Scientia Horticulturae 189 (2015) 159–167

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Global analysis of transcriptional response of Chinese cabbage to methyl jasmonate reveals JA signaling on enhancement of secondary metabolism pathways

Yunxiang Zang[a,1], Weiwei Zheng[a,1], Yong He[a], Seung-Beom Hong[b], Zhujun Zhu[a,*]

[a] The Key Laboratory for Quality Improvement of Agricultural Products of Zhejiang Province, College of Agriculture and Food Science, Zhejiang A & F University, Lin' an, Zhejiang 311300, China
[b] Department of Biotechnology, University of Houston Clear Lake, Houston, TX 77058-1098, USA

ARTICLE INFO

Article history:
Received 3 January 2015
Received in revised form 5 April 2015
Accepted 6 April 2015
Available online 20 April 2015

Keywords:
Brassica rapa
Transcriptome
Methyl jasmonate
Secondary metabolism
RNA-Seq

ABSTRACT

Methyl jasmonate (MeJA) is known to increase multiple resistance and secondary metabolism pathways in Brassicaceae (Cruciferae). However, the mechanisms of regulation at the transcriptomic level were not well studied. So, we conducted a RNA-Seq analysis of MeJA-treated Chinese cabbage leaf transcriptome. Among 27,461 detected genes, 1451 genes were up-regulated and 991 genes were down-regulated as differentially expressed genes (DEGs) ($\log_2$ of the fold change in gene expression $\geq$1, false discovery rate $\leq$0.001). More than 90% of the DEGs were between 2.0- and 8.0-fold changes. The most highly represented pathways by 1674 annotated DEGs were related to "metabolic pathways" (333 members). Fourteen genes involved in JA biosynthesis pathway were up-regulated. Forty genes for glucosinolate biosynthesis were induced, and the level of indole glucosinolate was highly increased by MeJA treatment. Several selected genes involved in JA signaling and indole glucosinolate synthesis pathways were validated by quantitative RT-PCR. The results expand our understanding of the complex molecular events on JA-induced plant resistance and accumulation of secondary metabolites.

1. Introduction

Plants in nature are exposed to diverse biotic or abiotic stresses, such as, pathogen infection, herbivore attack, wounding, water deficit, low temperature, and salinity. Responding to environmental cues, plants synthesize several key secondary signaling molecules, such as jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) (Balbi and Devoto, 2008; Mewis et al., 2005). They subsequently generate a signal transduction network leading to a cascade of events for the physiological adaptation of the plant to the external stress.

Jasmonic acid and its derivatives, methyl ester (MeJA), isoleucine conjugate (JA-Ile) etc., collectively known as jasmonates (JAs), have been confirmed to be powerful regulators of plant development as well as inducible resistance to insects, pathogens, wounding and environmental stresses (Farmer et al., 2003; Pauwels et al., 2009). JAs regulate a number of plant developmental processes, including root growth, senescence, pollen and trichome development (Reinbothe et al., 2009; Wasternack, 2007). Exogenous JA application on wild-type *Arabidopsis* plants either reduced root growth at high concentrations or increased the frequency of lateral root initiation and elongation at low concentrations (Staswick et al., 1992). In addition to their role in plant development, JAs play an important role in defense against bacterial and fungal infections, insect attack or wound response (Ellis et al., 2002; Li et al., 2002; Kempema et al., 2007). Exogenous JA application is known to enhance the resistance of *Arabidopsis* to both phloem-feeding and chewing insects by eliciting dramatic increase of indole glucosinolates (Mewis et al., 2005). Shoot application of JA, which mimics herbivory response, altered carbon partitioning into the lower stem or roots so that it can be used later for regrowth, reproduction, or synthesis of defense compounds (Henkes et al., 2008). Defense response largely depends on complex signaling networks that modulate the balance between growth and defense (Chini et al., 2009). Both microarray-based transcriptomic and proteomic analyses of MeJA-treated *Arabidopsis* rosette leaves showed that photosynthesis and carbohydrate anabolism were down-regulated, while carbohydrate catabolism, JA biosynthesis pathway, stress and defense, and secondary metabolism were up-regulated (Chen et al.,

* Corresponding author. Tel.: +86 571 6374 3001; fax: +86 571 6374 1276.
E-mail address: zhuzj@zafu.edu.cn (Z. Zhu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scienta.2015.04.008
0304-4238/

2011; Jung et al., 2007). Thus, JA signal appears to redirect growth-related metabolism to defense-related metabolism for a defensive action. The most recent research further confirmed that plants trigger defense response at the expense of growth (Yang et al., 2012).

JAs are synthesized from alpha-linolenic acid via the octadecanoid pathway involving at least seven enzymes (Wasternack and Hause, 2002). JA precursor OPDA (12-oxo-phytodienoic acid) and JA conjugate JA-Ile are known to have their own biological activities in jasmonate-regulated responses. OPDA acts as a signaling molecule to trigger plant defense responses independently of JA (Taki et al., 2005). Upon perception of JA-Ile signal, F-box protein COI1 recruits the Jasmonate-ZIM domain repressor proteins (JAZs) that interact with a series of transcription factors to SCFCOI1-E3 ubiquitin ligase complex and 26S proteasome for ubiquitination and degradation of JAZs (Sheard et al., 2010), and the released transcription factors activate JA-regulated genes (Chini et al., 2007).

As a close relative of model plant *Arabidopsis*, Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) is believed to undergo a whole genome triplication process soon after divergence from the *Arabidopsis* lineage approximately 17–20 million years ago, followed by genome shrinkage and differential loss of duplicated genes (Mun et al., 2009). This was recently confirmed by *B. rapa* whole genome sequence showing that it contains 41,174 genes, as compared to *Arabidopsis* 27,416 genes with an average 87% sequence homology in the coding regions of homologous genes (Wang et al., 2011). The comparative analysis of *B. rapa* and *Arabidopsis thaliana* genomes suggests that the ongoing processes of polyploidy and chromosomal diploidization stabilized the *B. rapa* genome and facilitated its evolution (Mun et al., 2009). In response to exogenous JA, *Brassicas* crops displayed enhanced glucosinolate accumulation and insect resistance similar to those of *Arabidopsis* (Fritz et al., 2010; van Dam and Oomen, 2008). Genome-wide gene expression of *Brassica* crops have been studied by a microarray analysis under abiotic stresses (Lee et al., 2008). The availability of Chinese cabbage whole genome sequence data and the recently developed massive parallel DNA sequencing-based RNA-Seq technology has provided us unprecedented opportunities to perform comprehensive transcriptome analysis that is more sensitive and accurate than microarray, especially for low-expressed genes (Garber et al., 2011).

Here, we present a comprehensive investigation of Chinese cabbage RNA-Seq transcriptome in response to MeJA application. A number of genes involved in the cell cycle, primary metabolism, and secondary metabolism for defense response were regulated in the MeJA-treated Chinese cabbage. More genes were regulated in Chinese cabbage than *Arabidopsis*, but not all of the duplicated genes were found to be up- or down-regulated, which may support a model of gene evolution by neo-functionalization or sub-functionalization (Kawaguchi et al., 2013). We also report that significantly increased accumulation of indole gluconsinolate was accompanied by up-regulation of its biosynthesis genes and sugar metabolic genes, whereas neither up-regulation of aliphatic and aromatic glucosinolates genes nor increased accumulation of their products was noted in response to MeJA treatment. The transcriptome profile and expression value for each gene will be useful for further research on individual genes.

2. Materials and methods

2.1. Plant material and sampling

Seeds of Chinese cabbage (*B. rapa* ssp. *pekinensis*, cv. ZSN5) were germinated in vermiculite in the greenhouse. The average temperature was 22–28 °C/15–20 °C (day/night) under natural light. Relative humidity fluctuated between 60% and 70%. Seedlings with one fully expanded true leaf were transferred into trough with 20 L of half-strength Hoagland's nutrient solution, aerated continuously with an air bubbler. The nutrient solutions were renewed every week. After grown in greenhouse for 30 days, Chinese cabbage plants with about 8–10 leaves were sprayed with 0.2 mM MeJA (dissolved in 1% ethanol) or 1% ethanol in aqueous solution as a control. After 24 h, the newly expanded leaves from three independent mock or MeJA treated plants were harvested and immediately frozen in liquid nitrogen. They were then brought to laboratory for RNA extraction.

2.2. RNA extraction and preparation of cDNA for RNA-Seq

Total RNA was isolated using Trizol reagent according to the manufacturer's protocol (Sigma–Aldrich, Inc., USA) and treated with RNase-free DNase to remove any genomic DNA contaminants. The purity of extracted RNA were assessed by ratio of absorbance A_{260} to A_{280}, using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

After extracting the total RNA from the samples, mRNA is enriched by using the oligo(dT) magnetic beads from the total RNA. Adding the fragmentation buffer, the mRNA is interrupted to short fragments (about 200 bp), and then the first strand cDNA is synthesized by random hexamer-primer using the mRNA fragments as templates. Buffer, dNTPs, RNase H and DNA polymerase I are added to synthesize the second strand. The double strand cDNA is purified with QiaQuick PCR extraction kit and washed with EB buffer for end repair and single nucleotide A (adenine) addition. Finally, sequencing adaptors are ligated to the fragments. The required fragments is purified by agrose gel electrophoresis and enriched by PCR amplification. The library products are ready for sequencing analysis via Illumina HiSeq™ 2000.

2.3. Mapping reads to the reference genome and annotated genes

The Chinese cabbage genome and gene information were downloaded from the *Brassica* Genome Sequencing Project (http://www.brassica.info/resource/sequencing.php). To get the clean reads for data analysis, raw sequence data were filtered by removing adaptor sequences and low quality reads (the percentage of the low quality bases of quality value ≤5 is more than 50% in a read). The clean reads were mapped to reference sequences using SOAPaligner/soap2 (Li et al., 2009), allowing up to 2 bases mismatch in the alignment.

2.4. Statistics of gene expression level from RNA-Seq

The gene expression is calculated by the numbers of reads mapped to the reference sequence and every gene. The gene expression level is calculated by using RPKM method (Reads Per kb per Million reads), and the formula is shown as follows:

$$\mathrm{RPKM} = \frac{10^9 C}{LN}$$

Given RPKM(A) to be the expression of gene A, C to be number of reads that uniquely aligned to gene A, N to be total number of reads that uniquely aligned to all genes, and L to be number of bases on gene A. The RPKM method is able to eliminate the influence of different gene length and sequencing discrepancy on the calculation of gene expression. Therefore, the calculated gene expression can be directly used for comparing the difference of gene expression among samples. If there is more than one transcript for a gene, the longest one is used to calculate its expression level and coverage.