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A proteomic analysis of *Arabidopsis thaliana* seedling responses to 3-oxo-octanoyl-homoserine lactone, a bacterial quorum-sensing signal

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ABSTRACT

N-acyl-homoserine lactones (AHLs) are a class of bacterial quorum-sensing (QS) signals that are commonly used by Gram-negative bacteria for cell-to-cell communication. Recently, it has become evident that AHLs can regulate plant root growth and trigger plant defense responses; however, little is known about the plant response mechanisms to bacterial QS signals. In this study, we used a proteomic approach to investigate the responses of *Arabidopsis thaliana* seedlings to *N*-3-oxo-octanoyl-homoserine lactone (3OC8-HSL), a bacterial QS signal. The results revealed that the abundance of 53 protein spots was significantly altered; two thirds of these proteins were found to be up-regulated after 3OC8-HSL treatment. Thirty-four proteins were identified using MALDI-TOF-MS. These 3OC8-HSL-responsive proteins, in addition to one protein of unknown function, are implicated in a variety of physiological processes, including metabolism of carbohydrate and energy, protein biosynthesis and quality control systems, defense response and signal transduction and cytoskeleton remodeling. Our bioinformatic analysis indicated that the chloroplasts are the intracellular organelles most influenced by the exposure to 3OC8-HSL. Our data indicate that plants have an extensive range of functional responses to bacterial AHLs that may play important roles in the interaction between plants and bacteria.

1. Introduction

It is important for plant hosts to detect the presence of bacteria quickly and reliably to respond appropriately to pathogens or symbionts. Many Gram-negative bacteria are known to utilize *N*-acyl-homoserine lactone (AHL) signals to monitor their local population and coordinately activate gene expression during host infect, a process that is commonly referred to as quorum sensing (QS) [1–3]. Bacterial QS is involved in many physiological processes [4,5], and, in recent years, accumulating evidence demonstrates that plants are able to sense and respond to bacterial AHLs. Mathesius et al. [6] showed that the model legume *Medicago truncatula* responds to two different types of AHLs, *N*-3-oxo-dodecanoyl-homoserine lactone (3OC12-HSL) and *N*-3-oxo-(tetrahydro-2-oxo-3-furanyl)-hexadecenamide (3OC16:1-HSL), and that low concentrations of these compounds elicit major changes in protein expression. Microarray experiments have revealed that *N*-hexanoyl-homoserine lactone (C6-HSL) induces the systemic expression of salicylic acid (SA)- and ethylene-dependent defense genes in tomato [7]. In *Arabidopsis thaliana* (hereafter referred to as Arabidopsis), treatment with a variety of AHLs affected primary root growth, lateral root formation and shoot development [8]. Recently, Schikora et al. found that the treatment of Arabidopsis roots with *N*-3-oxo-tetradecanoyl-homoserine lactone (3OC14-HSL) reinforced the systemic resistance to the obligate biotrophic fungus *Golovinomyces orontii* and suggested that the mitogen-activated protein kinase 6 (AtMPK6) is required for AHL-induced resistance [9]. Bai et al. reported that *N*-3-oxo-decanoyl-homoserine lactone (3OC10-HSL) can accelerate auxin-dependent adventitious root formation in mung bean (*Vigna radiata*) seedlings and that this regulation might be mediated by H_2O_2- and NO)-dependent cGMP signaling pathways [10]. In our previous studies, we found that G-protein signaling and Ca^{2+} signaling participate in the regulation of Arabidopsis root growth by bacterial QS signals [11–13]. The results of these studies implied that plants have evolved means to perceive AHLs and respond to them with changes in gene expression or modifications in development via a number of signal transduction pathways. However, the molecular basis of the influence of AHLs on plant growth and development remains unclear.

The transcriptomic analysis conducted by von Rad et al. has contributed to our understanding of the response of Arabidopsis to AHLs [8]. However, transcriptome profiling has some limitations because mRNA levels are not always correlated to those of the corresponding

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proteins due, in part, to post-transcriptional regulation. Furthermore, post-translational modifications, such as phosphorylation and glycosylation, can result in a dramatic increase in proteome complexity without a concomitant increase in gene expression. In this study, we performed an analysis of the response of Arabidopsis seedlings to 3OC8-HSL at the proteomic level and identified 34 responsive proteins that function in the following processes: energy metabolism; amino acid metabolism; photosynthesis; defense responses; signal transduction; protein translation, processing and degradation; cytoskeleton; and cellular transport. The results provide further insight into the molecular mechanism of the regulation of plant growth and development by bacterial AHLs.

2. Materials and methods

2.1. Plant growth conditions and treatments

The Arabidopsis (Col-0) seeds were surface sterilized with 75% (vol/vol) ethanol for 1 min and 30% (vol/vol) NaClO for 5 min. After washing with sterile distilled water five times, the seeds were germinated on MS medium (1% sucrose, 0.8% agar, 1×Murashige and Skoog salts and vitamins, pH 5.8) or 1/2 MS medium. The germinated seeds were transferred to a growth chamber at 22 °C with a 16 h light/8 h dark regime and a light intensity of 100 μmol m^{-2} s^{-1}. The 3OC8-HSL used in this study was purchased from Sigma–Aldrich (Taufkirchen, Germany), stored dry, diluted from a 10 mM stock solution using sterile distilled water and adjusted to pH 5.0 just prior to use. For the root elongation assays, the 1/2 MS nutrient medium was supplemented with 3OC8-HSL at concentrations ranging from 10 nM to 100 μM. The dissolved compound was added to the cooled (50 °C) molten medium and poured into plates. The control plates were supplied with the distilled water instead of the compound.

A hydroponic system was employed to cultivate Arabidopsis for the proteomic experiment. The seedlings were grown to the two-leaf stage and until the roots were 2 cm in length and were transplanted into a sterile plastic basin (18 × 11 cm) containing 500 mL of 1/2 MS medium and 10 μM 3OC8-HSL. The plants grown under these conditions were more vigorous than those grown in potting media, uniformly absorbing the AHL solution. To ensure that the hydroponic system was free of bacterial contamination, the medium and AHL solution were sterilized by passing them through a 0.22-μm filter just prior to use. Untreated plants were considered the control. After harvesting the seedlings, an aliquot of the growth medium was plated on a bacterial growth medium and incubated overnight to evaluate any contamination that occurred during the experiment; the contaminated samples were discarded.

2.2. Protein extraction and quantification

Total protein extracts were prepared from the seedlings essentially according to the method described by Wang et al. [14], with modifications. In brief, 2–4 g of 3OC8-HSL-treated and untreated seedlings were homogenized separately to a fine powder in liquid nitrogen and were transferred to 10 mL ice-cold protein extraction buffer (25 mM HEPES, 150 μM PVP, 5 mM EDTA, 25 mM NaF, 5 mM DTT, 1 μM E-64, 1 μM bestatin, 1 μM pepstatin, 2 μM leupeptin, 1 mM PMSF, 12 mM sodium molybdate, 50 mM ascorbic acid, 2 mM imidazole and 1 mM activated sodium vanadate). The tubes were centrifuged at 15,000*g* for 15 min, and the supernatants were collected into new tubes. The supernatants were then twice extracted using ice-cold Tris-buffered phenol (pH 7.5–7.9) and ice-cold acetone Tris–HCl (pH 8.0). The organic phase was precipitated with 0.1 M ammonium acetate/methanol, and the protein extract was washed twice with 1 mL ice-cold methanol. The protein extracts were lyophilized and stored at −80 °C. The protein extracts were quantified using a 2-D Quant kit (GE Healthcare, USA) using bovine serum albumin as the standard.

2.3. Two-dimensional gel electrophoresis

Three biological replicates per treatment were performed. Precast 17 cm, pH 3–10 unilinear gradient (Bio-Rad, USA) strips were rehydrated for 14 h with 500 μL buffer containing 7 M urea, 4% CHAPS, 65 mM DTT, 0.2% Bio-Lyte (Bio-Rad) and 0.001% bromophenol blue. Protein samples (1 mg) were loaded at the cathodic end of the strips and electrofocused (Bio-Rad Protean IEF Cell system, USA) at 20 °C using a gradually increasing voltage (250–10,000 V) reaching 90,000 V/h. Following the IEF, the IPG strips were equilibrated according to Görg et al. [15]. The strips were then transferred on to vertical slab 12% SDS–polyacrylamide gels (Bio-Rad PROTEAN Plus Dodeca Cell, USA) and electrophoresed at 50 mA/gel until the dye front reached the bottom of the gel. The gels were stained with CBB R-250. The 2-D gels were scanned using a UMAX PowerLook 2100XL scanner (AeroVision Avionics, Taiwan) and analyzed with the PDQuest software 8.0 (Bio-Rad) using tenfold over background as the minimum criterion for presence/absence. The analysis was re-evaluated by a visual inspection. The normalized spot volumes (individual spot intensity/normalization factor) calculated for each gel based on the total quantity in the valid spots were determined, and these values were used to designate the significant differentially expressed spots as calculated by the Student's *t*-test ($p \leqq 0.5$). The mean value for the normalized spot volume and the SD were determined for each spot.

2.4. MALDI-TOF/TOF mass spectrometry and protein identification

The spots from the CBB-stained gels were excised automatically (Investigator ProPic, Genomic Solutions), transferred to Multiwell 96 plates and digested with modified porcine trypsin (sequencing grade; Promega) using a ProGest (Genomics Solution) digestion station. The digestion protocol used was that of Schevchenko et al. [16], with minor variations. The gel plugs were destained by incubation (twice for 30 min each) in a solution containing 200 mM ammonium bicarbonate in 40% acetonitrile at 37 °C and then subjected to three consecutive dehydration/rehydration cycles with pure acetonitrile and 25 mM ammonium bicarbonate in 40% acetonitrile, respectively. The samples were then dried at room temperature for 10 min, and 20 μL trypsin, at a concentration of 12.5 ng μL^{-1} in 25 mM ammonium bicarbonate, was added to the dry gel pieces. The digestion proceeded at 37 °C for 12 h, and the peptides were extracted from the gel plugs by adding 10 μL of 1% (v/v) trifluoroacetic acid (TFA) and incubating for 15 min.

The samples (3 μL) were deposited on to MPep Chips pre-spotted with CHCA (Sunyx, Germany) using the thin layer affinity method [17], according to the manufacturer's instructions. The MALDI-TOF/TOF (UltrafleXtreme MALDI TOF/TOF, Bruker Daltonics) mass spectrometer was operated in the *m*/*z* range of 800–4000, with an accelerating voltage of 20 kV. The spectra were internally calibrated with peptides from trypsin autolysis ($M+H^+$ = 842.509, $M+H^+$ = 2211.104). The 5 most abundant peptide ions were then subjected to fragmentation analysis, providing information that can be used to determine the peptide sequence.

3. Results

3.1. Plant root growth response to 3OC8-HSL

Previously, it has been shown that several AHLs regulate the root system architecture of Arabidopsis in a dose- and structure-dependent manner [8,18]. Accordingly, we performed a root

elongation response assay to investigate the effect of 3OC8-HSL on Arabidopsis root growth and determine an appropriate 3OC8-HSL concentration for plant treatment prior to our proteomic analysis. It was found that the root length increased as the concentration of 3OC8-HSL increased from 10 nM to 10 μM. Because the maximum growth-promoting effect of 3OC8-HSL was observed at 10 μM [12], 10 μM was selected as the 3OC8-HSL concentration to be used in the present study.

3.2. Arabidopsis seedling proteome in response to 3OC8-HSL

The hydroponically grown Arabidopsis seedlings were exposed to 10 μM 3OC8-HSL for 24 h, and the proteins were extracted and separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Fig. 1 shows the representative 2-DE gel maps of the control and 3OC8-HSL-treated seedlings. Approximately 600 protein spots were resolved on Coomassie brilliant blue-stained gels, and the analysis of the 2-DE gels using PD Quest 8.0 software revealed a total of 53 protein spots significantly changed in abundance: 39 protein spots were found to be up-regulated, and 14 spots were found to be down-regulated after the 3OC8-HSL treatment (Fig. 1).

3.3. Identification and classification of the 3OC8-HSL-responsive proteins

The 53 variable spots were analyzed by MALDI-TOF-MS. The protein identification was accomplished by DMF, consulting the NCBInr and TAIR Arabidopsis databases (http://www.arabidopsis.org) and taking Arabidopsis as the taxonomy by using the MASCOT (Matric Science Ltd. London; http://www.matrixscience.com). Of the 53 gel plugs analyzed, this search resulted in 34 hits (Table 1), representing approx. 6.5% of the total resolved proteins.

These identified 3OC8-HSL-responsive proteins were found to be involved in diverse biological processes, comprising carbohydrate metabolism (spots 12, 14, 40 and 44), energy metabolism (spots 10, 16, 51 and 53), photosynthesis (spots 11, 25 and 49), amino acid metabolism (spots 18, 26 and 2940), defense response (spot 36), signal transduction (spots 39 and 52), cytoskeleton (spots 15, 18 and 27), protein translation, processing and degradation (spots 3, 6, 13, 20, 32, 34, 35, 37, 38, 42, 43 and 46), and cellular transport (spot 22) (Fig. 2). One protein with an unknown function (spot 30) was also identified as an AHL-responsive protein in this study. Our bioinformatic analysis indicated that 19 of the 34 identified proteins are located in the chloroplasts, implying that chloroplasts are highly affected by the exposure to 3OC8-HSL.

4. Discussion

In this study, we analyzed the proteomic profile of Arabidopsis seedlings in response to 3OC8-HSL, a bacterial QS signal molecule, using 2-DE and MALDI-TOF-MS and identified 34 3OC8-HSL-responsive proteins from approximately 600 resolved spots on 2-DE gels. The majority of the identified proteins were significantly up-regulated after the exposure to 3OC8-HSL (Table 1). These responsive proteins can be classified into the following diverse physiological processes: material metabolism; energy metabolism; protein translation, processing and degradation; photosynthesis; signal transduction; cytoskeleton; defense response and cellular transport. The present data provide insight into our understanding of the mechanism of plant responses to bacterial QS signals.

Recently, increasing evidence has indicated that plants can perceive and respond to bacterial QS signals. This is reasonable because plants and bacteria have co-existed for approximately a billion years during which time they have co-evolved a sophistical mechanism to sense and respond to each other's signals, a process referred to as cross-kingdom signaling. Mathesius et al. found that over 150 proteins of approx. 2000 resolved protein spots were significantly altered in their accumulation in *M. truncatula* roots after the treatment with low concentration of 3OC12-HSL and 3OC16:1-HSL [6]. In addition, von Rad et al. showed that the contact of Arabidopsis roots with C6-HSL resulted in distinct transcriptional change in the roots [8]. In our study, significant changes in protein accumulation were found for approx. 6.5% proteins of the total resolved proteins on 2-DE gels after the interaction of Arabidopsis roots with 3OC8-HSL. These results indicate that the responses of plants to AHLs are quite extensive in terms of the quantities of protein involved.

Plant defense or stress responses, protein degradation or processing, flavonoid synthesis, hormone response, cytoskeleton,

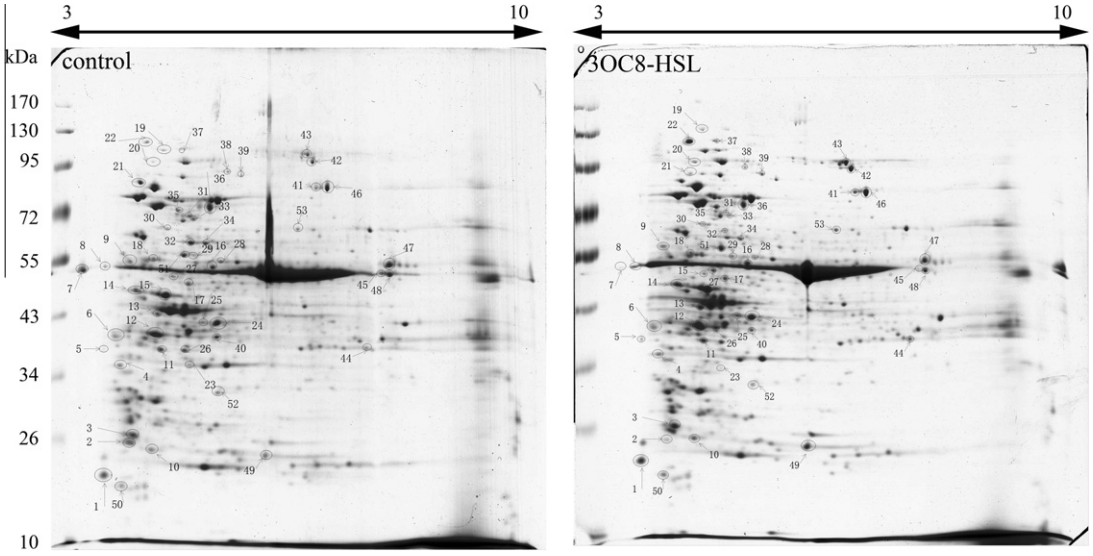

Fig. 1. Representative 2-DE gels of Arabidopsis seedling proteins. Fifty-three of the spots showing at least a 1.5-fold change after 3OC8-HSL treatment with $P < 0.05$ were analyzed by MALDI-TOF-MS. The proteins were extracted from Arabidopsis seedlings grown in 1/2 MS (control) or treated with 10 μM 3OC8-HSL for 24 h. The proteins were separated by 2-DE. In the first dimension (IEF), 1 mg of protein was loaded onto a 17 cm IPG strip with a linear gradient of pH 3–10; 12% SDS–PAGE gels were used for the second dimension. The proteins were visualized using CBB R-250. The proteins that were altered in abundance by 3OC8-HSL treatment are numbered on the 2-DE map.

Table 1
Arabidopsis seedling proteins responsive to 3OC8-HSL treatment identified by MALDI-TOF-MS.

Spot ID	Putative identity	Molecular weight (kDa) (theoretical/experimental)	Isoelectric point (theoretical/experimental)	Score	AGI	Location	Fold change
Primary carbon metabolism							
14	Fructose-1,6-bisphosphatase	45.59/53.66	5.25/4.3	82	AT3G54050	Chloroplast	2.36
12	Sedoheptulose-1,7-bisphosphatase	42.787/43.84	6.17/4.49	55	AT3G55800	Chloroplast	2.02
55	Glyceraldehyde-3-phosphate dehydrogenase	37.005/40.86	6.62/6.5	103	AT1G13440	Cytoplasm	9.56
51	Phosphoglycerate kinase	42.162/43.3	5.49/5.2	61	AT1G79550	Cytoplasm	0.72
Energy metabolism							
10	ATP synthase subunit alpha	55.351/25.93	5.19/4.45	79	AtCg00120	Chloroplast	1.95
17	ATP synthase subunit beta	53.957/59.4	5.38/5.1	118	AtCg00480	Chloroplast	1.97
63	ATP synthase subunit alpha	55.351/62.7	5.19/4.8	152	ATCG00120	Chloroplast	1.75
65	Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+)	64.771/81.4	6.01/6	65	AT5G11670		3.18
Photosynthesis							
11	Photosystem II stability/assembly factor HCF136	44.133/43	6.79/4.56	57	AT5G23120	Chloroplast	1.64
25	Ribulosebisphosphate carboxylase/ oxygenaseactivase	52.347/46.12	5.87/5.18	80	AT2G39730	Chloroplast	1.25
60	Carbonic anhydrase	37.825/27.51	5.74/5.71	98	AT3G01500	Chloroplast	27
Amino acid metabolism							
18	Glutamate-cysteine ligase	58.924/55.1	6.16/4.9	61	AT4G23100	Chloroplast	1.73
27	Glutamine synthetase cytosolic isozyme1-1	39.318/43.4	5.28/4.68	68	AT5G37600		4.5
40	Alanine aminotransferase 1	60.410/60.03	6.00/4.92	106	At1g17290	Mitochodrion	0.75
Defense response and signal cascade reaction							
47	Myrosinase 1	61.664/77.5	5.61/5.1	155	AT5G26000		1.94
3	14-3-3-Like protein GF14 lambda	29.227/27.66	4.94/4.27	68	AT5G10450	Cell wall, plasmembrane	2.41
50	Phospholipase D alpha 1	92.246/92.1	5.53/5.4	79	AT3G15730	Chloroplast, plasmembrane	3.2
64	Putative protein phosphatase 2C-like protein 44	29.878/33.6	7.01/5.2	59	AT3G23360		0.33
Cytoskeleton							
19	Tubulin beta-2/beta-3 chain	51.385/61.7	4.70/4.4	143	AT5G62700	Cell wall	4.19
28	Tubulin alpha-3/alpha-5 chain	50.250/56.65	4.95/4.63	90	AT5G19780		18.78
15	Tubulin alpha-2/alpha-4 chain	50.194/56.42	4.93/4.55	155	AT1G50010	Cell wall	4.44
Protein metabolism (translation, processing and degradation)							
6	Peptidyl-prolylcis–trans isomerase CYP38	48.180/44.61	5.06/4.26	108	AT3G01480	Chloroplast	6.37
46	Heat shock 70 kDa protein	71.456/75.85	5.14/4.7	118	At3g12580		2.38
21	Presequence protease 1	121.680/101.1	5.48/4.5	135	AT3G19170	Chloroplast	23.50
57	30S ribosomal protein S4	23.340/81.77	10.33/6.07	60	ATCG00380	Chloroplast	1.66
13	Elongation factor Tu	51.883/48.9	5.84/4.5	127	AT4G20360	Chloroplast	2.30
48	Ubiquitin-activating enzyme E1	121.202/108.32	5.11/4.69	95	AT2G30110	Plasmembrane	9.38
49	Chaperone protein ClpC1	103.616/97.7	6.36/5.1	112	At5g50920	Chloroplast	5.68
43	Chaperonin 60 subunit beta 1	64.169/66.35	6.21/4.89	113	At1g55490	Chloroplast	0.65
45	Chaperonin 60 subunit beta 2	63.702/66.25	5.60/5.05	108	At3g13470	Chloroplast	0.38
53	Elongation factor EF-2	95.098/93.11	5.89/5.91	90	AT1G56070	Chloroplast, or plasmembrane	6.25
54	Ubiquitin carboxyl-terminal hydrolase 27	56.647/96.04	6.20/6.2	63	AT4G39370		8.28
Cellular transport							
23	Patellin-1	64.121/115	4.82/4.4	98	AT1G72150		8.62
Function unknown							
41	Uncharacterized protein	139.324/71.1	5.04/4.52	70	AT4G27595		8.74

The fold change is expressed as a ratio of the vol.% between 10 μM 3OC8-HSL treated/control seedlings, and each value represents the mean value ± SD of three biologically independent experiments. The location of the identified protein was predicted by Target P (http://www.cbs.dtu.dk/service/TargetP).

energy and various primary metabolism processes were found to be involved in the response of *M. truncatula* to AHLs [6]. After treating Arabidopsis with 3OC6-HSL, Niu et al. found that the 3OC6-HSL-responsive proteins functioned in plant antioxidant activity, material metabolism and signal transduction [19]. In this study, we found that the identified 3OC8-HSL-responsive proteins were related to processes that included protein translation, processing and degradation, material metabolism, photosynthesis, defense response, cytoskeleton, signal translation, and cellular transport. All of these data suggest that the plant host has an extensive range of functional responses when plants detect AHLs and sense bacteria in the root rhizosphere.

In the present study, the majority of proteins responding to 3OC8-HSL were found to be associated with protein metabolism. Three of the proteins identified are factors for protein biosynthesis (spot 13, elongation factor Tu; 42, elongation factor EF-2; and 46, 30S ribosomal protein S4), and eight proteins are involved in protein folding, assembly, and degradation: peptidyl-prolyl cis–trans isomerase (spot 6); presequence protease (spot 20); heat shock protein (spot 35); chaperone protein C1PC1 (spot 38);

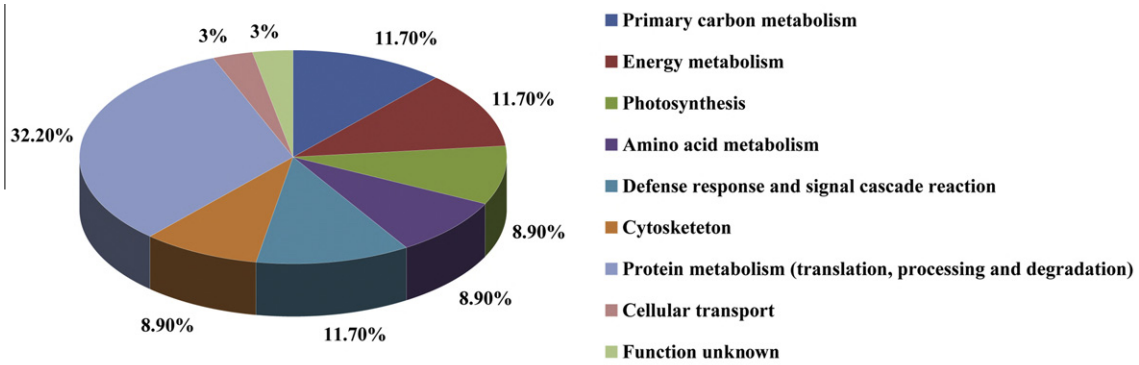

Fig. 2. Functional classification of the 3OC8-HSL-responsive proteins. The functional categories are named according to the major metabolic substrate, except for the categories representing the processes of signal transduction, transcriptional regulation (transcription), and unclassified or unknown (unclass. and unk.) proteins.

chaperone beta subunits 1 and 2 (spots 32 and 34); ubiquitin-activating enzyme E1 (spot 37); and ubiquitin carboxy L-terminal hydrolase (spot 43). The protein abundance ratio revealed that, except for chaperone β subunits 1 and 2 (spots 32 and 34), the expression of all of the other proteins mentioned above is enhanced remarkably, suggesting that novel protein biosynthesis is required when the plants respond to bacterial AHLs and that an active protein quality control system inside the cells is playing an important role in the sensing of bacterial QS signal molecules.

The proteins implicated in primary carbon metabolism and energy metabolism (e.g., glycolysis, citrate cycle, electron transport) comprised 23.4% of the proteins identified (Fig. 2). Three enzymes that are involved in carbohydrate metabolism, i.e., fructose-1, 6-bisphosphatase (spot 14), sedoheptulose-1, 7-bisphosphatase (spot 12), and glyceradehyde-3-phosphate dehydrogenase (spot 44), were up-regulated following the 3OC8-HSL treatment, indicating a fundamental metabolism alteration in Arabidopsis. In addition, we found that certain proteins, such as the ATP synthase α and β subunits (spots 10, 16 and 51), exhibited an increased accumulation after induction by 3OC8-HSL. This result implies that more energy was required for the plants to respond to 3OC8-HSL through root elongation or that 3OC8-HSL promotes the production of energy that can be used to enhance the biosynthesis of novel proteins for root growth.

Tubulin dynamics have important functions in cellular homeostasis: the cytoskeleton is rapidly remodeled by various endogenous and external stimuli, such as hormones, low temperature, and NaCl [20–22]. In this study, we found that the accumulation of three tubulins, i.e., tubulin β-2/β-3 chain (spot 18), tubulin α-3/α-5 chain (spot 27), and tubulin α-2/α-4 chain (spot 15), was significantly increased following the 3OC8-HSL treatment. Mathesius et al. also observed alterations in the expression level of some tubulin elements when *M. truncatula* roots were exposed to 3OC12-HSL or 3OC16:1-HSL [6]. Although the mechanistic significance is not fully understood, the AHL responsiveness of these common, cytoskeleton proteins calls into question their designation as house-keeping genes.

Three proteins (spots 11, 24 and 49) functioning in photosynthesis were found to be induced by 3OC8-HSL in Arabidopsis. It was noted that the accumulation of carbonic anhydrase increased up to 27-fold compared with that of the untreated Arabidopsis seedlings. Carbonic anhydrase plays an important role in photosynthetic carbon assimilation (Table 1), and it has been reported that Rubisco, photosystem II stability/assembly factor, and carbonic anhydrase are involved in the response to drought in sunflower (*Helianthus annuus*) and to NaCl stress in Arabidopsis roots [23,24]. These results implicate the involvement of photosynthesis in the plant response to various environmental stimuli, including AHLs.

Abscisic acid (ABA), jasmonic acid (JA), and brassinosteroids (BRs) are phytohormones with activity that has been correlated with environmental stress. In this study, we found that three identified proteins implicated in phytohormone responses were increased in their abundance after the 3OC8-HSL treatment: spot 3 (14-3-3-like protein GF14 lambda), spot 36 (myrosinase) and spot 39 (phospholipase D). The 14-3-3 proteins play essential roles in BR signal transduction in Arabidopsis [25]. Islam et al. reported that myrosinases TGG1 and TGG2 redundantly function in ABA and MeJA signaling in Arabidopsis guard cells [26]. Zhang et al. found that phospholipase D (PLD) and protein phosphatase 2C (PP2C) both play a role in mediating plant responses to ABA [27]. These results suggest the concept of cross-talk between various phytohormones and AHLs.

In addition, 14-3-3 proteins, myrosinase, and protein phosphatase 2C are reported to function in plant defense responses. Plant myrosinase is known as a defense enzyme against bacteria, pathogens, and herbivores; it hydrolyzes glucosinolates into a variety of toxic small molecules, including cyanate, isothiocyanate, and nitrile, which are active against intruders [28,29]. In Arabidopsis, a 14-3-3 protein positively regulates the RPW8-mediated resistance against the biotrophic fungal pathogens *Golovinomyces* spp. that cause powdery mildew disease on multiple plant species [30]. Wang et al. further found that PP2C interacts with RPW8.2 and negatively regulates SA-dependent defense responses in Arabidopsis [31]. Recently, a number of reports pointed to the induction of plant resistance against biotrophic and hemibiotrophic pathogens by AHLs [7,9,10]. Our findings in the present study might provide some clues for the molecular mechanism of increasing disease resistance by bacterial QS signals in plants.

In summary, the present proteomic investigation of Arabidopsis roots subjected to 3OC8-HSL treatment revealed a complex network influenced by 3OC8-HSL. The proteins identified in this study represent only a small portion of the Arabidopsis proteome that is responsive to bacterial QS signals, and many other AHL-responsive proteins still need to be identified. Considering the limitations of a proteomic study based on 2-DE gels, a complementary proteomic strategy should be used to gain more insight into the intricate network of plant responses to bacterial AHLs.

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