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Chemical Changes and Overexpressed Genes in Sweet Basil (*Ocimum basilicum* L.) upon Methyl Jasmonate Treatment

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The effects of methyl jasmonate (MeJA) on the production of bioactive chemicals and gene expression in sweet basil were investigated. The total amount of phenolic compounds significantly increased in sweet basil after 0.5 mM MeJA treatment. Among the phenolic compounds, rosmarinic acid (RA) and caffeic acid (CA) were identified, and their amounts increased by 55 and 300%, respectively. The total amount of terpenoids also significantly increased after the same treatment. Particularly, eugenol and linalool increased by 56 and 43%, respectively. To better understand the signaling effect of MeJA on sweet basil, suppression subtractive hybridization (SSH) was used to identify the MeJA up-regulated genes. Among the 576 cDNA clones screened from the forward SSH cDNA library, 28 were found to be up-regulated by the MeJA treatment. Sequencing of these cDNA clones followed by BLAST searching revealed six unique transcripts displaying high similarities to the known enzymes and peptide, that is, lipoxygenase (LOX), cinnamic acid 4-hydroxylase (C4H), prephenate dehydrogenase (PDH), polyphenol oxidase (PPO), acid phosphatase (APase), and pentatricopeptide repeat (PPR), which play significant roles in the formation of secondary metabolites in sweet basil. Northern blot further confirmed the increased production at transcriptional level of LOX, C4H, PDH, PPO, PPR, and APase.

KEYWORDS: Suppression subtractive hybridization (SSH); gene expression; methyl jasmonate (MeJA); phenolic compounds; terpenoids; sweet basil; *Ocimum basilicum*

INTRODUCTION

Many phytochemicals possess important beneficial pharmacological or nutraceutical traits, such as the abilities to serve as cellular antioxidants by maintaining low levels of reactive oxygen intermediates, as anti-inflammatory agents by inhibiting prostaglandin synthesis, or as anticancer agents by acting as inhibitors of cell proliferation and inducers of cell apoptosis. Such evidenced benefits of phyto-bioactive compounds prompt us to explore novel ways to increase the production of beneficial secondary metabolites in dietary vegetables. As is well-known, plant secondary metabolites are not only controlled by plant genotypes but also greatly affected by growth environmental conditions. Under various biotic and abiotic stresses such as wounding, pathogen attack, and UV-light exposure, plants respond to produce not only the direct defensive compounds such as proteinase inhibitors, polyphenol oxidase, and α-amylase inhibitors to protect themselves from stresses but also secondary metabolites such as health-benefiting phenolics, terpenoids, and alkanoids (*1*−*4*). In addition, it has been reported that various elicitors such as chitosan, *β*-glucan, and yeast extracts, as well as plant hormonal chemicals such as jasmonic acid (JA) and methyl jasmonate (MeJA), can act like biotic and abiotic stresses (*5*−*7*).

Endogenous MeJA is a signaling compound that modulates various physiological processes in plants (*8*). Under stresses such as wounding and pathogen attack, volatile MeJA can be released into the air from the wounded plants (*9*−*11*). The wounding signal can also prompt other healthy plants that receive the signal to increase their secondary metabolites and improve their defensive systems. Therefore, exogenous MeJA has been used to induce useful secondary metabolites such as alkaloids, terpenoids, and phenolics in some plants such as *Nicotina* species, *Hyoscyamus muticus*, Norway spruce stems, and some plant cell cultures (*12*−*18*). The increased amounts of secondary metabolites increase not only plants' disease resistance but also their market values regarding the increased health benefits. However, MeJA may act both synergistically and antagonistically in regulating stress-induced gene expression at the transcriptional level (*19*). Therefore, we selected sweet basil (*Ocimum basilicum* L.) as a simple model and aimed to (1) investigate the effect of MeJA on the induction of health-promoting compounds, that is, phenolic compounds and terpenoids and (2) isolate and identify the MeJA-up-regulated genes that relate to the aforementioned secondary metabolites by using suppression subtractive hybridization (SSH) technique.

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MATERIALS AND METHODS

Chemicals. MeJA, gallic acid, caffeic acid (CA), and Folin−Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO). Rosmarinic acid (RA) was obtained from Cayman Chemical Co. (Ann Arbor, MI). Eugenol and L-linalool were purchased from Aldrich Chemical Co. (Milwaukee, WI). Ampicillin and all HPLC analytical grade solvents were from Fisher Scientific (Suwanee, GA). X-Gal was from Gold Biotechnology (St. Louis, MO), and IPTG was from Molecula (Columbia, MD).

Plant Culture and MeJA Treatment. Sweet basil seeds purchased from a local grocery (Clemson, SC) were sown into 48-cell trays containing a commercial potting mixture (Fafard 3-B Mix, Fafard Inc., Anderson, SC) and grown under natural light conditions in the greenhouse located at Clemson University in Clemson, SC. The sweet basil plants were watered every other day with the addition of fertilizer once a week. Greenhouse cooling/heating set points were 27/25 °C. At the third leaf stage, 0.5 mM MeJA in 2% ethanol was sprayed onto the basil plants. The untreated basil (control) plants were sprayed with only 2% ethanol. Then the MeJA-treated and the control plants were zipped with a vinyl pack for 40 min. Before the vinyl pack was removed, the control plants were isolated from the treated plants, and the treated plants were left in the open air for 2 h to completely evaporate the remaining MeJA.

Extraction of Phenolic Compounds and Terpenoids from Sweet Basil. Fresh sweet basil plants harvested at 0, 1, 2, and 4 days after the treatment were ground in liquid nitrogen. Two grams of the sweet basil powder was mixed with 20 mL of 80% methanol to extract phenolic compounds or with 20 mL of methyl *tert*-butyl ether (MTBE) to extract terpenoids. The mixture was shaken at room temperature for 12 h and then centrifuged at 2000*g* for 20 min. After the centrifugation, the supernatant from methanol extraction was used for the determination of phenolic compounds, and the supernatant from MTBE extraction was used for terpenoid analysis.

Determination of Phenolic Compounds and Terpenoids from Sweet Basil. The total amount of phenolics was determined using Folin−Ciocalteu reagent according to the method of Singleton and Rossi (*20*). Fifty microliters of the methanolic extract was mixed with 450 μL of distilled water and 250 μL of 2 N Folin−Ciocalteu reagent; 1.25 mL of 20% Na_2CO_3 was added into the mixture, and the mixture was incubated at 25 °C for 20 min and then centrifuged at 2000*g* for 10 min. The absorbance of the supernatant was measured at 735 nm, and the standard curve was prepared using gallic acid. To separate and identify individual phenolic compounds in the sweet basil extract, reverse phase high-performance liquid chromatography (HPLC) was used. A Pinnacle II C_{18} column (150 × 4.6 mm, 5 μm; Restek, PA) was connected to the LC-10AT HPLC system (Shimadzu, Kyoto, Japan) with an SPD-M10V photodiode array detector (PDA). Fifty microliters of the methanolic extract was injected and eluted with 0.05% aqueous trifluoroacetic acid TFA (solvent A) and acetonitrile (solvent B) at a flow rate of 1 mL/min. Authentic standards were used to identify phenolic compounds of sweet basil. A GC (GC-17A)−mass spectrometer (QP 5050 MS) system (Shimadzu, Kyoto, Japan) with a DB-5 capillary column (60 m × 0.25 mm, thickness = 0.25 μm) was used to identify terpenoids in sweet basil. The detailed procedures for chemical identification and quantitation have been described in previous papers (*21*, *22*).

RNA Isolation and Purification. Leaves (1−2 g) from the untreated (control) and the MeJA-treated sweet basil plants were randomly sampled at 0, 1, 3, 6, 9, 12, 24, and 48 h after treatment. The leaves were immediately frozen in liquid nitrogen and stored at −80 °C prior to analysis. Total RNA was extracted respectively from the pooled leaves of the MeJA-treated and control plants using the Trizol method (Invitrogen) according to the manufacturer's recommendations. The concentration and total amount of RNA were determined using a Beckman DU 640B spectrophotometer. The integrity was determined by electrophoresis using formaldehyde agarose/EtBr gel (1.3%). Poly(A)+RNA was isolated from the total RNA using an Oligotex mRNA Midi Kit (Qiagen Inc., Valencia, CA). The poly(A)+RNA from both the MeJA-treated basil and the untreated basil leaves (1 μg from each) were used as templates for the reverse transcription using a cDNA synthesis kit according to the manufacturer's instructions (Clontech). The efficiency of the cDNA synthesis was monitored by electrophoresis using agarose/EtBr gel (1.3%).

Construction of the cDNA Library Using SSH. SSH was performed using a PCR Select cDNA Subtraction Kit (Clontech) according to the manufacturer's instructions. The cDNAs that contained transcripts from the MeJA-treated basil were referred to as "tester", whereas the cDNAs from the untreated basil control were referred to as "driver". Tester and driver were digested separately with *Rsa*I and ligated with different cDNA adaptors. Then, tester and driver cDNAs were hybridized. The hybrid sequences were removed, and the remaining unhybridized cDNAs represented the genes that were expressed in tester, but not in driver, mRNA. These differentially expressed transcripts were PCR amplified using primers directed to tester-ligated adaptor sequences for further enrichment of the target molecules.

For screening purposes, two subtractions were performed: the original intended subtraction (forward subtracted hybridization) and the reverse subtracted hybridization in which the tester serves as the driver and the driver as the tester. The positive control subtraction was done side by side by using skeletal muscle cDNA subtracted from skeletal muscle cDNA doped with *Hae*III-digested X174 DNA.

Cloning into a T/A Vector. The differentially expressed cDNAs (forward subtracted cDNAs) were cloned directly into the T/A cloning vector pCR2.1 (Invitrogen). Approximately 100 ng of freshly PCR-amplified forward subtracted cDNA was ligated into 50 ng of vector, and the ligation mixture was transformed into the Top 10F′ chemically competent cells (*Escherichia coli*). The transformed cells were plated onto 9 cm agar plates containing 150 μg/mL ampicillin, 100 μM IPTG, and 60 μg/mL X-Gal. Plates were incubated at 37 °C until small colonies became visible. They were further incubated at 4 °C until blue/white staining could be clearly distinguished. All of the recombinant clones were picked up to establish the subtracted cDNA library.

Colony Array and Differential Screening. Differential screening was performed using colony array (PCR-Select Differential Screening Kit, Clontech). A total of 576 colonies were arrayed on four identical nylon membranes (Amersham Biosciences) according to the method of Sambrook et al. (*23*). Four probes, that is., forward subtracted cDNAs, reverse subtracted cDNAs (obtained with the original tester cDNAs as a driver and the driver cDNAs as a tester), unsubtracted tester cDNAs, and unsubtracted driver cDNAs, were sequentially prepared by digestion with *Rsa*I, purified using phenol−chloroform, and labeled using Prime-It RmT random primer labeling kit (Stratagene, La Jolla, CA) with [^{32}P]dCTP (Amersham Biosciences). After removal of the unincorporated nucleotides by Stratagens's NucTrap probe purification columns, the probes were hybridized with the forward subtracted clones arrayed on nylon membranes, respectively. Prehybridization was performed at 72 °C in ExpressHyb hybridization solution (BD Biosciences) for 2 h, and hybridization was carried out at 72 °C for at least 16 h. The membranes were washed five times for 30 min each at 72 °C in 0.2× SSC containing 0.5% w/v SDS. After autoradiography, positive clones, which showed signal to the forward subtracted cDNA probe and tester cDNA probe but not to the reverse subtracted cDNA probe and driver cDNA probe, were picked up.

Sequencing. Recombinant plasmid DNAs from the positive clones were isolated using the Plasmid Miniprep kit from Qiagen (Valencia, CA). Sequencing was performed on an ABI fluorescent sequencer in the Clemson Sequence Center with the ABI Prism Big Dye Terminator 3.1 (Applied Biosystems, Foster City, CA).

Sequence Analysis. Homology searches were conducted using the BLAST program (TBLASTX and BLASTX) in three databases (GenBank non-redundant, EST and Swissprot) of the NCBI (http://www.ncbi.nlm.nih.gov). Multiple sequence alignments were performed with MULTALIN (http://prodes.toulouse.inra.fr/multalin/multalin.html).

Northern Analysis. Aliquots of 10 μg of total RNA from both MeJA-treated and untreated basil were separated on 1.3% denatured agarose gel and transferred to a Hybond-N+ nylon membrane (*23*). The positive clones were labeled with [^{32}P]dCTP using a Prime-It RmT random primer labeling kit. Northern prehybridization was performed at 68 °C in ExpressHyb hybridization solution for 2 h, and hybridization was carried out at 68 °C for at least 16 h. The membrane was washed

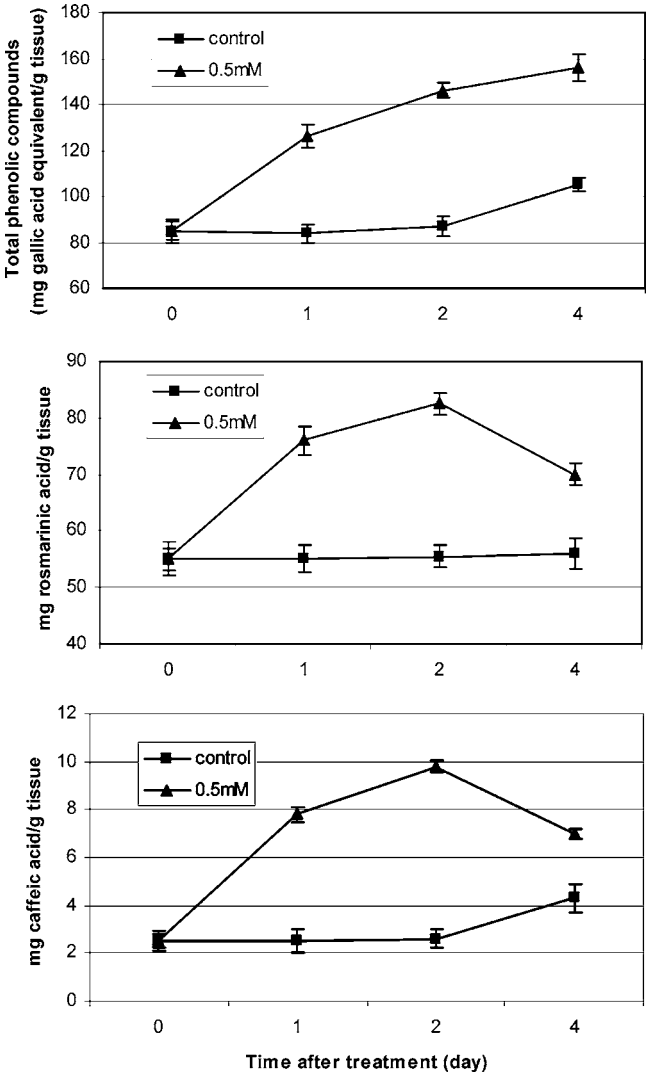

Figure 1. Time course of the effect of MeJA on phenolic contents in sweet basil. RA, rosmarinic acid; CA, caffeic acid.

four times for 15 min each at 68 °C in 0.1× SSC, 0.5% w/v SDS. The membrane was exposed to Kodak BioMax film for 48−72 h at −80 °C.

Experimental Design and Data Analysis. To investigate the effect of MeJA on sweet basil, MeJA treatment and control were adopted under a randomized complete block design. Fifteen sweet basil plants were used, respectively, from treatment and control for three replicates. Five sweet basil plants were randomly selected and placed at three randomly selected places in the greenhouse for each replicate. The sweet basil plants were harvested at 0, 1, 2, and 4 days after the treatment for chemical analyses. All experiments were performed in triplicate. Data were subjected to analysis of variance and were analyzed with nonlinear regressions (SAS 9.1, SAS Institute Inc., Cary, NC).

RESULTS

Effect of MeJA on Phenolic Compounds and Antioxidative Ability in Sweet Basil. Total phenolic content (TPC) of the sweet basil significantly increased after 0.5 mM MeJA treatment (**Figure 1**). The TPC of the sweet basil reached a maximal value of 158 mg of gallic acid equiv/g of tissue within the test period. To investigate the effect of MeJA on individual phenolic compounds in the sweet basil, the methanolic extract was analyzed by HPLC using a C_{18} column. Among the various phenolic compounds, rosmarinic acid (RA) and caffeic acid

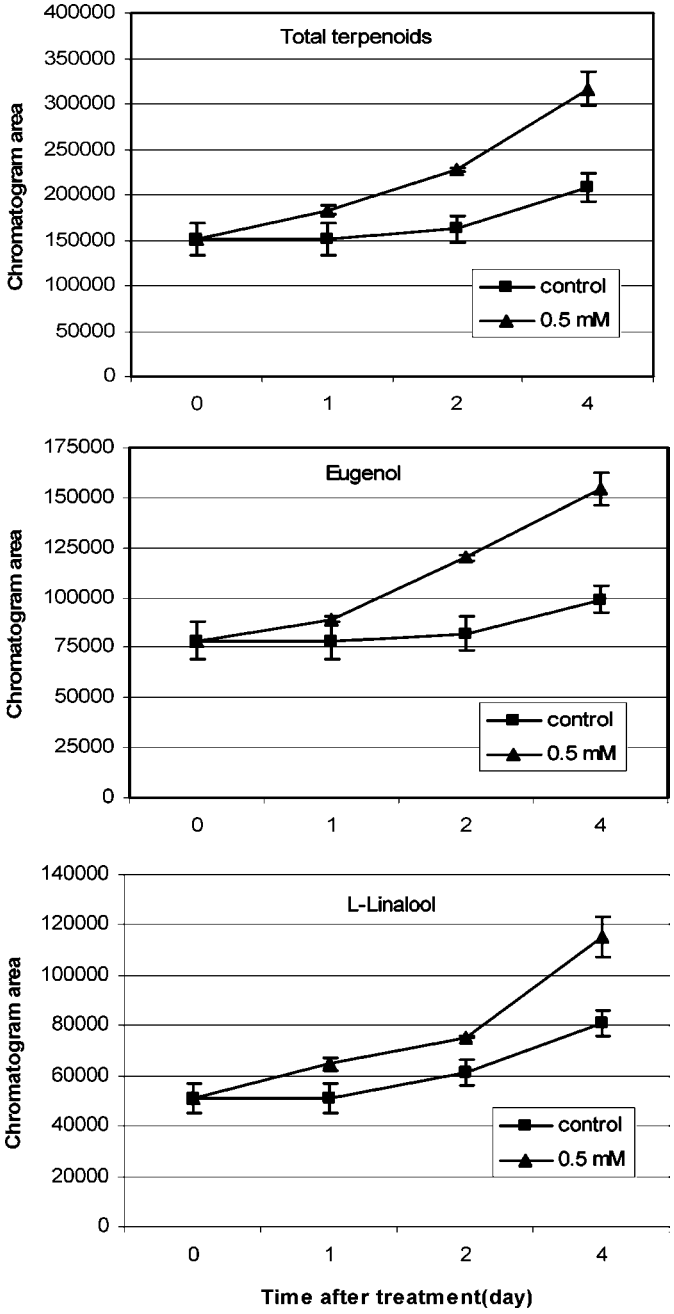

Figure 2. Time course of the effect of MeJA on terpenoid contents in sweet basil. Eugenol and L-linalool were quantitatively determined by GC-FID.

(CA) were identified, and their amounts increased by 55 and 300%, respectively, 2 days after the treatment (**Figure 1**).

Effect of MeJA on Terpenoid Content of Sweet Basil. The total amount of terpenoids also significantly increased after MeJA treatment. The amount at the fourth day was 58% greater than that of the control (**Figure 2**). This result was in agreement with the reports (*15*, *17*, *24*−*26*) that terpenoids could be positively affected by stresses and elicitors in various plants such as Norway spruce, tobacco plant, grand fir, lima bean, and tomato. Eugenol and L-linalool were identified as two major flavoring terpenoids in sweet basil. Compared with that of the control, the amounts of eugenol and L-linalool at the fourth day after the treatment increased by 56 and 43%, respectively. A similar phenomenon was observed in that eugenol and L-linalool in sweet basil were induced by UV-B treatment (*27*) due to its

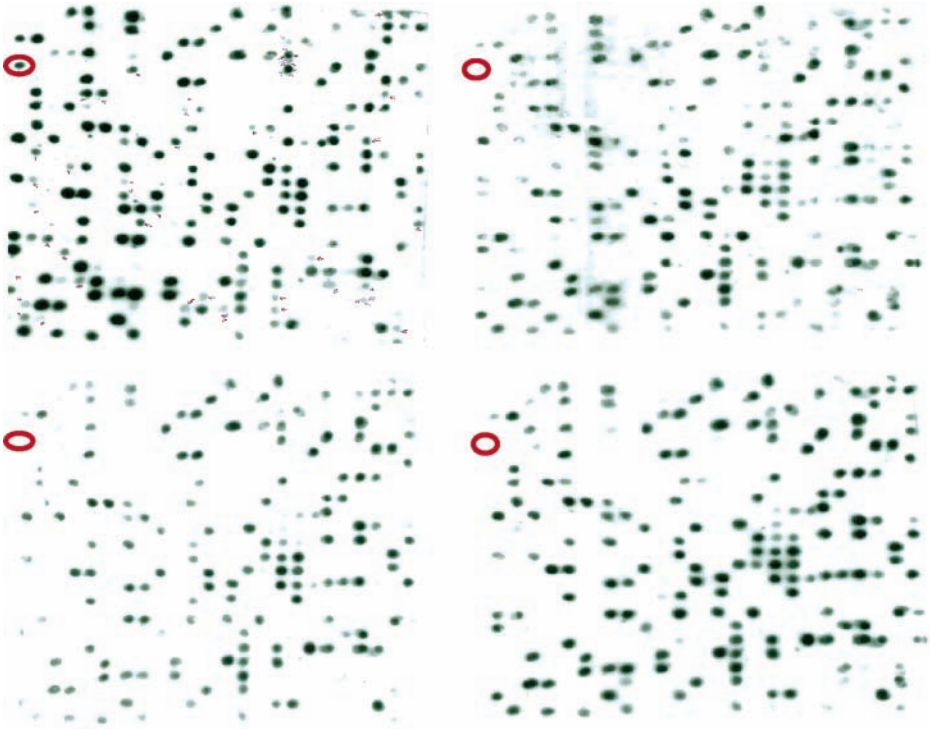

Figure 3. Differential screening of the suppression-subtracted cDNA library. Dot blots were hybridized with cDNA probes made from forward subtracted cDNA (**A**), forward unsubtracted cDNA (**B**), reverse subtracted cDNA (**C**), and reverse unsubtracted cDNA. Twenty-eight MeJA up-regulated cDNA clones were screened from 576 clones (the red circled one indicates the MeJA up-regulated clone).

regulatory effects on key enzymes such as phenylalaline ammonia-lyase.

Identification of Differentially Expressed Genes by MeJA. Upon MeJA treatment, the bioactive compounds such as phenolics and terpenoids significantly increased. This suggested that some genes involved in the biosyntheses of the aforementioned secondary metabolites were up-regulated by the MeJA. To understand the signaling effects of MeJA on basil, gene expression was further investigated by using the SSH method. Forward subtracted cDNAs were cloned into a plasmid vector and transformed into *E. coli*. Differential hybridization screening was performed to eliminate false-positive clones. Selection of differentially expressed cDNA clones was achieved by comparing signal intensities on the membranes with four probes prepared from the forward subtracted cDNAs, the unsubtracted tester cDNAs, the reverse subtracted cDNAs, and the unsubtracted driver cDNAs. The partial representative differential screening results are illustrated in **Figure 3**.

SSH coupled with high-throughput screening detected 28 differentially expressed clones. The nucleotide sequences of the 28 selected clones were analyzed, and their putative functions were identified by BLASTX and TBLASTX searching. As a result of homology searches, all of these sequences showed significant matches (significance *E* value $\leq$ 1E−10) to known plant genes (**Table 1**). Among these 28 genes, clones encoding lipoxygenase (LOX) appeared 18 times, and the deduced amino acid sequences displayed identity 66−84% to LOX in the GenBank database. Three clones, P1A10, P3E1, and P3E5, showed significant protein homology of 87−90% to cinnamic acid 4-hydroxylase (C4H). Four clones, P2B9, P2C7, P5H2, and P6E9, showed 30−37% identity to acid phosphatase (APase) genes in the GenBank SwissProt database. The deduced amino acid sequences of the other three differentially expressed clones, P1A3, P1B10, and P3B3, showed different similarities in identities of 60, 74, and 53% to pentatricopeptide repeat (PPR), prephenate dehydrogenase (PDH), and polyphenol oxidase (PPO), respectively. The fragment insert size ranged from 250 to 612 bp (see **Table 1** for detailed cDNA size, accession number, gene description, and *E*values), and these EST have been submitted to GenBank with accession numbers from EB741003 to EB741030.

Multiple sequence alignments with hierarchical clustering of two differential expression clones, P1A10 and P5A12, were performed using MULTALIN software. The results suggested that P1A10 showed significant matches to the C4H genes of roseus, *Arabidopsis*, and soybean, whereas P5A12 showed significant matches to the LOX genes of tomato, potato, and lemon (**Figure 4**). Except for the PDH, the other five genes up-regulated by the MeJA were also found to be induced or regulated by different stresses in different plants such as pepper (PPR, pathogen stress induced) (*28*), sugar beet (C4H, fungus infection induced) (*29*), cotton leaves (APase, water stress induced) (*30*), oilseed rape suspension cells (APase, phosphate starvation induced) (*31*), apple (PPO, wounding induced) (*32*), and *Arabidopsis thaliana* (LOX, pathogens, abscisic acid, and MeJA induced) (*33*).

Confirmation of Differential Expression Using the Northern Blot Analysis. To verify the elevated expression levels of the screened clones, the transcriptional patterns of six MeJA up-regulated clones (P1A3, P1A10, P1B10, P1B5, P2B9, and P3B3) that showed homology to PPR, C4H, PDH, LOX, APase, and PPO genes were analyzed in the Northern blot (**Figure 5**). The Northern blot further confirmed the increased production of LOX, C4H, PDH, APase, PPO, and PPR transcripts. These putative up-regulated genes have been found to play pivotal roles in secondary metabolite synthesis, plant defense system, signal transportation, and RNA editing in some plants.

Table 1. Similarity Searches of the Positive Clones Obtained by SSH Analysis of the Sweet Basil Induced by MeJA

clone	cDNA size[a] (bp)	database[b]	accession no.	species	gene description	identity (aa)[c] (%)	*E* value
P2B9	392	GenBank SwissProt	P27061	*Lycopersicon esculentum*	APase	33	2×10^{-10}
P2C7	351	GenBank SwissProt	P27061	*Lycopersicon esculentum*	APase	37	2×10^{-15}
P5H2	323	GenBank SwissProt	P27061	*Lycopersicon esculentum*	APase	36	6×10^{-14}
P6E9	382	GenBank SwissProt	P27061	*Lycopersicon esculentum*	APase	34	4×10^{-14}
P1A10	369	GenBank nr	P48522	*Catharanthus roseus*	C4H	90	1×10^{-50}
P3E1	398	GenBank nr	BAB71717	*Lithospermum erythrorhizon*	C4H	87	6×10^{-62}
P3E5	340	GenBank SwissProt	P48522	*Catharanthus roseus*	C4H	90	3×10^{-47}
P1B5	590	GenBank nr	BAB84352	*Citrus jambhiri*	LOX	74	1×10^{-86}
P1B6	402	GenBank nr	AAP83137	*Nicotiana attenuata*	LOX	76	6×10^{-57}
P1D1	589	GenBank nr	AAP83137	*Nicotiana attenuata*	LOX	74	7×10^{-85}
P1D7	359	GenBank nr	AAZ57445	*Populus deltoids*	LOX	84	6×10^{-48}
P2A8	324	GenBank EST	CO116430	*Gossypium raimondii*	LOX	81	1×10^{-51}
P2H10	361	GenBank EST	CN996565	*Malus* × *domestica*	LOX	83	4×10^{-27}
P2G10	403	GenBank EST	DR172071	*Triphysaria versicolor*	LOX	81	5×10^{-45}
P3B2	394	GenBank EST	DR992991	*Malus* × *domestica*	LOX	81	2×10^{-60}
P3C2	455	GenBank nr	AAZ57444	*Populus deltoids*	LOX	66	6×10^{-56}
P3G4	391	GenBank EST	CX290355	*Citrus clementina*	LOX	82	6×10^{-57}
P3H3	398	GenBank EST	CX290355	*Citrus clementina*	LOX	83	7×10^{-59}
P4B10	412	GenBank EST	DR172071	*Triphysaria versicolor*	LOX	82	4×10^{-48}
P4H10	410	GenBank EST	DR172071	*Triphysaria versicolor*	LOX	84	6×10^{-55}
P5A12	614	GenBank nr	AAP83137	*Nicotiana attenuata*	LOX	73	2×10^{-88}
P5G9	616	GenBank nr	AAP83137	*Nicotiana attenuata*	LOX	75	5×10^{-88}
P6B11	409	GenBank nr	AAB65766	*Lycopersicon esculentum*	LOX	76	4×10^{-48}
P6E7	612	GenBank nr	AAP83137	*Nicotiana attenuata*	LOX	74	7×10^{-70}
P6G6	611	GenBank nr	AAP83137	*Nicotiana attenuata*	LOX	72	6×10^{-79}
P1B10	407	GenBank nr	NP_173023	*Arabidopsis thaliana*	PDH	74	1×10^{-12}
P3B3	250	GenBank nr	AAK13242	*Trifolium pretense*	PPO	53	2×10^{-14}
P1A3	477	GenBank nr	BAC22241	*Oryza sativa*	PPR	60	5×10^{-27}

[a] Base pairs of inserts that were sequenced by using the primer Nest Primer 1: 5′-TCGAGCGGCCGCCCGGGCAGGT-3′. [b] Three databases were searched with both the BLASTX and TBLASTX algorithms: GenBank non-redundant (nr), EST, and SwissProt databases. [c] Amino acid.

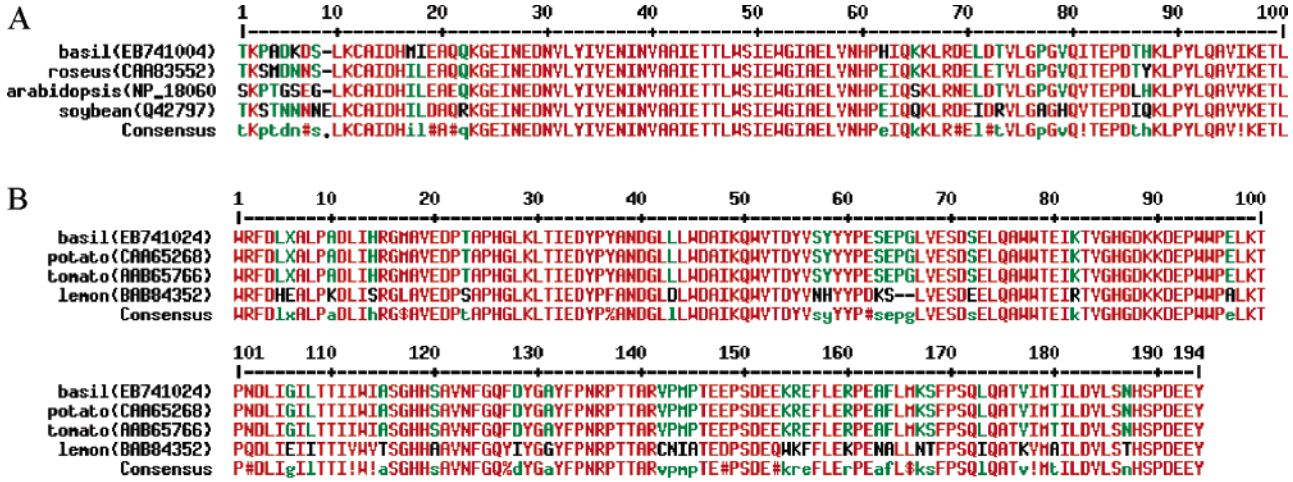

Figure 4. Sequences of the two cDNA clones aligned with MULTALIN followed by BLAST. (**A**) Clone P1A10: Alignment of deduced partial amino acid sequences of sweet basil and partial amino acid sequences of other plant cinnamate-4-hydroxylases. (**B**) Clone P5A12: Alignment of deduced partial amino acid sequences of sweet basil and partial amino acid sequences of other plant lipoxygenases. Red, high consensus (default, 90%); green, low consensus (default, 50%); black, neutral.

DISCUSSION

SSH is a powerful technique that enables researchers to compare two populations of mRNA and obtain clones of genes that are expressed in one population but not in the other. In the present investigation of the effect of MeJA on sweet basil, SSH coupled with high-throughput screening methods identified six unique overexpressed transcripts: LOX, C4H, PDH, APase, PPO, and PPR.

LOX is a key regulator in two distinct oxylipin pathways and is known to play a major role in plants' wound recognition and is involved in the biosynthesis of physiological active compounds. LOX catalyzes the oxygenation of polyunsaturated fatty acids, such as linoleic and linolenic acids, to produce fatty acid hydroperoxides, which serve as intermediates in the formation of bioactive compounds, such as jasmonic acid and traumatin, or convert to volatile short-chain aldehydes, esters, alcohols, etc. (*34*). Our results demonstrated that the LOX gene is dramatically induced in response to the MeJA treatment (**Figure 5**), which also had provoked higher flavor production in the elicited sweet basil than the control.

In this study, we found that the MeJA treatment could significantly increase the levels of RA and CA. RA, an ester of CA and 3,4-dihydroxyphenyllactic acid, has been reported to

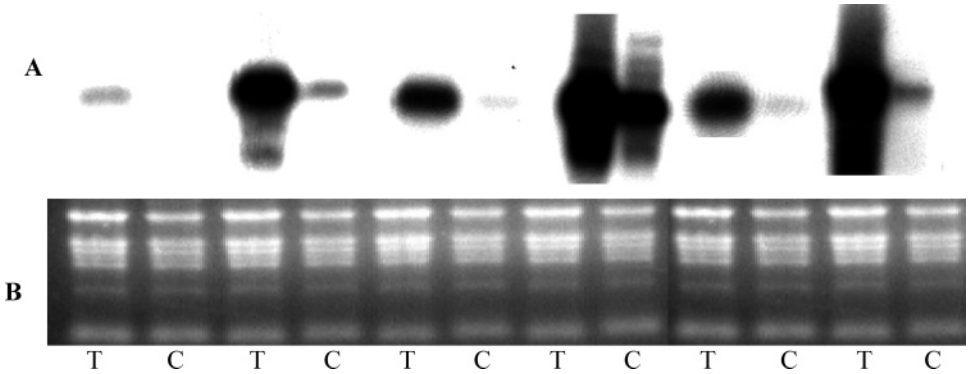

Figure 5. Effect of MeJA treatment on transcript levels of jasmonate-induced genes. From left to right (**A**) RNA gel blots were probed with ^{32}P-labeled insertions of clone P2B9 (deduced acid phosphatase, APase), P3B3 (deduced polyphenol oxidase, PPO), P1A3 (deduced pentatricopeptide repeat, PPR), P1A10 (deduced cinnamate-4-hydroxylase, C4H), P1B10 (deduced prephenate dehydrogenase, PDH), and P1B5 (deduced lipoxygenase, LOX), respectively. (**B**) Total RNAs were prepared from control and MeJA-treated sweet basils. Ten micrograms of total RNAs was loaded onto each lane, and the equal loading for each lane was confirmed by staining the gels with ethidium bromide before blotting.

have many bioactive properties such as antioxidative, anti-inflammatory, antimutagen, antibacterial, and antiviral activities (*35*). RA and CA were derivatized through the phenylpropanoid pathway initiated by the phenylalanine ammonia-lysase (PAL) (*35*). Also, the C4H is the first p450 upstream in the phenylpropanoid pathway resulting in the synthesis of a variety of compounds including lignin monomers, flavonoids, phenolics, and furocoumarins. C4H catalyzes the hydroxylation of cinnamic acid into coumaric acid and is involved in the biosynthesis of RA. The Northern blot confirmed that the C4H mRNA level was significantly induced upon the MeJA treatment (**Figure 5**). Activation of the enzyme C4H may have led to the higher production of RA and CA that can add to the herb's market value. From the results of higher C4H mRNA level in the elicited sweet basil, we may also predict that the amounts of flavonoids would increase, although this needs further analyses for confirmation.

It was reported that the PDH gene was involved in the production of tocotrienols and tocopherols (*36*), which are the two forms of vitamin E. Vitamin E is a natural antioxidant that has shown a wide spectrum of bioactivities, such as antiaging and lifestyle-related disease prevention. The MeJA treatment significantly stimulated the expression level of the PDH gene, which may result in the increased production of vitamin E. If the MeJA treatment can induce a higher production of vitamin E, it will benefit not only farmers and plant distributors for an expected extended shelf life of plants but also consumers due to the natural antioxidant.

The stress inducibility of PPO activity has been well documented (*37*, *38*). It has been suggested that PPO plays an important role in plant defensive reactions (*38*, *39*). It has been found that PPO gene expression can be induced in different plant species by wounding and pathogen attack (*32*, *40*). PPO can convert monophenols into *o*-diphenols and is responsible for hydroxylation of *p*-coumaric acid to CA in the phenylpropanoid pathway (*41*). The coincidence of the induced overexpressed PPO gene and higher production of CA in the MeJA-elicited sweet basil hinted that the former might be the causative factor of the latter.

Another transcript encoding for APase was also significantly activated by the MeJA treatment. APase is believed to be important for many physiological processes, such as regulating phosphorus efficiency (*42*). Secretion of APase under phosphorus stress is speculated to liberate phosphorus from organic sources (*43*). APase also has been shown to have alkaline peroxidase activity, implying a role for plants in defense against pathogen infection (*44*). The APase gene was induced by the MeJA in sweet basil leaves. However, the accumulation of transcript of APase could not be detected in the untreated basil, indicating a low (or no) expression of APase in the untreated sweet basil.

PPR was first discovered in 2000 by Small and Peeters (*45*). The PPR proteins have been shown to affect the stability or translation of specific organellar mRNAs (*45*, *46*). However, little is known about the functions of PPR protein. Although the gene encoding for PPR was found to be up-regulated by the MeJA treatment in this study, the mechanism of how MeJA acts on PPR needs more investigation.

Dietary vegetables containing high concentrations of phenolics and terpenoids have been recognized as important for human consumption and have higher economic value. Among the 576 cDNA clones screened from the forward SSH cDNA library, 6 unique transcripts were identified as up-regulated by the MeJA treatment. Although the amounts of eugenol and linalool significantly increased after MeJA treatment, we have not identified genes directly involved in the pathway for terpenoid production. Particularly, there was a lack of information of the well-known geranyl diphosphate synthase (GPPS) that is directly associated with the formation of linalool. Because evidence supported that eugenol was biosynthesized through the phenylpropanoid pathway (*47*), the induced increase of PAL might explain the increased production of eugenol (*22*). Nevertheless, we are continuing to screen the forward subtracted cDNA library to pull out more up-regulated genes, in which we expect certain genes would be responsible for the terpenoid production.

In conclusion, MeJA treatment could significantly increase the bioactive compounds in sweet basil rapidly and transitionally. Analysis of the forward subtracted cDNA library of sweet basil resulted in the identification of 28 clones that matched known genes. Northern hybridization confirmed that the transcriptional accumulations of LOX, C4H, PDH, APase, PPO, and PPR in the treated basil were significantly higher than those in the control. The information from this study has provided valuable information on how plant metabolism can be regulated to favor the biosynthesis of particular health-benefiting metabolites. We hope the aforementioned techniques can also be applied in the production of medicinal chemicals or their precursors in plants to benefit human health.

ABBREVIATIONS USED

MeJA, methyl jasmonate; SSH, suppression subtractive hybridization; LOX, lipoxygenase; C4H, cinnamic acid 4-hydroxylase; PDH, prephenate dehydrogenase; PPO, polyphenol oxidase; APase, acid phosphatase; PPR, pentatricopeptide repeat.

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