Physiological and Molecular Plant Pathology 102 (2018) 79–87

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

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

A tale of survival: Molecular defense mechanisms of soybean to overcome *Soybean mosaic virus* infection

Gustavo A. Díaz-Cruz, Bryan J. Cassone*

Department of Biology, Brandon University, Brandon, MB R78 6A9, Canada

ARTICLE INFO

Article history:
Received 21 July 2017
Received in revised form
3 November 2017
Accepted 14 November 2017
Available online 20 November 2017

Keywords:
Transcriptomics
Metabolomics
Soybean mosaic virus
Soybean infection

ABSTRACT

Plant viruses represent some of the greatest contributors to crop losses worldwide. Viral disease symptoms often include mosaic leaf patterns, crinkled and yellowed leaves, plant stunting and even necrosis. As intracellular pathogens, viruses hijack host cell machinery for their own replication, which can elicit dramatic changes to their hosts at the cellular, molecular and physiological levels. *Soybean mosaic virus* (SMV) is a widely distributed soybean pathogen, which can cause severe stunting and yield losses to infected plants. A better understanding of the underlying interactions between SMV and soybean will aid in the establishment of disease management plans. In these studies, we implemented high throughput RNA sequencing approaches and targeted metabolite profiling to describe transcriptomic and metabolic changes occurring in soybean leaves ten days post-infection with SMV. A massive defense response was detected by the increased accumulation of transcripts associated with biotic stresses, including those involved in pathogen recognition, autophagy and defense inductors. Moreover, significant decreases in expression were detected for transcripts associated with fundamental growth and development processes, such as nutrient transport and photosynthesis. At the metabolomic level, significant changes were identified in correspondence with viral infection, particularly in amino acid concentrations. Overall, it appears as though SMV inoculated plants reroute their limited resources as a survival strategy, favoring defense over plant development.

© 2017 Published by Elsevier Ltd.

1. Introduction

The interactions between microorganisms and their host plants are continually shaped and moulded throughout evolutionary time, which often results in very different biological outcomes for each member involved [1–3]. Indeed, a given microorganism may provide a fitness advantage, cost or neutral effect to its host [4]. As emphasis continues to be placed on improving agricultural production, microbe-plant assemblages have become more closely scrutinized, especially among pathogenic microbes and economically important crops [5]. Disease-causing pathogens act rapidly at the molecular and microscopic levels, diminishing the chances of success for plants. This is particularly true of viruses, which are obligate specialists in cellular machinery hijacking, rerouting the normal processes and products in plant cells for its own benefit [6]. However, the changes on the host may vary dramatically when different viruses are infecting, and these alterations are important to understand each pathosystem, and subsequently design control strategies [7].

Like other major crops, soybean (*Glycine max* (L.) Merr.) is a host to diverse microbial pathogens [8]. This includes several viruses that can cause substantial yield losses and/or seed quality deteriorations [9–12]. *Soybean Mosaic Virus* (SMV) is one of the most ubiquitous soybean viruses, established in nearly every growing area around the world [13–18]. SMV is a member of the genus *Potyvirus*, with a single-stranded positive-sense RNA genome, classified in strains, which differ depending on the classification system used [16]. SMV is responsible for causing different symptoms on soybean, which vary in severity in different cultivars, but have highly relevant effects on production. The main symptom is the mosaic pattern on leaves, but necrosis and crinkling can also be seen [8,19]. In terms of plant development, SMV infection results in stunted growth, with some varieties showing reductions up to 57% on plant height and 68% on the number of pods [20–22].

The underlying basis of morphological changes brought about by virus infection are often observable at the biochemical, cellular, molecular and physiological levels, and this has been well

* Corresponding author.
E-mail address: cassoneb@brandonu.ca (B.J. Cassone).

https://doi.org/10.1016/j.pmpp.2017.11.006
0885-5765/© 2017 Published by Elsevier Ltd.

documented in infected host plants [23]. At the transcriptomic level, viral pathogen infection has produced up- and down-regulation of different genes combinations [24]. Differential transcriptomic responses have been obtained by exposing the same virus to different ecotypes of *Arabidopsis* [25], by combining different cultivars and different viral strains [26] and even using strains of the same virus with different aggressiveness [27]. In turn, these transcriptomic changes impact the production of metabolites [28]. For instance, an increase in foliar sugar production after viral infections has been recorded, and its significance has been hypothesized to be a vector-attraction strategy by the virus to increase its dissemination [29], or as compensation for decreased photosynthetic rates [30]. Other primary and secondary metabolites, including amino acids and hormone precursors, have been reported to change significantly in as little as one day after inoculation, showing an acute response to viral infections [31].

In soybean, viral infections have yielded significant changes to the transcriptomic and metabolomic profiles [32–34]. Previous microarray analysis have indicated SMV infection significantly altered the gene expression profiles of soybean, with a delayed defense response between 7 and 14 days post inoculation (dpi) [35]. To further explore the mechanisms by which SMV modulates host plant chemistry and to better characterize the immune response of soybean to viral infection, we carried out global transcriptomic and targeted metabolomic analyses on soybean leaves 10 dpi – a time point where this information is considerably lacking for SMV. Further, this time point elicited a substantial reaction in soybean inoculated with another prevalent virus, *Bean pod mottle virus* [29]. Our results reveal a massive upregulation of defense genes coupled with the downregulation of genes involved in energy production and nutrient transport, as well as significant changes in the concentrations of multiple metabolites. At the morphological level, SMV-infected plants showed classic foliar symptoms (e.g., mosaic patterns and crinkling) and severely stunted growth. Taken collectively, it appears as though inoculated plants shift their usage of the finite cellular resources from vegetative growth and other regular processes to a vastly heightened defense response in order to combat the viral infection.

2. Methods

2.1. Virus maintenance

The SMV isolate was maintained in 'Sloan' cultivar soybean through serial leaf-rub inoculation with inoculum made from leaves of infected plants [36]. To generate SMV-infected experimental plants, inoculum was made by grinding infected leaf tissue into 10 mM KHPO4, pH 7 (1:4 w/v) with Carborundum to induce wounding. The inoculum was mechanically inoculated onto 10 day old soybean plants (20 μL per plant). Prior to collection (see below), the height of experimental plants were recorded (Table S1).

2.2. Transcriptional responses of soybean to SMV infection

Sample collections. The youngest trifoliate leaf of both the experimental V2-stage (20 day old) SMV-infected and healthy (control) 'Sloan' soybean plants were collected 10 days post-inoculation. All collections were carried out at the same time (2 p.m. EDT) to circumvent potential diurnal changes in mRNA accumulation profiles. Both treatments were replicated four times using seedlings from different stocks planted at different times (8 samples total).

RNA extraction and cDNA library synthesis. For each replicate, total RNA was isolated from pools of 5 leaves sampled from different plants using the RNeasy Plant Mini Kit (QIAGEN, Germantown, MD). RNA quality was assessed on the Nanophotometer NP80 (Implen Inc., Westlake Village, CA), and quantity was determined using the Qubit 3.0 fluorometer using the RNA HS assay kit (Thermo Fisher Scientific, Waltman, MA). RNA (1 μg/sample) was used to generate adaptor-ligated double-stranded cDNA libraries for RNA sequencing using the TruSeq Sample Prep Kit V1 (Illumina, San Diego, CA) following the manufacturer's protocol. Quantity and quality of each cDNA library was evaluated using the BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA). Samples were diluted to 50 fmoles and pooled to generate the multiplexed cDNA library.

Illumina sequencing and read preprocessing. The multiplexed cDNA library was sequenced in 100-bp paired-end fashion on a partial flow cell lane using the Illumina HiSeq 2500 platform at the Génome Québec Innovation Centre at McGill University. Illumina Analysis Package CASAVA 1.8.2 was used to perform bcl conversion and demultiplexing. Image deconvolution and quality value calculations were carried out using the Illumina GA pipeline v1.6. Raw reads were imported into CLC Genomics Workbench (v7.5; CLC bio, Aarhus, Denmark) where adapter indexes and poly (A) tails were trimmed (Ambiguous limit = 2, quality limit = 0.05). Quality assessment of the data file included hierarchical clustering of samples (Measure: Euclidean distance, Clusters: Average linkage) and principle component analyses (PCA) in CLC bio. The raw sequence reads can be retrieved from the NCBI short sequence read archive under the accession numbers SRR5466715 to SRR5466722 (study SRP104733).

Differential gene expression analysis. The most recent *Glycine max* [Glyma2.0; 56,044 genes] reference cDNA database was retrieved using the BioMart tool in Phytozome [37]. Preprocessed reads were aligned to the cDNA database using the map to reference function in CLC bio Genomics Workbench and the following parameters: Similarity Fraction = 0.96; Length Fraction = 0.65; default settings herein. Only unique reads (i.e., mapping to only one cDNA in the *Glycine max* database) were counted for a given sample.

Genes differentially expressed between SMV-infected and healthy (control) soybean were identified using the DESeq2 package [38] in Bioconductor [39]. This software computes relative differences in mRNA abundance based on mapped reads counts by using a negative binomial distribution model. Significance was defined at FDR <0.05 and FDR <0.01 [40], and only genes with a minimum of 10 reads across the 8 samples were included in the analysis (i.e., the subset of expressed genes). GO Enrichment Analysis was then performed on the differentially expressed genes (DEGs) using Soybase [41]. Significant GO terms ($P < 0.05$) were then manually placed into broader categories based on their function. To complement this analysis, DEGs were manually annotated using the PANTHER (Protein ANalysis THrough Evolutionary Relationships) classification system and gene enrichment assessed using Pearson's chi-squared tests ($\chi 2$).

2.3. Targeted metabolomics analyses of soybean leaves

Metabolite extraction. Collection of experimental leaves was done identical to the transcriptional analysis (see above). Leaves were lyophilized, weighed (between 23 and 45 mg DW) and ground in 2 ml tubes containing a 5 mm tungsten bead for 3 min at 30 Hz in a mixer mill. Internal standards were added into each sample tube consisting of 500, 500, and 1000 nmol of U-^{13}C-labeled glucose, glycine and fumarate, respectively. Water-soluble metabolites (sugars, sugar alcohols, amino acids, organic acids and phosphorylated compounds) were extracted using 1 ml of boiling water and incubated for 10 min. Extracts were centrifuged at 14,000 × g for 5 min at 4 °C and the supernatants were filtered

Download English Version:

https://daneshyari.com/en/article/8649215

Download Persian Version:

https://daneshyari.com/article/8649215

Daneshyari.com