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Jasmonic acid causes short- and long-term alterations to the transcriptome and the expression of defense genes in sugarbeet roots

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ABSTRACT

Jasmonic acid (JA) induces native defense responses in plants and increases the resistance of postharvest sugarbeet roots to three common storage-rot causing organisms. To gain insight into the defense responses induced by JA in harvested sugarbeet roots, RNA was isolated from roots treated with water or 10 μM JA and incubated for 2 or 60 d post-treatment. RNA was sequenced, and sequence data was analyzed for short-term (2 d) and long-term (60 d) effects of JA on the sugarbeet root transcriptome. A total of 283 and 326 differentially expressed unigenes were identified in JA-treated roots at 2 and 60 d after treatment, respectively. Of these, >96% were uniquely altered in expression at 2 or 60 d, indicating that long-term JA-induced changes to the sugarbeet root transcriptome were substantially different from short-term JA-induced changes. Categorization of differentially expressed unigenes by gene ontology (GO) or Kyoto Encyclopedia of Genes and Genomes (KEGG) identifiers indicated that JA altered expression of a large number of unigenes involved in metabolism, including many unigenes encoding enzymes and unigenes that participate in secondary metabolism. In addition, 88 unigenes, or 15% of all unigenes whose expression were altered by JA, were putatively involved in plant defense. Included were unigenes for pathogenesis-related proteins, regulatory proteins, plant disease resistance proteins, secondary metabolite biosynthetic enzymes, oxidases, and plant cell wall-modifying proteins. Detailed, time-course expression analysis of 19 JA-altered defense unigenes revealed similarities in expression for three plant disease resistant (R) genes and three laccase genes, indicating possible co-regulation of these genes. No relationships, however, were observed between enzyme activities and transcript levels for any of four major families of upregulated defense genes for which enzyme activities were determined, consistent with JA functioning as a primer, rather than an inducer, of plant defenses. Overall, this research supports JA's role as a signaling molecule for plant defense and provides evidence that a variety of defense mechanisms, including the production of antifungal and antimicrobial compounds, stimulation of antioxidant defenses, and stiffening and strengthening of cell walls, may contribute to JA-induced storage rot resistance in sugarbeet roots.

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1. Introduction

Jasmonic acid (JA) and its derivatives, collectively termed jasmonates, are naturally occurring hormones that function as signaling compounds for the induction of native plant defense responses (Ballaré, 2011; Dar et al., 2015; Pozo et al., 2005). Synthesized in response to physical injury, herbivory, pathogen attack, and environmental stresses including drought, cold, and heat (Creelman and Mullet, 1997; Sharma and Laxmi, 2016; Wasternack, 2014), jasmonates have been found to increase plant tolerance to a variety of biotic and abiotic stresses including fungal, bacterial and viral pathogens, herbaceous and piercing-sucking insects, drought, high and low temperatures, heavy metals, and saline soils (Brossa et al., 2011; Dar et al., 2015; Haggag et al., 2010; Meir et al., 1996; Rohwer and Erwin, 2008). JA-induced tolerance to biotic and

Abbreviations: ABST, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonate); AP2/ERF, APETELA2/ethylene responsive factor; C4H, cinnamate 4-hydroxylase; CC, N-terminal coiled-coil; COG, clusters of orthologous groups; FC, fold change; FDR, false discovery rate; GO, gene ontology; JA, jasmonic acid; KEGG, Kyoto Encyclopedia of Genes and Genomes; MeJA, methyl jasmonate; NBS-LRR, nucleotide-binding site-leucine-rich repeat; POD, peroxidase; PR, pathogenesis-related; PVP-40, polyvinylpyrrolidone-40; R protein, plant disease resistance protein; SA, salicylic acid; SAMT, salicylic acid carboxyl methyltransferase.

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abiotic stresses has been correlated to changes in gene expression or protein concentration for pathogenesis-related proteins (e.g., chitinase, glucanase, and protease inhibitors), enzymes that detoxify reactive oxygen species (e.g., catalase, superoxide dismutase, and peroxidase), cell wall-modifying proteins (e.g., peroxidase and pectin methylesterase), and enzymes involved in the phenylpropanoid pathway and other secondary metabolite biosynthetic pathways. Therefore, jasmonates are thought to protect by promoting the synthesis of proteins and metabolites which are toxic, harmful, or anti-nutritive to insects or plant pathogens, by altering concentrations of reactive oxygen species, and by modifying cell wall properties (Cao et al., 2010; Denness et al., 2011; Ding et al., 2002; Gundlach et al., 1992; Rohwer and Erwin, 2008; Sun et al., 2013a). The combination of genes and proteins that are altered in expression by jasmonates, however, differs between plant species, plant organs, developmental stages, and the environmental conditions under which plants are grown (Bailey et al., 2005; Neller et al., 2016; Parra-Lobato et al., 2009; Rohwer and Erwin, 2008). The defense mechanisms induced by jasmonates, therefore, are dependent on a plant's genetics, age, development, production environment, and the structural organ under investigation.

Sugarbeet (*Beta vulgaris* L.) roots treated with jasmonic acid have improved resistance to the storage pathogens *Botrytis cinerea*, *Penicillium claviforme*, and *Phoma betae* (Fugate et al., 2012). Although susceptible to infection by these pathogens, JA-treated roots restrict the advancement of storage rot symptoms more effectively than untreated roots. The defense mechanisms responsible for this improved resistance are presently unknown. For other postharvest plant products, jasmonate-induced protection against storage rots has been linked to activity increases for enzymes involved in antioxidant defenses, including peroxidases, superoxide dismutases and catalases, and by defense-related proteins such as chitinases and glucanases (Cao et al., 2008; Haggag et al., 2010; Wang et al., 2009; Yao and Tian, 2005). However, no increases in these enzyme activities have been found in JA-treated sugarbeet roots (Ferrareze et al., 2013).

To provide insight into the mechanisms by which JA protects postharvest sugarbeet roots against storage rots, JA-induced alterations in the sugarbeet transcriptome at 2 and 60 d after treatment were determined. Two time points were chosen to identify both short-term and long-term transcriptome changes, since JA affords both rapid and long-lasting protection to sugarbeet roots against storage rots (Fugate et al., 2012). From these transcriptome changes, short- and long-term JA-induced defense genes were identified. A subset of these defense genes, chosen for high levels of expression, large induction by JA, and their putative functions, were characterized for temporal changes in expression and enzyme activity during the 60 d after treatment. From this research, we provide the first description of JA effects on the sugarbeet root transcriptome and identify defense genes that may contribute to storage rot resistance in postharvest roots.

2. Materials and methods

2.1. Plant material and postharvest treatment

Sugarbeet hybrid VDH66156 (SESVanderHave, Tienen, Belgium) was greenhouse grown in Sunshine Mix #1 (Sun Gro Horticulture, Vancouver, Canada) in 15-L pots with supplemental light under a 16 h light/8 h dark regime. Taproots were harvested 16–18 weeks after planting, all leaf and petiole material was removed, and roots were gently washed to remove potting media. Roots were submerged in water or aqueous 10 μM JA (Cayman Chemical, Ann Arbor, MI, USA) for 1 h at room temperature, then stored at 20 °C and 90% relative humidity for up to 60 d in a controlled environment chamber (Conviron, model MTR30, Winnipeg, Canada). JA was readily soluble in water. Root samples were acquired 0, 1, 2, 3, 10, 30, and 60 d post-treatment by collecting tissue from the main portion of the root, free of crown or tail tissue, with the epidermis and approximately 2 mm of subepidermal tissue excluded. Samples were flash frozen in liquid N_2, lyophilized, ground to a powder, and stored at −80 °C. Individual roots were the experimental unit with four replicates per treatment per time point. The experiment was repeated twice.

2.2. RNA isolation

RNA for RNA sequencing experiment was isolated from tissue collected from water and JA-treated roots at 2 and 60 d after treatment. These time points were selected as representative times to evaluate short-term and long-term JA effects. RNA for qRT-PCR experiments was isolated from tissue collected from water and JA-treated roots at 0, 1, 2, 3, 10, 30, and 60 d post-treatment. Both experiments (RNA sequencing and qRT-PCR experiments) used tissue collected from the same roots. Prior to RNA extraction, replicates for each time point and treatment within an experiment were pooled using an equal weight of lyophilized tissue from each root. Total RNA was extracted from 20 mg samples using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) with an on-column DNase digestion. An Agilent Technologies 2100 Bioanalyzer (Pal Alto, CA, USA) was used to confirm RNA quality. Repetitions of the experiment served as replicates for RNA sequencing and qRT-PCR experiments.

2.3. RNA sequencing

RNAs from roots treated with water or 10 μM JA and stored for 2 and 60 d were converted to cDNAs and sequenced. cDNA library preparation and sequencing were performed by BGI Americas (Cambridge, MA, USA). cDNAs were sequenced using an Illumina, Inc. HiSeq 2000 system (San Diego, CA, USA).

2.4. Bioinformatics

Raw sequence data was cleaned to remove reads with adapters, reads with >10% unknown bases, and low quality reads. Clean reads were mapped to a sugarbeet reference transcriptome (Fugate et al., 2014) using SOAPaligner/soap2 (Li et al., 2009). Mismatches of no more than 2 bases were permitted. Differential gene expression between water and JA-treated roots at 2 d post-treatment and between water and JA-treated roots at 60 d post-treatment were determined, using RobiNA software (Lohse et al., 2012). Only unigenes with an absolute value of $\log_2$ (fold change) ≥ 1 and a false discovery rate (FDR) ≤ 0.001 were considered differentially expressed. WEGO software (Ye et al., 2006) was used for functional classification of differentially expressed genes using gene ontology (GO) identifiers. Unigenes were assigned to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways using the Search Pathway module of KEGG Mapper (Kanehisa et al., 2006).

2.5. Quantitative real-time PCR (qRT-PCR)

cDNA was synthesized from total RNA using oligo(dT) primers, dNTPs, and SuperScript III reverse transcriptase (Invitrogen, Foster, CA, USA). Primer pairs were designed with Primer3Plus (Untergasser et al., 2007) as reported in Table 1. qRT-PCR was performed with a MJ Research PTC-200 thermal cycler (Watertown, MA, USA), equipped with a Chromo 4 detector (Bio-Rad Laboratories, Hercules, CA, USA) using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA). Samples were denatured for 10 min at 95 °C and amplified by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. Three replicate qRT-PCR reactions were performed for each gene and the average Ct from the three replicates was used to calculate expression. Expression level changes were determined by the method of Pfaffl (2001), using β-actin (GenBank accession DQ866829) as a reference gene (Li and Smigocki, 2016). Melting curves confirmed that single products were amplified.