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Microarray analysis of the phytoremediation and phytosensing of occupational toxicant naphthalene

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ABSTRACT

Naphthalene is of global environmental concern because it is assumed to contribute considerably to human cancer risk. Plants are important in removing naphthalene from the atmosphere and soil. However, there remains insufficient knowledge on plant response to this compound. To determine the mechanism of naphthalene uptake and transduction in plants, as well as plant response to this compound, a microarray system was used to analyze gene expression patterns in *Arabidopsis thaliana* after irrigation with 2.0 mM naphthalene. A total of 247 differentially expressed genes were identified as upregulated by naphthalene. These genes might specifically contribute to naphthalene uptake, transformation, conjugation, and compartmentalization in the plant. The potential role of upregulated genes in plant defense to naphthalene and the use of phytosensing for naphthalene detection were also discussed.

1. Introduction

Naphthalene is the most volatile member of polycyclic aromatic hydrocarbons (PAHs). It is ubiquitously discharged to the environment by incomplete combustion of fossil fuel from industrial, domestic, and natural sources (motor vehicles, air traffic, residential heating with fossil fuel, gasoline burning, industrial plants, forest fires, etc.) [1–3]. Since 2000, when the US National Toxicology Program confirmed the carcinogenic activity of naphthalene in rats, the International Agency for Research on Cancer and the US Environmental Protection Agency have reclassified naphthalene as a possible human carcinogen. Therefore, the industrial and domestic production of naphthalene has led to an environment burden for the general population [4].

Microbial degradation of PAHs is thought to be the major process involved in effective site bioremediation. Numerous bacteria can degrade PAHs, and some can utilize naphthalene as their sole carbon source [5,6]. However, microbial degradation of naphthalene in aquatic and terrestrial ecosystems is influenced strongly by a wide variety of abiotic and biotic factors, such as temperature, pH, soil type, aeration, and nutrients, among others [7]. Phytoremediation for the removal of naphthalene pollutants can be a supplementary method because plants can grow independently using sunlight, water, and inorganic ions, and they can be cultivated by germination of seeds or by vegetative propagation, causing the least disturbance to the contaminated sites [8–10]. Polycyclic aromatic hydrocarbons uptake in upland plants has been detected. For example, many vegetables grown in garden plots contaminated with PAHs may uptake PAHs, such as naphthalene [11]. The mechanisms for the transfer of organic contaminants from soil to plant tissue include uptake in the transpiration stream, volatilization and subsequent re-deposition on leaves, and sorption from direct contact with soil particles [12–14].

Hazardous pollutants like PAHs are stress inducers for plants. For example, phenanthrene can induce many morphological symptoms in Arabidopsis, such as growth reduction of the root and shoot, deformed trichomes, reduced root hairs, chlorosis, late flowering, and appearance of necrotic lesions [15]. Naphthalene is structurally similar to plant hormones and secondary metabolites, so plants treated with naphthalene are expected to exhibit altered growth, morphology, and gene expression [15]. However, the plant genes responsible for naphthalene uptake, degradation, and conjugation are mostly unknown. The mechanisms of naphthalene toxicity in plants are poorly understood. Research on plant stress response and defense mechanisms to naphthalene at the molecular level is also rare. Therefore, understanding plant transcriptional responses to naphthalene is necessary and useful in searching for phytoremediators. In this paper, the transcriptional changes in Arabidopsis in response to naphthalene were investigated using a microarray.

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Several naphthalene-induced and naphthalene-repressed genes were identified, and the results were discussed in the context of the diverse biological functions of naphthalene in plants.

2. Materials and methods

2.1. Plants and phytotoxicity studies

Arabidopsis thaliana ecotype Columbia were grown on half-strength Murashige and Skoog (MS) medium supplemented with 1% sucrose at pH 5.8. The Arabidopsis seeds were sterilized using 20% bleach and 0.1% Tween-20. Surface-sterilized seeds at a density of 60 seeds per Petri dish were placed on solid 1/2 MS medium containing naphthalene (Sigma, St. Louis, MO, USA) at concentrations of 0, 0.1, 0.25, 0.50, 1.00, and 2.00 mM. Each concentration of naphthalene was replicated thrice. The Petri dishes were prepared by adding 10–200 μL of 1 M naphthalene in acetone to 100 mL aliquots of 1/2 MS medium to achieve the required concentrations of naphthalene. The seeds were then cold stratified at 4 °C for 3 days and then incubated vertically under long-day conditions (16/8 h photoperiod day and night 23 ± 1 °C). The growth responses and phytotoxicity tolerance thresholds of the wild-type Arabidopsis plants to naphthalene were analyzed by observing the germination rate and measuring the primary root length after germination.

For the assay on the effect of naphthalene on photosynthesis, 30-day-old plants grown in soil were transferred into liquid 1/2 MS medium containing naphthalene and grown for a week. Then 3–5 leaves from Arabidopsis were used for photosynthesis analysis. Chlorophyll was extracted from individual leaves with 95% ethanol. The chlorophyll content was determined spectrophotometrically at 470, 649, and 665 nm following the method of Lichtenthaler [16]. The photosynthetic parameters were detected by an LI-6200 portable photosynthesis system [(Li-Cor Inc., Lincoln, NE) and calculated as described [17]]. The total variable fluorescence (Fv) and the maximum fluorescence yield (Fm) were determined after 30 min in the dark, and the light-adapted values (Fv′ and Fm′) were measured after 30 min of illumination with 500 μmol m^{-2} s^{-1}.

2.2. RNA preparation

For naphthalene treatment, seedlings grown in the pots (placed in MS media for 4 days, then removed to soil for 30 days) were irrigated with 2.0 mM naphthalene for 4 days and harvested. The plants were treated during the light period, and three replications with 12 plants in each were included. Plants irrigated with 2 mL/L acetone were used as controls. Total RNA from pooled leaf tissue was isolated and purified using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RNA quality was assessed through agarose gel electrophoresis for two-color microarrays using the Agilent Bioanalyzer for gene expression microarrays. mRNA was extracted from the total RNA pools using Oligotex mRNA mini kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol. Ten micrograms of mRNA was reverse-transcribed using SuperScript II RT (Invitrogen, Carlsbad, CA, USA) and T7-(dT)24 primer. All first-strand cDNA was used for double-strand cDNA synthesis. After purification, one-half of the purified double-strand cDNA was used to generate biotin-labeled cRNA. The reaction was performed in a solution containing dNTP mix, cyanine 3-dCTP (for treated samples) or cyanine 5-dCTP (for untreated samples; Perkin-Elmer), and T7 RNA Polymerase, and incubated at 40 °C for 2 h. To remove unincorporated nucleotides, the labeled cRNA was purified using RNeasy mini kit (Qiagen). The biotin-labeled targets were hybridized into an Arabidopsis 2 oligo-microarray (Agilent Technologies, Inc.) for 17 h at 60 °C in a Hybridization Oven (Agilent Technologies, Inc.) and then washed. The content of this microarray was derived from the ATH1 v. 3 database of The Institute for Genomic Research representing 21,500 genes. The processed arrays were scanned in an Agilent GeneArray Scanner (Agilent, Palo Alto, CA, USA).

2.3. Data analysis

The hybridization signals were quantified and analyzed using Agilent Feature Extraction software (Agilent Technologies, Inc.). Statistical analysis was performed using Cluster 3.0 software (University of Tokyo). Transcripts that had a detection call "*P*," a signal value ≥25, and a change in the *P* value <0.05 in all three replicates of each treatment were selected for the identification of naphthalene-related genes. Only the transcripts with a minimum twofold increase or decrease in signal over the control in at least two of the three biological replicates and a coefficient of variation (CV) < 10% were identified as naphthalene-related genes. The signal log ratio is the change in expression level of a transcript between the control and the experimental samples, expressed as the log 2 ratio. The fold change was considered 2 (signal log ratio) when the signal log ratio was ≥0.

2.4. Verification of the array result by RT-PCR

Gene-specific primers were synthesized for selected probe sets, and RT-PCR was carried out to verify the microarray results. Equal amounts (1 μg) of purified total RNA were reverse-transcribed. Subsequent semi-quantitative PCR was performed using a limited number of PCR cycles kept in the exponential phase of amplification. Actin2 gene was amplified in parallel and used for normalization. Quantifications were based on ethidium bromide fluorescence. Real-time PCR was performed in a Mini Option Real-time PCR System (Bio-Rad, CA, USA). The reaction mix (10 μL) contained cDNA with 20 ng total RNA, 0.2 μM of each primer, 0.2 μM SYBR, 3 mM $MgCl_2$, 200 μM each of dATP, dCTP, and dGTP, 400 μM dUTP, and 1 unit Taq DNA polymerase. The fold change in the expression of RNA was estimated using threshold cycles. All analyses were performed in triplicate. Mean values were calculated for relative expression ratios.

3. Results and discussion

3.1. Growth of plants in naphthalene condition

Arabidopsis exhibited many stress characteristics, such as inhibition of seed germination and reduction of root growth, when grown in a medium containing a high concentration of naphthalene (Fig. 1). Treatment with high concentrations of naphthalene also decreased the photosynthetic efficiency of seedlings grown in pots (Fig. 2). Based on the growth of four-week-old seedlings, 2 mM was considered a sub-lethal concentration of naphthalene and was used for the subsequent microarray experiments.

3.2. Naphthalene-responsive genes of Arabidopsis

A coefficient of variation <10% and a fold change >2.0 indicated that 247 differentially expressed genes upregulated, and 140 genes downregulated in response to 2 mM naphthalene. RT-PCR was carried out for 20 putative naphthalene upregulated genes to confirm the microarray data. The expression patterns obtained by RT-PCR were consistent with those obtained by microarray analysis (Fig. 3). For example, *CYP96A12* and *CYP706A6* genes were induced by naphthalene, whereas the induction of ubiquitin-protein ligase was expressed higher than both cytochrome P450 genes. The fold-change in expression was verified using real-time PCR, with the tested genes exhibiting approximately 2- to 25-fold changes in the microarray. In all transcripts tested, the average fold-change

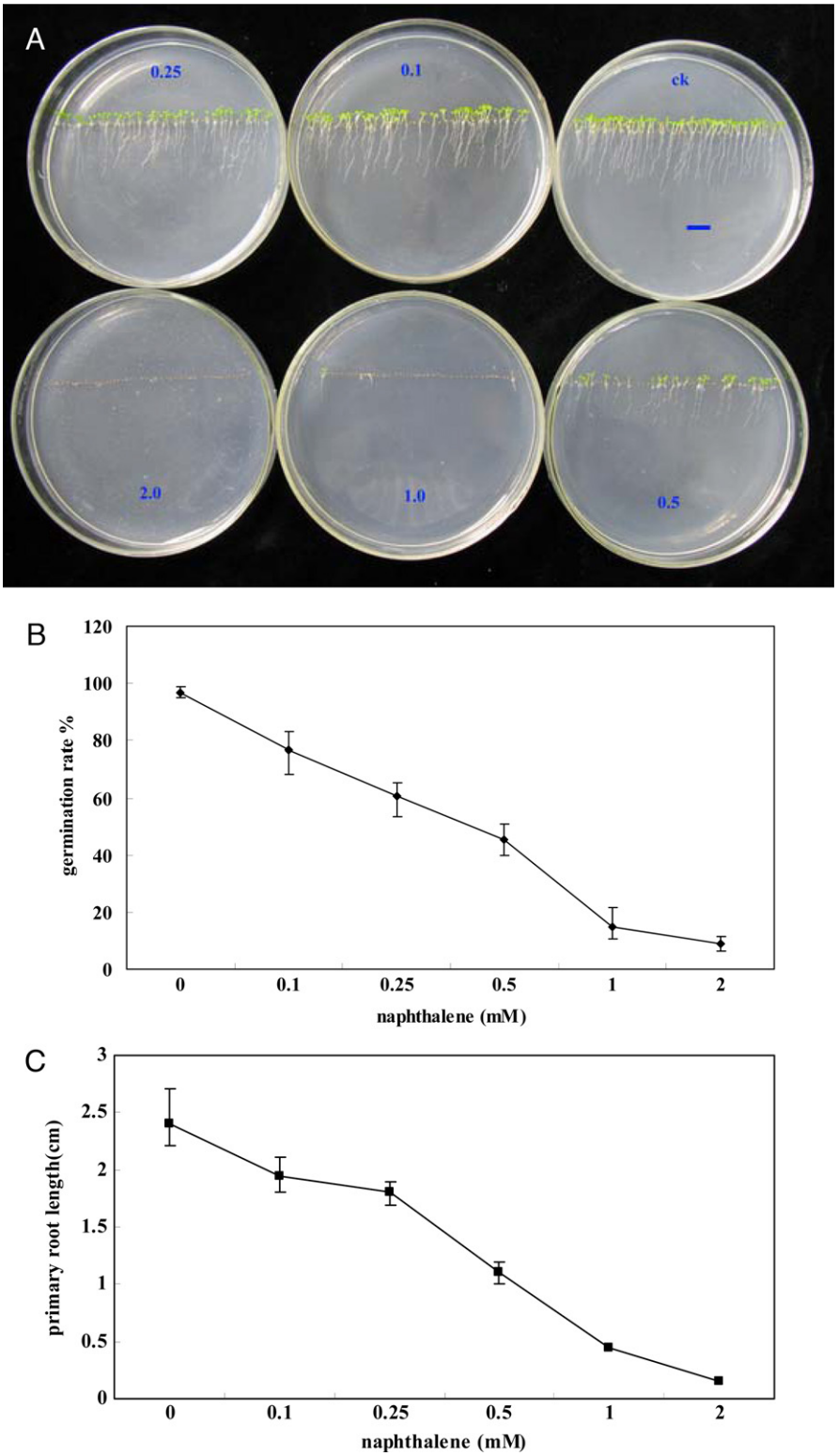

Fig. 1. Effect of naphthalene treatment on Arabidopsis seed germination and seedling growth. (A) Arabidopsis was grown on medium supplemented with 0–2 mM naphthalene. Scale bars 10 mm; affect of naphthalene on (B) seed germination and (C) root development. Seeds were sowed on 0, 0.1, 0.25, 0.5, 1 or 2 mM naphthalene plate. On the *Y*-axis is the primary root length in centimeters and on the *X*-axis is the concentration in millimolar for naphthalene. Data points show mean standard deviation, $n = 3$ for every concentration.

estimated from real-time PCR data was higher than that from microarray data (Fig. 4).

The current data demonstrated that naphthalene affected the transcript levels in numerous genes related to pollutants remediation. These transcripts can be related to the metabolism of the chemical, the conjugation of the chemical, and the movement of the contaminants. Numerous genes related to plant defense responses, signal transduction, and senescence were also induced. However, there were 71 genes (41 upregulated and 30 downregulated) coding for proteins with unknown functions. A selective list of genes upregulated by naphthalene is given in Table 1, and the complete list is available as Supplementary Material Tables S1 and S2.

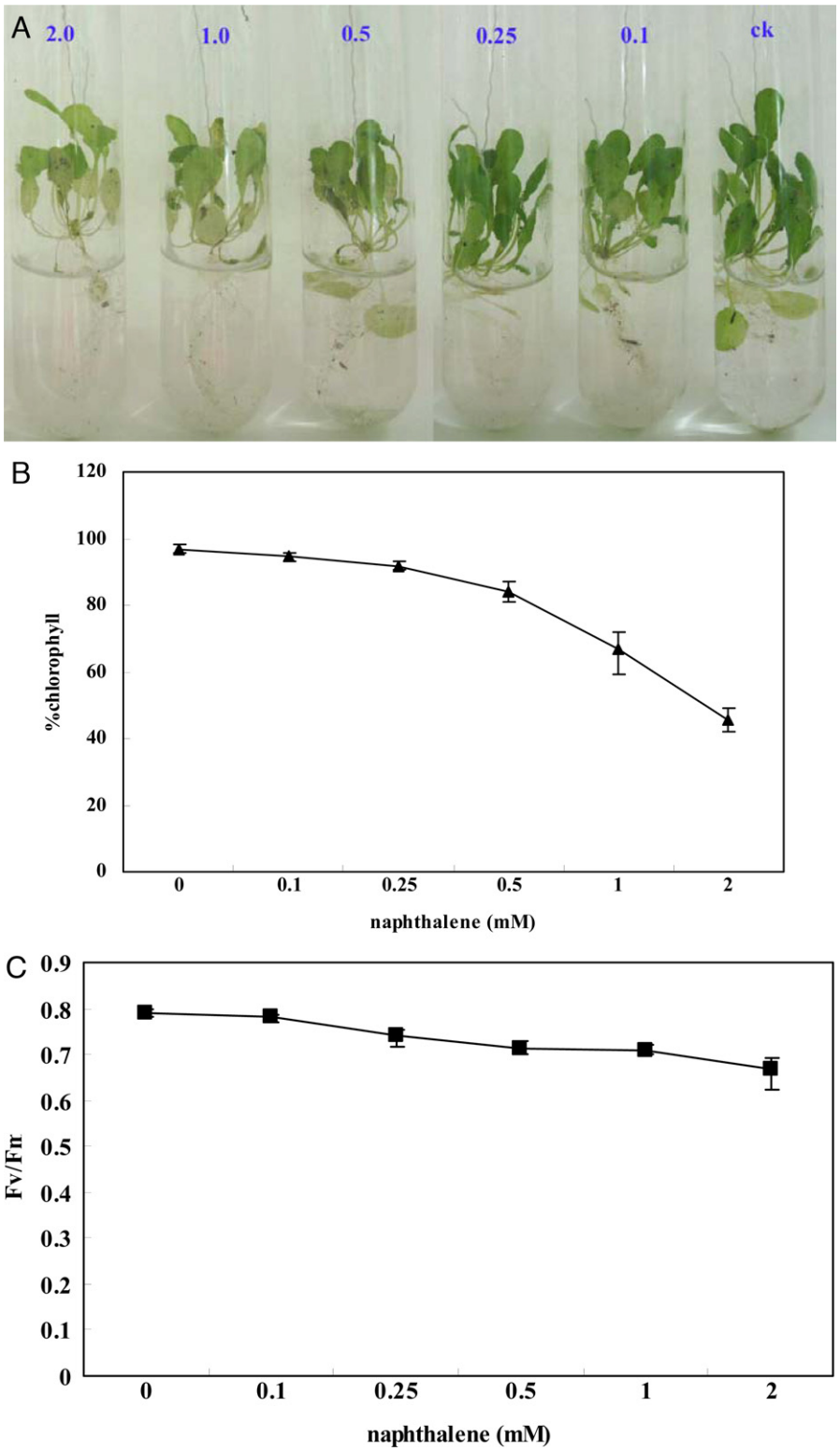

Fig. 2. Effect of naphthalene treatment on photosynthetic activities of Arabidopsis. (A) Arabidopsis was grown on soil for 30 days then transferred to liquid medium supplemented with 0–2 mM naphthalene; naphthalene promotes (B) chlorophyll degradation and (C) Fv/Fm decrease. Plants grown in soil for 30-day-old then transferred to liquid 1/2 MS medium containing 0, 0.1, 0.25, 0.5, 1 or 2 mM naphthalene for a week. Values shown are means ± SE of three experiments.

3.3. Genes involved in the metabolism of naphthalene in plants

Xenobiotics can be degraded chemically and ultimately mineralized into harmless biological compounds in plants. Initially, xenobiotics must be efficiently extracted from contaminated sediments and water. Lipophilic organic pollutants, such as PAHs, are firmly associated with soil organic fraction and are not expected to be susceptible to plant uptake and translocation [18]. Studies have shown that plant lipids are the major factor in the plant uptake of lipophilic contaminants from the soil [19,20]. In this research, a lipid synthase-related protein, monogalactosyldiacylglycerol synthase (MGDGS), was induced by naphthalene. This

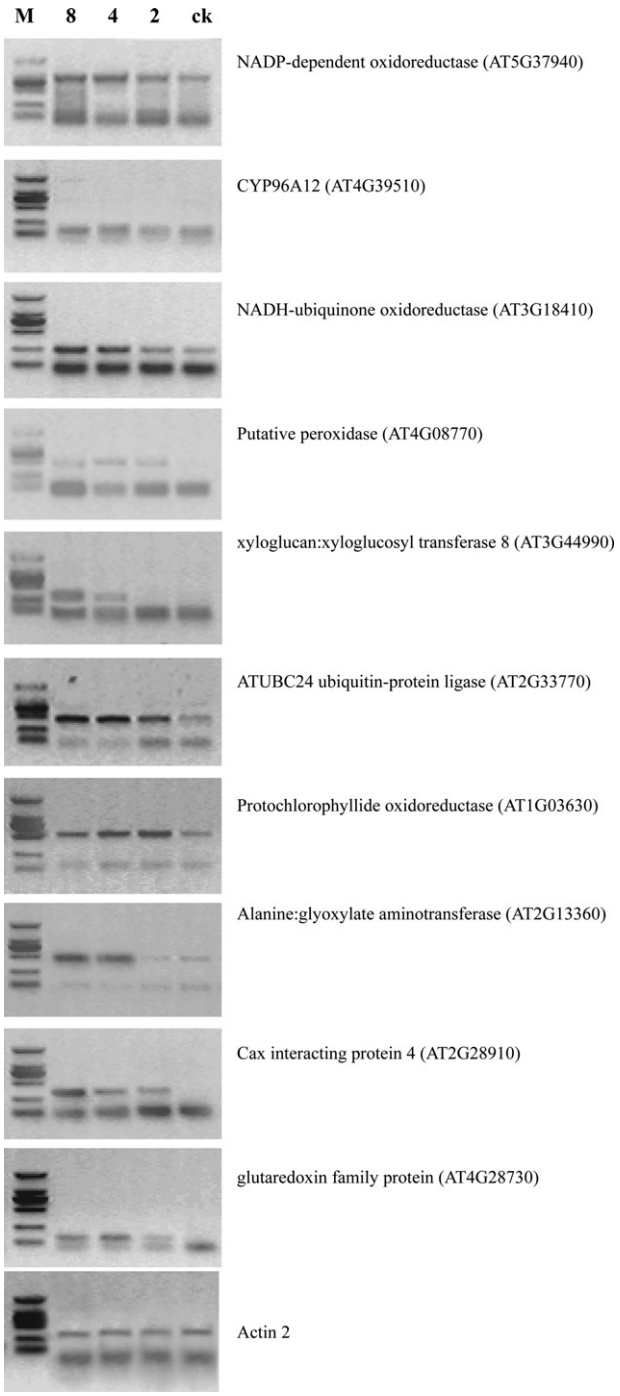

Fig. 3. Reverse transcriptase polymerase chain reaction (RT-PCR) analyses of changes in gene expression in response to naphthalene. Arabidopsis plants were treated with 2 mM naphthalene for 2, 4 and 8 days. The control plants were treated with 0.5% acetone for 8 days. The RT-PCR products were separated in 1.5% agarose gels, and a 2 kb DNA ladder (Takara) was used as a marker.

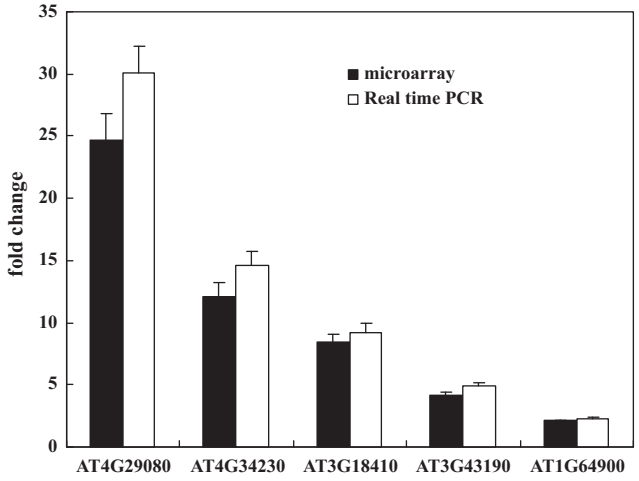

Fig. 4. Comparison of gene expression data from microarray hybridization and real-time PCR. Changes in gene expression were estimated as the fold-change over the control. The genes tested are indicated below each group of bars. The bars represent the average fold-change and standard error in transcript changes estimated from three biological replications for both real-time PCR and microarray hybridization.

enzyme catalyzes the formation of monogalactosyldiacylglycerol (MGDG). In higher plants, about 50% (wt/wt) of the membrane lipids of chloroplasts are composed of two major galactolipids, MGDG and DGDG (digalactosyldiacylglycerol) synthesized by the dismutation of two molecules of MGDG [21]. An approximately twofold increase in lipid transfer protein (LTP) was observed in response to naphthalene treatments. LTP can enhance the shuttling of phospholipids and the transfer of phospholipids between cell membranes; it can also bind acyl chains. As a result, LTPs were assumed to be responsible for membrane biogenesis and the regulation of intracellular fatty acid pools [22]. The lipids in the membrane might help naphthalene enter Arabidopsis both through direct contact with the tissue and from the air without any carrier. This hypothesis is supported by the observations of Wild et al. who traced the movement of anthracene in maize leaves using two-photon excitation microscopy [14].

Once plants have absorbed the xenobiotics, these chemical contaminants are metabolized based on three sequential phases [23]. The first phase is the transformation of the xenobiotics. The progress generally involves oxidation, hydrolysis, or reduction reactions, where functional groups such as hydroxyl (–OH) and carboxyl (–COOH) are added to the pollutant through the enzymatic involvement of cytochrome P450 monooxygenases, esterases, and oxidoreductases [24]. Among the naphthalene upregulated genes, eight P450 monooxygenase genes are induced with a 2- to 22-fold increase in expression. The biological functions of the cytochrome P450 monooxygenases are based on their capability to catalyze the insertion of oxygen into a wide variety of compounds. Mammalian P450 plays key roles in the detoxification of PAHs [25]. In Arabidopsis, the 272 annotated P450 genes formed one of the largest families. Their catalytic functions are extremely diverse [26]. Some of the above P450 monooxygenases might act on naphthalene and catabolize the compound to more hydrophilic materials. In addition, 13 oxidoreductase (including 5 peroxidases) genes were induced with a 2- to 8-fold increase in expression. Although there is still no information to correlate plant oxidoreductases to PAH dissipation, some peroxidases (e.g., horseradish peroxidase) along with the mediator 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) are capable of oxidizing at least PAHs with a lower ionization potential [27]. Many fungi are potent mediators of PAH degradation due to the action of their oxidoreductases [28]. Therefore, in the presence of natural mediators, plant peroxidases can potentially attack a number of PAHs. We also observed a carboxylesterase (AT1G57590) induced twofold. Carboxylesterase activity in the intestinal mucosa was reported to increase after oral administration of anthracene or phenanthrene to rats [29]. We surmised that carboxylesterase might be modulated by PAHs in plants as in animals.

The hydrophilic compounds transformed in the first phase were then introduced to moieties such as glutathione or glucuronate during the second phase. These are often conjugated

Table 1
List of potential genes suggesting for naphthalene response and metabolism from microarray experiments along with their fold change.

Primary accession	Gene name	Fold change	*p*-Value
Uptake			
AT3G58550.1	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2.468	0.011
AT2G11810.1	MGDC; 1,2-diacylglycerol 3-beta-galactosyltransferase	3.014	0.008
Degradation			
AT3G18410.2	NADH-ubiquinone oxidoreductase-related	8.406	0.016
AT5G38000.1	NADP-dependent oxidoreductase, putative	4.472	0.001
AT5G37960.1	Oxidoreductase-related	4.403	0.005
AT4G03060.1	AOP2 (Alkenyl Hydroxalkyl Producing 2); oxidoreductase	4.217	0.001
AT5G37940.1	NADP-dependent oxidoreductase, putative	3.75	0.004
AT1G03630.1	POR C (Protochlorophyllide Oxidoreductase); NADPH dehydrogenase	3.089	0.001
AT1G14345.1	Oxidoreductase	2.274	0.012
AT3G19000.1	Oxidoreductase, 2OG-Fe(II) oxygenase family protein	2.087	0.003
AT1G57750.1	CYP96A15/MAH1 (MID-CHAIN ALKANE HYDROXYLASE 1)	22.314	0.031
AT4G12320.1	CYP706A6 (cytochrome P450, family 706, subfamily A, polypeptide 6)	5.244	0.005
AT4G39510.1	CYP96A12 (cytochrome P450, family 96, subfamily A, polypeptide 12)	2.859	0.025
AT3G26290.1	CYP71B26 (cytochrome P450, family 71, subfamily B, polypeptide 26)	2.815	0.049
AT4G12310.1	CYP706A5 (cytochrome P450, family 706, subfamily A, polypeptide 5)	2.682	0.032
AT3G61880.1	CYP78A9 (CYTOCHROME P450 78A9)	2.407	0.029
AT2G45560.1	CYP76C1 (cytochrome P450, family 76, subfamily C, polypeptide 1)	2.303	0.011
AT1G64900.1	CYP89A2 (CYTOCHROME P450 89A2)	2.099	0.001
AT1G57590.1	Carboxylesterase	2.238	0.001
Conjugation			
AT3G43190.1	SUS4; UDP-glycosyltransferase/sucrose synthase/transferase, transferring glycosyl groups	4.167	0.001
AT4G01070.1	GT72B1; UDP-glucosyltransferase/UDP-glycosyltransferase	3.951	0.009
AT4G09500.1	Glycosyltransferase family protein	2.147	0.002
AT5G17220.1	ATGSTF12 (Glutathione S-Transferase 26); glutathione transferase	2.884	0.033
AT1G09350.1	ATGOLS3; transferase, transferring glycosyl groups	3.633	0.006
2AT2G43910.1	Thiol methyltransferase, putative	5.189	0.021
AT3G44990.1	XTR8 (xyloglucan:xyloglucosyl transferase 8); hydrolase, acting on glycosyl bonds	19.388	0.003
AT4G23990.1	ATCSLG3 (Cellulose synthase-like G3); transferase/transferase, transferring glycosyl groups	8.773	0.005
AT5G63560.1	Transferase family protein	2.45	0.003
Transporter			
AT3G55100	ABC transporter family protein	5.421	0.046
AT4G02050.1	Sugar transporter, putative	2.643	0.003
AT3G21670.1	Nitrate transporter (NTP3)	3.482	0.032
Cell death			
AT3G57810.2	OTU-like cysteine protease family protein	2.136	0.016
AT2G33770.1	ATUBC24/PHO2/UBC24 (PHOSPHATE 2); ubiquitin-protein ligase	24.419	0.011
AT5G06730.1	Peroxidase, putative	4.284	0.014
AT4G08770.1	Peroxidase, putative	4.859	0.003
AT3G32980.1	Peroxidase 32 (PER32) (P32) (PRXR3)	2.153	0.008
AT2G38390.1	Peroxidase, putative	6.694	0.012
AT2G38380.1	Peroxidase 22 (PER22) (P22) (PRXEA)/basic peroxidase E	4.947	0.006
AT2G20140.1	26S protease regulatory complex subunit 4, putative	13.273	0.02
Plant defense			
AT2G33150.1	KAT2/PED1 (PEROXISOME DEFECTIVE 1); acetyl-CoA C-acyltransferase	20.03	0.002
AT5G48880.1	KAT5/PKT1/PKT2; acetyl-CoA C-acyltransferase	2.173	0.008
AT4G25480.1	DREB1A (DEHYDRATION RESPONSE ELEMENT B1A)	6.27	0.047
AT2G31380.1	STH (salt tolerance homologue); transcription factor/zinc ion binding	3.598	0.002
AT5G22570.1	WRKY38 (WRKY DNA-binding protein 38); transcription factor	2.866	0.023
AT4G29080.1	PAP2 (PHYTOCHROME-ASSOCIATED PROTEIN 2); transcription factor	24.734	0.01
AT1G56650.1	PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1); DNA binding/transcription factor	4.809	0.001
Auxin related			
AT1G70940.1	PIN3 (PIN-FORMED 3); auxin:hydrogen symporter/transporter	15.123	0.018
AT3G23050.1	IAA7 (AUXIN RESISTANT 2); transcription factor	16.675	0.031
AT3G61900.1	Auxin-responsive family protein	2.512	0.003
DR368506	Auxin-responsive family protein	2.324	0.034

and sequestered by glutathione-transferases (GST) and UDP-glucoronosyl/UDP-glucosyltransferases (UGTs) [30]. UGTs transfer carbohydrate residues onto hydrophilic compounds containing an available nucleophilic center, such as a hydroxyl, carboxyl, amino, or thiol group, to regulate their activity, toxicity, or amenability to transport [31]. Similar to UGTs, GSTs play an important role in the detoxification of endogenous and xenobiotic compounds. Soluble GSTs form dimers, each subunit of which contains active sites that bind glutathione and hydrophobic ligands [32]. Both UGTs and GSTs are encoded by large and diverse gene families. However, there is only limited information on the function of individual enzymes. Three UGTs and one GST gene were upregulated by naphthalene. Among these genes, *UGT72B1* (AT4G01070) is highly active in conjugating the persistent pollutants, such as 3,4-dichloroaniline (DCA) and 2,4,5-trichlorophenol (TCP), whereas the *ATGSTF12* (AT5G17220) gene is conjugated with anthocyanins [33,34]. UGT72B1-catalyzed and ATGSTF12-catalyzed conjugations may occur for naphthalene.

In the end, the conjugated xenobiotics are exported to either the vacuole or the apoplast using ATP-binding cassette transporters (ABC transporters) or multidrug and toxic compound extrusion (MATE) transporters [30]. Arabidopsis harbors 105 predicted members of the ABC transporters, characterized by the presence of a specific transmembrane and signature ATP-binding cassette domain [35]. Three transporter proteins were found to be induced by naphthalene, in which an ABC transporter was induced with about a fivefold increase in expression (Table 1).

3.4. Genes involved in stress responses to naphthalene

In addition to detoxification of PAHs, the stress response and defense mechanisms of plants to PAH toxicity are poorly understood. Plant cells responding to PAH exposure were characterized by localized cell death in Arabidopsis [15]. Treatment with high concentrations of naphthalene also promoted plant death (Fig. 2A). Programmed cell death is mediated by local increases in ROS levels. Among the five naphthalene upregulated peroxidase genes, peroxidase 22 (AT2G38380) was increased to nearly fivefold. This peroxidase is involved in ROS generation, both locally and systemically, to activate cell death as a response to pathogen invasion and xenobiotic uptake [36]. We also found a cysteine protease (AT3G57810) induced threefold by naphthalene. Cysteine proteases play many roles in plant physiology and development, including senescence and programmed cell death [37]. On the other hand, the ubiquitin–proteasome system plays a prominent role in the control of apoptosis by conjugating many proteins and committing them for breakdown [38]. Among the upregulated genes, a ubiquitin-protein ligase (AT2G33770) increased 24-fold [39]. This protein is related to phosphate transport in the phloem between the shoots and the roots. The ubiquitin-protein ligase induces cell death by destroying the phosphate transporter and preventing phosphate accumulation in cells. The 26S protease (AT2G20140), substantially upregulated by naphthalene, might also be involved in the degradation of the target protein through the ubiquitin–proteasome system.

Plants have evolved a number of mechanisms to cope with different environmental stresses. However, the mechanism of plant responses to naphthalene has not been investigated to date. According to gene chip data, a number of putative stress defense genes were changed significantly after treatment with naphthalene. Among these, the gene encoding for phytochrome-associated protein 2 (PAP2; AT4G29080) was the most upregulated with an approximately 24-fold increase in expression. PAP2 belongs to the MYB transcription factor family and is a senescence-associated protein. Another MYB transcription factor PAP1 (AT1G56650), involved in flavonoid biosynthesis [40], was also induced fourfold. Characterization of the genes in the anthocyanin pathway unambiguously proved the role of flavonoids in resistance to abiotic stresses [41]. Expression of a putative acetyl-CoA C-acyltransferase gene *KAT5* (AT5G48880) induces anthocyanin biosynthesis. Another acetyl-CoA C-acyltransferase gene *KAT2* (AT2G3315) is required for the efficient mobilization of triacylglycerol (TAG); it can reduce the fatty acid chain length by successively cleaving two carbons at each turn of the cycle to yield acetyl-CoA. *KAT2* plays a major role in driving wound-activated responses by participating in the biosynthesis of jasmonic acid in wounded Arabidopsis plants [42]. Expression of both putative acetyl-CoA C-acyltransferase genes has been shown to be inducible by naphthalene in Arabidopsis seedlings.

An abiotic-associated transcription factor, DREB1A, was also induced by naphthalene. This transcription factor binds to the cold-responsive *cis*-element *CRT/DRE* and activates the expression of target genes, which encode proteins functioning in stress tolerance such as late embryogenesis abundant proteins, antifreeze proteins, RNA-binding proteins, and protease inhibitors. Overexpression of DREB1A in Arabidopsis results in enhanced tolerance to drought, salt, and freezing [43]. Other stress-defense-related transcription factors were also induced in response to naphthalene exposure in Arabidopsis seedlings. For example, an apparent increase in the level of a salt-tolerance transcription factor, STH, was observed. Salt tolerance produced by STH appeared to be partially dependent on an ATPase required for Na^+ efflux.

3.5. Genes involved in auxin response

Interestingly, some auxin relative genes were upregulated by naphthalene. A regulator of auxin efflux PIN3 was increased nearly 15-fold and a member of the IAA family of auxin-inducible genes *IAA7* up to 16-fold after treatment with naphthalene (Table 1). *IAA7* was induced by auxin and controlled the development of light-grown seedlings. The dominant gain-of-function *IAA7* mutation of Arabidopsis causes agravitropic root and shoot growth, short hypocotyls and stems, and auxin-resistant root growth [44]. Naphthalene is similar to the plant growth regulator naphthalene acetic acid, so the upregulated auxin-related protein can possibly serve as a common factor for plants in response to naphthalene and auxin. This protein might interact with the absorbed naphthalene and help plants respond to pollutant stress.

4. Conclusion

In conclusion, we identified a number of genes encoding known or putative proteins induced under naphthalene stress conditions. With the development of Arabidopsis functional genomics, the role of each protein identified through expression profiling analysis can be tested rapidly. These genes were classified according to their function in response to naphthalene treatment. The potential involvement of a number of genes in naphthalene metabolism and plant defense was illustrated by the induced expression of genes during the stress treatments. The characterization of differential gene regulation in this study provides relevant background data to help choose phytoremediation and phytosensing components. Further studies using other approaches, such as reverse genetics, are necessary to verify the roles of these genes in the metabolism of naphthalene and in plant stress responses. A widespread analysis of the promoter regions of these potential gene targets is useful to identify novel *cis*-regulatory elements responsive to naphthalene and product recombinant plants acting as biomarkers.

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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.jhazmat.2010.12.114.

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