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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.

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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 halfstrength 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 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 μ mol 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 Super-Script 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 $(1 \mu g)$ 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 μ L) contained cDNA with 20 ng total RNA, 0.2 μ M of each primer, 0.2 μ M SYBR, 3 mM MgCl₂, 200 μ M each of dATP, dCTP, and dGTP, 400 μ M 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 \pm 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.

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



Fig. 4. Comparison of gene expression data from microarray hybridization and realtime 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.

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.

Uprobe Uprobe ATGCR5550. Protexae inhibitor/seed storage/lip/d transfer protein (LTP) family protein 2.468 0.011 ATGCR5550. Protexae inhibitor/seed storage/lip/d transfer protein (LTP) family protein 2.468 0.011 ATGCR550.01 NADP-dependent codoreductase, related 8.406 0.011 ATGCR550.11 NADP-dependent codoreductase, related 4.407 0.000 ATGCR550.11 NADP-dependent codoreductase, invariative 4.407 0.000 ATGCR550.11 NADP-dependent codoreductase, invariative 4.407 0.000 ATGCR550.11 NADP-dependent codoreductase, invariative 2.087 0.003 ATGCR550.11 CVPCR50.11/00.01/01/01/01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01/01.01/01/01/01.01/01.01/01.01/01.01/01.01/01.01/01.01/01/01.0	Primary accession	Gene name	Fold change	<i>p</i> -Value
AT3C6850.1 Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein 2.468 00.11 AT3C11810.2 NADH-ubiquinone oxidoreductase-related 8.406 0.016 AT3C181810.2 NADH-dependent oxidoreductase, putative 4.472 0.001 AT3C181810.2 NADH-dependent oxidoreductase, putative 4.472 0.001 AT3C18190.1 DAMD-dependent oxidoreductase, putative 4.07 0.005 ATG37390.1 NADH-dependent oxidoreductase, putative 3.75 0.001 ATG37390.1 NADH-dependent oxidoreductase, putative 2.047 0.002 ATG37390.1 NADH-dependent oxidoreductase, butative 2.047 0.003 ATG37390.1 CVPSA515/MAH1 (MD-CHAIN ALKANE HYDROXLASE 1) 2.2314 0.003 ATG3250.1 CVPSA615/Geptatine P450, family 70, subfamily A, polypeptide 2) 2.859 0.025 ATG26280.1 CVPSA612 (cytochrome P450, family 70, subfamily A, polypeptide 2) 2.851 0.049 ATG26280.1 CVPSA612 (cytochrome P450, family 70, subfamily A, polypeptide 2) 2.682 0.022 ATG26280.1 CVPSA612 (cytochrome P450, family 70, subfamily A, polypeptide 2) 2.681 0.026 ATG262500.1	Uptake			
AT2C11810.1 MGDC: 1.2-disc/glg/cord 3-beta-glaactosyltransferase 3.014 0.008 AT3C181410.2 NDAP-disputnene oxidereductase-related 8.4466 0.016 AT3C181410.2 NDAP-disputnene oxidereductase-related 4.4407 0.001 AT3C137900.1 Dottoreductase related 4.4407 0.001 AT3C137900.1 NDAP-dependent oxidereductase; 1: NADPI debydrogenase 3.089 0.001 AT3C137900.1 Dottoreductase; nutative 2.274 0.003 AT3C137900.1 Oxidoreductase; 2.0C-FC(1) oxygenase family protein 2.087 0.003 AT3C13200.1 CVPT0866 (synchrome P450, family 7, oxybenide 50) 5.244 0.003 AT4C3250.1 CVPT0866 (synchrome P450, family 7, synthamily 7, oxybenide 50) 2.815 0.049 AT4C3250.1 CVPT386 (synchrome P450, family 7, synthamily 7, oxybenide 50) 2.815 0.049 AT4C33550.1 CVPT386 (synchrome P450, family 7, synthamily 7, oxybenide 50) 2.815 0.049 AT3C35590.1 CVPT386 (synchrome P450, family 7, synthamily 7, oxybenide 50) 2.815 0.049 AT3C43590.1 CVPT386 (synchrome P450, family 7, synthamily 7, oxybenide 50) 2.815 0.049 AT3C43590.1	AT3G58550.1	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	2.468	0.011
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AT4G20800.1 ADPC (Alkernyl Hydroxaleyl Producing 2): oxidoreductase 4.217 0.004 AT5G37940.1 NDP-dependent oxidoreductase, INADPH dehydrogenase 3.089 0.001 AT5G37940.1 DOR C (Protechlorophyllife Oxidoreductase): INADPH dehydrogenase 2.274 0.012 AT3G19400.1 Oxidoreductase, 20C-Fe(II) oxygenase family protein 2.087 0.003 AT1G43251.01 CYP706AG (cyrechrome P450, family 706, subfamily A, polypeptide 12) 2.859 0.025 AT1G43251.01 CYP706AG (cyrechrome P450, family 706, subfamily A, polypeptide 12) 2.859 0.025 AT1G43251.01 CYP706AG (cyrechrome P450, family 706, subfamily A, polypeptide 5) 2.682 0.032 AT1G47351.01 CYP70AG (cyrechrome P450, family 706, subfamily A, polypeptide 5) 2.682 0.032 AT1G47351.01 CYP70AG (cyrechrome P450, family 706, subfamily A, polypeptide 5) 2.682 0.032 AT1G473570.01 CYP70AG (CYPCHROME P450 70AG) 2.692 0.031 AT1G473570.01 CYP70AG (CYPCHROME P450 70AG) 2.692 0.031 AT1G473570.01 CUP70AG (CYPCHROME P450 70AG) 2.683 0.033 AT1G473570.01 CUP70AG (CYPCHROME P450 70AG) 2.645 0.033	AT5G37960.1	Oxidoreductase-related	4.403	0.005
ATSG37940.1 NADP-dependent oxidoreductase, putative 3.75 0.001 ATIGG380.1 D04 (CProtochlorophyllide Oxidoreductase); NADPI dehydrogenase 3.089 0.001 ATIGG380.1 D04 doreductase; COC-Fe(II) oxygenase family protein 2.087 0.003 ATIGG380.1 CYPS6A15 (MAH1 (MID-CHAN ALKANE HYDROXYLASE 1) 2.3214 0.0015 ATIGG380.1 CYPS6A15 (cytochrome P450, family 905, subfamily A, polypeptide 26) 2.815 0.049 ATIGG380.1 CYPS6A12 (cytochrome P450, family 706, subfamily A, polypeptide 12) 2.859 0.022 ATIGG480.1 CYPS6A12 (cytochrome P450, family 706, subfamily A, polypeptide 5) 2.682 0.032 ATIGG4800.1 CYPS6A12 (cytochrome P450, family 706, subfamily A, polypeptide 5) 2.682 0.032 ATIGG4800.1 CYPS6A12 (cytochrome P450, family 706, subfamily A, polypeptide 5) 2.089 0.001 ATIGG4800.1 CYPS6A12 (cytochrome P450, family 706, subfamily A, polypeptide 5) 2.081 0.001 ATIGG4800.1 CYPSCE1 (cytochrome P450, family 706, subfamily A, polypeptide 5) 2.081 0.001 ATIGG4800.1 CYPSCE1 (cytochrome P450, family 706, subfamily A, polypeptide 5) 2.082 0.001 ATIGG4800.1 CYPSCE1 (cytochrom	AT4G03060.1	AOP2 (Alkenyl Hydroxalkyl Producing 2); oxidoreductase	4.217	0.001
ATI-G0380.1 PDR C (Protochlorophyllide Oxidoreductase): NADPH dehydrogenase 3.089 0.001 ATIG-G1380.1 Doidoreductase, 20C-Fe(I) oxygenase family protein 2.087 0.003 ATIG-G1380.1 CYPPO6A6 (cytochtome P450, family VDR, subfamily A, polypeptide 5) 2.2314 0.003 ATIG-G7250.1 CYPPO6A6 (cytochtome P450, family VDR, subfamily A, polypeptide 5) 2.855 0.025 ATIG-G2380.1 CYPPO6A6 (cytochtome P450, family VDR, subfamily A, polypeptide 5) 2.682 0.032 ATIG-G2630.1 CYPPO6A6 (cytochtome P450, family VDR, subfamily A, polypeptide 5) 2.682 0.032 ATIG-G4880.1 CYPPO6A6 (cytochtome P450, family VDR, subfamily A, polypeptide 5) 2.682 0.032 ATIG-G4890.1 CYPRSA6 (cytochtome P450, family 70, subfamily A, polypeptide 5) 2.682 0.032 ATIG-G4890.1 CYPRSA6 (cytochtome P450, family 70, subfamily A, polypeptide 5) 2.682 0.032 ATIG-G4890.1 CYPRSA6 (cytochtome P450, family 70, subfamily A, polypeptide 5) 2.633 0.001 ATIG-G4900.1 CYPRSA6 (cytochtome P450, family 70, subfamily A, polypeptide 5) 2.633 0.001 ATIG-G4900.1 CYPRSA6 (cytochtome F450, family protein	AT5G37940.1	NADP-dependent oxidoreductase, putative	3.75	0.004
AT1C14345.1 Oxidoreductase 2.274 0.012 AT3C1900.1 Oxidoreductase 2.007 0.003 AT1C37750.1 CVP96A15 MAH1 (MID-C14N1 ALKABE HYDROXYLASE 1) 2.2314 0.003 AT1C37750.1 CVP96A12 (cytochrone P450, family polyseptide 5) 5.244 0.005 AT4C12320.1 CVP96A12 (cytochrone P450, family 905, subfamily A, polypeptide 2) 2.859 0.022 AT3C62830.1 CVP796A12 (cytochrone P450, family 905, subfamily A, polypeptide 5) 2.882 0.032 AT3C61830.1 CVP79CA12 (cytochrone P450, family 976, subfamily A, polypeptide 5) 2.089 0.011 AT1C64390.1 CVP79CG1 (cytochrone P450, family 976, subfamily A, polypeptide 1) 2.303 0.011 AT1C64390.1 CVP7SC1 (cytochrone P450, family 976, subfamily A, polypeptide 1) 2.039 0.001 AT1C64390.1 CVP7SC1 (cytochrone P450, family 976, subfamily A, polypeptide 1) 2.033 0.001 AT1C64390.1 SUS4: UDP, glycocyttransferase future set transferase transfering glycosyl groups 4.167 0.0001 AT4C00501. Cytocyttransferase family protein 2.147 0.0022 AT4C0051.1 Tansferase family	AT1G03630.1	POR C (Protochlorophyllide Oxidoreductase): NADPH dehydrogenase	3.089	0.001
ATIG: 19000.1 Oxidiaredicase. 20C-Fe(II) expgenase family protein 2.087 0.003 ATIG: 577.50.1 CYP806A15 (MAH HURD-CHAN NLKAR HYDROXYLASE 1) 22.314 0.031 ATIG: 577.50.1 CYP806A5 (cytochrome P450, family 96, subfamily A, polypeptide 6) 5.244 0.005 ATIG: 527.50.1 CYP806A3 (cytochrome P450, family 96, subfamily A, polypeptide 2) 2.859 0.022 ATIG: 527.50.1 CYP70EA5 (cytochrome P450, family 705, subfamily R, polypeptide 2) 2.862 0.032 ATIG: 628.0.1 CYP78A9 (CYTOCHROME P450, family 705, subfamily C, polypeptide 2) 2.089 0.001 ATIG: 658.0.1 CYP78A9 (CYTOCHROME P450, family 75, subfamily C, polypeptide 1) 2.303 0.011 ATIG: 654900.1 CYP88A2 (CYTOCHROME P450 89A2) 2.089 0.001 Conjugation	AT1G14345.1	Oxidoreductase	2.274	0.012
ATLGS750.1 CYPB6AL SMAHL (MD-CHAIN ALKANE HYDRXYLASE 1) 22.314 0.031 ATG22520.1 CYPB6AD (STORME ASS, DAMAR), A polypeptide 6) 5.244 0.005 ATG26250.1 CYPB6AD (STORME ASS, DAMAR), A polypeptide 61) 2.859 0.025 ATG26250.1 CYP706AS (STOCHTORME P450, DAMIY 70, subfamily A, polypeptide 2) 2.851 0.049 ATG613210.1 CYP706AS (STOCHTORME P450, TARA) 2.662 0.032 ATG643560.1 CYP76AS (CYTOCHTORME P450, TARA) 2.033 0.011 ATG643560.1 CYP76AS (CYTOCHTORME P450, TARA) 2.039 0.001 ATG643560.1 CYP76C1 (STOCHTORME P450, TARA) 2.033 0.001 ATG643190.1 SUS4; UDP-glycosyltransferase/sucrose synthase/transferase 2.238 0.001 ATG601950.1 Galvosyltransferase/sucrose synthase/transferase 3.951 0.009 ATG601950.1 Glycosyltransferase/sucrose/synthaser/tansferase 2.147 0.002 ATG601950.1 Glycosyltransferase/sucrose/synthaser/tansferase 2.147 0.002 ATG601950.1 Glycosyltransferase/sucrose/synthaser/tansferase 2.147 0.002 ATG601950.1 Glycosyltransferase/sucrose/synthaser/tansferase <td< td=""><td>AT3G19000 1</td><td>Oxidoreductase 20G-Fe(II) oxygenase family protein</td><td>2.087</td><td>0.003</td></td<>	AT3G19000 1	Oxidoreductase 20G-Fe(II) oxygenase family protein	2.087	0.003
r14G12320.1 CYP706A6 (cyrochrome P450 family 706, subfamily A, polypeptide 6) 5.244 0.005 r14G39510.1 CYP70FA65 (cyrochrome P450 family 70, subfamily A, polypeptide 12) 2.859 0.025 r14G32510.1 CYP70FA65 (cyrochrome P450 family 70, subfamily A, polypeptide 25) 2.862 0.032 r14G12310.1 CYP70FA65 (cyrochrome P450 family 76, subfamily A, polypeptide 5) 2.467 0.029 r14G64500.1 CYP78A9 (CyroCHROME P450 F8A0) 2.069 0.001 r1G64500.1 CYP78A9 (CyroCHROME P450 F8A0) 2.033 0.001 r1G57590.1 CYP89A2 (CyroCHROME P450 58A2) 2.099 0.001 r1G57590.1 CYP80A2 (CyroCHROME P450 58A2) 2.033 0.001 r1G57590.1 CYP80A2 (CyroCHROME P450 58A2) 2.033 0.001 r1G57590.1 CSUB-US-Systemasferase/stansferase/stansferase 3.51 0.009 r1G50590.1 GYroSystamsferase/stansferase/stansferase 2.147 0.021 r1G5172.01.1 GYroSystamsferase patiative 5.189 0.021 r1G5172.01 ATGCASSI0.1 ATGCASSI0.1 5.183 0.021 r1G5172.01 ATGCASSI0.1 ATGCASSI0.1 5.421 0.033	AT1G57750.1	CYP96A15/MAH1 (MID-CHAIN ALKANE HYDROXYLASE 1)	22.314	0.031
TAG293101 CYP96A12 (cyrochrome P430, family 96, subfamily A, polypeptide 12) 2.859 0.025 ATG262500 CYP71826 (cyrochrome P430, family 706, subfamily A, polypeptide 5) 2.682 0.032 ATG612310.1 CYP706A5 (cyrochrome P430, family 706, subfamily A, polypeptide 5) 2.682 0.032 ATG61880.1 CYP72A9 (CYP0CH (Kobe P450, family 706, subfamily A, polypeptide 1) 2.033 0.011 ATIG64900.1 CYP8A3 (CYP0CH KObe P450, family 76, subfamily C, polypeptide 1) 2.038 0.001 ATIG64900.1 CYP8A3 (CYP0CH KObe P450, gasA2) 2.038 0.001 ATIG64900.1 CYP8A3 (CYP0CH KObe P450, gasA2) 2.338 0.001 ATIG63750.1 Carboxylestrease 2.338 0.001 ATIG64900.1 CT281; UDP-glycosyltransferase/UDP-glycosyltransferase 3.951 0.009 ATIG64930.1 Grupsyltransferase family protein 2.147 0.002 ATGC613910.1 ATIGFT261, (Lytathione S-Transferase 26); glutathione transferase 2.884 0.033 ATIG643910.1 Thiol methyltransferase, transferring glycosyl groups 8.773 0.005 ATSG4390.1 ATIG1853 (ransferase 51); hydrolase, acting on glycosyl groups 8.773 0.005	AT4G123201	CYP706A6 (cytochrome P450) family 706 subfamily A polypeptide 6)	5244	0.005
T3C262901 CYP71B26 (cytochrome P430, family 7L, subfamily K, polypeptide 26) 2.815 0.049 TAGL2101 CYP76A5 (cytochrome P430, family 7D, subfamily A, polypeptide 5) 2.682 0.032 TAGE45860.1 CYP76AC (cytochrome P430, family 7D, subfamily C, polypeptide 1) 2.039 0.011 ATIGE45860.1 CYP76C1 (cytochrome P430, family 7D, subfamily C, polypeptide 1) 2.039 0.001 ATIGE45860.1 CYP78AC (cytochrome P430, family 7D, subfamily C, polypeptide 1) 2.039 0.001 ATIGE45860.1 CYP78AC (cytochrome P430, family 7D, subfamily C, polypeptide 1) 2.039 0.001 ATIGE45800.1 CYP78AC (cytochrome P430, family 7D, subfamily C, polypeptide 2) 2.038 0.001 ATIGE4590.1 Stuby Cytochrome P430, family 7D, subfamily 7D, subfami	AT4G395101	CYP96A12 (cytochrome P450 family 96 subfamily A polypeptide 12)	2,859	0.025
AT4G12310.1 CYP20685 (cytochrome P450, family 70s subfamily A, polypeptide 5) 2.682 0.032 AT3G61880.1 CYP20A0 (CYTOCHROME P450 78A9) 2.407 0.029 AT3G61880.1 CYP26.1 (cytochrome P450, family 76, subfamily C, polypeptide 1) 2.099 0.001 AT1G64900.1 CYP28.0 (CYTOCHROME P450 88A2) 2.338 0.001 Conjugation 2.338 0.001 AT3G4180.1 SUS4; UDP-glycosyltransferase/sucrose synthase/transferase 3.051 0.009 AT4G05100.1 GT2281; UDP-glycosyltransferase/glycosyltransferase 3.051 0.009 AT4G0500.1 GT2281; UDP-glycosyltransferase/glycosyltransferase 3.851 0.009 AT4G0500.1 GT281; UDP-glycosyltransferase glycosyltransferase 3.833 0.006 ATG61390.1 GTC6132; transferase family protein 3.633 0.005 AT3G4390.1 ATGS (syloca) transferase gly: hydrolase, acting on glycosyl groups 8.773 0.005 AT3G4390.1 ATGS (syloca) transferase gly: thydrolase, acting on glycosyl groups 8.773 0.005 AT3G4390.1 ATGS (syloca) transferase gly: thydrolase, acting on glycosyl groups 8.773 0.005 AT3G43990.1 ATGS (syloca) transfer	AT3C26290 1	CYP71B26 (cytochrome P45, family 71, subfamily 8, polyperide 26)	2.815	0.049
ATGG1801 CVP78A9 (CYDCLIROME P450 78A9) 2.407 0.029 ATGG1801 CVP78A9 (CYDCLIROME P450 78A9) 2.407 0.029 ATGG1801 CVP78A9 (CYDCLIROME P450 78A9) 2.099 0.001 ATGG45001 CVP78A2 (CYDCLIROME P450 89A2) 2.099 0.001 ATGG45001 CVP78A2 (CYDCLIROME P450 89A2) 2.019 0.001 ATGG45101 GryBaz (CYDCLIROME P450 89A2) 2.019 0.001 ATGG45101 GryBaz (CYDCLIROME P450 89A2) 2.147 0.001 ATGG01701 GTZ72B1; UDP-glycosyltransferase (Juptycosyltransferase) 2.834 0.033 ATGG01701 GTS712 (Clutathione S-Transferase (Juptycosyl groups 3.633 0.006 ATGG19301 ATGC15712 (Clutathione S-Transferase (Juptycosyl groups 3.633 0.006 ATGG4900.1 XTR8 (vyloglucan:xyloglucosyl transferase, transferring glycosyl groups 3.73 0.005 ATGG3500.1 Transferase family protein 2.45 0.003 ATGG2050.1 Start ansporter family protein 2.45 0.006 ATGG2050.1 Transferase family protein 3.422 0.014 ATGC3570.1 </td <td>AT4C12310.1</td> <td>CVP706A5 (cytochrome P450, family 70, subfamily A, polypeptide 5)</td> <td>2.613</td> <td>0.032</td>	AT4C12310.1	CVP706A5 (cytochrome P450, family 70, subfamily A, polypeptide 5)	2.613	0.032
ATSAMU00.1 C1PPSG1 (Cytochrome P450, family 76, subfamily C, polypeptide 1) 2.303 0.011 ATIG45560.1 CYPSG2 (Cytochrome P450, family 76, subfamily C, polypeptide 1) 2.099 0.001 ATIG57590.1 Carbox/jesterase 2.238 0.001 Conjugation ATIG45790.1 Carbox/jesterase 3.951 0.001 ATIG67590.1 GTycosyltransferase/UDP-glycosyltransferase 3.951 0.002 ATG0170.1 GT7281; UDP-glycosyltransferase/UDP-glycosyltransferase 3.853 0.006 ATG0570.1 ATGST12 (Clutathione S-Transferase) (30; glutathione transferase 3.884 0.003 ATIG43910.1 Thio methyltransferase, putative 5.189 0.001 ATGC43910.1 Thio methyltransferase, putative 5.189 0.002 ATG24390.1 ATRSK1y0glucan:xyloglucosyl transferase[transferase, transferring glycosyl groups 8.773 0.005 ATG2590.1 ATGS15010 ABC transporter family protein 2.45 0.003 ATG42390.1 Transferase family protein 2.45 0.003 ATG62501.0 Sugar transporter, putative 2.643 0.003 ATG625701.0 Nitrate transporter family protein <	AT3C61880.1	CVD7840 (CVTOCHPOME 1450, failing 760, sublating 7, polypeptide 5)	2.002	0.032
Tracescolor. C/YB9A2 (CYTOCHROME P450 89/2) 2.099 0.001 ATTGG49001 C/YB9A2 (CYTOCHROME P450 89/2) 2.099 0.001 ATTGG49001 Carboxyltransferase/sucrose synthase/transferase, transferring glycosyl groups 4.167 0.001 ATGG43190.1 SUS4: UDP-glycosyltransferase/sucrose synthase/transferase 2.147 0.002 ATGG0170.1 GTSTP21; UDP-glucosyltransferase (Juptersterase 2.147 0.002 ATGG0170.1 GTSTP21; Clutathione S-transferase (Juptersterase) 2.884 0.033 ATGG0350.1 ATGCSTP12; Clutathione S-transferase (Juptersterase) 5.189 0.021 ATGC4390.1 Thiol methyltransferase, transferring glycosyl groups 5.189 0.021 ATGC4390.1 ATGC439.10 Transferase family protein 2.45 0.006 ATGC4200.1 XTR8 (vyloglucan-xyloglucosyl transferase, transferring glycosyl groups 8.773 0.005 ATGC4390.1 Transferase family protein 2.45 0.003 ATGC4200.1 Sugar transporter (MTP3) 3.482 0.032 ATGC4201.1 Nitrate transporter (MTP3) 3.482 0.032	AT2C/5560 1	CVP76C1 (outochrome P450, family 76, subfamily C, polyneptide 1)	2.407	0.025
ATIGS730.1 Citrobia (CitroEnder Fub OSEC) 2.33 0.001 Conjugation	AT1C6/900 1	CVDSQA2 (CVTCCHDOME D450 802)	2.000	0.011
NT101301. Callouplextense 0.001 AT3C43190.1 SUS4; UDP-glycosyltransferase/sucrose synthase/transferrase transferring glycosyl groups 4.167 0.001 AT3C401070.1 GTZ2B1; UDP-glycosyltransferase/UDP-glycosyltransferase 3.951 0.009 AT4G01070.1 GTZ2B1; UDP-glycosyltransferase (JUDP-glycosyltransferase 3.951 0.009 AT4G01070.1 GTZ2B1; UDP-glycosyltransferase (JUDP-glycosyltransferase 2.884 0.033 ATGG9350.1 ATGCSTE12 (Gutathione S-Transferase 2b; glutathione transferase 3.633 0.006 ATGC4390.1 ATGCLG3 (Cellulose synthase-like G3); transferase, transferring glycosyl groups 8.773 0.005 ATGC4390.1 ATGLG3 (Cellulose synthase-like G3); transferase (transferase, transferring glycosyl groups 8.773 0.006 ATGC4390.1 ATGLG3 (Cellulose synthase-like G3); transferase (transferase, transferring glycosyl groups 8.773 0.005 ATGC4670.1 Nitrate transporter 2.45 0.003 ATGC4701.0 Nitrate transporter, MTP3) 2.45 0.032 ATGC4701.1 Nitrate transporter, MTP3) 3.482 0.032 ATGC47810.2 OTU-like cysteine protease family protein 5.136 0.006 <	AT1C57500.1	Carboviletorso	2.035	0.001
Cabigation 4.167 0.001 AT3GA1190.1 SUS4; UDP-glycosyltransferase/sucrose synthase/transferase 3.951 0.009 AT4G01070.1 GT7281; UDP-glycosyltransferase cabigy protein 2.147 0.002 AT4G0950.01 GLycosyltransferase family protein 2.147 0.002 ATGC1301 ATGC0153; transferase, transfering glycosyl groups 3.633 0.006 ATIC030301 ATGC0153; transferase, pratese 8); hydrolase, acting on glycosyl groups 8.773 0.003 AT4G2390.1 Transferase family protein 2.45 0.003 ATGC3570.0 ABC transporter family protein 5.421 0.046 AT4G02050.1 Sugar transporter, putative 2.643 0.003 ATGC3370.01 ABC transporter family protein 3.482 0.032 ATGC33770.1 Nitrate transporter (NTP3) 3.482 0.032 ATGC43770.1 ATUBC24(PH05/HATE 2); ubiquitin-protein ligase 2.4419 0.011 ATGC63770.1 Peroxidase, putative 4.284 0.004 ATGC33770.1 Peroxidase, putative 6.694 0.012	Conjugation	Caliboxylestelase	2.230	0.001
AT3G01701 GT72B1: UPD-glucosyltransferase/UD-glucosyltransferase 3.851 0.001 AT4G010701 GT72B1: UPD-glucosyltransferase/UD-glucosyltransferase 2.844 0.002 AT3G1021021 ATGSTF12 (Utathinos 5-Transferase) 2.844 0.003 ATGC102101 ATGST512 (Utathinos 5-Transferase) 2.844 0.003 ATGC102101 ATGST512 (Utathinos 5-Transferase); glutathione transferase 3.833 0.006 ATGC40930.1 ATGSL32 (Utathinos 5-Transferase); glutathione transferase 5.189 0.021 ATGC30101 Transferase family protein 2.45 0.003 ATGC2050.1 Transferase family protein 5.421 0.046 ATGC2050.1 Sugar transporter, putative 2.643 0.003 ATGC2050.1 Sugar transporter (NTP3) 3.482 0.003 Cell death		SUS4. UDD glugogultangeforage/gugages suppliese/transforage_transforage_transforage	4 167	0.001
AT4G0950.1 G1/201; UDP-gutusylitalisterase/UDP-gytusylitalisterase 3.931 0.009 AT4G0950.01 G1/yosylitanisterase/Bytualisterase/UDP-gytusylitalisterase 2.147 0.002 AT4G0950.01 ATGOTSTF12 (Glutathione 5-Transferrase 26); glutathione transferase 2.884 0.033 ATGO4050.1 ATGOTSTF12 (Glutathione 5-Transferrase 26); glutathione transferase 5.189 0.001 ATGC43910.1 Thiol methyltransferase, putative 5.189 0.003 AT4G23990.1 ATGSLG3 (Cellulose synthase-like G3); transferase, transferring glycosyl groups 8.773 0.005 ATGC3550.01 Transferase family protein 2.45 0.003 ATGC2050.1 Sugar transporter, putative 2.643 0.003 ATGC2050.1 Sugar transporter, putative 2.643 0.003 ATGC2050.1 Sugar transporter, putative 2.136 0.016 ATGC33770.1 ATUBC24/FH02/UBC24 (PHOSPHATE 2); ubiquitin-protein ligase 24.419 0.011 ATGC60730.1 Peroxidase, putative 4.284 0.014 ATGC3370.1 ATUBC24/FH02/UBC24 (PHOSPHATE 2); ubiquitin-protein ligase 24.419 0.011 ATGC60730.1 Peroxidase, putative 4.	AT4C01070.1	CT2011, UDD elivery/iterar/ference/UDD elivery/iterar/ference	4.107	0.001
ATGG9300.1 CityOsyntanseetas laming protein 2.147 0.002 ATGG712.01 ATGGF12.01 ATGGF12.01 0.033 ATGG730.1 ATGGF12.01 0.033 0.006 ATGG702.01 ATGGV12.01 0.033 0.006 ATGG702.01 Thiol methyltransferase. transferring glycosyl groups 3.633 0.006 ATGG4900.1 XTR8 (xyloglucan:xyloglucosyl transferase 8); hydrolase, acting on glycosyl groups 8.773 0.005 ATGG501.0 Transferase family protein 2.45 0.003 Transporter 7 0.046 0.046 ATGC57810.0 ABC transporter family protein 5.421 0.046 ATGC57810.1 Sigar transporter, putative 2.643 0.003 ATGC57810.2 OTU-like cysteine protease family protein 2.136 0.011 ATGC57810.2 OTU-like cysteine protease family protein 2.136 0.014 ATGC57810.2 OTU-like cysteine protease family protein 2.135 0.008 ATGC57810.2 OTU-like cysteine protease family protein 2.136 0.011 ATGC57810.2 OTU-like cysteine protease family protein 2.133 0.026	AT4G01070.1	G172B1; ODP-glucosylitansietase/ODP-glycosylitansietase	3.951	0.009
ATISG 17220.1 ATISG 1712 (Juitationine 5-1 ransfertase 2b); guitatione transfertase 2.884 0.033 ATIG09350.1 ATIGOS S.633 0.006 2ATIC43910.1 Thiol methyltransferase, putative 5.189 0.021 ATIG09350.1 ATIGOS 19.388 0.003 ATIG423990.1 ATRS (x)Bigulexas; X)Bigulexas; YL ansferase 8; hydrolase, acting on glycosyl groups 8.773 0.005 ATIG0550.1 Transferase family protein 2.45 0.003 ATGC2050.1 Sugar transporter, putative 2.643 0.003 ATIG055100 ABC transporter (NTP3) 3.482 0.032 ATIGC57810.2 OTU-like cysteine protease family protein 2.136 0.016 ATIG23770.1 ATUBC24(PHO2/UBC24 (PHOSPHATE 2); ubiquitin-protein ligase 2.4419 0.011 ATIGC9370.1 Peroxidase, putative 4.859 0.003 ATIG23830.1 Peroxidase 22 (PER22) (P22) (PRXR3) 2.153 0.006 ATIG238380.1 Peroxidase 22 (PER22) (P22) (PRXEA)/basic peroxidase E 4.947 0.006 ATIG238380.1 Peroxidase 22 (PER22) (P22) (PRXEA)/basic peroxidase E 2.033 0.002 ATIG238380.1	A14G09500.1	Giycosyltransterase family protein	2.147	0.002
ATIC0350.1 ATGOLS3 (Transferase, transferring glycosyl groups 3.633 0.006 ATICG43910.1 Thiol methyltransferase, putative 5.189 0.021 ATGC43910.1 TRIK (xyloglucan:xyloglucosyl transferase 8); hydrolase, acting on glycosyl bonds 19.388 0.003 ATGC63910.1 Transferase family protein 2.45 0.003 Transporter 73 0.066 ATGC43910.1 Staga transporter, putative 2.643 0.003 ATGC2050.1 Sugar transporter, putative 2.643 0.003 ATGC357810.2 OTU-like cysteine protese family protein 2.136 0.016 ATGC357810.2 OTU-like cysteine protese family protein 2.136 0.016 ATGC357810.2 OTU-like cysteine protese family protein 2.136 0.008 ATGC3370.1 ATUBC24/(PHO2/UBC24 (PHOSPHATE 2); ubiquitin-protein ligase 24.419 0.011 ATGC3370.1 Peroxidase, putative 4.284 0.003 ATGC33830.1 Peroxidase 22 (PER22) (PZ2) (PXR3) 2.153 0.008 ATGC3370.1 Peroxidase 22 (PER22) (PZ2) (PRXR3) 2.153 0.002 ATGC33830.1 Peroxidase 22 (PER22) (PZ2) (PRXA3/basi	AT1 C00250 1	AIGSTF12 (Glutatnione S-Transferase 26); glutatnione transferase	2.884	0.033
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AT2G20140.1 Plant defense26S protease regulatory complex subunit 4, putative13.2730.02Plant defense	AT2G38380.1	Peroxidase 22 (PER22) (P22) (PRXEA)/basic peroxidase E	4.947	0.006
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AT3G61900.1Auxin-responsive family protein2.5120.003DR368506Auxin-responsive family protein2.3240.034	AT3G23050.1	IAA7 (AUXIN RESISTANT 2); transcription factor	16.675	0.031
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	DR368506	Auxin-responsive family protein	2.324	0.034
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and sequestered by glutathione-transferases (GST) and UDPglucoronosyl/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 3,4dichloroaniline (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 ATPbinding 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-defenserelated 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 lightgrown 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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