated spots have the same protein abundance; microarray data: fold change and statistical significance ($p < 0.01$) of transcripts corresponding to the identified proteins; SUBA (the *Arabidopsis* sub-cellular database, Heazlewood et al. [21]): sub-cellular localization, obtained from experimental evidences as reported in the SUBA database; organelle targeting prediction, obtained from prediction software in the SUBA database.

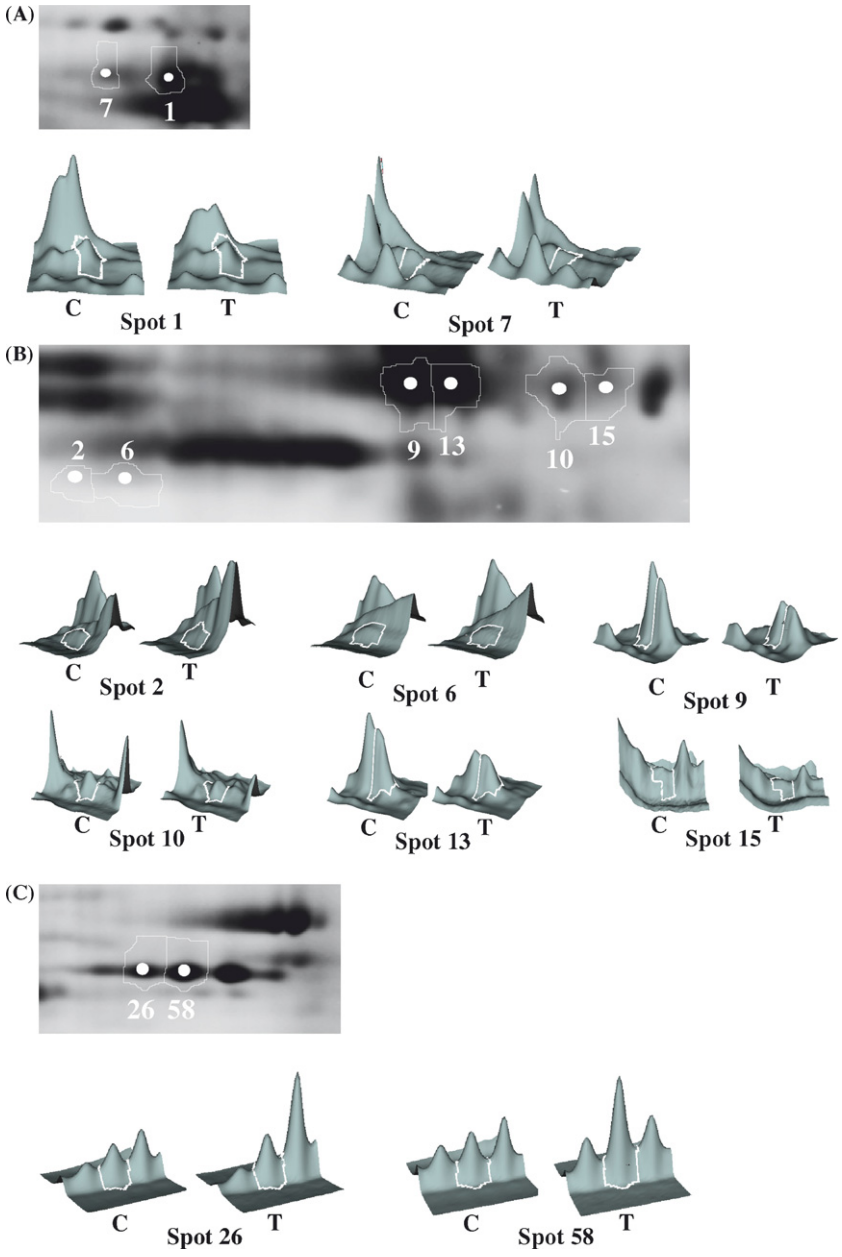

Fig. 3. 2D spots and corresponding peaks in control (C) and OG-treated (T) samples of eIF4A-1 (A), EF-tu (B) and the putative lectin (C). Spots corresponding to eIF4A-1 (1 and 7) and to EF-tu (2, 6, 9, 10, 13 and 15) significantly decreased in abundance after the OG treatment, whereas spots 26 and 58, corresponding to the putative lectin, increased in abundance after the treatment.

nuclear proteome of cold stressed *Arabidopsis* plants [30]. Lectins are carbohydrate-binding proteins, many of which have insecticidal activities [32]. This protein is also referred to as myrosinase-binding protein (MBP) [33], potentially involved in the myrosinase-glucosinolate system. Glucosinolates are well characterized defense compounds that upon tissue damage are hydrolyzed by specific thioglucosidases called myrosinases, releasing an array of toxic compounds, such as isothiocyanates, thiocyanates, nitriles, or epithionitriles [34]. Myrosinases and MBPs are physically separated and can only form complexes after tissue disruption [35]. Some MBPs are induced by jasmonic acid [36], and levels of specific indole glucosinolates can also increase upon treatment with non-specific elicitors, MeJA, or salicylic acid [37,38].

In conclusion, our data indicate that OGs repress the synthesis of proteins likely required for translation, such as an initiation factor, an elongation factor and ribosomal proteins, suggesting a general down-regulation of the plant protein biosynthetic machinery. We believe that OG signalling may initiate a general reprogramming of the cell from basal metabolism to defense, in order to prevent the spread of pathogens beyond the infection site. Global transcript profiling shows a dramatic change in gene expression profiles occurring in *Arabidopsis* seedlings treated with these elicitors [20] and also suggests that primary metabolism is “switched off” upon OG per-

ception, while secondary metabolism is "switched on" and many genes involved in protein trafficking and secretion are induced. During pathogen infection, it is likely that OGs do not block protein synthesis during the early stage of the response or in non-infected leaf tissues. Under these conditions, plants thus can retain the full capacity of responding to an attempted invasion by the rapid induction of defense proteins. In long-term stressed tissues, however, OGs may mediate the general down-regulation of the protein biosynthetic machinery.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2007.07.007.

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