Master's Thesis

Characterization of Defensive Response Influenced by Volatile Oxylipin in Arabidopsis

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Department of Biotechnology

GRADUATE SCHOOL

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(Supervised by professor Dong-Sun Lee and Pyung-Ok Lim)

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for the degree of Master of Biotechnology

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ABBREVIATIONS

ABA	Abscisic acid
ACC2	1-aminocyclopropane-1-carboxylate synthase 2
ACT2	Actin 2
AOS	Allene oxide synthase
C6	Six carbon
CHS	Chalcone synthase
DES	Divinyl ether synthase
DFR	Dihydroflavonol reductase
DOX	Dioxygenase
EAS	Epoxy alcohol synthase
ESTs	Expressed sequence tags
ET	Ethylene
ERF	Ethylene response factor
FC	Fold change
GLVs	Green leaf volatiles
GO	Gene ontology
HPL	Hydroperoxide lyase
JA	Jasmonic acid
LOX	Lipoxygenase

MAPK	Mitogen activated protein kinase
MeJA	Methyl jasmonic acid
MeSA	Methyl salicylic acid
MEV	Multi experimental viewer
MS	Murashige and Skoog
PDF1.2	Plant defensin gene 1.2
PR1	Pathogenesis-related gene class 1
PR gene	Pathogenesis-related gene
Q-RT-PCR	Quantitative real-time polymerase chain reaction
R gene	Resistance gene
SA	Salicylic acid
TAIR	The arabidopsis information resource
THI2.1	Thionin 2.1

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SUMMARY

Oxylipins are oxygenated natural products derived from fatty acids and widespread in aerobic organisms such as plant, animal and fungi. The important function of these compounds in plant is the regulation of many defense responses and developmental processes. However, although the role of JA in defense responses and signalling, and cross-talk between JA and other signal molecules such as salicylic acid (SA), ethylene (ET) and abscisic acid (ABA) have been extensively investigated in plant, other oxylipins including cis-3-hexenal presented in most plants and 1-octen-3-ol known as fungal oxylipin discovered in some plants have not been fully investigated at molecular level.

Thus, in this study, we focused on identification of transcriptional responses in Arabidopsis after treatment of cis-3-hexenal and 1-octen-3-ol to understand defense response and signal transduction by them.

As the results, transcriptional responses by vapor treatments of oxylipin, 3-hexenal and 1-octen-3-ol, in Arabidopsis were identified through microarray analysis, and total numbers of 414 and 547 genes were up- or down- regulated more than 2 folds in the treatments of each 3-hexenal and 1-octen-3-ol, respectively. About 30% of those genes 124/414 genes in 3-hexenal treatment and 167/547 genes in 1-octen-3-ol, were related to biotic/abiotic responses. In the clustering of expression patterns, genes related to biotic/abiotic responses revealed two representative patterns, up-regulation consistently during 24 hr or progressive increase until 12 hr time point in each treatment of 3-hexenal and 1-octen-3-ol. Furthermore, the genes related to biotic/abiotic responses were mapped into biotic stress pathway regarding to R and PR genes, hormone (auxins, ABA, ethylene and JA) signaling, cell wall, proteolysis, redox state, peroxidases, glutathione-S-transferase, signaling, MAPK, transcription factors (ERF

and WRKY), heat shock proteins and secondary metabolism. The number of genes mapped into biotic stress pathway was 85 of 414 genes (about 20%) and 117 of 547 genes. Interestingly, almost of genes up- or down- regulated more than two folds in each 3-hexenal and 1-octen-3-ol treatment were overlapped, and rate of overlapping genes was more than 85% in up-regulated genes, and more than 60% in down-regulated genes.

Based on these results, it was assumed that when plant recognizes 3-hexenal or 1-octen-3-ol, various biotic stress genes including R and PR genes, hormone signaling, redox state, cell wall, proteolysis, signaling, and transcription factors related genes might be up-regulated during 12 hr to increase defense system in the cell.

INTRODUCTION

Oxylipins are an important class of biologically active compounds derived from the catabolism of fatty acids, which regulate many defense responses and developmental processes in plant [Creelman and Mulpuri, 2002; Eckardt, 2008]. These compounds are not pre-formed but rather are synthesized de novo in response to mechanical injury, herbivore and pathogen attack, and other environmental and developmental inputs [Howe and Schilmiller, 2002]. Representative compounds of oxylipins are jasmonic acid (JA), 3-hexenal, α- & γ-ketol, colneleric acid, etherolenic acid, 1-octen-3-ol, etc [Blee, 1998; Feussner and Wasternack, 2002; Grechkin, 1998].

The biosynthesis of most plant oxylipins is initiated through releasing fatty acid from membrane lipids by lipase, and then oxidative reaction is followed by lipoxygenase (LOX) or dioxygenase (DOX) to generate hydroperoxy fatty acid [Feussner *et al.*, 2001]. Hydroperoxy products are metabolized to an array of oxylipins by several enzymes, including allene oxide synthase (AOS), hydroperoxide lyase (HPL), divinyl ether synthase (DES), epoxy alcohol synthase (EAS), peroxygenase, alkyl hydroperoxide reductase, and LOX itself [Howe and Schilmiller, 2002]. General biosynthetic pathway of oxylipins in plant and enzyme in each step are described in Figure 1.

Most of oxylipin compounds in plant are synthesized through the four major metabolic pathway, namely AOS, HPL, DES and EAS, and representative active compounds produced via these pathway are JA, C6 or C9 aldehydes, divinyl ether and epoxy alcohol, respectively [Gardner, 1991; Hamberg, 1988, 1999; Matsui, 1998]. In addition to those, one more branch pathway is known to present in several plant, producing octenol such as 1-octen-3-ol. However, the biosynthetic pathway has not been studied well in plant, althrough several

investigations have been performed in mushroom and fungi, suggesting pathway that 10-DOX catalyses oxygenation of linoleic acid into a hydroperoxyoctadecadienoic acid, and then subsequent enzymatic cleavage by 10-HPL produce 1-octen-3-ol [Borjesson *et al.*, 1990; Mau *et al.*, 1992; Tuma *et al.*, 1989].

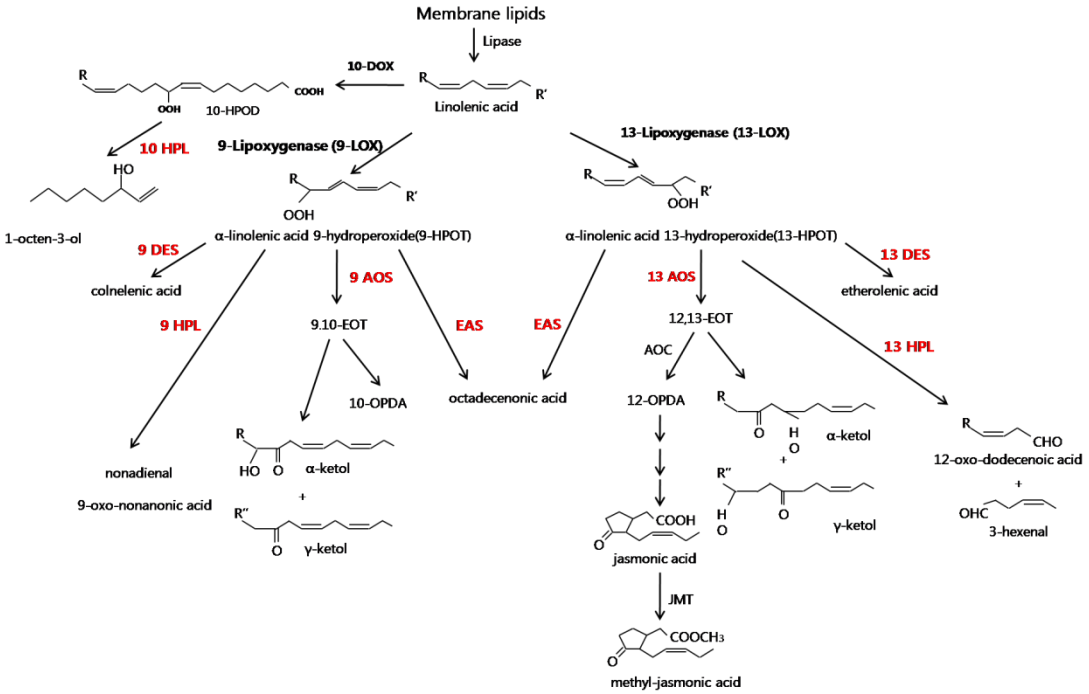


Figure 1. General biosynthetic pathway of oxylipin in plant and enzyme in each step.

The best characterized oxylipin in plant defense response is JA which is biosynthesized through AOS pathway and accumulated in response to various stresses, hervibore and pathogen attack [Block *et al.*, 2005]. The role of JA in stress signaling has been investigated extensively and their results have been supporting that its important role is key regulators in plant defense response against biotic/abiotic stress such as hervibore and pathogen attack, wounding and water deficiency [Farmer and Ryan, 1990; Hermsmeier *et al.*, 2001; Reymond *et al.*, 2000]. Furthermore, cross-talk between JA and other signal molecules possessing an

important function in plant defense response such as salicylic acid (SA), ethylene (ET) and abscisic acid (ABA) have been studied, suggesting antagonistic and synergistic interactions between these signals might contribute towards the specificity of the final defense response [Anderson *et al.*, 2004; Sasaki *et al.*, 2001; Schenk *et al.*, 2000].

Comparing to JA, defense mechanism and signal transduction in plant by other oxylipins, including cis-3-hexenal presented in most plants and 1-octen-3-ol known as fungal oxylipin discovered in some plants, have not been fully investigated at molecular level.

Cis-3-hexenal, one of the representative green leaf volatiles (GLVs) consisted of C6, are formed through the HPL pathway of oxylipin metabolism [Matsui, 2006]. Generally these compounds are released by damaged leaf tissue and biotic/abiotic stresses. Particularly, cis-3-hexenal is produced in most plants at small amount level, and there are some evidences that this compound has an important role in defense signaling, suggesting contributions to direct defense by inhibition of pathogen growth and/or to indirect defense by attraction of predatory insects [Engelberth *et al.*, 2004; Kishimoto *et al.*, 2005; Matsui *et al.*, 2012; Vancanneyt *et al.*, 2001]. However, detailed information for defense mechanism and signaling within and between plants by this compound have not been investigated yet, although it is demonstrated that aerial treatment of 2-hexenal to Arabidopsis induce the transcription of defense-related genes such as *lox*, *chs* and *dfr* [Bate and Rothstein, 1998].

In the case of 1-octen-3-ol, it is known as fungal aroma, especially most important flavor in mushrooms. This compound consists of eight-carbon and is used as a spoilage indicator in stored cereals [Borjesson *et al.*, 1990; Mau *et al.*, 1992; Tuma *et al.*, 1989]. Moreover, this compound is known as self-inhibitor and has anti-fungal activity against *Penicillium expansum* and *penicillium paneum* [Chitarra *et al.*, 2005; Okull *et al.*, 2003]. In plant, emission of this compound after infection of fungi have been reported, however, researches regarding to production and defense mechanism in plant have not been performed because it

is not major oxylipin [Yue *et al.*, 2001]. Recently, induction of some defense genes by treatment of 1-octen-3-ol in Arabidopsis was reported, suggesting that Arabidopsis can recognize this molecule released from fungal pathogen [Kishimoto *et al.*, 2007]. This report led us to study gene expression change in Arabidopsis after treatment of 1-octen-3-ol.

Microarray analysis has been used for genome-wide expression analysis of genes and should be a powerful tool to discover all defense related genes in Arabidopsis [Mahalingam *et al.*, 2003; Maleck *et al.*, 2000; Schena *et al.*, 1995; Tao *et al.*, 2003]. This technology has also been served to identify oxylipin-responsive genes in Arabidopsis, and regulations of large number of genes have been observed [Mahalingam *et al.*, 2003; Mandaokar *et al.*, 2003; Oztur *et al.*, 2002; Reymond *et al.*, 2000, 2004; Sasaki *et al.*, 2001; Schenk *et al.*, 2000]. Addition to that, defense and hormonal responses against biotic/abiotic stress have been analyzed using the microarrays.

Thus, in this study, to understand defense response and signal transduction by oxylipin we focused on identification of transcriptional responses in Arabidopsis after treatment of cis-3-hexenal and 1-octen-3-ol, which are major oxylipin in plant and fungi, respectively, and determined the change of gene expressions in the tissue at each time points, 1, 6, 12 and 24 hr by microarray analysis.

MATERIALS AND METHODS

Plant material and grown condition

Arabidopsis (Columbia ecotypes) wild type plants were grown on 1/2 Murashige and Skoog (MS) medium in square Petri dishes (70 x 70 x 100 mm), supplemented with 3% sucrose and solidified with 0.8% phytoagar. Seeds were washed once with 70% ethanol containing 0.003% of Triton X-100 and 2 - 3 times with 70% ethanol, and then dried on filter paper in clean bench. Eight seeds were plated on each Petri dishes. Seeds were incubated for 2 to 3 days at 4^{o}C in the dark and thereafter transferred to controlled environment at 23 - 25^{o}C under 16 hr light and 8 hr dark.

Treatment of oxylipins

Treatment of oxylipins into plants was conducted at 14 days after germination of Arabidopsis seeds.

For chemical treatments to plants, solutions containing 10% 3-hexenal and 10% 1-octen-3-ol (Sigma-Aldrich, Germany) emulsified in deionized sterile water were prepared, respectively. Then, 10 ul of solutions were applied to each Petri dishes by dropping on paper disc (8 mm) placed in center. Control plants were constructed with 10 ul of deionized sterile water. Treated plants were placed on controlled environment at 23 - 25^{o}C under light, and harvested at 1, 6, 12, and 24 hr after treatment. Control and treatment plants of Arabidopsis harvested at each time course were shown in Figure 2. The harvested samples were immediately frozen in liquid nitrogen and stored at -80 ^{o}C.

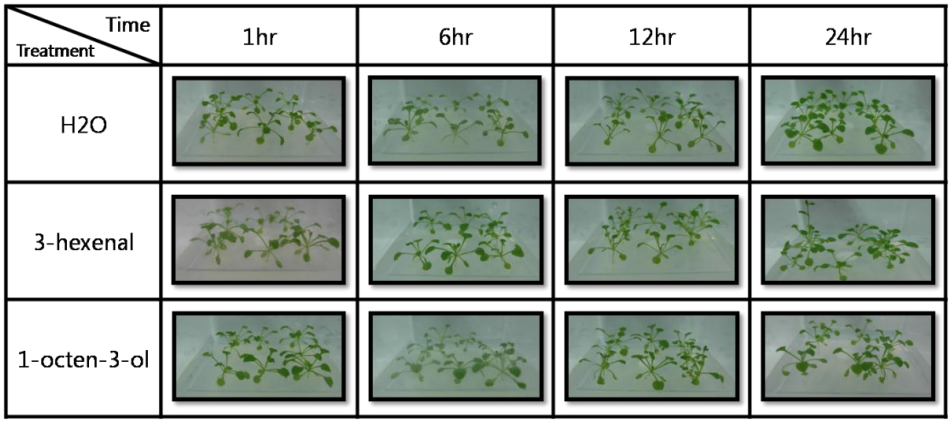


Figure 2. Control and treatment plants of Arabidopsis harvested at each time course.

RNA isolation and cDNA synthesis

Total RNA was isolated from Arabidopsis using RiboEx^{TM} (GeneAll, Korea) and Hybrid-R^{TM} kit (GeneAll, Korea) according to the manufacturer's instructions. RNA concentration was measured in spectrophotometer (SHIMADZU UV-1800, Japan). For elimination of genomic DNA, total RNA (1 ug) was added to 2 ul 7x gDNA wipeout buffer (QIAGEN, Germany) and the volume was adjusted to 14 ul with deionized sterile water. The solution was incubated at 42^{o}C for 2min then immediately transferred on ice. Then, 6.5 ul of reverse transcriptase master mix (Enzynomics, Korea) containing 2 ul 10x M-MLV RT buffer, 2 ul dNTPs Mixture (2.5 mM each), 1 ul oligo(dT)$_{18}$ (10 pmole), 0.5 ul RNase inhibitor (40 unit/ul) and 1 ul M-MLV reverse transcriptase (200 units/ul) were added to reaction. The reaction was incubated at 42^{o}C for 30 min before heat inactivation of reverse transcriptase by incubation at 90^{o}C for 3min. The efficiency of cDNA synthesis was assessed by real-time PCR amplification of control genes encoding Actin 2.

Pre-check of sample by Q-RT-PCR before microarray analysis

The cDNA was diluted 1/5 with deionized sterile water. Polymerase chain reactions

were performed in a 96-well Alpha™ unit with a Chromo4™ Real-time Detection system (Bio-Rad, USA) and a 96-well block with a iQ5 Multicolor Real-time Detection system (Bio-Rad, USA). Q-RT-PCR reactions contained 5 ul diluted cDNA template, 2 ul of gene specific primer mix (containing 10 pmole of each forward and reverse primer), 10 ul 2x iQ™ SYBR Green supermix (Bio-Rad, USA), and 3 ul deionized sterile water to make a total volume of 20 ul. For best results, the tubes were briefly spun in a microcentrifuge at low speed to remove any bubbles.

Gene information, primers' sequences and product size for Q-RT-PCR were described in Table 1.

A Chromo4™ Real-time Detection system was used for Q-RT-PCR, and thermal cycling conditions consisted of 2 min at 94°C and 40 cycles of 30 sec at 94°C, 30 sec at 55°C and 30 sec 72°C. Melting curve conditions consisted of starts at 60°C and ends at 95°C with temperature increment of 0.2°C and a hold time of 2 sec.

Data were analyzed using the Opticon Monitor™ version 3.1.

Table 1. Gene information, primers' sequences and product size for Q-RT-PCR

Gene	Function	Genebank ID	Primer	Size of PCR product(bp)
ACT2	Actin	AT3G18780	5′-GGCTCCTCTTAACCCAAAGG-3′ 5′-ACCCTCGTAGATTGGCACAG-3′	234
THI2.1	Pathogenesis-Related(PR) Proteins	AT1G72260	5′-TCTGGTCATGGCACAAGTTC-3′ 5′-TTGCAGTGCTCATTGGTAGC-3′	204
PDF1.2		AT5G44420	5′-TGCTTCCATCATCACCCTTA-3′ 5′-ACACTTGTGTGCTGGGAAGA-3′	190
PR1		AT2G14610	5′-CTTCCCTCGAAAGCTCAAGA-3′ 5′-CTCGCTAACCCACATGTTCA-3′	243
LOX1	Oxylipin synthesis	AT1G55020	5′-CATCGATTGAACCGTTTGTG-3′ 5′-TAATGCTTGGTCAGGGAAGG-3′	233
HPL		AT4G15440	5′-CAATACTTGGCTTGCGTTGC-3′ 5′-AATAGCCTCATCTCGGGTCAAC-3′	211
ACC2	Ethylene synthesis	AT1G36180	5′-TGGGAAAAGCTAGAGGTGGAAG-3′ 5′-TCGGAGCTTGAACAAGGAAC-3′	211

Microarray analysis

RNA quality check. For quality control, RNA purity and integrity were evaluated by OD 260/280 ratio, and analyzed on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA).

cDNA synthesis and purification. cDNA synthesis was performed with the SuperScript double-stranded cDNA synthesis kit (Invitrogen Life Technologies, Carlsbad, USA) according to the Nimblegen Expression protocol. Briefly, 10 ug of total RNA was reverse-transcribed to cDNA using a oligo dT primer. Second-strand cDNA was synthesized and thereafter the cDNA was quantified using the ND-1000 Spectrophotometer (NanoDrop, Wilmington, USA).

Labeling and purification. cDNA was labeled using the One-Color Labeling Kit (Nimblegen, Madison, USA) according to the Nimblegen Expression protocol. One ug of cDNA samples were labeled with Cy3 using Cy3-random nonamer. After purification, the labeled cDNA was quantified using the ND-1000 Spectrophotometer (NanoDrop, Wilmington, USA).

Hybridization and data export. Labeled cDNA samples were hybridized to Nimblegen Expression array for 16 - 20 hr at 42℃, according to the manufacturer's instructions (Nimblegen, Madison, USA). Arrays were scanned with a NimbleGen MS 200 Microarray scanner set at 532 nm with a resolution of 2 um to produce images in TIFF format according to the manufacturer's instructions. Array data export processing and analysis was performed using NimbleScan v2.5

Raw data preparation. Raw data were extracted using the software provided by the manufacturer NimbleScan v2.5 (Gene Expression RMA algorithm). For filter non-biological experimental variation (batch effects), we adjusted batch effects in data (http://biosun1.harvard.edu/complab/batch/). A single raw intensity value was determined for each gene in each array by averaging spot replicates of all about 3 probes for each of the 39640 genes. Gene signal value was transformed by logarithm (based 2). NimbleScan v2.5 software was used for quantification, image analysis of mRNA data. R scripts were used for all other analytical process.

Data analysis. The genes that revealed significant changes in treatment when compare with mock treatment were collected from normalized data. Cutoff value of significant change was more than 2-fold, which is commonly used for microarray data analysis. Gene ontology analysis was carried out with GO analysis program in TAIR (The Arabidopsis Information Resource, http://www.arabidopsis.org/index.jsp). Clustering of expression patterns was conducted by Multi-Experimental Viewer (MEV) program, and hierarchical and K-mean clustering methods were used. Pathway mapping of the genes related to biotic/abiotic stress and calculation of number of overlapping genes between 3-hexenal and 1-octen-3-ol treatments were performed with MapMan program. Flow chart for data analysis after normalization was shown in Figure 3.

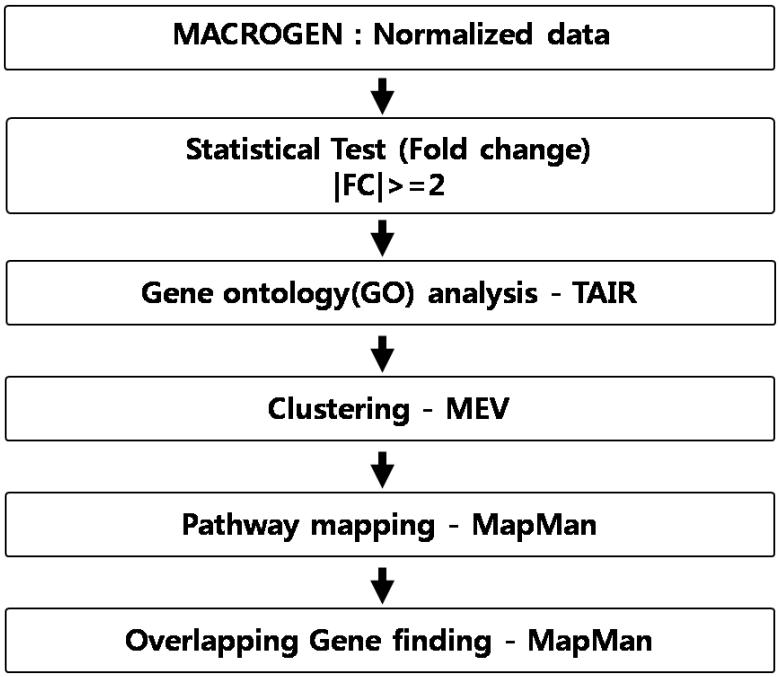


Figure 3. Flow chart for microarray data analysis after normalization.

RESULTS AND DISCUSSION

Pre-check of oxylipin treatment condition

To check oxylipin treatment condition, we conducted preliminary experiment with methyl jasmonic acid (MeJA) and methyl salicylic acid (MeSA) of which vapor were treated in closed square Petri dishes during 24 hr after emulsification with water to final concentration of 10%. Then, marker genes up-regulated by those compounds were analyzed by Q-RT-PCR. Actin2 (ACT2) gene in Arabidopsis was used as internal control and relative expression was determined as ratio of signal intensity between any treatment and mock treatment.

As the results, THI2.1 and PDF1.2, and PR1 known as marker genes for MeJA and MeSA, respectively, were highly up-regulated in each treatment of MeJA or MeSA as our expectation, suggesting the oxylipin treatment condition might be proper to generate sample to analyze transcript profile by microarray analysis (Figure 4).

Therefore, emulsified oxylpin in the water to final concentration of 10% was used to construct samples for microarray analysis.

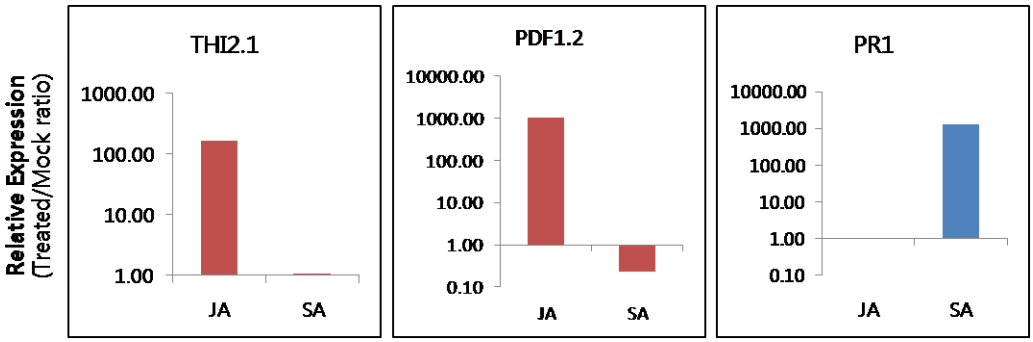


Figure 4. Relative expression levels of marker genes up-regulated by MeJA or MeSA treatment. THI2.1 and PDF1.2 are known as marker genes for MeJA, and PR1 for MeSA. Relative expression is determined as ratio of signal intensity between each treatment and the control mock treatment at each indicated treatment.

RNA extraction & quality check for microarray analysis

As shown in Figure 5, when RNA extracts were checked by agarose gel electrophoresis, major bands existing in total RNA extract, 28S and 18S ribosomal RNA, were detected in the gel after staining with ethidium bromide. Furthermore, density of 28S band in each extract was higher than that of 18S. So, we proceeded to next step to check RNA quality by Agilent 2100 Bioanalyzer.

As the results, all of RNA extracts were evaluated as high quality consisting of more than 2.0 of 260/280 nm and 260/230 nm ratios. The electropherograms associated with each RNA extract sample were shown in Figure 6.

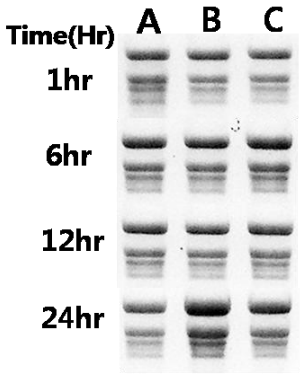


Figure 5. Agarose gel electrophoresis of total RNA extracts. A: Mock treatments, B: 3-hexenal treatments, C: 1-octen-3-ol treatments.

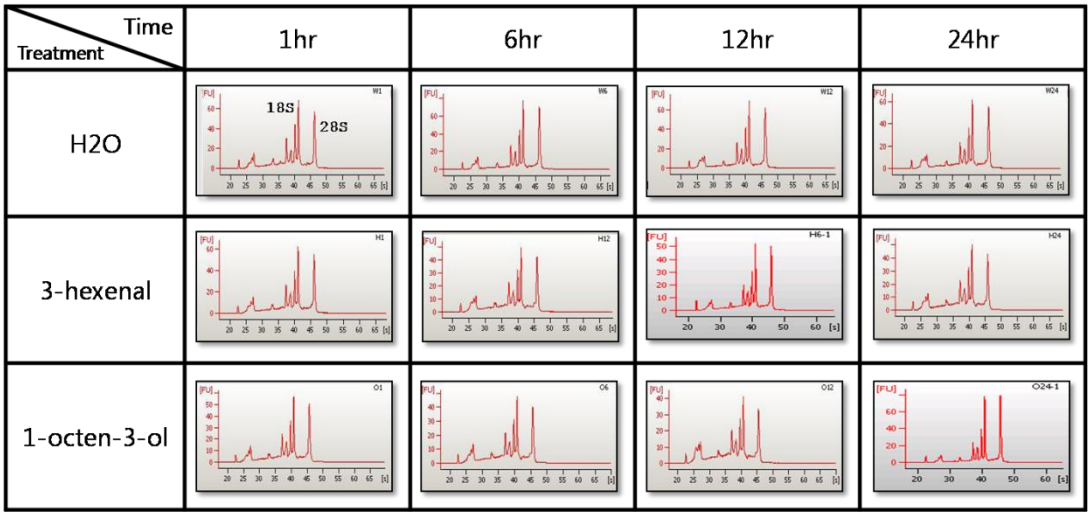


Figure 6. The electropherograms associated with each RNA extract sample treatments.

Analysis of transcript level for representative defense genes before microarray analysis

Prior to microarray analysis, expression levels of transcripts related to representative defense genes (THI2.1, PDF1.2 and PR1), oxylipin biosynthesis genes (LOX1 and HPL) and ethylene biosynthesis gene (ACC2) were analyzed to determine the effect of oxylipin on Arabidopsis by Q-RT-PCR. ACT2 gene in Arabidopsis was used as internal control and

relative expression is determined as ratio of signal intensity between any treatment and mock treatment.

As the results, patterns of expression for each gene in each 3-hexenal and 1-octen-3-ol treatment were changed similarly each other and variously depending on gene, suggesting that transcripts in Arabidopsis were influenced by treatment of 3-hexenal and 1-octen-3-ol (Figure 7). In the defense-related genes, expression pattern of THI2.1 gene was highly up-regulated at 1 hr time point, and thereafter decreased continuously in each 3-hexenal and 1-octen-3-ol treatment. In contrast to that, expressions of PDF1.2 and PR1 genes were highly up-regulated at 12 hr time point in each treatment. HPL gene related to oxylipin biosynthesis showed similar expression pattern with PDF1.2 and PR1. However, the other one, LOX1, revealed different expression pattern, showing down-regulation. In the case of ACC2 associated with the ethylene biosynthesis, the expression was highly down-regulated at the initial time point, then return to normal condition.

This result supposed that the samples might be available for microarray analysis. Therefore, we used total RNA of those samples for first strand cDNA synthesis, and then proceeded to a series of microarray chip analysis.

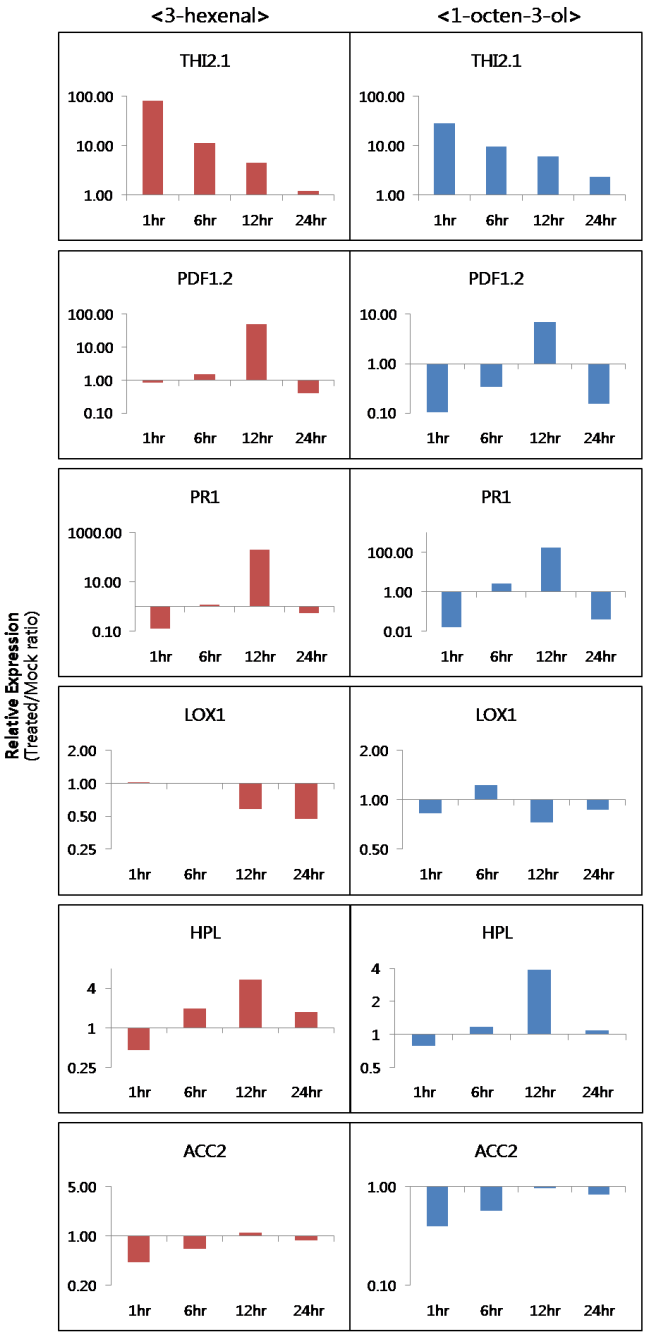


Figure 7. Expression levels of transcripts related to representative defense genes, oxylipin biosynthesis genes and ethylene biosynthesis gene. Representative defense genes: THI2.1, PDF1.2 and PR1, oxylipin biosynthesis genes: LOX1 and HPL, ethylene biosynthesis gene: ACC2. Relative expression is determined as ratio of signal intensity between each treatment and the control mock treatment at each indicated time.

Plot of expression level in microarray analysis

Scatter plot analysis was firstly conducted with normalized data of microarray chip analysis, and the results revealed that expression levels of the highest number of genes were changed more than 2 folds in each 3-hexenal and 1-octen-3-ol treatment at 12 hr after treatment and followed by 1 hr treatment (Figure 8).

The number of genes changed more than 2 folds at each treatment time was counted and shown in Figure 9. The number of genes distributed at 12 hr time point was composed that 299 and 15 genes were up- and down- regulated by 3-hexenal, and 406 and 35 genes were up- and down- regulated by 1-octen-3-ol, respectively. And the next was at 1 hr time point, comprising that 79 and 8 genes were up- and down- regulated by 3-hexenal, and 128 and 10 genes were up- and down- regulated by 1-octen-3-ol, respectively.

These results supposed that more than 6 hr later after treatment of each 3-hexenal and 1-octen-3-ol might affect to transcriptional change of the highest number of genes, and transcription levels of some genes might be changed within 1 hr after treatments.

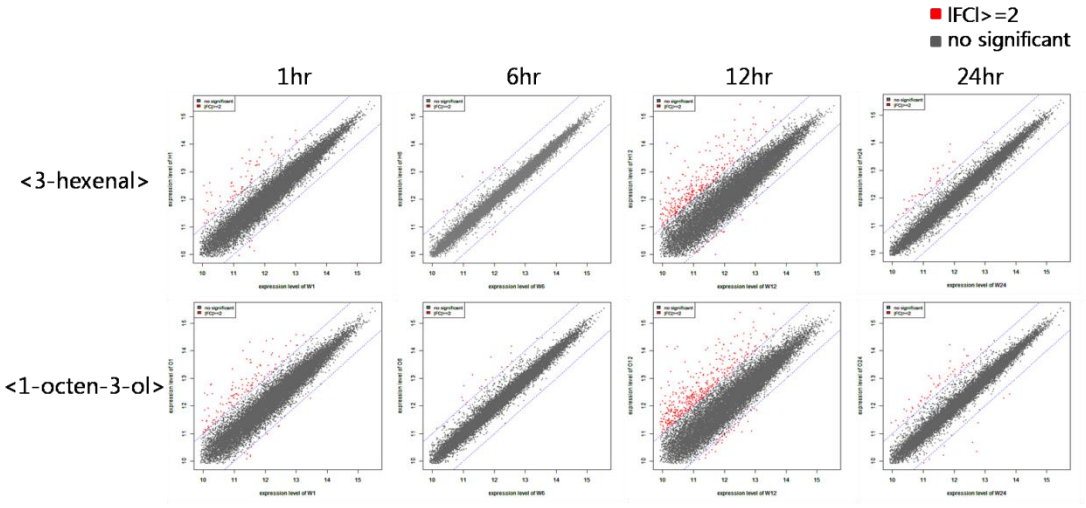


Figure 8. Overview of scatter plot graphs of transcripts expression levels after microarray chip analysis with cDNA probes obtained from any treatment and mock treatment.

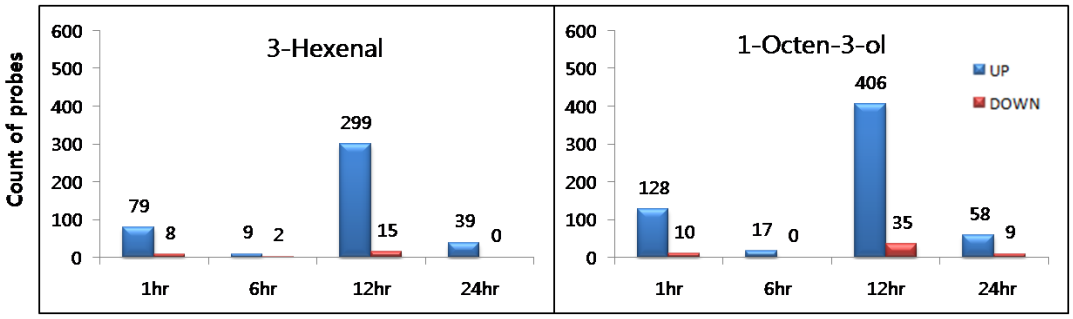


Figure 9. The number of genes changed more than 2 folds at each treatment time.

Construction of dataset

For more detail analysis, we constructed dataset using genes changed more than 2 folds in transcription level at any time point. The total number of genes were 414 and 547 genes up- or down- regulated more than 2 folds by 3-hexenal and 1-octen-3-ol treatment, respectively(Table2). Thus, these genes were used as dataset for further analysis including gene ontology, clustering by expression pattern, mapping into biotic/abiotic stress and finding overlapping genes regulated by each of 3-hexenal and 1-octen-3-ol.

Table 2. Total number of genes changed more than 2 folds in transcription level at any time point by 3-hexenal or 1-octen-3-ol.

Treated compound	No. of up- or down- regulated gene (|FC|>=2)
3-hexenal	414
1-octen-3-ol	547

Analysis of transcriptional change after treatment of 3-hexenal

Gene ontology (GO) analysis. To identify gene function, total number of 414 genes regulated up or down by 3-hexenal were classified into functional categories according to GO analysis program in TAIR (The Arabidopsis Information Resource, http://www.arabidopsis.org/index.jsp). Although GO terms were separated to 3 independent sets such as cellular components, molecular function and biological process, our analysis was focused on biological process due to that objectives of this study was to understand defense mechanism influenced by oxylipin in plant. When dataset was classified into categories in part of biological process, the highest number of genes was distributed in cellular processes with 20% and followed by metabolic processes with 19% (Figure 10). Total percent of genes classified into categories related to defense mechanism including response to stress and response to biotic/abiotic stimulus was 14%. In terms of number of genes, 102 genes were concerned with response to stress and 87 with response to biotic/abiotic stimulus. Thus, without excluding multifunctional gene, about 30% of dataset (124/414) was related to response of biotic/abiotic stress. These results supposed that high number of genes among changed transcripts more than 2 folds were concerned with defense mechanism.

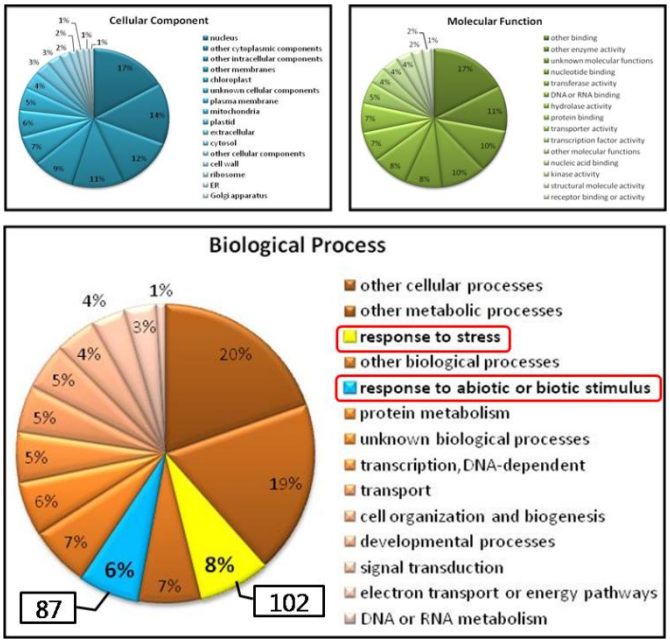


Figure 10. GO analysis of total number of 414 genes regulated up or down by 3-hexenal.

Expression profiling by clustering. To analyze gene expression patterns, we performed hierarchical and K-means clustering analyzes using 414 genes regulated up or down by 3-hexenal (Figure 11). It showed that a number of genes were differentially regulated by 3-hexenal treatment. The highest number of genes was belonged to Cluster 8, comprising 121 genes, and followed by Cluster 10 with 112 genes. These two clusters were representative clusters containing high number of genes, more than 100 genes. Expression pattern of Cluster 8 was typical cluster of up-regulated genes consistently during treatment. Cluster 10 showed a progressive increase in expression level until 12 hr time point. Gene belonging to Cluster 1, 2, 7 and 9, containing 3, 1, 83 and 31 genes, respectively, showed up-regulation at 12 hr time points. In contrast to those, 18 genes in Cluster 5 showed a progressive decrease in expression level. Cluster 3, 4 and 6, containing 1, 14 and 30 genes, respectively, exhibited down-regulation at 12 hr time points. In conclusion, representative clusters, containing high

number of genes, showed up-regulation consistently during treatment or increasingly until 12 hr time points.

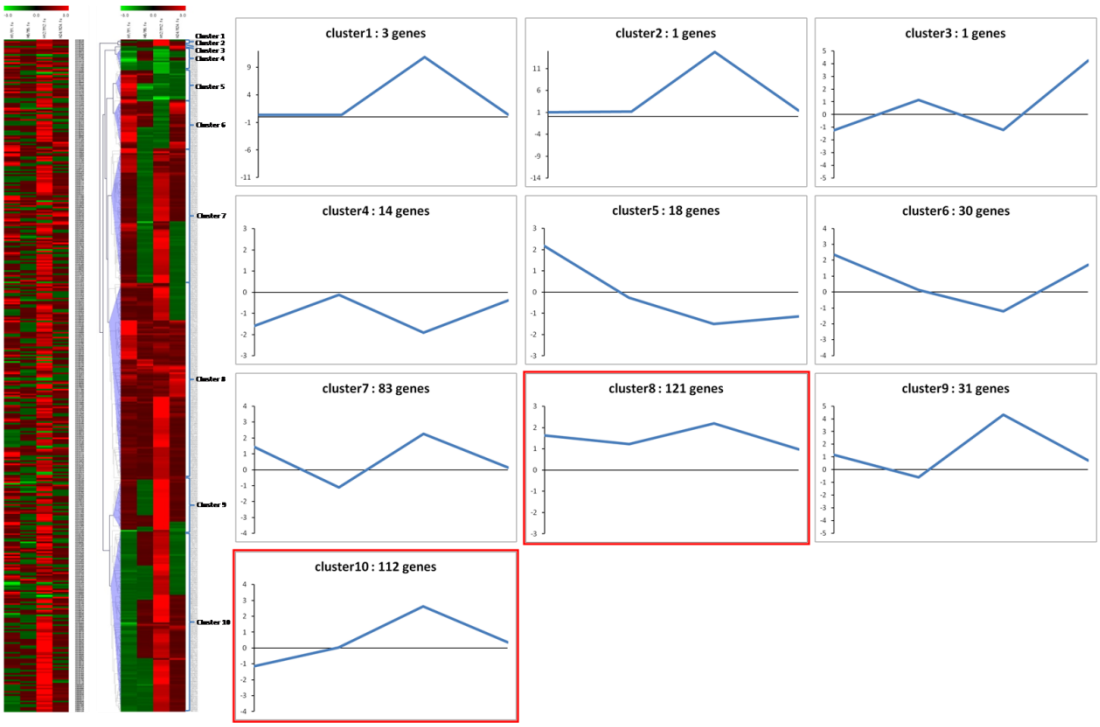


Figure 11. Hierarchical and K-means clustering of 414 genes regulated up or down by 3-hexenal.

Analysis of stress related genes in representative clusters. To identify genes related to biotic/abiotic stress, GO analysis of representative clusters, Cluster 8 and 10, were carried out. As the results shown in Figure 12, 11 and 9% of genes in Cluster 8 were related to response to stress and response to abiotic or biotic stimulus, respectively. In number, 47 genes were concerned with response to stress and 39 genes with response to abiotic or biotic stimulus. Cluster 10, around 5% of this cluster gene, 17 and 15 genes, were concerned with response to stress and response to abiotic or biotic stimulus, respectively. Additionally, we analyzed other clusters to identify genes related to biotic/abiotic stress, however, a few genes

were identified to concern with that (Data not shown). Those results meant that expression pattern of most genes regarding biotic/abiotic stress exhibited up-regulation consistently during treatment or progressive increase until 12 hr time point.

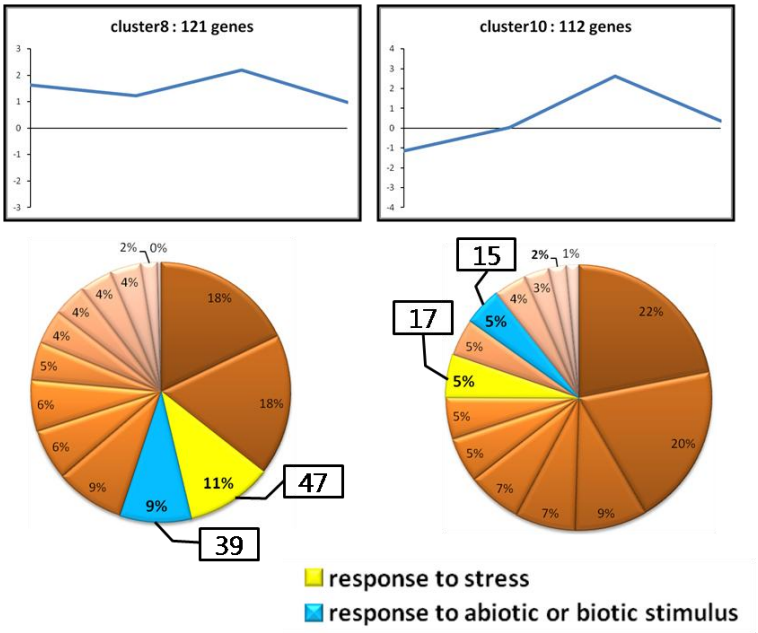


Figure 12. GO analysis of representative clusters, Cluster 8 and 10 shown in Figure 11.

Mapping into biotic stress pathway. Through the GO and cluster analyzes, we were able to suppose that large number of genes might be related to biotic stress. Thus, we performed pathway mapping of the genes in dataset into biotic stress pathway to identify related genes in this pathway and its detailed expression patterns. For pathway mapping, expression levels at 12 hr time point in dataset were used, because the highest number of genes was changed in this point in expression level. As the results, 85 of 414 genes (about 20%) were mapped into biotic stress pathway including R and PR genes, hormone (auxins, ABA, ethylene and JA) signaling, cell wall, proteolysis, redox state, peroxidases, glutathione-S-transferase, signaling, MAPK, transcription factors (ERF and WRKY), heat shock proteins and secondary

metabolites pathways (Figure 13). Detailed expression patterns of those genes were described in Appendix. The highest number of genes (19 genes) were mapped into proteolysis pathway and followed by signaling pathway (15 genes). Especially, ABA and ethylene signaling genes, ERF gene, and WRKY genes were also included in our dataset, which were known as cross-talking genes with JA signaling in plant. These results suggested that signal transduction of 3-hexenal might be similar with one of JA.

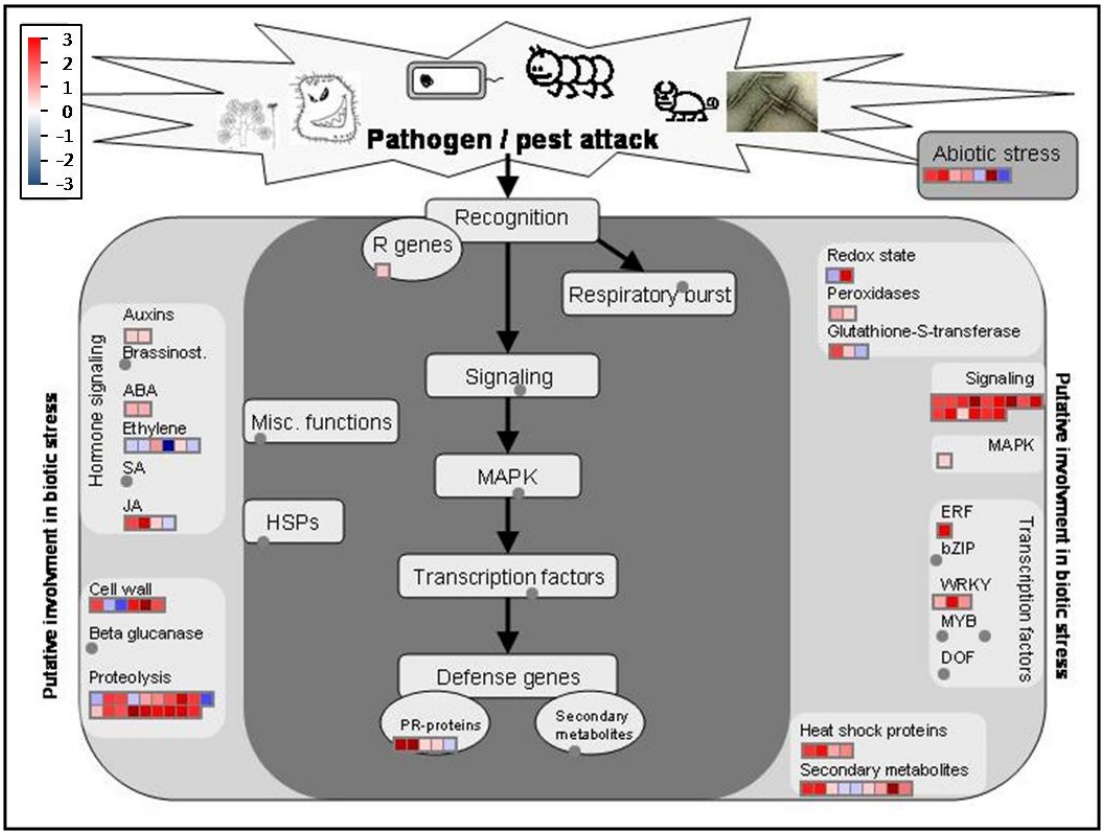


Figure 13. Pathway mapping of the genes regulated more than 2 folds by 3-hexenal treatment into biotic stress pathway, and its expression pattern at 12 hr time point.

Analysis of transcriptional change after treatment of 1-octen-3-ol

Gene ontology (GO) analysis. As the same manner as 3-hexenal dataset, total number of 547 genes regulated by 1-octen-3-ol were classified into functional categories using GO analysis program in TAIR. When dataset was classified into categories in part of biological process, the highest number of genes was distributed in cellular processes with 20% and followed by metabolic processes with 19% (Figure 14). Total percent of genes classified into categories related to defense mechanism including response to stress and response to biotic/abiotic stimulus was 15%. In terms of number of gene, 122 genes were concerned with response to stress and 140 with response to biotic/abiotic stimulus. Thus, without excluding multifunctional gene, about 30% of dataset (167/547) was related to response of biotic/abiotic stress. These results were very similar with 3-hexenal dataset and supposed that high number of genes among changed transcripts more than 2 folds were concerned with defense mechanism.

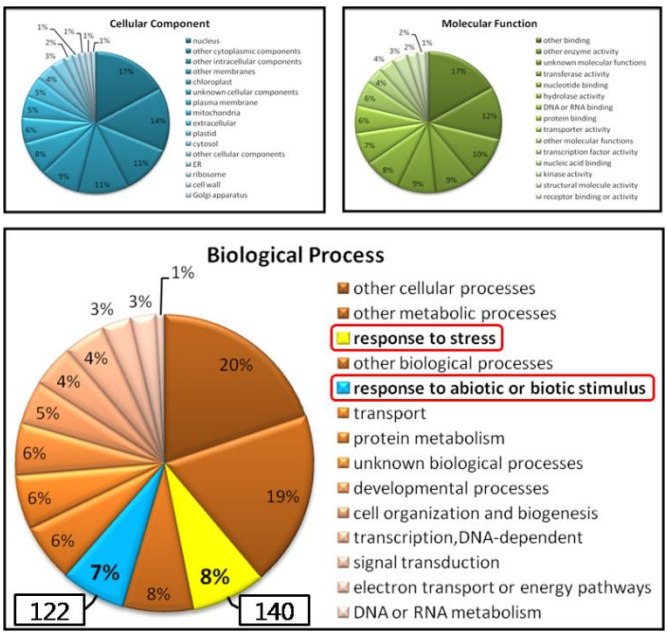


Figure 14. GO analysis of total number of 547 genes regulated up or down by 1-octen-3-ol.

Expression profiling by clustering. When we performed hierarchical and K-means clustering analyzes using 547 genes regulated by 1-octen-3-ol (Figure 15), similarly with 3-hexenal treatment, a number of genes were differentially regulated. The highest number of genes belonging to Cluster 9, comprising 274 genes, was representative clusters of up-regulated genes consistently during treatment. The next was Cluster 10, containing 129 genes, showed a progressive increase in expression level until 12 hr time point. These two clusters were representative clusters containing high number of genes, more than 100 genes. Cluster 2 containing 14 genes exhibited similar expression pattern with Cluster 9, showing up-regulation consistently during treatment. Gene belonging to Cluster 1, 6 and 8, containing 3, 34 and 27 genes, respectively, showed up-regulation at 12 hr time points. In contrast to those, Cluster 3 and 5, comprising 1 and 11 genes, showed a progressive decrease in expression level. Cluster 4 and 7, containing 33 and 21 genes, respectively, exhibited down-regulation at 12 hr time points. Consequentially representative clusters, containing high number of genes, showed similar expression pattern with 3-hexenal treatment, up-regulation consistently during treatment or increasingly until 12 hr time points.

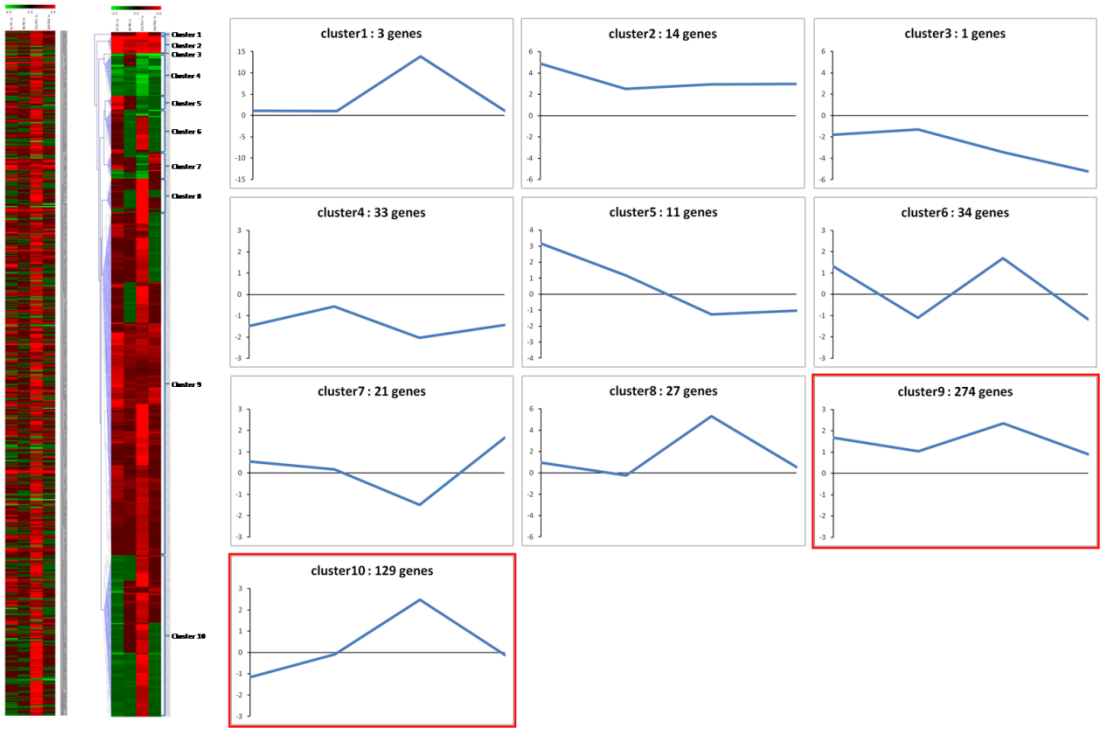


Figure 15. Hierarchical and K-means clustering of 547 genes regulated up or down by 1-octen-3-ol.

Analysis of stress related genes in representative clusters. To identify gene related to biotic/abiotic stress, GO analysis of representative clusters, Cluster 9 and 10, were carried out. As the results, in case of Cluster 9, 9 and 7% of genes were distributed into categories, response to stress and response to abiotic or biotic stimulus, respectively (Figure 16). In terms of number, 82 genes were concerned with response to stress and 62 genes with response to abiotic or biotic stimulus. In case of Cluster 10, 6 and 7%, containing 27 and 28 genes, respectively, were concerned with response to stress and response to abiotic or biotic stimulus, respectively. In contrast to those, a few genes were identified to concern with that in other clusters (Data not shown). Those results were very similar with 3-hexenal treatment, and suggested that expression pattern of most genes regarding to biotic/abiotic stress

exhibited up-regulation consistently during treatment or progressive increase until 12 hr time point by each treatments of 3-hexenal and 1-octen-3-ol.

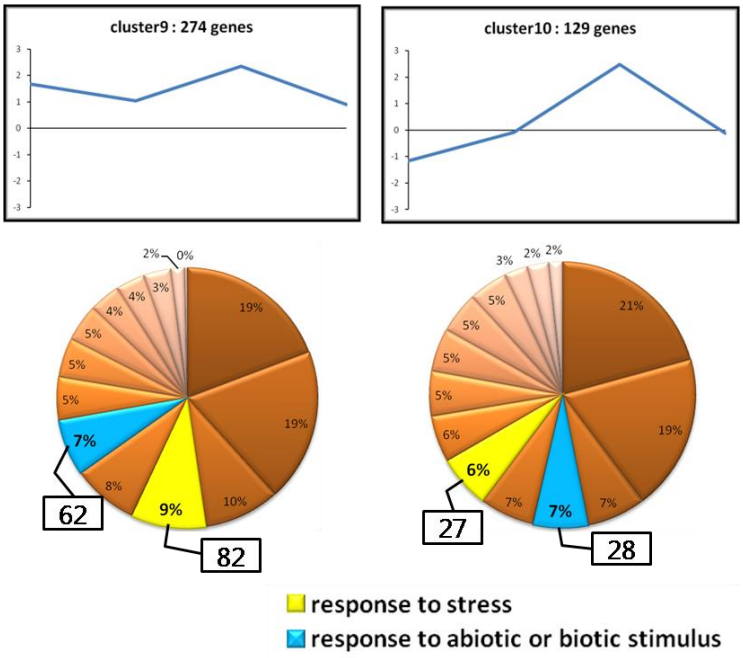


Figure 16. GO analysis of representative clusters, Cluster 9 and 10 shown in Figure 15.

Mapping into biotic stress pathway. The results in the GO and cluster analyzes were consistent with those of 3-hexenal dataset. Thus, we performed pathway mapping of the genes in dataset into biotic stress pathway to identify related genes in this pathway and its detailed expression patterns. In the same way with 3-hexenal dataset analysis, expression levels at 12 hr time point in dataset were used, and 117 of 547 genes (about 20%) were mapped into biotic stress pathway such as R and PR genes, hormone (auxins, ABA, ethylene and JA) signaling, cell wall, proteolysis, redox state, peroxidases, glutathione-S-transferase, signaling, MAPK, transcription factors (ERF and WRKY), heat shock proteins and secondary metabolites pathways (Figure 17). Detailed expression patterns of those genes

were described in Appendix. The highest number of genes (26 genes) were mapped into proteolysis pathway and followed by signaling pathway (19 genes). The results were also almost the same with 3-hexenal treatment. Furthermore, ABA and ethylene signaling genes, ERF gene, and WRKY genes which were known as cross-talking genes with JA signaling in plant were also included in our dataset. These results suggested that signal transduction of 1-octen-3-ol as well as 3-hexenal might be similar with those of JA.

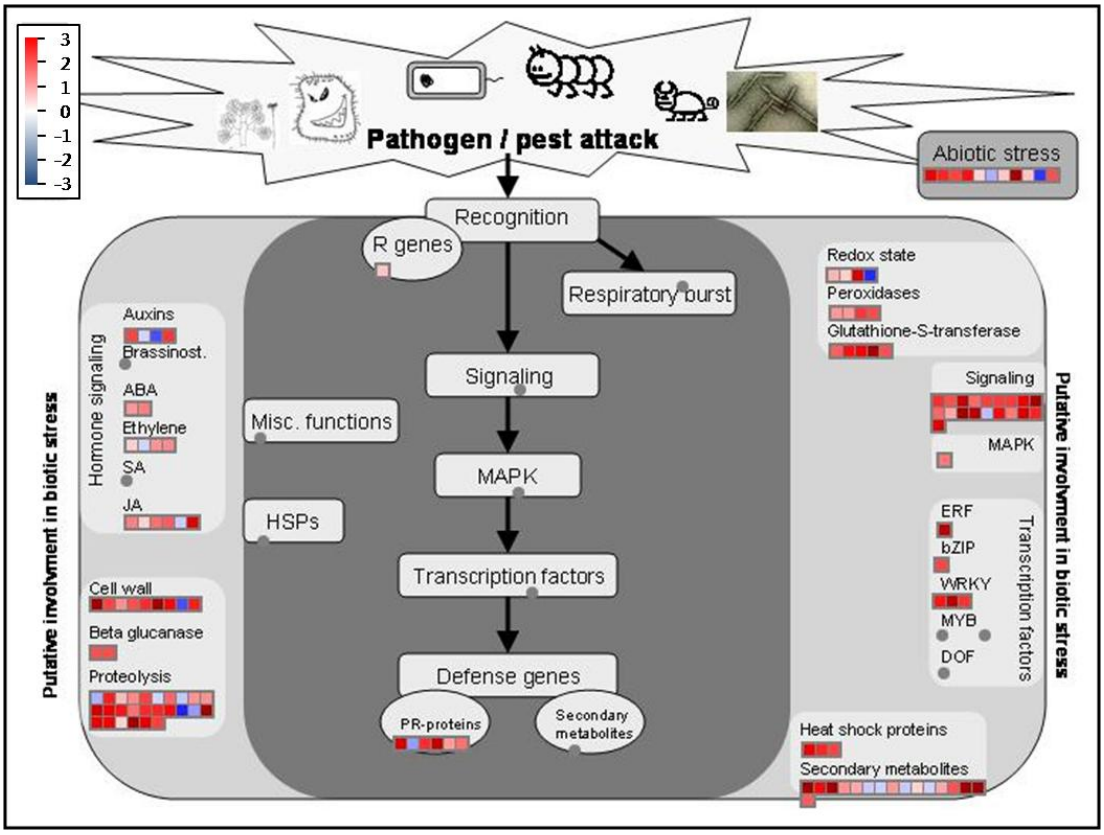


Figure 17. Pathway mapping of the genes regulated more than 2 folds by 1-octen-3-ol treatment into biotic stress pathway, and its expression patterns at 12 hr time point.

Analysis of overlapping gene regulated by each treatment of 3-hexenal and 1-octen-3-ol

Through previous analysis, we were able to suppose that large number of genes regulated by each 3-hexenal and 1-octen-3-ol treatment might be overlapped. So, in order to confirm our expectation, we carried out overlapping genes finding using MapMan program. When we carried out this analysis, we used two time point dataset, 1 hr and 12 hr, because transcriptional changes were occurred frequently in these time points.

The results were shown in Figure 18 with Venn diagrams. In this analysis, it was revealed that almost of genes regulated by each 3-hexenal and 1-octen-3-ol treatment were overlapped in both 1 hr and 12 hr time points. The rate of overlapping genes was more than 85% in up-regulated genes, and more than 60% in down-regulated genes. The results implicated that signal transduction or defense response in plant by even different oxylipins might be going through similar pathway.

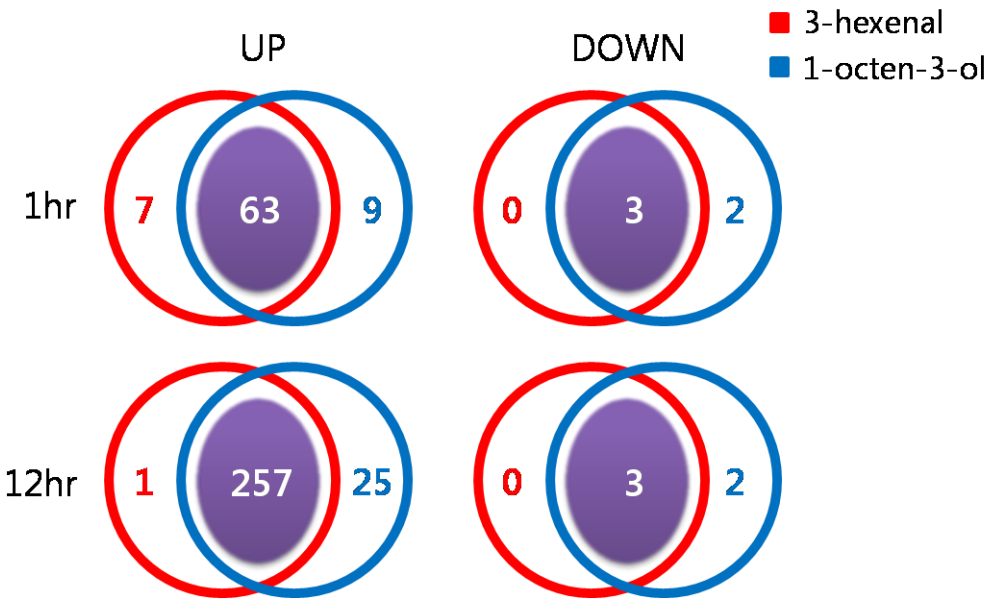


Figure 18. The numbers of overlapping and nonoverlapping up- or down- regulated genes at 1 hr and 12 hr time points after treatment of each 3-hexenal and 1-octen-3-ol.

CONCLUSION

In this study, transcriptional responses by vapor treatments of oxylipin, 3-hexenal and 1-octen-3-ol, in Arabidopsis were identified through microarray analysis, suggesting that Arabidopsis recognizes 3-hexenal and 1-octen-3-ol as signal molecules. In the treatments of each 3-hexenal and 1-octen-3-ol to Arabidopsis, total numbers of 414 and 547 genes were up- or down- regulated more than 2 folds, respectively, and those genes were used as datasets for further analysis.

When the datasets were classified into functional categories in part of biological process, high number of genes were distributed in the categories related to defense mechanism including response to stress and response to biotic/abiotic stimulus. In each 3-hexenal and 1-octen-3-ol treatment, about 30% of dataset, 124/414 and 167/547, respectively, were related to biotic/abiotic responses. These results supposed that high number of genes among changed transcripts more than 2 folds were concerned with defense mechanism.

In the clustering of expression patterns with the datasets, genes related to biotic/abiotic responses revealed two representative patterns, up-regulation consistently during treatment or progressive increase until 12 hr time point by each treatment of 3-hexenal and 1-octen-3-ol.

The genes related to biotic/abiotic responses were mapped into biotic stress pathway including R and PR genes, hormone (auxins, ABA, ethylene and JA) signaling, cell wall, proteolysis, redox state, peroxidases, glutathione-S-transferase, signaling, MAPK, transcription factors (ERF and WRKY), heat shock proteins and secondary metabolites pathways. The number of genes mapped into biotic stress pathway was 85 of 414 genes (about 20%) and 117 of 547 genes (about 20%). These results suggested that signal

transduction of 3-hexenal and 1-octen-3-ol might be similar with those of JA, because particular genes including ABA and ethylene signaling genes, ERF gene, and WRKY genes, which were known as cross-talking genes with JA signaling in plant, were regulated at transcription level by each treatment of 3-hexenal and 1-octen-3-ol.

Furthermore, when we performed overlapping genes finding using two time point dataset, 1 hr and 12 hr, in each 3-hexenal and 1-octen-3-ol treatment, it was revealed that almost of genes regulated by each 3-hexenal and 1-octen-3-ol treatment were overlapped in both time points. The rate of overlapping genes was more than 85% in up-regulated genes, and more than 60% in down-regulated genes, suggesting that signal transduction or defense response in plant by even different oxylipins might be similar.

Taken together, it was assumed that when plant recognizes 3-hexenal or 1-octen-3-ol, various biotic stress genes, including R and PR genes, hormone signaling, redox state, cell wall, proteolysis, signaling, and transcription factors related genes, might be up-regulated during 12 hr to increase defense system in the cell (Figure 19).

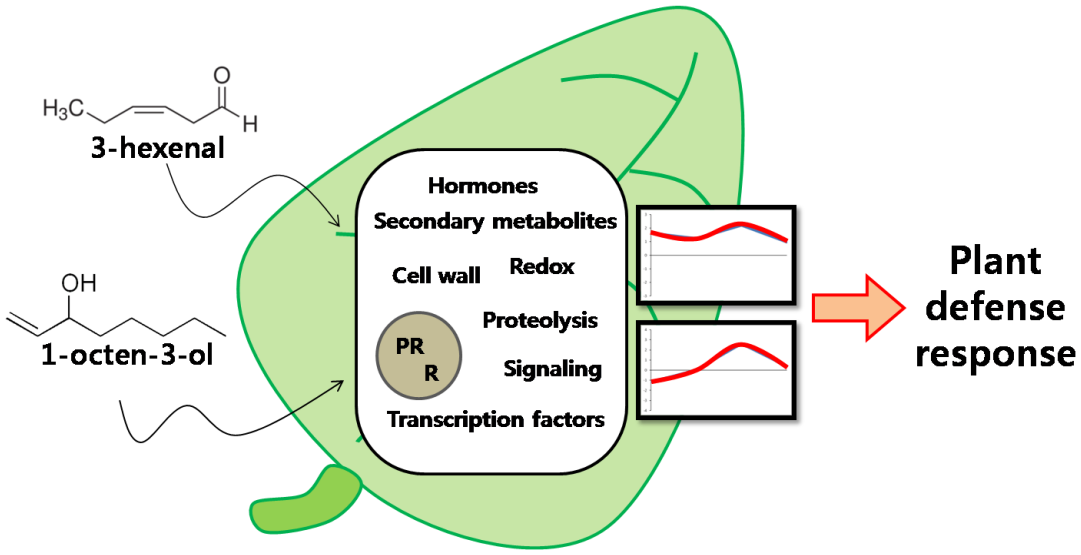


Figure 19. Proposed defense response in plant by 3-hexenal and 1-octen-3-ol.

The results in this study might be useful to have an insight into defense response in the plant cell occurred by oxylipin compounds, especially 3-hexenal and 1-octen-3-ol. However, further investigations are needed to clarify the physiological function and signaling of up-regulated biotic stress related genes in detail.

REFERENCES

Anderson, J.P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Maclean, D. J., Ebert, P. R. and Kazan, K. (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in arabidopsis. *Plant Cell,* **16**(12), 3460-3479.

Bate, N.J. and Rothstein, S. J. (1998) C6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. *Plant J.,* **16**(5), 561-569.

Blee, E. (1998) Phytooxylipins and plant defense reactions. *Prog. Lipid Res.,* **37**(1), 33-72.

Block, A., Schmelz, E., Jones, J. B. and Klee, H. J. (2005) Coronatine and salicylic acid: The battle between arabidopsis and pseudomonas for phytohormone control. *Mol. Plant. Pathol.,* **6**(1), 79-83.

Borjesson, T., Stollman, U. and Schnurer, J. (1990) Volatile metabolites and other indicators of penicillium aurantiogriseum growth on different substrates. *Appl. Environ. Microbiol.,* **56**(12), 3705-3710.

Chitarra, G.S., Abee, T., Rombouts, F. M. and Dijksterhuis, J. (2005) 1-octen-3-ol inhibits conidia germination of penicillium paneum despite of mild effects on membrane permeability, respiration, intracellular pH, and changes the protein composition. *FEMS Microbiol. Ecol.,* **54**(1), 67-75.

Creelman, R.A. and Mulpuri, R. (2002) The oxylipin pathway in arabidopsis. *Arabidopsis Book*, **1**, e0012.

Eckardt, N.A. (2008) Oxylipin signaling in plant stress responses. *Plant Cell*, **20**(3), 495-497.

Engelberth, J., Alborn, H. T., Schmelz, E. A. and Tumlinson, J. H. (2004) Airborne signals prime plants against insect herbivore attack. *Proc. Natl. Acad. Sci. U. S. A.*, **101**(6), 1781-1785.

Farmer, E.E. and Ryan, C. A. (1990) Interplant communication: Airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proc. Natl. Acad. Sci. U. S. A.*, **87**(19), 7713-7716.

Feussner, I. and Wasternack, C. (2002) The lipoxygenase pathway. *Annu. Rev. Plant. Biol.*, **53**, 275-297.

Feussner, I., Kuhn, H. and Wasternack, C. (2001) Lipoxygenase-dependent degradation of storage lipids. *Trends Plant Sci.*, **6**(6), 268-273.

Gardner, H.W. (1991) Recent investigations into the lipoxygenase pathway of plants. *Biochim. Biophys. Acta*, **1084**(3), 221-239.

Grechkin, A. (1998) Recent developments in biochemistry of the plant lipoxygenase pathway. *Prog. Lipid Res.*, **37**(5), 317-352.

Hamberg, M. (1999) An epoxy alcohol synthase pathway in higher plants: Biosynthesis of antifungal trihydroxy oxylipins in leaves of potato. *Lipids*, **34**(11), 1131-1142.

Hamberg, M. (1988) Biosynthesis of 12-oxo-10,15(Z)-phytodienoic acid: Identification of an allene oxide cyclase. *Biochem. Biophys. Res. Commun.*, **156**(1), 543-550.

Hermsmeier, D., Schittko, U. and Baldwin, I. T. (2001) Molecular interactions between the specialist herbivore manduca sexta (lepidoptera, sphingidae) and its natural host nicotiana attenuata. I. large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiol.*, **125**(2), 683-700.

Howe, G.A. and Schilmiller, A. L. (2002) Oxylipin metabolism in response to stress. *Curr. Opin. Plant Biol.*, **5**(3), 230-236.

Kishimoto, K., Matsui, K., Ozawa, R. and Takabayashi, J. (2007) Volatile 1-octen-3-ol induces a defensive response in arabidopsis thaliana. *J Gen Plant Pathol*, **73**(1), 35-37.

Kishimoto, K., Matsui, K., Ozawa, R. and Takabayashi, J. (2005) Volatile C6-aldehydes and allo-ocimene activate defense genes and induce resistance against botrytis cinerea in arabidopsis thaliana. *Plant Cell Physiol.*, **46**(7), 1093-1102.

Mahalingam, R., Gomez-Buitrago, A., Eckardt, N., Shah, N., Guevara-Garcia, A., Day, P., Raina, R. and Fedoroff, N. V. (2003) Characterizing the stress/defense transcriptome of arabidopsis. *Genome Biol.*, **4**(3), R20.

Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K. A., Dangl, J. L. and Dietrich, R. A. (2000) The transcriptome of arabidopsis thaliana during systemic acquired resistance. *Nat. Genet.*, **26**(4), 403-410.

Mandaokar, A., Kumar, V. D., Amway, M. and Browse, J. (2003) Microarray and differential display identify genes involved in jasmonate-dependent anther development. *Plant Mol. Biol.*, **52**(4), 775-786.

Matsui, K. (1998) Properties and structures of fatty acid hydroperoxide lyase. *Belgian Journal of Botany*, **131**(1), 50-62.

Matsui, K. (2006) Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Curr. Opin. Plant Biol.*, **9**(3), 274-280.

Matsui, K., Sugimoto, K., Mano, J., Ozawa, R. and Takabayashi, J. (2012) Differential metabolisms of green leaf volatiles in injured and intact parts of a wounded leaf meet distinct ecophysiological requirements. *PLoS One*, **7**(4), e36433.

Mau, J.L., Beelmann, R. B. and Ziegler, G. R. (1992) 1-octen-3-ol in the cultivated mushroom, agaricus bisporus. *J. Food Sci*, **57**(3), 704-706.

Okull, D.O., Beelman, R. B. and Gourama, H. (2003) Antifungal activity of 10-oxo-trans-8-decenoic acid and 1-octen-3-ol against penicillium expansum in potato dextrose agar medium. *J. Food Prot.*, **66**(8), 1503-1505.

Oztur, Z.N., Talame, V., Deyholos, M., Michalowski, C. B., Galbraith, D. W., Gozukirmizi, N., Tuberosa, R. and Bohnert, H. J. (2002) Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. *Plant Mol. Biol.*, **48**(5-6), 551-573.

Reymond, P., Weber, H., Damond, M. and Farmer, E. E. (2000) Differential gene expression in response to mechanical wounding and insect feeding in arabidopsis. *Plant Cell*, **12**(5), 707-720.

Reymond, P., Bodenhausen, N., Van Poecke, R. M., Krishnamurthy, V., Dicke, M. and Farmer, E. E. (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell*, **16**(11), 3132-3147.

Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., Amagai, M., Kuwata, C., Tsugane, T., Masuda, T., Shimada, H., Takamiya, K., Ohta, H. and Tabata, S. (2001) Monitoring of methyl jasmonate-responsive genes in arabidopsis by cDNA macroarray: Self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. *DNA Res.*, **8**(4), 153-161.

Schena, M., Shalon, D., Davis, R. W. and Brown, P. O. (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, **270**(5235), 467-470.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C. and Manners, J. M. (2000) Coordinated plant defense responses in arabidopsis revealed by microarray analysis. *Proc. Natl. Acad. Sci. U. S. A.*, **97**(21), 11655-11660.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H. S., Han, B., Zhu, T., Zou, G. and Katagiri, F. (2003) Quantitative nature of arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen pseudomonas syringae. *Plant Cell*, **15**(2), 317-330.

Tuma, D., Sinha, R. N., Muir, W. E. and Abramson, D. (1989) Odor volatiles associated with microflora in damp ventilated and non-ventilated bin-stored bulk wheat. *Int. J. Food Microbiol.*, **8**(2), 103-119.

Vancanneyt, G., Sanz, C., Farmaki, T., Paneque, M., Ortego, F., Castanera, P. and Sanchez-Serrano, J. J. (2001) Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. *Proc. Natl. Acad. Sci. U. S. A.*, **98**(14), 8139-8144.

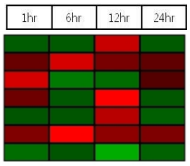
Yue, Q., Wang, C., Gianfagna, T. J. and Meyer, W. A. (2001) Volatile compounds of endophyte-free and infected tall fescue (festuca arundinacea schreb.). *Phytochemistry*, **58**(6), 935-941.

APPENDIX

[Expression patterns of genes mapped into biotic/abiotic stress]

<3-hexenal>

Abiotic stress



-3.0 0.0 3.0

Genebank ID	Putative Function
AT1G62970	DNAJ heat shock N-terminal domain-containing protein
AT3G12580	HSP70 (heat shock protein 70); ATP binding
AT3G62020	GLP10 (GERMIN-LIKE PROTEIN 10); manganese ion binding
AT5G15780	pollen Ole e 1 allergen and extensin family protein
AT5G27240	DNAJ heat shock N-terminal domain-containing protein
AT5G52640	ATHSP90.1 (HEAT SHOCK PROTEIN 90.1); ATP binding
AT5G66590	allergen V5/Tpx-1-related family protein

R(resistance) gene

Genebank ID	Putative Function
AT5G45070	AtPP2-A8 (Phloem protein 2-A8); carbohydrate binding

PR(Pathogenesis related) genes

Genebank ID	Putative Function
AT1G17860	trypsin and protease inhibitor family protein
AT1G72910	disease resistance protein (TIR-NBS class)
AT1G72940	disease resistance protein (TIR-NBS class)
AT3G25020	AtRLP42 (Receptor Like Protein 42)
AT4G19510	disease resistance protein (TIR-NBS-LRR class)

Hormone Signaling

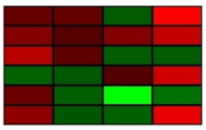
Auxin pathway

Genebank ID	Putative Function
AT1G05560	UGT75B1; UDP-GLUCOSYLTRANSFERASE 75B1
AT2G23170	GH3.3; indole-3-acetic acid amido synthetase

ABA(Abscisic acid) responsive

Genebank ID	Putative Function
AT3G14440	NCED3; NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3)
AT5G59220	protein phosphatase 2C

Ethylene pathway

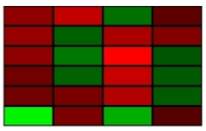


Genebank ID	Putative Function
AT4G11280	ACS6; 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6
AT4G17500	ATERF-1; ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 1
AT5G43450	2-oxoglutarate-dependent dioxygenase
AT5G47220	ERF2; ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 2
AT5G61590	AP2 domain-containing transcription factor family protein
AT5G61600	ethylene-responsive element-binding family protein

JA(Jasmonic acid) pathway

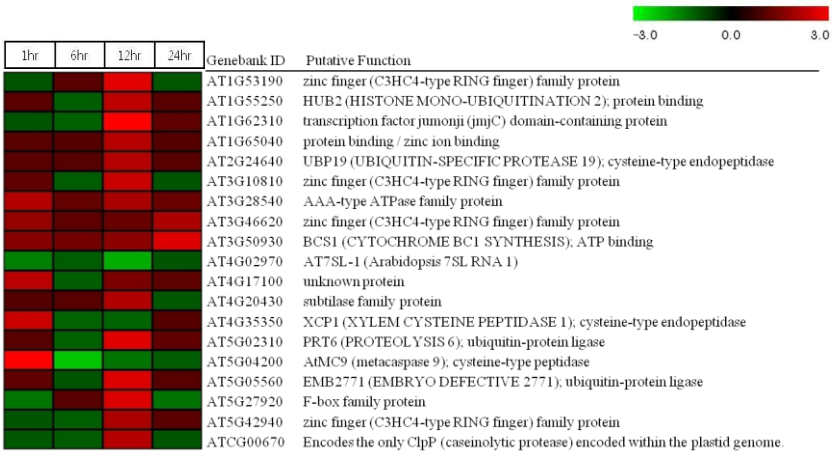
Genebank ID	Putative Function
AT1G17420	LOX3; lipoxygenase
AT1G76680	OPR1; 12-oxophytodienoate reductase
AT1G76690	OPR2; 12-oxophytodienoate reductase
AT5G42650	AOS; allene oxide synthase

Cell wall modification



Genebank ID	Putative Function
AT2G20870	cell wall protein precursor, putative
AT3G19620	glycosyl hydrolase family 3 protein
AT4G08400	proline-rich extensin-like family protein
AT4G38770	PRP4 (PROLINE-RICH PROTEIN 4)
AT5G44480	DUR (DEFECTIVE UGE IN ROOT)
AT5G53250	AGP22 (ARABINOGALACTAN PROTEIN 22)

Proteolysis



Genebank ID	Putative Function
AT1G53190	zinc finger (C3HC4-type RING finger) family protein
AT1G55250	HUB2 (HISTONE MONO-UBIQUITINATION 2); protein binding
AT1G62310	transcription factor jumonji (jmjC) domain-containing protein
AT1G65040	protein binding / zinc ion binding
AT2G24640	UBP19 (UBIQUITIN-SPECIFIC PROTEASE 19); cysteine-type endopeptidase
AT3G10810	zinc finger (C3HC4-type RING finger) family protein
AT3G28540	AAA-type ATPase family protein
AT3G46620	zinc finger (C3HC4-type RING finger) family protein
AT3G50930	BCS1 (CYTOCHROME BC1 SYNTHESIS); ATP binding
AT4G02970	AT7SL-1 (Arabidopsis 7SL RNA 1)
AT4G17100	unknown protein
AT4G20430	subtilase family protein
AT4G35350	XCP1 (XYLEM CYSTEINE PEPTIDASE 1); cysteine-type endopeptidase
AT5G02310	PRT6 (PROTEOLYSIS 6); ubiquitin-protein ligase
AT5G04200	AtMC9 (metacaspase 9); cysteine-type peptidase
AT5G05560	EMB2771 (EMBRYO DEFECTIVE 2771); ubiquitin-protein ligase
AT5G27920	F-box family protein
AT5G42940	zinc finger (C3HC4-type RING finger) family protein
ATCG00670	Encodes the only ClpP (caseinolytic protease) encoded within the plastid genome.

Redox state

Genebank ID	Putative Function
AT1G75270	DHAR2(DEHYDROASCORBATE REDUCTASE 2); glutathione binding
AT3G10130	SOUL heme-binding family protein

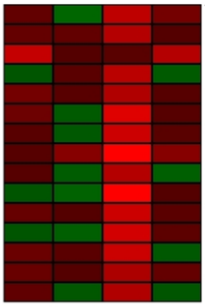
Peroxidases

Genebank ID	Putative Function
AT1G44970	peroxidase
AT5G51890	peroxidase

Glutathione-S-transferase

Genebank ID	Putative Function
AT1G02930	ATGSTF6 (GLUTATHIONE S-TRANSFERASE); copper ion binding
AT1G17170	ATGSTU24 (GLUTATHIONE S-TRANSFERASE TAU 24); glutathione binding
AT2G29420	ATGSTU7 (ARABIDOPSIS THALIANA GLUTATHIONE S-TRANSFERASE TAU 7)

Signaling



Genebank ID	Putative Function
AT1G35710	leucine-rich repeat transmembrane protein kinase
AT1G66880	serine/threonine protein kinase family protein
AT1G73500	MKK9 (MAP KINASE KINASE 9); MAP kinase kinase
AT1G80010	FRS8 (FAR1-related sequence 8); zinc ion binding
AT2G29120	ATGLR2.7; intracellular ligand-gated ion channel
AT2G46700	calcium-dependent protein kinase
AT3G06540	GDP dissociation inhibitor family protein
AT3G63380	calcium-transporting ATPase, plasma membrane-type
AT4G01090	extra-large G-protein-related
AT4G08850	kinase
AT4G11900	S-locus lectin protein kinase family protein
AT4G32160	phox (PX) domain-containing protein
AT4G38180	FRS5 (FAR1-related sequence 5); zinc ion binding
AT5G66210	CPK28; ATP binding / calcium ion binding
AT5G67200	leucine-rich repeat transmembrane protein kinase

MAPK

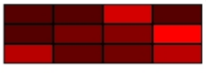
Genebank ID	Putative Function
AT1G73500	MKK9 (MAP KINASE KINASE 9); MAP kinase kinase

Transcription factors

ERF(ethylene response factors)

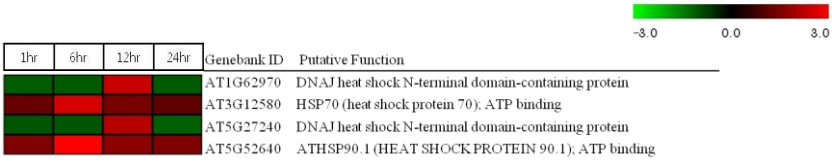
Genebank ID	Putative Function
AT5G10510	AIL6 (AINTEGUMENTA-LIKE 6); DNA binding

WRKY



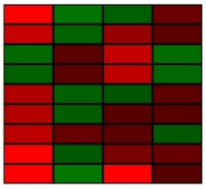
Genebank ID	Putative Function
AT1G13960	WRKY4; DNA binding / transcription factor
AT2G38470	WRKY33; transcription factor
AT3G56400	WRKY70; transcription factor/ transcription repressor

Heat shock proteins



Genebank ID	Putative Function
AT1G62970	DNAJ heat shock N-terminal domain-containing protein
AT3G12580	HSP70 (heat shock protein 70); ATP binding
AT5G27240	DNAJ heat shock N-terminal domain-containing protein
AT5G52640	ATHSP90.1 (HEAT SHOCK PROTEIN 90.1); ATP binding

Secondary metabolites

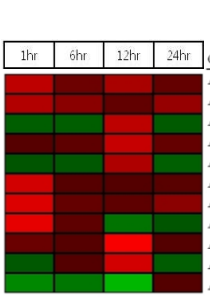


Genebank ID	Putative Function
AT1G32100	PRR1 (PINORESINOL REDUCTASE 1); pinoresinol reductase
AT2G29130	LAC2 (laccase 2); laccase
AT3G25820	ATTPS-CIN (terpene synthase-like sequence-1,8-cineole); (E)-beta-ocimene synthase
AT3G25830	ATTPS-CIN (terpene synthase-like sequence-1,8-cineole); (E)-beta-ocimene synthase
AT5G07860	transferase family protein
AT5G07870	transferase family protein
AT5G22300	NIT4 (NITRILASE 4); 3-cyanoalanine hydratase
AT5G39050	transferase, transferring acyl groups other than amino-acyl groups
AT5G60020	LAC17 (laccase 17); laccase

[Expression patterns of genes mapped into biotic/abiotic stress]

<1-octen-3-ol>

Abiotic stress

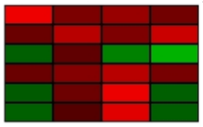


Genebank ID	Putative Function
AT1G18980	germin-like protein, putative
AT1G35260	MLP165 (MLP-LIKE PROTEIN 165)
AT1G62970	DNAJ heat shock N-terminal domain-containing protein
AT1G69450	HYP1 (HYPOTHETICAL PROTEIN 1)
AT1G79030	DNAJ heat shock N-terminal domain-containing protein
AT3G25760	AOC1 (ALLENE OXIDE CYCLASE 1)
AT3G50970	LTI30 (LOW TEMPERATURE-INDUCED 30)
AT3G62020	GLP10 (GERMIN-LIKE PROTEIN 10); manganese ion binding
AT5G15780	pollen Ole e 1 allergen and extensin family protein
AT5G27240	DNAJ heat shock N-terminal domain-containing protein
AT5G66590	allergen V5/Tpx-1-related family protein

R(resistance) gene

Genebank ID	Putative Function
AT5G45070	AtPP2-A8 (Phloem protein 2-A8); carbohydrate binding

PR(Pathogenesis related) genes



Genebank ID	Putative Function
AT1G17860	trypsin and protease inhibitor family protein
AT1G72900	disease resistance protein (TIR-NBS class)
AT3G04210	disease resistance protein (TIR-NBS class)
AT3G05360	AtRLP30 (Receptor Like Protein 30); kinase
AT3G25020	AtRLP42 (Receptor Like Protein 42); protein binding
AT4G19510	disease resistance protein (TIR-NBS-LRR class)

Hormone Signaling

Auxin pathway00

Genebank ID	Putative Function
AT1G05560	UGT75B1 (UDP-GLUCOSYLTRANSFERASE 75B1)
AT1G44350	ILL6; IAA-amino acid conjugate hydrolase
AT5G13370	auxin-responsive GH3 family protein
AT5G20730	NPH4 (NON-PHOTOTROPHIC HYPOCOTYL); DNA binding

ABA(Abscisic acid) responsive

Genebank ID	Putative Function
AT3G14440	NCED3 (NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3)
AT5G59220	protein phosphatase 2C

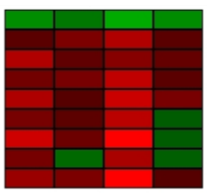
Ethylene pathway

Genebank ID	Putative Function
AT4G11280	ACS6 (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) SYNTHASE 6)
AT4G17500	ATERF-1 (ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 1)
AT5G43450	2-oxoglutarate-dependent dioxygenase
AT5G61600	ethylene-responsive element-binding family protein

JA(Jasmonic acid) pathway

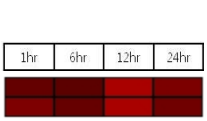
Genebank ID	Putative Function
AT1G17420	LOX3; electron carrier/ lipoxygenase
AT1G76680	OPR1; 12-oxophytodienoate reductase
AT1G76690	OPR2; 12-oxophytodienoate reductase
AT2G06050	OPR3; 12-oxophytodienoate reductase
AT2G25980	jacalin lectin family protein
AT3G25770	AOC2 (ALLENE OXIDE CYCLASE 2)

Cell wall modification



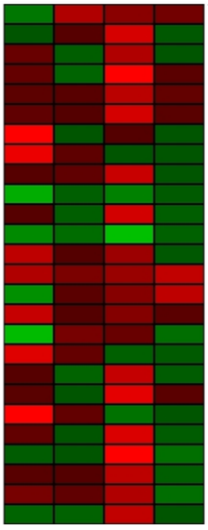
Genebank ID	Putative Function
AT1G11545	xyloglucan:xyloglucosyl transferase
AT2G15390	FUT4; fucosyltransferase/ transferase, transferring glycosyl groups
AT2G22470	AGP2 (ARABINOGALACTAN PROTEIN 2)
AT2G45220	pectinesterase family protein
AT3G19620	glycosyl hydrolase family 3 protein
AT3G22800	leucine-rich repeat family protein / extensin family protein
AT4G08400	proline-rich extensin-like family protein
AT4G38770	PRP4 (PROLINE-RICH PROTEIN 4)
AT5G44480	DUR (DEFECTIVE UGE IN ROOT); UDP-glucose 4-epimerase

Beta glucanase



Genebank ID	Putative Function
AT2G27500	glycosyl hydrolase family 17 protein
AT4G16260	catalytic/ cation binding / hydrolase

Proteolysis



Genebank ID	Putative Function
AT1G32960	SBT3.3; identical protein binding / serine-type endopeptidase
AT1G53190	zinc finger (C3HC4-type RING finger) family protein
AT1G55250	HUB2 (HISTONE MONO-UBIQUITINATION 2); protein binding
AT1G62310	transcription factor jumonji (jmjC) domain-containing protein
AT1G65040	protein binding / zinc ion binding
AT2G24640	UBP19 (UBIQUITIN-SPECIFIC PROTEASE 19); cysteine-type endopeptidase
AT2G44130	kelch repeat-containing F-box family protein
AT2G44140	autophagy 4a (APG4a)
AT2G45920	U-box domain-containing protein
AT2G46160	zinc finger (C3HC4-type RING finger) family protein
AT3G10810	zinc finger (C3HC4-type RING finger) family protein
AT3G11110	zinc finger (C3HC4-type RING finger) family protein
AT3G19390	cysteine proteinase, putative / thiol protease, putative
AT3G46620	zinc finger (C3HC4-type RING finger) family protein
AT3G50930	BCS1 (CYTOCHROME BC1 SYNTHESIS); ATP binding
AT4G17100	unknown protein
AT4G25110	AtMC2 (metacaspase 2); cysteine-type endopeptidase
AT4G35350	XCP1 (XYLEM CYSTEINE PEPTIDASE 1); cysteine-type endopeptidase
AT4G36550	binding / ubiquitin-protein ligase
AT5G02310	PRT6 (PROTEOLYSIS 6); ubiquitin-protein ligase
AT5G04200	AtMC9 (metacaspase 9); cysteine-type peptidase
AT5G05560	EMB2771 (EMBRYO DEFECTIVE 2771); ubiquitin-protein ligase
AT5G27920	F-box family protein
AT5G42940	zinc finger (C3HC4-type RING finger) family protein
AT5G59090	ATSBT4.12; identical protein binding / serine-type endopeptidase
ATCG00670	ClpP (caseinolytic protease)

Redox state

Genebank ID	Putative Function
AT1G75270	DHAR2 (DEHYDROASCORBATE REDUCTASE 2); glutathione binding
AT2G47880	glutaredoxin family protein
AT3G10130	SOUL heme-binding family protein
AT4G11600	ATGPX6 (GLUTATHIONE PEROXIDASE 6)

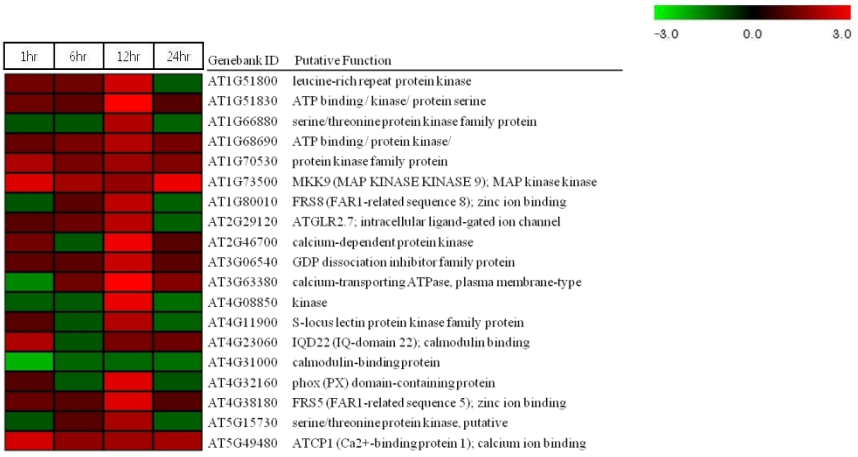
Peroxidases

Genebank ID	Putative Function
AT1G30870	cationic peroxidase, putative
AT2G18980	peroxidase, putative
AT2G41480	electron carrier/ heme binding
AT5G51890	peroxidase

Glutathione-S-transferase

Genebank ID	Putative Function
AT1G02920	GSTF7; copper ion binding / glutathione binding
AT1G02930	ATGSTF6 (GLUTATHIONE S-TRANSFERASE); copper ion binding
AT1G17170	ATGSTU24 (GLUTATHIONE S-TRANSFERASE TAU 24); glutathione binding
AT2G29420	ATGSTU7 (ARABIDOPSIS THALIANA GLUTATHIONE S-TRANSFERASE TAU 7)
AT3G09270	ATGSTU8 (GLUTATHIONE S-TRANSFERASE TAU 8); glutathione transferase

Signaling

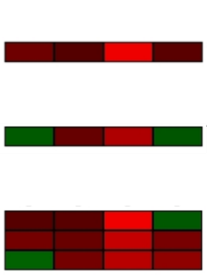


Genebank ID	Putative Function
AT1G51800	leucine-rich repeat protein kinase
AT1G51830	ATP binding / kinase/ protein serine
AT1G66880	serine/threonine protein kinase family protein
AT1G68690	ATP binding / protein kinase/
AT1G70530	protein kinase family protein
AT1G73500	MKK9 (MAP KINASE KINASE 9); MAP kinase kinase
AT1G80010	FRS8 (FAR1-related sequence 8); zinc ion binding
AT2G29120	ATGLR2.7; intracellular ligand-gated ion channel
AT2G46700	calcium-dependent protein kinase
AT3G06540	GDP dissociation inhibitor family protein
AT3G63380	calcium-transporting ATPase, plasma membrane-type
AT4G08850	kinase
AT4G11900	S-locus lectin protein kinase family protein
AT4G23060	IQD22 (IQ-domain 22); calmodulin binding
AT4G31000	calmodulin-binding protein
AT4G32160	phox (PX) domain-containing protein
AT4G38180	FRS5 (FAR1-related sequence 5); zinc ion binding
AT5G15730	serine/threonine protein kinase, putative
AT5G49480	ATCP1 (Ca2+-binding protein 1); calcium ion binding

MAPK

Genebank ID	Putative Function
AT1G73500	MKK9 (MAP KINASE KINASE 9); MAP kinase kinase

Transcription factors



ERF(ethylene response factors)

Genebank ID	Putative Function
AT5G10510	AIL6 (AINTEGUMENTA-LIKE 6); DNA binding

bZIP

Genebank ID	Putative Function
AT1G08320	bZIP family transcription factor

WRKY

Genebank ID	Putative Function
AT1G13960	WRKY4; DNA binding / transcription factor
AT1G62300	WRKY6; transcription factor
AT2G38470	WRKY33; transcription factor

Heat shock proteins

Genebank ID	Putative Function
AT1G62970	DNAJ heat shock N-terminal domain-containing protein
AT1G79030	DNAJ heat shock N-terminal domain-containing protein
AT5G27240	DNAJ heat shock N-terminal domain-containing protein

Secondary metabolites

Genebank ID	Putative Function
AT1G32100	PRR1 (PINORESINOL REDUCTASE 1); pinoresinol reductase
AT1G72680	cinnamyl-alcohol dehydrogenase, putative
AT2G29130	LAC2 (laccase 2); laccase
AT3G25820	ATTPS-CIN (terpene synthase-like sequence-1,8-cineole); (E)-beta-ocimene synthase
AT3G25830	ATTPS-CIN (terpene synthase-like sequence-1,8-cineole); (E)-beta-ocimene synthase
AT3G51450	strictosidine synthase family protein
AT3G57010	strictosidine synthase family protein
AT4G23600	CORI3 (CORONATINE INDUCED 1); cystathionine beta-lyase/ transaminase
AT4G35160	O-methyltransferase family 2 protein
AT5G05600	oxidoreductase, 2OG-Fe(II) oxygenase family protein
AT5G07860	transferase family protein
AT5G07870	transferase family protein
AT5G22300	NIT4 (NITRILASE 4); 3-cyanoalanine hydratase/ cyanoalanine nitrilase
AT5G39050	transferase, transferring acyl groups other than amino-acyl groups
AT5G48010	THAS1 (THALIANOL SYNTHASE 1); catalytic/ thalianol synthase
AT5G60020	LAC17 (laccase 17); laccase

요약문

옥시리핀 화합물은 지방산으로부터 만들어지는 산화물로서 식물, 동물, fungi에 널리 분포되어 있다. 이런 옥시리핀 화합물은 식물에서 식물의 방어반응과 분화를 조절하는 중요한 역할을 하고 있다.

옥시리핀 화합물 중 주로 jasmonic acid에 대한 연구가 활발히 이루어져 있는데 식물 방어 기작 연구는 물론 더 나아가 다른 식물 방어 경로인 ABA, ET, SA pathway와의 cross talking까지도 연구가 되어 있다.

따라서 본 연구에서는 이외에 다른 옥시리핀 화합물에 대해 좀 더 자세히 알아보고자 옥시리핀 화합물 중 식물에서 보편적으로 생성된다고 알려진 3-hexenal과 식물에서 거의 드물게 생성된다고 알려진 1-octen-3-ol을 대상으로 진행하였다. 이 두 옥시리핀 화합물에 대해서는 아직 gene profiling이 이루어져 있지 않아 Microarray 분석을 통해 두 옥시리핀 화합물 처리에 따라 변화하는 방어 반응에 관련하는 유전자의 발현 변화를 알아 보았다.

Microarray 분석결과, 3-hexenal 또는 1-octen-3-ol 처리시 유전자의 발현이 2배이상 변화된 유전자는 3-hexenal 처리구는 414개, 1-octen-3-ol 처리구는 547개의 유전자가 발현 변화한 것을 확인하였다. 각 처리구의 전체 발현 변화된 유전자에서 biotic/abiotic 반응에 관련하는 유전자는 약 30%나 차지하는 것을 확인하였고 이 유전자의 발현패턴은 24시간 동안 지속적으로 발현을 유지하거나 12시간 이내에 지속적으로 발현을 증가하는 패턴을 띠는 것을 확인하였다. 또한 biotic/abiotic 반응에 관련하는 유전자는 R gene, PR gene,

hormone signaling, cell wall, proteolysis, transcription factor, signaling, redox state, peroxidases, glutathione-S-transferase, signaling, MAPK, transcription factor, heat shock protein, secondary metabolism 등과 관련된 유전자들이 발현한다는 것을 확인하였다. 두 처리구인 3-hexenal이나 1-octen-3-ol 처리시 발현 변화된 biotic/abiotic 반응에 관련하는 유전자를 overlapping 해본 결과 다수의 biotic/abiotic 반응유전자가 공통적으로 발현 변화한다는 것을 확인하였다.

따라서 본 연구에서 내린 결론은 아마도 3-hexenal과 1-octen-3-ol이 식물에 영향을 미치게 되면 이 두 화합물이 signal molecular로 작용하여 다양한 biotic stress 반응에 관여하는 유전자의 발현을 12시간 이내에 증가시켜 식물의 defense 반응을 일으킬 것으로 예상된다.

ACKNOWLEDGEMENT

朱子曰

勿謂今日不學而有來日**하며** 勿謂今年不學而有來年**하라**

日月逝矣**라** 歲不我延**이니** 嗚呼老矣**라** 是誰之愆**고**.

사람이 배우는 것은 다 때가 있다.
그 때를 놓치게 되면 배우지 못하고 만다.

오늘 배우지 아니하고서 내일로 미루고
금년에 배우지 아니하고 내년으로 미루어서

세월이 하루하루 덧없이 흘러가고 보면
어느덧 나이가 들어 늙어 버린다.

배우고 싶어도 이미 때는 늦었다.
그것은 어디까지나 자신의 잘못이다.

누구를 원망할 것인가?
또 후회한들 무엇 하랴