Available online at www.sciencedirect.com
ScienceDirect
Plant Science 173 (2007) 12–24
PLANT SCIENCE
www.elsevier.com/locate/plantsci

MeJA-induced transcriptional changes in adventitious roots of *Bupleurum kaoi*

Li-Ru Chen [a], Yi-Jiun Chen [a], Cherng-Yu Lee [b], Tsai-Yun Lin [a,*]

[a] *Institute of Bioinformatics and Structural Biology and Department of Life Science, National Tsing Hua University, No. 101, Sec. 2, Kuang Fu Road, Hsinchu 30013, Taiwan, Republic of China*

[b] *Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, No. 321, Sec. 2, Kuang Fu Road, Hsinchu 30013, Taiwan, Republic of China*

Received 8 January 2007; received in revised form 22 March 2007; accepted 22 March 2007
Available online 4 April 2007

Abstract

The plant regulator methyl-jasmonate (MeJA) mediates diverse developmental processes and defense responses which induce a variety of secondary metabolites. This study reports the regulatory network of MeJA-induced genes in adventitious root cultures of the medicinal herb *Bupleurum kaoi*. A cDNA microarray containing 465 unique expressed sequence tags (ESTs) as probe sets, generated with a PCR-Select cDNA Subtraction Kit, was employed to profile the transcriptome of MeJA induction at thirteen points in time. Real-time PCR was performed to verify changes in expression of 36 unique ESTs (uniESTs), detected via microarray. Genes upregulated by MeJA include those involved in saikosaponin, amino acid, phenylpropanoid and jasmonate biosynthesis, defense signaling, plant hormone regulation and stress protection; indicating that the JA signaling pathway interacts with other signaling pathways leading to transcriptional reprogramming in *B. kaoi* adventitious roots. Some of these MeJA-induced genes have applications in agricultural, pharmaceutical and food industries.

Keywords: Adventitious root; *Bupleurum kaoi*; cDNA microarray; Methyl jasmonate (MeJA); Relative quantification in real-time PCR (qRT-PCR)

1. Introduction

The fragrant volatile compound methyl-jasmonate (MeJA), initially identified in flowers of *Jasminum grandiflorum*, is ubiquitous in the plant kingdom [1]. MeJA and jasmonic acid (JA) are plant hormones involved in regulating plant–herbivore interactions [2] and plant response to environmental stress through the modulation of gene expression [3,4]. For example, exogenous application of JAs inhibits primary root growth [5] and causes premature senescence in leaves [6] of *Arabidopsis*. MeJA also promotes the accumulation of vegetative storage protein (VSP) in taproots of *Brassica napus* [7] and increases levels of VSP transcript and protein in *Medicago sativa* [8]. Biosynthesis of many classes of secondary plant metabolites is induced by JA [9]. JA regulates the accumulation of phenolics, terpenes and alkaloids as a stress response [10].

Bupleurum kaoi Liu, Chao et Chuang is a species endemic to Taiwan that has 12 pairs of chromosomes and a genome size of about 7.3×10^8 bp per copy [11]. *Bupleurum* roots have been reported to possess anti-inflammatory activity [12] and anti-hepatotoxic effects [13]. Extracting pharmacological agents from roots of intact plants or from tissue-cultured roots is of interest. Saikosaponins which are triterpenoid glycosides (saponins) in *Bupleurum* have considerable commercial value and are exploited as drugs and medicines. The enzymes, genes, and biochemical pathways involved in saikosaponin biosynthesis are largely uncharacterized. In higher plants, oxidosqualene is a precursor common to the biosynthesis of both steroids and triterpenoids [14]. The first committed step in triterpene biosynthesis in *Medicago* is catalyzed by β-amyrin synthase (β-AS) [15]. The downstream reactions may include a set of cytochrome P450-dependent (P450) hydroxylations/oxidations and several glycosyl transfer reactions catalyzed by glycosyltransferases [14]. Glycosyltransferase activity has been shown to correlate with saponin production in root cultures of *Gypsophila paniculata* [16], but triterpene glycosyltransfereases remain to be

* Corresponding author. Tel.: +886 3 5742758; fax: +886 3 5715934.
E-mail address: tylin@life.nthu.edu.tw (T.-Y. Lin).

0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2007.03.013

characterized at the molecular level. A more detailed understanding of the genes that are involved in saikosaponin biosynthesis would facilitate the development of plants with altered saikosaponin content, either by breeding or by transgenic modification. Optimizing levels of therapeutic compounds is important and requires knowledge of relevant genes and their regulation. Exogenously applied MeJA induced biosynthesis of many secondary metabolites and stimulated saikosaponin production in cultured *B. falcatum* root fragments [17]. Although the mechanism of action is not known, JA and MeJA stimulation of particular genes has been hypothesized [15,18–20].

The objective of this research is to profile the regulatory network of MeJA-induced genes in *B. kaoi* cultured roots. Partial *B. kaoi* cDNA libraries from adventitious roots were constructed using PCR-select cDNA subtraction. Transcript levels of selected genes were analyzed with a small scale cDNA microarray and relative quantification with real-time PCR (qRT-PCR). Here we report the identification of 36 *B. kaoi* genes that are upregulated by MeJA in adventitious roots.

2. Materials and methods

2.1. Plant growth and treatment

Adventitious root cultures were induced from the roots of germ-free seedlings of *B. kaoi* and were subcultured every 6 weeks by adding 0.2 g roots to a 125 ml flask containing 50 ml of B5 liquid medium [21] supplemented with 2 mg l^{-1} NAA. Cultures were shaken using a rotary shaker at 100 rpm at 25 °C in the dark. To quantify the effect of MeJA on saikosaponin level, adventitious roots were treated with 500 and 1000 μM MeJA 2 weeks after subculture. Total saikosaponin was measured 2 weeks after MeJA treatment. To monitor expression of MeJA-responsive genes, the nutrient medium was refreshed 4 weeks after subculture and MeJA (500 μM) was applied 2 weeks after refreshment. Roots were harvested at 13 time points (5 min; 1, 2, 8, 24 h; 2, 4, 6, 8 days; 2, 3, 4, 5 weeks) after the addition of the MeJA.

2.2. Measurement of H_2O_2 and malondialdehyde (MDA)

To test whether MeJA caused oxidative stress, the H_2O_2 level was measured according to Jena and Choudhuri [22] with some modifications. H_2O_2 was extracted by homogenizing 0.3–0.5 g of adventitious roots with 3 ml phosphate buffer (50 mM, pH 6.8) and centrifuged at 6000 × *g* for 25 min. A 2 ml extract was mixed with 1 ml 0.1% titanium sulfate in 20% H_2SO_4 (v/v), and centrifuged at 6000 × *g* for 15 min. The optical density of the supernatant was measured at 410 nm using an equal volume of phosphate buffer as a blank. The H_2O_2 level was determined with an extinction coefficient of 0.28 $\mu mol^{-1}\ cm^{-1}$. The MDA level was colorimetrically measured as described by Heath and Packer [23] with some modifications. MDA was extracted by homogenizing 0.3–0.5 g adventitious roots with 2 ml 5% trichloroacetic acid, and centrifuged at 10,000 × *g* for 5 min at 20 °C. A mixture of 1 ml supernatant with 4 ml 0.5% thiobarbituric acid in 20% trichloroacetic acid was heated in a 95 °C water bath for 30 min and centrifuged for 10 min at 2000 × *g* to remove haziness. Optical density of the supernatant was measured at 532 nm and 600 nm using 1 ml 5% trichloroacetic acid to replace supernatant as a blank. The concentration of MDA was calculated with an extinction coefficient of 155 $mmol^{-1}\ cm^{-1}$.

2.3. Quantification of total saikosaponins

The saikosaponins level was measured according to Li et al. [24] with some modifications. Freeze-dried adventitious roots were ground into a fine powder and extracted with 70% methanol at a ratio of 10:1 (v/w) at 25 °C with gentle shaking for 24 h. After centrifugation at 10,000 × *g* for 10 min, the supernatant was filtered through a 0.45 μm filter. The amount of total saikosaponins (saikosaponin-a, saikosaponin-c, and saikosaponin-d) in each extract was quantified using a high performance liquid chromatograph (HPLC) (Waters 600 controller autoinjector) with a C18 Inertsil 5 ODS-2 column (4.6 mm × 250 mm) and a mobile phase of 45% acetonitrile/55% H_2O. The flow rate was 1 ml min^{-1}, the injection volume was 20 μl, and the eluent was monitored at 210 nm using a Waters 996 photodiode array detector.

2.4. Total cellular RNA extraction

Total cellular RNA was extracted as described by Chang et al. [25] with some modifications. Three to five grams of tissue was frozen in liquid nitrogen and ground to a fine powder with mortar and pestle. The powder was added to 15 ml of pre-warmed (65 °C) extraction buffer (2%, v/v hexadecyltrimethylammonium bromide (CTAB), 2%, v/v polyvinylpyrrolidinone K 30 (PVP), 100 mM Tris–HCl, pH 8.0, 25 mM EDTA, 2 M NaCl, 0.5 mg ml^{-1} spermidine, 2% β-mercaptoethanol), and mixed completely by vigorous shaking. The mixture was extracted twice with an equal volume of chloroform:isoamyl alcohol (24:1). The RNA was precipitated by adding 1/4 volume of cold 10 M LiCl to the aqueous phase and held for 12–18 h at −20 °C. After centrifugation at 19,800 × *g* at 4 °C, the RNA was dissolved in 500 μl sterile DEPC H_2O. The resuspended RNA was reextracted with chloroform:isoamyl alcohol (24:1). Three volumes of 100% ethanol and 1/10 volume of 3 M sodium acetate (pH 5.2) were added to the aqueous phase, and the solution was precipitated with liquid nitrogen for 15 min. The RNA was spun down at 19,800 × *g* for 30 min at 4 °C and washed with 80% ethanol. The dried pellet was resuspended in sterile DEPC H_2O.

2.5. Preparation of PCR-select cDNA subtraction library and DNA sequencing

Three PCR-select cDNA subtraction libraries were constructed with a MeJA-treated sample as tester and control as driver at 13 time points. RNA for construction of libraries I, II and III was prepared from samples at three sets of time points with set I of 5 min, 1, 2, 8 and 24 h, set II of 2, 4, 6 and 8 days and set III of 2, 3, 4 and 5 weeks, respectively. Within each set, an equivalent amount of RNA from each time point was added to a total of