Pak. J. Bot., 47(1): 177-188, 2015.

DEFENSIVE RESPONSES OF *POPLUS DELTOIDES* 895 SEEDLINGS AGAINST EXOGENOUS METHYL JASMONATE

QUN LIU, YANQIONG ZHOU, JUAN CHEN AND DEJUN HAO*

College of Forest Resources and Environment, Nanjing Forestry University, Nanjing 210037, P.R. China
**Corresponding author's e-mail: djhao@njfu.edu.cn*

Abstract

Methyl jasmonate (MeJA) is a chemical elicitor that induces a series of defensive responses in plants and has been widely used to simulate pathogens and herbivore attacks for several decades. This study aimed to determine the effect of exogenous MeJA on one-year-old *Populus deltoids* 895 seedlings. MeJA solution (10μM) was sprayed on the leaves, and the activities of sugar, free amino acid, four defensive enzymes, tannin, flavone, phenolic acid and volatile compounds were examined at different time periods. Sugar, free amino acid, and protein contents decreased to some extent regardless of the type of treatment (direct MeJA-treated leaves or indirect contralateral non-MeJA-treated leaves), whereas tannin, flavone, and phenolic acid increased. PPO, LOX, TI, and CI were activated by the treatment. MeJA also affected emissions of the leaf volatiles. These results indicate that MeJA could generate or trigger defensive responses by *P. deltoids* seedlings. This study provided basic information as reference for further studies on defoliators performance after feeding on MeJA-treated poplar leaves.

Key words: Methyl jasmonate, *Populus deltoids*, Defensive responses.

Introduction

Plants have developed complex defensive mechanisms involving direct and indirect strategies to survive biotic and abiotic environmental stresses (Walters & Heil, 2007; Champigny & Cameron, 2009; Feng *et al*., 2012b; Martin *et al*., 2012). Direct plant defense requires altering the palatability and/or toxicity of leaf tissues to prevent herbivores from feeding on plants (Agrawal, 1999; Graves *et al*., 2008; Simons *et al*., 2008). In contrast, indirect plant defense involves attracting predators and parasitoids of herbivores by emitting special volatile signal substances immediately after an attack (Kessler & Baldwin, 2001; Degenhardt *et al*., 2003; Williams *et al*., 2008). Both strategies are inducible in the presence of potent elicitors or signaling agents.

Jasmonic acid (JA) and its volatile derivative, methyl ester methyl jasmonate (MeJA) have been proven as effective elicitors that generate defensive responses by coordinating plant growth, resistance, and senescence (Creelman & Mullet, 1997; Rohwer & Erwin, 2008). JA and MeJA are involved in signal transduction via the jasmonate signaling pathway. However, these compounds interact with other elicitors such as ethylene, salicylic acid and nitric oxide (Zhao *et al*., 2005). A phosphorylation cascade is triggered when plants are infected with pathogens, wounded, or attacked by insects, thereby leading to JA biosynthesis (Carvalhais *et al*., 2013). The JA signal is detected by receptors and JA-responsive genes become activated and expressed, thereby producing physical and chemical barriers against pathogens or herbivores, but this reaction leads to growth reduction (Walters & Heil, 2007; Kazan & Manners, 2008) and down-regulation of primary metabolism (Hermsmeier *et al*., 2001). At high volatility, MeJA is free to move from the plant stomata to the inner part of the cytoplasm and becomes hydrolyzed to form JA as catalyzed by esterase, suggesting their potential function in interplant communication (Farmer & Ryan, 1990; Karban & Baldwin, 1997; Farmer, 2001).

Many researchers have investigated induced defensive responses by treating plants with exogenous MJ and MeJA to simulate pathogen and herbivorous insect attack (Maksymiec & Krupa, 2007; Feng *et al*., 2012b; Carvalhais *et al*., 2013). Heil (2004) found that MeJA-elicited response is more generalized than the damage caused by any specific herbivore. When applied on conifers, MeJA increases chemical defense, induces the formation of traumatic resin ducts, accelerates resin flow, and stimulates continuous accumulation of terpenoids (Zeneli *et al*., 2006). MeJA stimulates defense-associated proteins when sprayed on broad-leaved plants such as tobacco, soybean, corn, grapevine, radish, sweet pepper, tomato, water spinach, and others (Wu *et al*., 2008; Anjum *et al*., 2011; Tan *et al*., 2011; Feng *et al*., 2012b). MeJA can also mediate plant responses to drought stress (Anjum *et al*., 2011), cadmium damage (Keramat *et al*., 2010), and cold injury (Jin *et al*., 2009a; Jin *et al*., 2009b).

Poplar trees (*Populus* spp.) are economically important and widely planted worldwide, and are often seriously attacked by various defoliators (Coyle *et al*., 2003; Zhang *et al*., 2005; Rashmi *et al*., 2011). This study aimed to determine if MeJA induces defensive reactions in poplar seedlings. In this study, the leaves of *Populus deltoides* 895, one of the most commonly planted poplar tree species in China, was sprayed with 10μM MeJA. The responses affecting trade-offs in poplar were also determined. Three components of the leaves were examined: (1) Essential nutrients including sugars, amino acids, and proteins, along with three secondary metabolites (tannin, flavone, and phenolic acid) were analyzed to investigate the mechanism by which MeJA induces plant resistance for the purpose of breeding resistant cultivars in the future, (2) Defense enzymes, including polyphenol oxidase (PPO), lipoxygenase (LOX), trypsin inhibitor (TI), and chymotrypsin inhibitor (CI), were determined to assess the relationship between defensive enzymes and secondary metabolites, (3) Volatile compounds from both healthy and MeJA-treated leaves were analyzed by

headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) to clarify the effect of MeJA application on poplar volatile terpenoids.

Materials and Methods

Plant materials: *P. deltoids* 895 seedlings (1 year old) were obtained from Jurong Forest Farm in Zhenjiang City (31°57′N, 119°10′E), Jiangsu Province, P. R. China in March 2012. The seedlings were cut into smaller stems with sizes ranging from 20cm to 30cm and then soaked in water for 2 to 3 d. The stems were planted individually in plastic pots (28cm in diameter, 40cm height) with orchard soil and grown under natural light. The cutting seedlings were watered every day and weeded when necessary.

MeJA treatments and material collection: MeJA solution (10μM) was freshly prepared using distilled water. Once the cutting seedlings reached a height of 50cm, 100mL of MeJA solution was successively sprayed twice on the leaves, with the second spray made just after the initial spray was totally absorbed. The same volume of distilled water was applied on the control leaves. The leaves on a MeJA-treated branch and those on a contralateral non-MeJA-treated branch of the same plant were collected and analyzed in the laboratory.

Chemical analyses: The leaves were detached from the branches after being treated with MeJA or distilled water for 4, 12, 24, 48, and 72 h. The leaves were then cleaned with distilled water and naturally air dried. Afterward, the leaves were dried in an oven for 12h at 50°C. The dried leaves were ground using a pulverizer. The leaf powders leaves were then passed through a 60-mesh sieve. The screened powders were collected in a conical flask, sealed with parafilm, and set aside in a shaded area. The same procedure was performed on leaves from the control group. Three important nutrients (sugars, amino acids, and proteins) and three kinds of secondary metabolites (tannin, flavone, and phenolic acid) were quantified.

Sugar: To construct a standard curve to assess total sugar, the anthrone colorimetric method was used following Edwards *et al*. (2011). Pure analytical glucose was dried at 80°C until a constant weight was obtained. Afterward, 1g of dried pure glucose was transferred to a 100mL volumetric flask and dissolved in distilled water. Concentrated sulfuric acid (0.5mL) and water were added to form a volume of 100 mL. Eleven graduated test tubes were successively numbered 0 to 10, and the added reagents are listed in Table 1. After the contents were fully mixed, the tube was incubated in boiling water for 1 min and then cooled to room temperature. The optical density (OD) value at a wavelength of 630 nm was measured using a blank sample as contrast. Based on the obtained results, a standard curve for the total sugar was plotted, in which the sugar contents were placed on the horizontal axis and OD_{630} values were placed on the vertical axis. Afterward, 0.1 g of poplar foliar powder was placed in a test tube. Distilled water (10 mL) was added, sealed with plastic film, and bathed in boiling water twice (each for 30 min). The solution was filtered and placed in a 25 mL volumetric flask; the tube and the residue were rinsed three times. Distilled water was then added to obtain a constant volume, thereby producing the extracting solution. The extracting solution (0.5 mL) was transferred to 20 mL graduated test tubes, and 1.5 mL of distilled water was added. The following procedure was the same as that of drawing the standard curve.

Free amino acid: The ninhydrin method (Chutipongtanate, 2012) was used to draw a standard curve for the assessment of free amino acids. The reagents listed in Table 2 were added to six 20mL test tubes and mixed. The tube was shaken well, sealed with plastic film, and bathed in boiling water for 15 min. Then the tube was removed from the water bath and cooled rapidly in cold water with frequent agitation to facilitate oxidation, in which the color of the mixture changed from red to blue. Ethanol (60%) was added to a final volume of 20mL. OD value at a wavelength of 570nm was measured and a standard curve for the total free amino acid was illustrated based on the obtained result. Poplar foliar powder (0.5 g) was weighed and placed in a 100 mL volumetric flask. Acetic acid (5 mL of 50% solution) was added and mixed with distilled water to obtain a constant volume. The filtrate (1 mL) was transferred to a drying tube (20 mL) with 1.0 mL of ammonia-free distilled water. The remaining procedure was the same as that of drawing the standard curve. The free amino acid was estimated by OD_{570} values positioned on the standard curve.

Table 1. Reagents used to establish a standard curve to assess total sugar.

Tube number	0	1, 2	3, 4	5, 6	7, 8	9, 10
100 μg·L^{-1} glucose (mL)	0	0.2	0.4	0.6	0.8	1
Distilled water (mL)	2	1.8	1.6	1.4	1.2	1
Anthrone ethyl acetate (mL)	0.5	0.5	0.5	0.5	0.5	0.5
Concentrated sulfuric acid (mL)	5	5	5	5	5	5
Total glucose (μg)	0	20	40	60	80	100

Table 2. Reagents used to establish a standard curve for free amino acid assessment.

Tube number	1	2	3	4	5	6
Standard amino acid solution (mL)	0	0.2	0.4	0.6	0.8	1
Distilled water without ammonia (mL)	2	1.8	1.6	1.4	1.2	1
Ninhydrin (mL)	3	3	3	3	3	3
Ascorbic acid (mL)	0.1	0.1	0.1	0.1	0.1	0.1
Nitrogen content of each tube (μg)	0	1	2	3	4	5

Table 3. Reagents used to establish a standard curve for protein assessment.

Tube number	1	2	3	4	5	6	7
BSA standard (mL)	0	0.2	0.4	0.6	0.8	1	
Samples (mL)	-	-	-	-	-	-	0.1
Distilled Water (mL)	3	3	3	3	3	3	0.9
Coomassie brilliant blue G250 (mL)	0.1	0.1	0.1	0.1	0.1	0.1	
Concentration ($\mu g \cdot mL^{-1}$)	0	20	40	60	80	100	

Table 4. Reagents used to establish a standard curve for tannin assessment.

Tube number	0	1	2	3	4
Tannic acid solution (mL)	0	0.5	1	1.5	2
Methanol (mL)	2	1.5	1	0.5	0
4% vanillin methanol solution (mL)	2	2	2	2	2
Concentrated hydrochloric acid (mL)	1	1	1	1	1
Standard content (mg)	0	0.5	1	1.5	2

Protein: The method of Coomassie brilliant blue G250 was performed according to Liang & Li (2011) to draw a protein standard curve. The reagents were prepared in advance. BSA standard solution had a concentration of $100\mu g \cdot mL^{-1}$. Coomassie brilliant blue G250 was prepared using ethanol and *o*-phosphoric acid. To draw a standard curve, the reagents listed in Table 3 were combined and mixed well for 2 min. OD_{595} was then obtained to draw a standard curve. Afterward, 0.04g of foliar powder was grounded in a mortar and a homogenate was made by adding distilled water. Supernatant (0.1 mL) and Coomassie brilliant blue G250 (5 mL) were used to determine OD_{595}. The protein was estimated at OD_{595} positioned on the standard curve.

Tannin: The vanillin colorimetric method of Karamac & Pegg (2009) was used to construct a standard curve for tannin. The standard solution was initially prepared by dissolving 100mg of tannic acid in 100mL of methanol. The reagents (Table 4) were added to five test tubes (10mL) numbered 0 to 4. The samples were incubated in a water bath at 30°C for 20min, cooled, and tested for OD_{510}. A standard curve for tannin was plotted with OD_{510} on the horizontal axis against the quality in mg on the vertical axis. Foliar powder (0.5 g) was dissolved in methanol (50mL) and centrifuged. Afterward, 2.0 mL of supernatant was used to determine OD_{510} according as above.

Flavone: An ethanol extraction method was used to plot a standard curve by preparing rutin standard solutions (0.01 $mg \cdot mL^{-1}$; 0, 1, 3, 5, 10, and 15 mL) and measuring OD_{420}. Foliar powder (0.5 g) was immersed 70% ethanol (50 mL) overnight and bathed in boiling water for 10 min the next day. The resulting mixture was cooled, filtered, and extracted by adding distilled water to reach a final volume of 50 mL. Then 1 mL of extracting solution was transferred to a test tube containing 1% $AlCl_3$ (9 mL). The mixture was allowed to stand for 10 min before OD_{420} was determined.

Phenolic acid: The ferric trichloride-potassium ferricyanide colorimetric method was used to determine the amount of phenolic acid. Protocatechuic acid (2 mg) was weighed, dissolved in absolute ethanol, and diluted to obtain a final volume of 50 mL. Different volumes (1, 2, 3, 4, and 5 mL) of 20.8 $\mu g \cdot mL^{-1}$ of protocatechuic acid solution were prepared. Absolute ethanol (5 mL), sodium dodecyl sulfate (SDS; 2mL of 0.3%), 0.06% ferric trichloride -0.9% potassium ferricyanide (1 mL) were added to each solution. The solution was placed in the dark for 5 min. Hydrochloric acid ($0.1 mol \cdot L^{-1}$) was added and the resulting mixture was maintained in the dark for 20 min. OD_{720} was examined and the standard curve was plotted in which the concentration was placed on the horizontal axis against OD_{720} on the vertical axis. Afterward, 30mL of absolute ethanol was added to 0.1g of foliar powder and placed in an ultrasonic bath for 30min. The mixture was transferred to a 50mL volumetric flask and diluted with absolute ethanol. The second filtrate (1 mL) was collected to determine OD_{720}.

Enzyme activity: The leaves were detached from the branches after MeJA or distilled water was applied for 4, 12, 24, 48, and 72 h. These leaves were placed in an ice cooler immediately after they were separated from the branches, and they were stored at –20°C in the laborator before further analyses. Four enzymes (PPO, LOX, TI, and CI) were analyzed.

PPO: The method described by Bogdanovic *et al*. (2007) was modified and used to measure PPO. Poplar leaves (0.25 g), polyvinylpyrrolidone (PVP; 0.3g), and quartz sand (0.2g) were ground and added to pre-cooled citric acid buffer (pH 5.6) to produce homogenate and then placed in a refrigerator at 4°C for 12 h of extraction. After filtration and centrifugation at 4000 rpm for 15min, the supernatant was used as a substrate. The substrate (1 mL) and a mixed solution (3mL) containing 0.1 M citric acid buffer (pH 5.6)/0.1% proline/1% pyrocatechol (10:2:3) were incubated at 37°C in a water bath for 10min. Afterward, 3mL of 1 N metaphosphoric acid was added. OD_{460} was examined with a blank contrast by using pyrocatechol for substitution.

LOX: Linoleic acid (0.2 mL) was added dropwise to a mixture of 0.2mL of Tween and 20.4mL of borate buffer (pH 9.0). Then 0.6mL of 1 N NaOH and 36 mL of borate buffer were mixed. Distilled water was added to the system to obtain a final volume of 80 mL and adjusted to pH 6.5 using hydrochloric acid, which was considered as a substrate. Preserved leaves (0.25g) at –20°C were placed in 1.5mL of phosphate buffer (pH 8.04), ground with liquid nitrogen, and centrifuged at 4000 rpm for 20 min. The supernatant was used for LOX activity by modifying the method described by Surrey (1964). In brief, 1 mL of the substrate and 0.5mL of the supernatant were mixed, shaken, and maintained in flowing oxygen for 10 min. Absolute ethanol (0.4 mL) was added to the mixture (0.2 mL) and diluted with 60% ethanol to obtain a final volume of 10 mL. The final mixture was used to determine OD_{234}.

TI: Tris (0.3025 g) was dissolved in distilled water to obtain a final solution of 50mL. HCl (0.225mL) was also diluted to obtain a final volume of 50 mL. Afterward, 50mL of Tris solution, 34.5 mL of HCl solution, and 0.222g of $CaCl_2$ were mixed and distilled water was added to obtain a total volume of 100 mL. This mixture was considered as the extraction solution. Tripsin (0.8 g) was dissolved in 0.05 M Tris-HCl buffer (pH 7.8) to a final volume of 100 mL. BAEE (0.03428 g) was dissolved and the same buffer was added to obtain a final volume of 100 mL. Poplar leaves (1 g) were ground to a powder in liquid nitrogen with quartz sand. The extraction solution (6 mL) was added and ground in an ice bath. After the resulting mixture was centrifuged at 12,000rpm at 4°C for 10min, the supernatant was considered as TI crude extract and stored at 4°C. Then 100μL of tripsin solution and 20μL of inhibitor extract were stored in a water bath at 25°C for 60 min. BAEE (2.9 mL) was added and the solution was placed in a quartz cuvette and allowed to stand for 1 min. OD_{256} was determined using an ultraviolet spectrophotometer. The value was recorded five times at an interval of 60s. Tripsin inhibitory capacity (TIC) was calculated as follows:

$$TIC = \frac{E_{256}}{0.01 \times V \times Ew}$$

where E_{256} is the variation value of OD_{256} in every minute; 0.01 is the variation value of OD_{256} in every minute; V is the total volume of the system solution (mL); and Ew is the enzyme content of the tripsin solution.

CI: The extraction solution was prepared by dissolving 7 g of PVP, 0.0256g of phenylthiourea, 0.222g of $CaCl_2$, and 0.0072g of Vc. Tris-HCl buffer (0.05 M, pH 8.0) was added to obtain a final volume of 100 mL. Chymotrypsin (0.01851g) was dissolved in the same buffer to obtain a final volume of 100 mL. Phosphate buffer (pH 8.0) was prepared. The buffer was mixed with 40% methanol at a ratio of 1:1, to prepare a substrate solvent. The substrate was prepared by dissolving 0.01617g of BTEE in 100 mL of the substrate solvent. Poplar leaves (0.5 g) were ground in liquid nitrogen, added to 6mL of extraction solution, and ground in an ice bath. After the mixture was centrifuged at 12,000rpm at 4°C for 10 min, the supernatant was taken as the CI crude extract. Chymotrypsin solution (50μL) and 80μL of CI crude extract were mixed and incubated in a water bath at 25°C for 20min. The mixture (100μL) and the BTEE substrate (2.9mL) were transferred to a quartz cuvette and left standing for 1min. OD_{253} was determined using an ultraviolet spectrophotometer. OD_{253} was recorded five times at an interval of 60s. Chymotrypsin inhibitory capacity (CIC) was calculated as follows:

$$CIC = \frac{E_{253}}{0.964 \times V \times Ew}$$

where E_{253} is the variation in OD_{253} in every minute; 0.964 is the absorbance of BTEE; V is the total volume of the system solution (mL); and Ew is the enzyme content of the chymotripsin solution.

Analysis of leaf volatiles: The leaves were removed from seedling branches 36 h after MeJA or distilled water treatment. The leaves were placed in an ice cooler immediately after they were separated from the branches. HS-SPME combined with GC-MS was performed for analysis according to previously described methods (Feng *et al*., 2012a; Lin *et al*., 2012; Papotti *et al*., 2012; Wang *et al*., 2013).

SPME was performed using a fused silica fiber coated with polydimethylsiloxane-divinylbenzene with a thickness of 100 μm (Supelco Co., Bellefonte, PA, USA). The fiber was activated before sampling by introducing to a GC-heated injector port at 250°C for 15 min. Powdered fresh *P. deltoides* leaves (2 g) were placed in a 20mL screw-cap vial hermetically sealed with PTFE/silicon septum (Supelco, Bellefonte, PA, USA). The sample was kept in a water bath at a constant temperature of 50°C for 20min. The SPME fiber was inserted into the vial and exposed to the headspace during extraction for 25min.

The headspace poplar volatile compounds were analyzed by a Finnigan trace DSQ GC-MS spectrometer equipped with a DB-5MS (30 m × 0.25 mm i.d., 0.25μm film thickness) capillary column (Thermo Electron Co., USA). After sampling, the SPME fiber was immediately withdrawn from the vial and inserted into the GC injector (250°C) where the volatiles were thermally desorbed in splitless mode. Helium was used as a carrier gas at a constant column flow of 0.9mL/min. Injector and detector temperatures were set at 250°C. The oven temperature was programmed from 50°C for 2min, increased to 250°C at a rate of 5°C/min, and held for 5 min. Transfer line and ion source temperatures were set at 250°C. Electron impact mass spectra were recorded at an ionization voltage of 70 eV and in scan mode (30-350amu). Individual volatile compounds were identified by comparing retention times and mass spectra with those of authentic standards, the literature, and the NIST02 standard mass spectral database. The relative contents were determined by area normalization.

Statistical analysis: Data were generated and analyzed three replicates. Statistical tests were performed in SPSS 16.0 for Windows (SPSS Inc., Chicago, USA). Graphical presentation was prepared in Microsoft Excel 2007. The one-sample *t*-test was used to examine the treatment means of three replications. One-way ANOVA was performed for treatments effect. Significant differences among means were detected by Duncan's test ($p<0.05$).

Results

Chemical changes induced by MeJA treatment

Sugar: Sugar content changed with time (Fig. 1a). Initially, there were no significant differences among the treatments and control. At 4 h after MeJA treatment, the sugar content of both treatments and the control increased by approximately 2% but were not significantly different from the initial level. After 4 h, the sugar content of the MeJA-treated and contralateral non-MeJA-treated leaves started to decrease and at 24, 48, and 72 h, the sugar contents of MeJA-treated group were significantly different from that of the control ($F = 4.908$, $P = 0.008$; $F = 8.477$, $P = 0.001$; $F = 4.244$, $P = 0.013$; respectively). The MeJA-treated leaves decreased by 15.2%, 18.9%, 16.5%, and 21.9% at 12, 24, 48, and 72 h, respectively, compared with the control group. The sugar contents of the contralateral non-MeJA-treated leaves decreased by 11.5%, 20.4%, 11.4%, and 18.4%, at 12, 24, 48, and 72 h, respectively. Sugar from contralateral non-MeJA-treated leaves showed slightly less change than the leaves treated directly with MeJA.

Free amino acid: As shown in Fig. 1(b), the amino acid content of leaves treated with MeJA increased by 17.4%, 25.7%, 22.1%, and 3.8%, at 4, 12, 24, and 48 h after treatment, respectively, compared to the control group. A significant difference was observed at 4, 12, and 24 h ($F = 5.518$, $P = 0.005$; $F = 3.519$, $P = 0.024$; $F = 3.264$, $P = 0.031$; respectively). A slight increase was detected at 48 h but was not statistically significant ($F = 0.5$; $P = 0.643$). At 72 h, the amino acid content had decreased by 4.4%, not significantly less than that of the control group ($F = 1.559$; $P = 0.194$). In general, the amino acid content of contralateral non-MeJA-treated leaves showed a similar trend to that of MeJA-treated leaves. However, the observed changes were less than those of the non-MeJA-treated leaves, in which the amino acid contents increased by 14.1, 3.9, 2.7, and 2.9% at 4, 12, 24, and 48 h, respectively. A decrease of 10.1% at 72 h was observed. Thus, the amino acid content increased in the first 48 h and then decreased thereafter. Given that amino acids participate in several metabolic pathways, variations in their abundance may influence growth and resistance.

Protein: At 4 h after MeJA treatment, the protein contents of MeJA-treated leaves and contralateral non-MeJA-treated leaves decreased by 13.7 and 18.5%, respectively, compared with control (Fig. 1c). After another 4 h, the protein contents of MeJA-treated leaves increased by 12.5, 15.5, 8.8, and 19.8% compared to control. The observed differences were significant at 24 and 72 h ($F = 2.909$, $P = 0.044$; $F = 3.487$, $P = 0.025$; respectively). The protein content of the contralateral non-MeJA-treated leaves also increased beginning at 12 h but to a lesser extent than the MeJA-treated leaves. The differences were not significant ($F = 0.614$; $P = 0.573$).

Tannin: At 4, 12, 24, 48, and 72 h following MeJA application, the tannin content of the MeJA-treated leaves increased by 21.3, 1.8, 7.2, 0.4, and 2.0%, respectively, compared to control (Fig. 1d). The differences was significant at 4 h ($F = 3.661$, $P = 0.022$). Tannin from the contralateral non-MeJA-treated leaves increased by 15.2%, 1.6%, 8.7%, and 4.9% at 4, 12, 24, and 72 h, respectively, compared to control. By contrast, tannin decreased by 1.7% at 48 h.

Flavone: Figure 1 (e) shows the influence of exogenous MeJA on flavone content. The flavone content of MeJA-treated leaves increased following MeJA application by 17.8, 17.3, 14.7, 11.6, and 7.9% compared to control at 4, 12, 24, 48, and 72 h, respectively. The differences were significant at 12, 24, and 48 h ($F = 14.934$, $p<0.001$; $F = 10.599$, $p<0.001$; $F = 3.063$, $P = 0.038$; respectively). The flavone contents of the contralateral non-MeJA-treated leaves increased by 15.9, 9.5, and 7.7% at 4, 12, and 48 h, respectively, although a significant difference was detected only at 12 h ($F = 9.762$, $P = 0.001$). At 48 h and 72 h, the flavone content decreased by 4.0% and 8.0%, respectively, compared to the control.

Phenolic acid: The phenolic acid content of MeJA-treated leaves showed an increasing trend after application of MeJA (Fig. 1f). In particular, the phenolic acid contents increased by 2.0, 4.9, 40.9, and 35.1% at 4, 12, 24, and 48 h, respectively. The peak phenolic acid content was 10.82 $mg \cdot g^{-1}$. Significant differences were observed at 24 and 48 h ($F = 24.346$, $p<0.001$; $F = 12.448$, $p<0.001$; respectively). The content decreased by 0.5% at 72 h with a slightly significant difference. The phenolic acid content of contralateral non-MeJA-treated leaves increased by 4.8, 2.9, 11.3, 13.2, and 5.2% at 4, 12, 24, 48, and 72 h, respectively.

Variation in enzymatic activities after MeJA treatment

PPO: Elevated levels of PPO activity were observed from 4 h to 48 h, but then PPO activity declined starting at 72 h (Fig. 2a). PPO activities of MeJA-treated leaves were 31.4, 31.1, 6.7, and 1.7% higher than the untreated control at 4, 12, 24, and 48 h, respectively. Significant differences were observed at 12 and 24 h ($F = 6.839$, $P = 0.002$; $F = 4.487$, $P = 0.011$; respectively). The PPO activities of the contralateral non-MeJA-treated leaves were higher at 4 and 12 h (by 23.4 and 15.1%, respectively) and significance was observed at 12h ($F = 3.529$, $P = 0.024$) than those of the untreated control group. By contrast, the PPO activities of contralateral non-MeJA-treated leaves began to decrease after 24 h and reached the lowest level at 72 h.

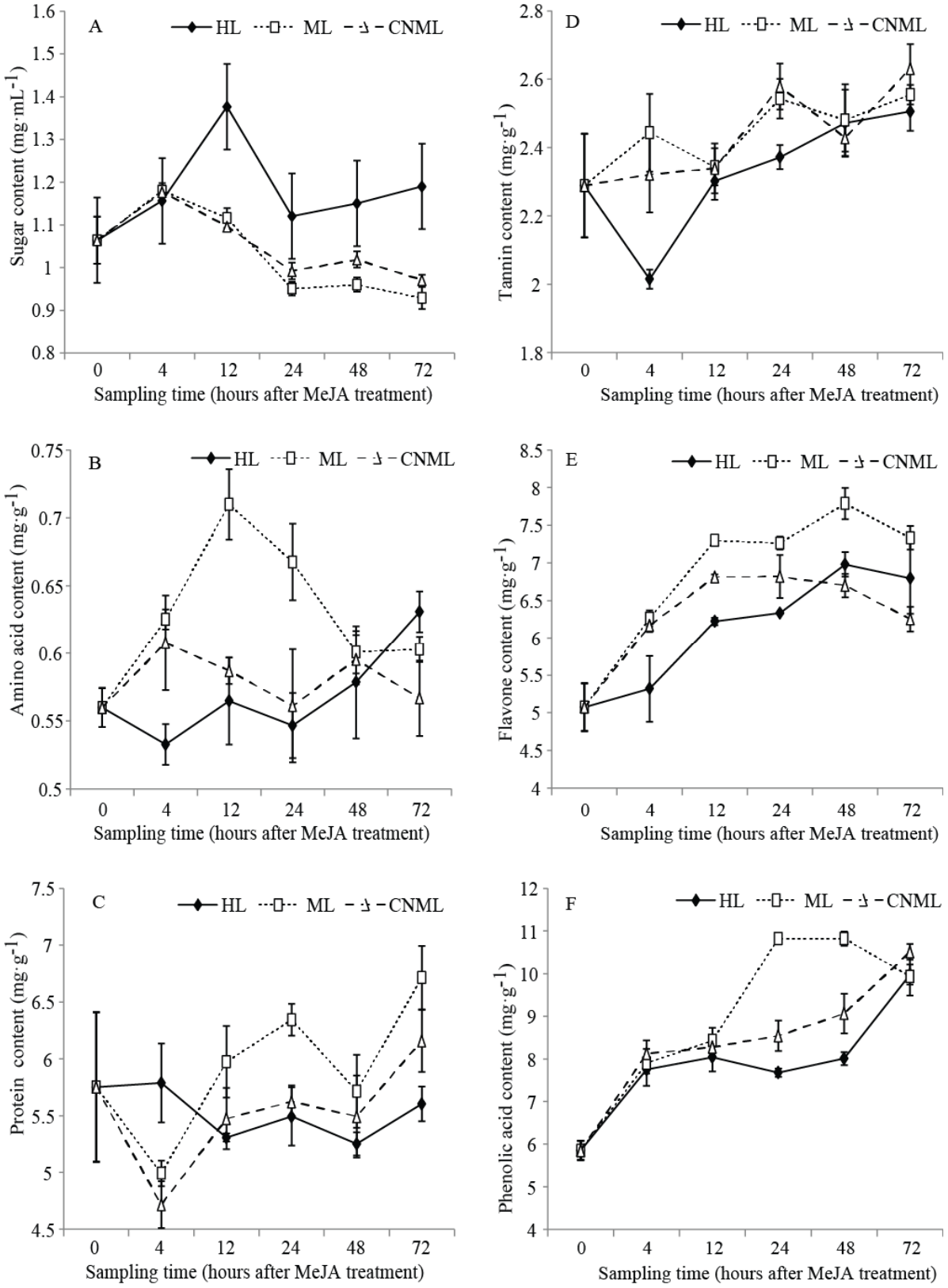

Fig. 1. Effect of MeJA application on six biochemical substances: three major nutrients, (a) sugars, (b) amino acids, and (C) proteins; and three secondary metabolites, (d) tannin, (e) flavone, and (f) phenolic acid. Error bars represent average SE of three replications. HL represents the leaves from healthy plants and considered the control treatment. ML represents the leaves from MeJA-treated branch. CNML represents the leaves from the contralateral non-MeJA-treated branch.

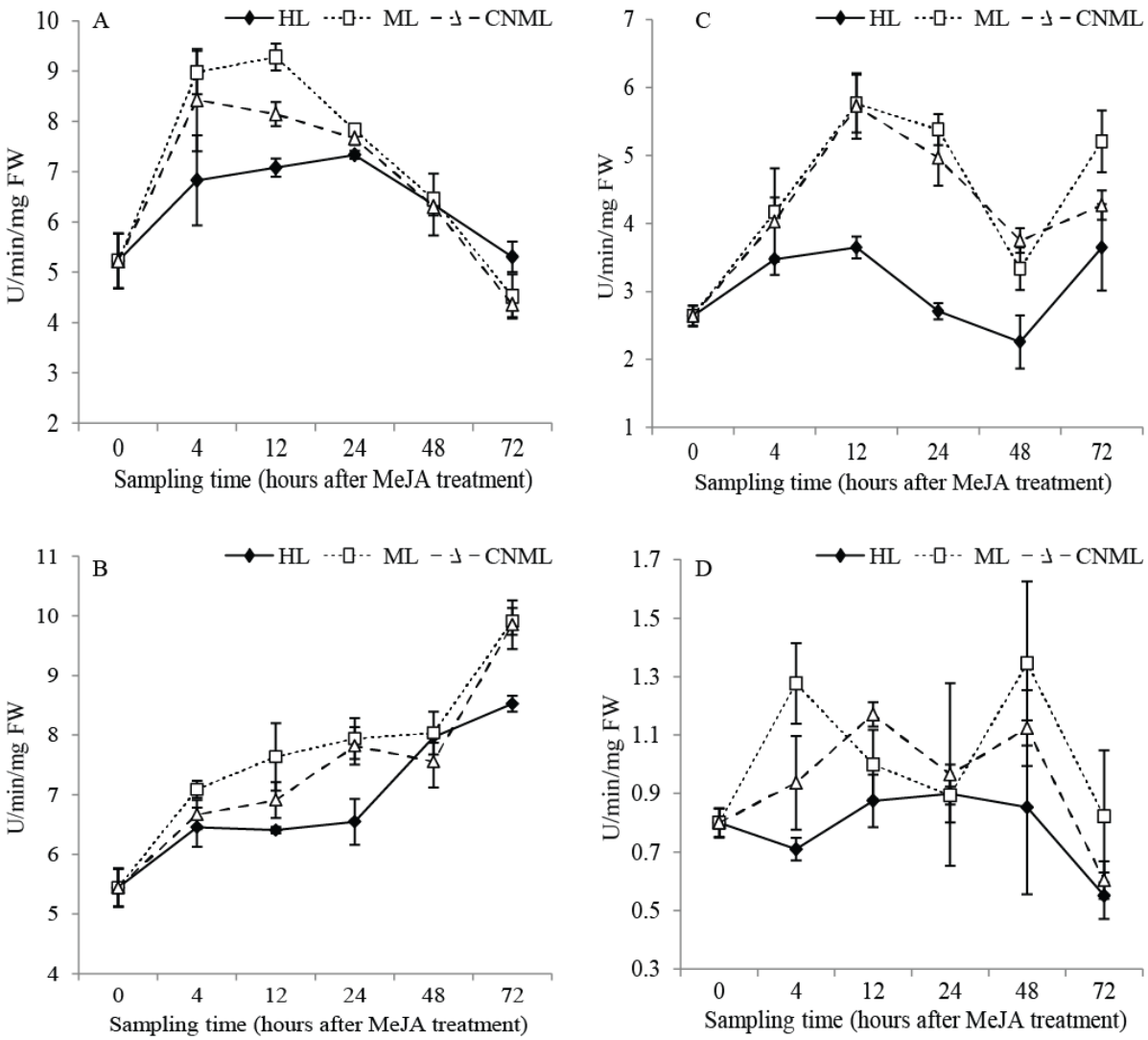

Fig. 2. Effect of MeJA application on four defense enzymes: (a) PPO, (b) LOX, (c) TI, and (d) CI. Error bars represent SE of means (n=3). HL represents the leaves from a healthy plant and used as the control group. ML represents the leaves from MeJA-treated branch. CNML represents the leaves from the contralateral non-MeJA-treated branch.

LOX: After MeJA was applied, LOX activity increased by 9.8, 19.2, 21.3, 0.9, and 16.2% compared to the untreated control group at 4, 12, 24, 48, and 72 h, respectively (Fig. 2b). Significant differences were only observed at 72 h ($F = 5.259$, $P = 0.006$). In the contralateral non-MeJA-treated leaves, LOX activities increased by 3.3, 7.9, 19.5, and 15.5% at 4, 12, 24, and 72 h, respectively, compared with the untreated control group. Significant differences were noted at 72 h ($F = 3.096$, $P = 0.036$). A surprise decrease of 5.1% occurred at 48 h.

TI: In response to exogenous MeJA, TI activity was consistently higher than that of control (Fig. 2c). Both MeJA-treated and contralateral non-MeJA-treated leaves exhibited elevated TI activities. For the MeJA-treated leaves, TI activities increased by 20, 58.1, 98.7, 47.7, and 42.9% above the control group at 4, 12, 24, 48, and 72 h, respectively. Significant differences were observed at 4, 12, and 24 h ($F = 3.162$, $P = 0.034$; $F = 4.651$, $P = 0.010$; $F = 10.383$, $p<0.001$; respectively). For the contralateral non-MeJA-treated leaves, TI activities increased by 16, 57.1, 83.3, 66.2, and 17.1% above the control group at the same observation times. Significant differences were observed at 12, 24, and 48 h ($F = 4.111$, $P = 0.015$; $F = 5.290$, $P = 0.006$; $F = 3.465$, $P = 0.026$; respectively).

CI: The CI activity was observed a significant difference at 4 h for the MeJA-treated leaves ($F = 3.959$, $P = 0.017$), and at 12 h for the contralateral non-MeJA-treates leaves ($F = 2.967$, $P = 0.041$), 79.8 and 32.0% above the control group, respectively (Fig. 2d).

Influence of MeJA application on volatile compounds: Thirty one volatile compounds were identified from healthy and MeJA-treated poplar leaves (Figs. 3 and 4). The volatiles were compared before and after MeJA application, indicating that volatile composition was affected by the treatment (Table 5).

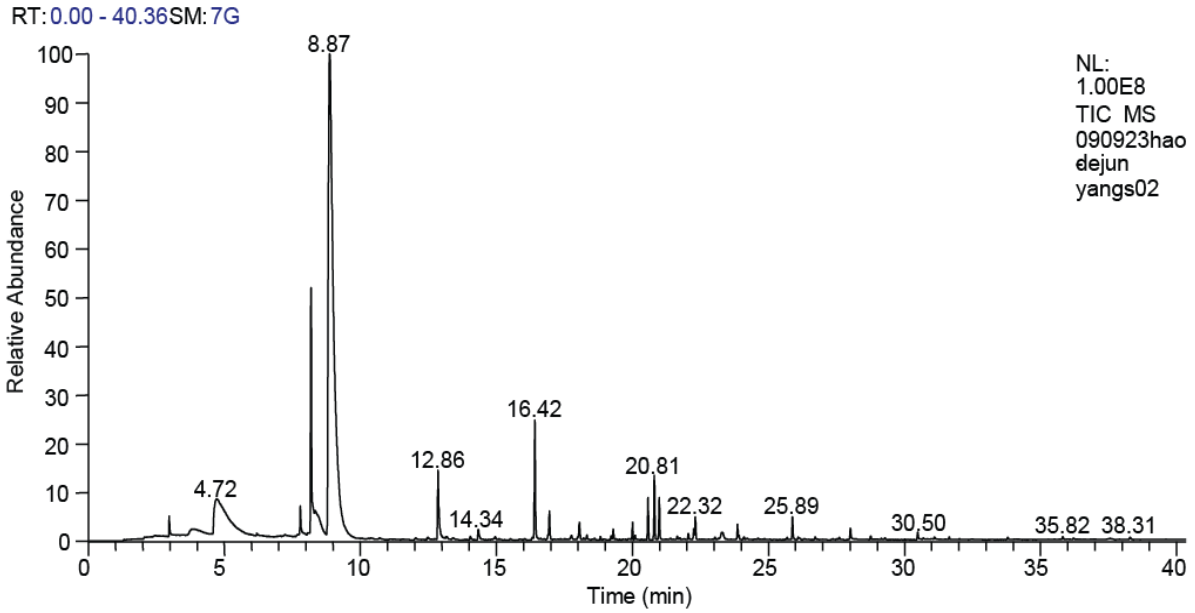

Fig. 3. Total ion chromatogram of volatiles from healthy poplar leaves.

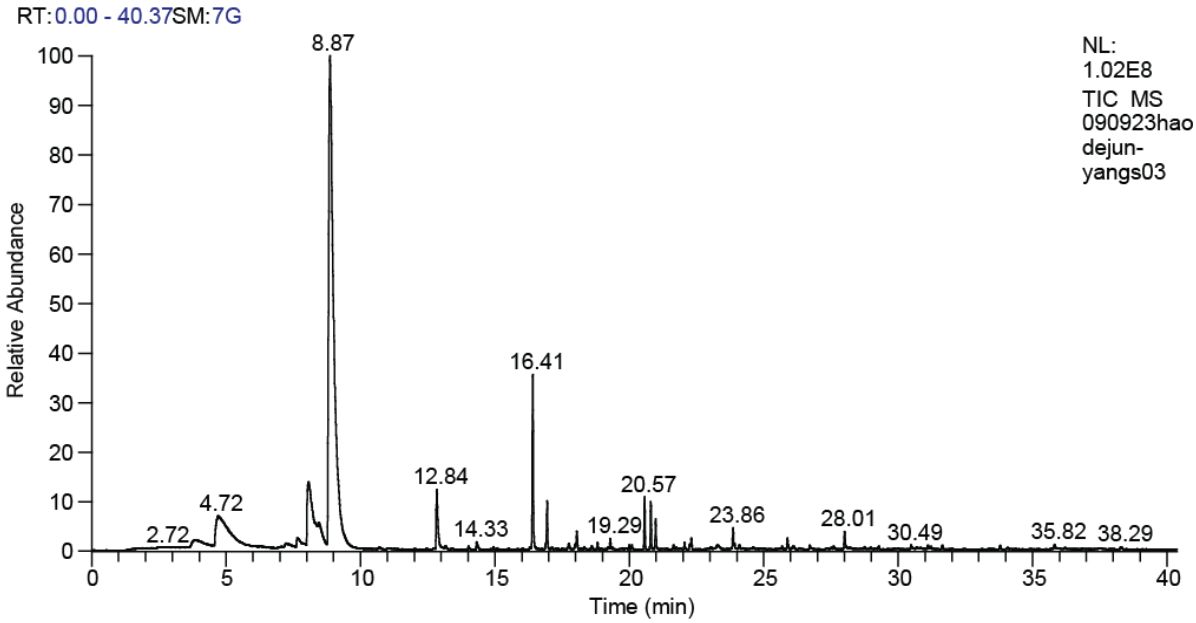

Fig. 4. Total ion chromatogram of volatiles from MeJA-treated poplar leaves.

Sixteen compounds were found in fresh, healthy *P. deltoides* 895 leaves, including three alcohols, five alkanes, four aldehydes, two ketones, two phenols, three alkenes, one ester, and one acid. Fourteen volatiles, including one alcohol, three alkanes, three aldehydes, one ketone, two phenols, two alkenes, one ester, and one acid were found in MeJA treated leaf tissue. After MeJA was applied, several new volatiles were produced and identified, and the relative quantities of the shared compounds also changed. Four compounds were newly produced after MeJA application, namely, geraniol, pentadecane, propyl-cyclopentane and hexanal. Six compounds disappeared in response to MeJA: phytol, 3,7-dimethyl-2,6-octadien-1-ol, nonadecane, (*E*)-2-heptenal, β-ionone, and caryophyllene. After exogenous MeJA application, 10 compounds were preserved, although changes in their percentage compositions were noted before and after treatment. The compounds were hexadecane, 2-hydroxy-benzaldehyde, 1,2-benzenediol, eugenol, decahydro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene, and *n*-hexadecanoic acid. These compounds increased by 16.67, 7.50, 8.08, 46.69, 7.78, and 59.62%, respectively. The other four compounds, namely, 2-hexenal, 2-cyclohexen-1-one, α-farnesene, and isopropyl myristate decreased by 42.22, 29.92, 75.38, and 41.49%, respectively.

Table 5. Comparison of foliar volatile compounds between healthy and MeJA-treated poplar leaves.

No.	Compounds	Molecular weight	Formula	Relative contents/%		Variation
				H	M	
1.	hexanal	100	$C_6H_{12}O$	-	1.65	-
2.	2-hexenal	98	$C_6H_{10}O$	10.28	5.94	-42.22%
3.	propyl-cyclopentane	112	C_8H_{16}	-	0.81	-
4.	(E)-2-heptenal	112	$C_7H_{12}O$	1.18	-	-
5.	2-cyclohexen-1-one	96	C_6H_8O	9.86	6.91	-29.92%
6.	2-hydroxy-benzaldehyde	122	$C_7H_6O_2$	54.29	58.36	7.50%
7.	1,2-benzenediol	110	$C_6H_6O_2$	3.59	3.88	8.08%
8.	geraniol	154	$C_{10}H_{18}O$	-	0.51	-
9.	3, 7-dimethyl-2, 6-octadien-1-ol	154	$C_{10}H_{18}O$	0.6	-	-
10.	eugenol	164	$C_{10}H_{12}O_2$	5.44	7.98	46.69%
11.	decahydro-4, 8, 8-trimethyl-9-methylene-1, 4-methanoazulene	204	$C_{15}H_{24}$	0.9	0.97	7.78%
12.	caryophyllene	204	$C_{15}H_{24}$	0.23	-	-
13.	β-ionone	192	$C_{13}H_{20}O$	0.4	-	-
14.	α-farnesene	204	$C_{15}H_{24}$	0.65	0.16	-75.38%
15.	pentadecane	212	$C_{15}H_{32}$	-	0.19	-
16.	hexadecane	226	$C_{16}H_{34}$	0.24	0.28	16.67%
17.	nonadecane	268	$C_{19}H_{40}$	0.24	-	-
18.	isopropyl myristate	270	$C_{17}H_{34}O_2$	0.94	0.55	-41.49%
19.	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	0.52	0.83	59.62%
20.	phytol	296	$C_{20}H_{40}O$	0.37	-	-

H represents volatiles released from healthy (untreated) poplar leaves; M represents volatiles released from MeJA-treated poplar leaves

Discussion

In this study, of *P. deltoides* 895 seedlings, especially the changes in biochemicals and production of defensive enzymes as well as volatiles, caused by directly spraying exogenous MeJA on the leaves were studied in vivo. Earlier studies (Walters & Heil, 2007; Gould *et al*., 2008; Feng *et al*., 2012b) have shown that plant defense is induced at the expense of growth and development, thereby resulting in resource reallocation.

After the leaves were treated with MeJA for 4, 12, 24, 48, and 72 h, the levels of the nutrients and secondary metabolites, changed both on the leaves where MeJA was directly sprayed and on contralateral non-MeJA-treated leaves. Such similar systematic alteration indicated that MeJA may function as a signal molecule, inducing systemic resistance in the whole plant.

Sugar is an essential nutrient that provides energy for plants. In this study, the amount of soluble sugar decreased 4 h after the MeJA treatment, and it was significantly lower than that of the untreated control group 12 h after the treatment. The decrease in sugar content may have resulted from defensive responses induced by exogenous MeJA, in which sugar is transformed to secondary metabolites such as phenols, ketones, and others. Similar variations in sugar content were reported from larch damaged by larch case-bearers (Xue & Xie, 2000) and rice cultivars under herbivore stress (Chen *et al*., 2006). However, the concentrations of soluble sugars in *Pinus pinaster* stems are not affected by MeJA (Sampedro *et al*., 2011). By contrast, MeJA can increase sugar content when applied to harvested fruits (Gonzalez-Aguilar *et al*., 2004; Meng *et al*., 2012). Thus, application of MeJA in *P. deltoides* could induce a similar defensive response against mechanical damage and herbivore stress in the inner part of the plant at the expense of consuming its own nutrients.

Free amino acids, another vital nutrient of plants, are involved in protein and fatty acid syntheses as well as energy metabolism. This study showed that the amount of amino acid initially increased and then decreased after MeJA treatment, suggesting MeJA application induced systematic resistance. MeJA-treated leaves exhibited a higher amino acid content than the contralateral non-MeJA-treated leaves, considering that time is needed for signal transduction. Given that plants have essential and non-essential amino acids, different constituents and contents stimulate diverse metabolic reactions (Wang *et al*., 2005). Therefore, the total amino acid content does not completely reflect resistance. However, the variation in amino acid content could affect the nutritive proportion, causing metabolic stress for herbivores. This variation could be considered as a factor affecting herbivore resistance. In addition, amino acids are related to secondary metabolite synthesis to some extent. For example, the synthesis of alkaloids, a group of substances that are toxic to insect pests, is partially associated with lysine and histidine (Luo & Mu, 1997).

In response to MeJA, the protein content decreased relative to the control group within 4 h after the MeJA treatment, but was higher than that of the control group thereafter. The decrease in protein content was an immediate stress reaction to MeJA, but numerous defensive proteins were subsequently produced, including some pathogenesis-related proteins that can improve plant resistance and activator proteins that can promote growth (Qiu, 2004).

The amount of secondary metabolites, such as tannin, flavone, and phenolic acid increased at different degrees as a response to MeJA treatment. The concentrations of condensed tannins and total polyphenolics of *P. pinaster* increase after MeJA treatment (Sampedro *et al*., 2011). In *Vitis vinifera*, exogenous MeJA increased the contents of flavonoid and phenolic compounds (Ruiz-Garcia *et al*.,

2012). In *Fucus vesiculosus*, phlorotannin concentrations are increased by 1.6 times after exposure to airborne MeJA (Arnold *et al*., 2002). Alkaloids can be produced transiently after plant cell suspension cultures of *Rauvolfia canescens* and *Eschscholtzia californica* are treated with MeJA (Gundlach *et al*., 1992).

Under natural conditions, nutrients and defensive substances are in a state of dynamic metabolic balance. Different signals, concentrations, and application times can induce various defensive actions.

Four defensive enzymes, including PPO, LOX, TI, and CI were selected to determine enzymatic activities in response to exogenous MeJA. These defensive enzymes increased to a certain extent.

PPO, a copper-binding oxidative enzyme, catalyzes the conversion of monophenols and *o*-diphenols to *o*-quinones in the presence of molecular oxygen, causing browning and cross-linking in wounded tissues (Queiroz *et al*., 2008; Tran & Constabel, 2011). This enhanced PPO activity in response to MeJA is consistent with that reported in previous studies (Li *et al*., 2007a; Darras *et al*., 2011; Tan *et al*., 2011). In postharvest sugarbeet roots, PPO activity is not affected by jasmonic acid (Ferrareze *et al*., 2013). PPO activity is inhibited by MeJA application in *Agaricus bisporus* fruit bodies (Meng *et al*., 2012) and postharvest loquat fruit (Cao *et al*., 2009).

LOX, a non-heme iron-containing enzyme, catalyzes regio- and stereo-specific dioxygenation of polyunsaturated fatty acids by transforming particular regioisomers to generate a series of bioactive compounds (Pérez Gilabert & Carmona, 2002). The study showed an increasing LOX activity in response to exogenous MeJA, and is supported by other studies (Li *et al*., 2007b; Marla & Singh, 2012; Szczegielniak *et al*., 2012).

Protease inhibitors (PI), including TI and CI, are produced when plants encounter pest or pathogen attacks. PIs are of great importance in endogenous and exogenous defense systems (Habib & Fazili, 2007; Lomate & Hivrale, 2012). In this study, TI and CI activities were enhanced on both the MeJA-treated leaves and the contralateral non-MeJA-treated leaves, in which the former had a higher concentration than the latter. By comparison, TI activity was enhanced at a higher extent than CI but with fewer fluctuations, which may be attributed to different optimum MeJA concentrations of the two PIs. Given that a high PI activity indicates defensive protection (Lomate & Hivrale, 2012; Tan *et al*., 2012), the present study confirmed the function of MeJA in inducing defensive responses.

In addition to biochemical substances and defensive enzymes, volatiles of *P. deltoides* 895 seedlings were also affected by exogenous MeJA. Degenhardt & Lincoln (2006) classified plant volatiles in three groups: constitutive volatile organic compounds (VOCs), induced VOCs, and novel VOCs. The volatile compounds of healthy and MeJA-treated *P. deltoides* leaves were compared. We found four newly produced compounds, including geraniol, pentadecane, propyl-cyclopentane, and hexanal, and these compounds were classified as induced VOCs. Hexadecane, 2-hydroxy-benzaldehyde, 1, 2-benzenediol, eugenol, decahydro-4,8,8-trimethyl-9-methylene-1, 4-methanoazulene, and *n*-hexadecanoic acid were also considered as induced VOCs. The six compounds that disappeared, including phytol, 3,7-dimethyl-2, 6-octadien-1-ol, nonadecane, (*E*)-2-heptenal, β-ionone, and caryophyllene, as well as the remaining four compounds, including 2-hexenal, 2-cyclohexen-1-one, α-farnescene, and isopropyl myristate, were considered as constitutive VOCs. As a stress reaction, the variation was protective and may be involved in intra- and inter-plant communication (Tamogami *et al*., 2008; Karban *et al*., 2010; Das *et al*., 2013). In addition, the alteration resembles the defensive reaction to herbivore attacks (Engelberth *et al*., 2004; Degenhardt & Lincoln, 2006), indicating the efficiency of MeJA simulation for herbivores and the potential of MeJA in stimulating a defensive reaction to herbivores.

In conclusion, the study demonstrated the effect of exogenous MeJA on *P. deltoides* 895 seedlings. The production of sugars, free amino acids, and proteins as well as three secondary metabolites, including tannin, flavone, and phenolic acid were increased by exogenous MeJA. Four defensive enzymes, including PPO, LOX, TI, and CI were activated. The volatiles produced by the leaves were changed. Our results indicated that exogenous MeJA could cause defensive responses of *P. deltoides* 895 seedlings. Further investigations on physiological and behavioral responses of a major poplar defoliator, *Clostera anachoreta* (Lepidoptera: Notodontidae) feeding on MeJA-treated *P. deltoides* 895 leaves are in progress to characterize MeJA-induced systematic resistance and to provide a potential new MeJA-based method against herbivore attacks.

Acknowledgement

The authors are grateful to National Natural Science Foundation of China (#31170606) and Priority Academic Program Development of Jiangsu province, China (PAPD) for the financial support.

References

Agrawal, A.A. 1999. Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. *Ecol.*, 80(5): 1713-1723.

Anjum, S.A., L. Wang, M. Farooq, I. Khan and L. Xue. 2011. Methyl jasmonate-ınduced alteration in lipid peroxidation, antioxidative defence system and yield in soybean under drought. *J. Agron. Crop Sci.*, 197(4): 296-301.

Arnold, T.M., N.M. Targett, C.E. Tanner, W.I. Hatch and K.E. Ferrari. 2002. Evidence for methyl jasmonate-induced phlorotannin production in Fucus vesiculosus (Phaeophyceae). *J. Phycol.*, 37(6): 1026-1029.

Bogdanovic, J., N. Milosavic, R. Prodanovic, T. Ducic and K. Radotic. 2007. Variability of antioxidant enzyme activity and isoenzyme profile in needles of Serbian spruce (Picea omorika (Panc.) Purkinye). *Biochem. Syst. Ecol.*, 35(5): 263-273.

Cao, S.F., Y.H. Zheng, Z.F. Yang, K.T. Wang and H.J. Rui. 2009. Effect of methyl jasmonate on quality and antioxidant activity of postharvest loquat fruit. *J. Sci. Food Agric.*, 89(12): 2064-2070.

Carvalhais, L.C., P.G. Dennis, D.V. Badri, G.W. Tyson, J.M. Vivanco and P.M. Schenk. 2013. Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. *PLoS ONE*, 8(2): e56457.

Champigny, M.J. and R.K. Cameron. 2009. Action at a distance: Long-distance signals in ınduced resistance. In: *Plant Innate Immunity*. (Ed.): L.C. VanLoon, Advances in Botanical Research. Academic Press Ltd-Elsevier Science Ltd, London, pp. 123-171.

Chen, W., Q. Zhou, X. Li and G.F. He. 2006. Physiological responses of different rice cultivars under herbivore stress. *Acta Ecol. Sinica*, 26(7): 2161-2166.

Chutipongtanate, S., K. Watcharatanyatip, T. Homvises, K. Jaturongkakul and V. Thongboonkerd. 2012. Systematic comparisons of various spectrophotometric and colorimetric methods to measure concentrations of protein, peptide and amino acid: Detectable limits, linear dynamic ranges, interferences, practicality and unit costs. *Talanta*, 98: 123-129.

Coyle, D.R., J.D. McMillin, R.B. Hall and E.R. Hart. 2003. Effects of cottonwood leaf beetle (Coleoptera : Chrysomelidae) larval defoliation, clone, and season on Populus foliar phagostimulants. *Environ. Entomol.*, 32(3): 452-462.

Creelman, R.A. and J.E. Mullet. 1997. Biosynthesis and action of jasmonates in plants. *Ann. Rev. Plant Biol.*, 48(1): 355-381.

Darras, A.I., D.C. Joyce and L.A. Terry. 2011. Methyl jasmonate and acibenzolar-S-methyl protect cut Freesia hybrida inflorescences against Botrytis cinerea, but do not act synergistically. *J. Hort. Sci. Biotech.*, 86(1): 74-78.

Das, A., S.H. Lee, T.K. Hyun, S.W. Kim and J.Y. Kim. 2013. Plant volatiles as method of communication. *Plant Biotech. Reports*, 7(1): 9-26.

Degenhardt, D.C. and D.E. Lincoln. 2006. Volatile emissions from an odorous plant in response to herbivory and methyl jasmonate exposure. *J. Chem. Ecol.,* 32(4): 725-743.

Degenhardt, J., J. Gershenzon, I.T. Baldwin and A. Kessler. 2003. Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. *Curr. Opin. Biotechnol.*, 14(2): 169-176.

Edwards, E.J., A.F. Downie and P.R. Clingeleffer. 2011. A Simple Microplate Assay to Quantify Nonstructural Carbohydrates of Grapevine Tissues. *Amer. J. Enol. Vitic.*, 62(1): 133-137.

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

Farmer, E.E. 2001. Surface-to-air signals. *Nature*, 411(6839): 854-856.

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

Feng, D., L. Liu, L.Y. Zhao, Q.F. Zhou and T.W. Tan. 2012a. Evaluation of simulant migration of volatile nitrosamines from latex gloves and balloons by HS-SPME-GC-MS. *J. Chromatogr. Sci.*, 50(8): 733-738.

Feng, Y.J., J.W. Wang, S.M. Luo, H.Z. Fan and Q. Jin. 2012b. Costs of jasmonic acid ınduced defense in aboveground and belowground parts of corn (*Zea mays* L.). *J. Chem. Ecol.,* 38(8): 984-991.

Ferrareze, J.P., K.K. Fugate, M.D. Bolton, E.L. Deckard, L.G. Campbell and F.L. Finger. 2013. Jasmonic acid does not increase oxidative defense mechanisms or common defense-related enzymes in postharvest sugarbeet roots. *Postharvest Biol. Tech.*, 77: 11-18.

Gonzalez-Aguilar, G.A., M.E. Tiznado-Hernandez, R. Zavaleta-Gatica and M.A. Martinez-Tellez. 2004. Methyl jasmonate treatments reduce chilling injury and activate the defense response of guava fruits. *Biochem. Biophys. Res. Commun.,* 313(3): 694-701.

Gould, N., T. Reglinski, M. Spiers and J.T. Taylor. 2008. Physiological trade-offs associated with methyl jasmonate-induced resistance in Pinus radiata. *Can. J. Forest Res.*, 38(4): 677-684.

Graves, A.D., E.H. Holsten, M.E. Ascerno, K.P. Zogas, J.S. Hard, D.P.W. Huber, R.A. Blanchette and S.J. Seybold. 2008. Protection of spruce from colonization by the bark beetle, *Ips perturbatus*, in Alaska. *Forest Ecol. Manag.*, 256(11): 1825-1839.

Gundlach, H., M.J. Müller, T.M. Kutchan and M.H. Zenk. 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proc. Natl. Aca. Sci.*, 89(6): 2389-2393.

Habib, H. and K.M. Fazili. 2007. Plant protease inhibitors: a defense strategy in plants. *Biotech. Mol. Biol. Rev.*, 2(3): 68-85.

Heil, M. 2004. Induction of two indirect defences benefits Lima bean (Phaseolus lunatus, Fabaceae) in nature. *J. Ecol.*, 92(3): 527-536.

Hermsmeier, D., U. Schittko and I.T. Baldwin. 2001. Molecular Interactions between the Specialist HerbivoreManduca 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.

Jin, P., K.U. Wang, H.T. Shang, J.M. Tong and Y.H. Zheng 2009a. Low-temperature conditioning combined with methyl jasmonate treatment reduces chilling injury of peach fruit. *J. Sci. Food Agric.*, 89(10): 1690-1696.

Jin, P., Y.H. Zheng, S.S. Tang, H.J. Rui and C.Y. Wang. 2009b. A combination of hot air and methyl jasmonate vapor treatment alleviates chilling injury of peach fruit. *Postharvest Biol. Tech.*, 52(1): 24-29.

Karamac, M. and R.B. Pegg. 2009. Limitations of the Tetramethylmurexide Assay for Investigating the Fe(II) Chelation Activity of Phenolic Compounds. *J. Agri. Food Chem.*, 57(14): 6425-6431.

Karban, R. and I.T. Baldwin. 1997. *Induced responses to herbivory*. University of Chicago Press, Chicago.

Karban, R., K. Shiojiri and S. Ishizaki. 2010. An Air Transfer Experiment Confirms the Role of Volatile Cues in Communication between Plants. *Am. Nat.*, 176(3): 381-384.

Kazan, K. and J.M. Manners. 2008. Jasmonate signaling: toward an integrated view. *Plant Physiol.*, 146(4): 1459-1468.

Keramat, B., K.M. Kalantari and M.J. Arvin. 2010. Effects of methyl jasmonate treatment on alleviation of cadmium damages in soybean. *J. Plant Nutr.*, 33(7): 1016-1025.

Kessler, A. and I.T. Baldwin. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, 291(5511): 2141-2144.

Li, Z., X. Wang, F. Chen and H.J. Kim. 2007a. Chemical changes and overexpressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. *J. Agric. Food Chem.*, 55(3): 706-13.

Li, Z.G., X. Wang, F. Chen and H.J. Kim. 2007b. Chemical changes and overexpressed genes in sweet basil (*Ocimum basilicum* L.) upon methyl jasmonate treatment. *J. Agric. Food Chem.*, 55(3): 706-713.

Liang, Q.Q. and Y.S. Li. 2011. Rapid determination of real protein content in dairy products by flow ınjection-spectrophotometry. *Chin. J. Anal. Chem.*, 39(9): 1412-1417.

Lin, J., Y. Dai, Y.N. Guo, H.R. Xu and X.C. Wang. 2012. Volatile profile analysis and quality prediction of Longjing tea (*Camellia sinensis*) by HS-SPME/GC-MS. *J. Zhejiang Univer. Sci. B*, 13(12): 972-980.

Lomate, P.R. and V.K. Hivrale. 2012. Wound and methyl jasmonate induced pigeon pea defensive proteinase inhibitor has potency to inhibit insect digestive proteinases. *Plant Physiol. Biochem.*, 57: 193-199.

Luo, W.C. and L.Y. Mu. 1997. Toxic Effects of Alkaloids from Plants against Insect Pests. *Pesticide*, 36(7): 11-15.

Maksymiec, W. and Z. Krupa. 2007. Effects of methyl jasmonate and excess copper on root and leaf growth. *Biol. Plantarum*, 51(2): 322-326.

Marla, S.S. and V.K. Singh. 2012. LOX genes in blast fungus (*Magnaporthe grisea*) resistance in rice. *Funct.Integr. Genomic.*, 12(2): 265-275.

Martin, J.A., A. Solla, M.C. Garcia-Vallejo and L. Gil. 2012. Chemical changes in Ulmus minor xylem tissue after salicylic acid or carvacrol treatments are associated with enhanced resistance to Ophiostoma novo-ulmi. *Phytochem.*, 83: 104-109.

Meng, D.M., T.Z. Song, L. Shen, X.H. Zhang and J.P. Sheng. 2012. Postharvest Application of Methyl Jasmonate for Improving Quality Retention of Agaricus bisporus Fruit Bodies. *J. Agric. Food. Chem.*, 60(23): 6056-6062.

Papotti, G., D. Bertelli and M. Plessi. 2012. Use of HS-SPME-GC-MS for the classification of Italian lemon, orange and citrus spp. honeys. *Int. J. Food Sci.Tech.,* 47(11): 2352-2358.

Pérez Gilabert, M. and F.G.A. Carmona. 2002. Chromatographic analysis of lipoxygenase products. *Anal. Chim. Acta*, 465(1): 319-335.

Qiu, D.W. 2004. Microbe Protein Pesticide and It's Prospect. *Chin. J. Biol. Control*, 20(2): 91-94.

Queiroz, C., M.L.M. Lopes, E. Fialho and V.L. Valente-Mesquita. 2008. Polyphenol oxidase: Characteristics and mechanisms of browning control. *Food Rev. Int.*, 24(4): 361-375.

Rashmi, Singh, K.P. and S. Arya. 2011. Phytochemical profile and evaluation of insecticidal efficacy of *Calotropis procera* against defoliators. *J. Med. Plant Res.*, 5(31): 6738-6743.

Rohwer, C. and J. Erwin. 2008. Horticultural applications of jasmonates: A review. *J. Hortic. Sci. Biotechnol.*, 83: 283-304.

Ruiz-Garcia, Y., I. Romero-Cascales, R. Gil-Munoz, J.I. Fernandez-Fernandez, J.M. Lopez-Roca and E. Gomez-Plaza. 2012. Improving grape phenolic content and wine chromatic characteristics through the use of two different elicitors: Methyl jasmonate versus benzothiadiazole. *J. Agric. Food Chem*., 60(5): 1283-1290.

Sampedro, L., X. Moreira and R. Zas. 2011. Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *J. Ecol.*, 99(3): 818-827.

Simons, L., T.L. Bultman and T. Sullivan. 2008. Effects of methyl jasmonate and an endophytic fungus on plant resistance to insect herbivores. *J. Chem. Ecol.*, 34(12): 1511-1517.

Surrey, K. 1964. Spectrophotometric method for determination of lipoxidase activity. *Plant Physiol.*, 39(1): 65.

Szczegielniak, J., L. Borkiewicz, B. Szurmak, E. Lewandowska-Gnatowska, M. Statkiewicz, M. Klimecka, J. Ciesla and G. Muszynska. 2012. Maize calcium-dependent protein kinase (ZmCPK11): local and systemic response to wounding, regulation by touch and components of jasmonate signaling. *Physiol. Plant*, 146(1): 1-14.

Tamogami, S., R. Ralkwal and G.K. Agrawal. 2008. Interplant communication: Airborne methyl jasmonate is essentially converted into JA and JA-Ile activating jasmonate signaling pathway and VOCs emission. *Biochem. Biophys. Res. Commun.*, 376(4): 723-727.

Tan, C.W., J.C. Lo, J. Yadav, K.T. Ravuiwasa and S.Y. Hwang. 2011. Methyl jasmonate induced responses in four plant species and its effect on Spodoptera litura Fab. performance. *J. Asia-Pacific Entomol.*, 14(3): 263-269.

Tan, C.W., S.Y. Chiang, K.T. Ravuiwasa, J. Yadav and S.Y. Hwang. 2012. Jasmonate-induced defenses in tomato against Helicoverpa armigera depend in part on nutrient availability, but artificial induction via methyl jasmonate does not. *Arthropod-Plant Inte.*, 6(4): 531-541.

Tran, L.T. and C.P. Constabel. 2011. The polyphenol oxidase gene family in poplar: phylogeny, differential expression and identification of a novel, vacuolar isoform. *Planta*, 234(4): 799-813.

Walters, D. and M. Heil. 2007. Costs and trade-offs associated with induced resistance. *Physiol. Mol. Plant P.*, 71(1-3): 3-17.

Wang, D.J., X.K. Shu, X.E. Zhao, X. Wang, S.B. Li and J.H. Du. 2013. Comparative analysis of volatile oils from *Lonicera japonica* Thunb. var. *chinensis* wakey by HS-SPME and GC-MS. *Asian J.Chem*., 25(2): 803-806.

Wang, G.C., Q. Fu, F.X. Lai, M.X. Chen, R.X. Mou and Z.T. Zhang. 2005. Relationship between yeast-like symbiotes and amino acid requirements in the rice brown planthopper, *Nilaparvata lugens* Stal (Homoptera: Delphacidae). *Acta Entomol. Sinica*, 48(4): 483-490.

Williams, L., C. Rodriguez-Saona, S.C. Castle and S. Zhu. 2008. EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. *J. Chem.Ecol.,* 34(9): 1190-1201.

Wu, J.S., L. Wang and I.T. Baldwin. 2008. Methyl jasmonate-elicited herbivore resistance: does MeJA function as a signal without being hydrolyzed to JA? *Planta,* 227(5): 1161-1168.

Xue, J.L. and Y.P. Xie. 2000. A study on the variation of some chemical substances in the body of larch induced by the damage from the larch case-bearer. *Sci. Silvae Sinica,* 36(4): 46-50.

Zeneli, G., P. Krokene, E. Christiansen, T. Krekling and J. Gershenzon. 2006. Methyl jasmonate treatment of mature Norway spruce (Picea abies) trees increases the accumulation of terpenoid resin components and protects against infection by Ceratocystis polonica, a bark beetle-associated fungus. *Tree Physiol.,* 26(8): 977-988.

Zhang, Q., Z.Y. Zhang, S.Z. Lin and Y.Z. Lin. 2005. Resistance of transgenic hybrid triplolds in Populus tomentosa Carr. against 3 species of lepidopterans following two winter dormancies conferred by high level expression of cowpea trypsin inhibitor gene. *Silvae Genet.*, 54(3): 108-116.

Zhao, J., L.C. Davis and R. Verpoorte. 2005. Elicitor signal transduction leading to production of plant secondary metabolites. *Biotechnol. Adv.*, 23(4): 283-333.

(Received for publication 29 July 2013)