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 μ M 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, more than 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.

Keywords: *Beta vulgaris*; gene expression; postharvest; priming; RNA sequencing; storage

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.

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 Lazmi, 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 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 μ M 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 N₂, 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 μ M 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 minutes 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.

2.6. Protein extraction and enzyme activity assays

Laccase, peroxidase (POD), and cinnamate 4-hydroxylase (C4H) activities were determined by the methods of De Marco and Roubelakis-Angelakis (1997), Fugate et al. (2016), and Bi et al. (2007), respectively. Protein extracts were prepared by adding five volumes (w/v) of an extraction buffer to lyophilized tissue. Extraction buffers used were, laccase: 0.1 M sodium acetate, pH 5.0 and 1% polyvinylpyrrolidone-40 (PVP-40); POD: 0.1 M potassium phosphate buffer, pH 7.0, 10 mM sodium bisulfite, and 0.5 M NaCl; C4H: 0.1 M potassium phosphate, pH 7.5 and 2 mM 2-mercaptoethanol. Suspensions of tissue and extraction buffer were sonicated for 15 minutes at 4 °C, filtered over Miracloth (EMD Millipore, Billerica, MA, USA), and centrifuged at 20,000 *g* for 20 minutes at 4 °C. Supernatants were used for laccase, POD and total soluble protein assays. For C4H activity assays, supernatants were passed over Sephadex G-25 columns (GE Life Sciences, Uppsala, Sweden) equilibrated with 0.1 M potassium phosphate, pH 7.5.

Enzyme activities were measured spectrophotometrically using a Shimadzu model UV-1601 dual beam spectrophotometer (Kyoto, Japan). Laccase activity assays contained protein

extract, 0.05 M sodium acetate, pH 5.0, 0.03% (w/v) 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonate) (ABST), 0.1 mM tropolone, and 60 U mL⁻¹ catalase. Activity was determined by the change in absorbance at 420 nm after incubation at 25 °C for 0.5 h. POD activity assays contained protein extract, 0.1 M potassium phosphate, pH 6.5, 10 mM guaiacol, and 4 mM hydrogen peroxide. Activity was determined at 25 °C using the maximum change in absorbance at 470 nm during the first 3 minutes of the reaction and an extinction coefficient of 26.6 mM⁻¹ cm⁻¹ (Koduri and Tien, 1995). C4H activity assays contained desalted protein extract, 50 mM potassium phosphate, pH 7.5, 2 mM 2-mercaptoethanol, 2 mM *trans*-cinnamic acid, and 0.5 mM NADPH. Reactions were incubated at 37 °C for 1 h and terminated by lowering the pH to 1.0 with 6 N HCl. Absorbance at 340 nm was measured after adjusting the pH to 11 with 6 N NaOH, and activity was determined by the change in absorbance relative to controls that lacked *trans*-cinnamic acid using a *p*-coumaric acid standard curve. Total soluble protein concentrations were determined using Bio-Rad Protein Assay Reagent with bovine serum albumin as a standard.

3. Results

3.1. Differential expression of sugarbeet root unigenes

Postharvest treatment with 10 µM JA caused both short-term and long-term alterations to the sugarbeet root transcriptome. The cDNA libraries generated from water and JA-treated roots at 2 and 60 d after treatment contained an average of 72,955 expressed unigenes per

library and ranged from 72,150 to 73,647 unigenes per library. From these libraries, 283 differentially expressed unigenes were identified in JA-treated roots 2 d after treatment, and 326 differentially expressed unigenes were identified in JA-treated roots 60 d after treatment (Table 2). Of the differentially expressed unigenes, approximately 65% were up-regulated and 35% were down-regulated by JA at both 2 and 60 d. From a BLASTx search against GenBank nr, Swiss-Prot, COG, and KEGG protein databases (Fugate et al., 2014), 139 (49%) of the differentially expressed unigenes 2 d after JA treatment and 178 (54%) of the differentially expressed unigenes 60 d after treatment were annotated. Most annotations indicated putative functions for unigenes. However, approximately 14% of unigene annotations at both time points were to proteins of unknown function.

Transcriptome changes occurring 2 d after treatment were generally different from those occurring at 60 d (Fig. 1). Greater than 96% of differentially expressed unigenes were uniquely altered in expression at 2 d or 60 d after JA treatment, with only 9 unigenes up-regulated and 12 unigenes down-regulated at both time points. The dissimilarity in differentially expressed unigenes for JA-treated roots at 2 and 60 d indicates that long-term JA effects were substantially different from short-term effects and were not merely a continuation of early JA-induced changes.

The magnitude of JA-induced gene expression changes varied from a logarithm (base 2) of the fold change in expression or log (FC) of -13 to +11 (Fig. 2), equivalent to expression changes of 0.0001 to 2050-fold. The majority of differentially expressed genes were moderately altered in expression; nearly 80% of differentially expressed unigenes had log (FC) values of -5

to +5. Differentially expressed unigenes at 2 d and 60 d after JA treatment, with annotations and expression level changes, are available (Tables 1 and 2, in Oliveira et al., in press).

3.2. Functional classification of differentially expressed unigenes

3.2.1. Gene ontology classifications

A total of 61 and 72 differentially expressed unigenes from roots 2 and 60 d after JA treatment, respectively, had associated gene ontology terms, and these were used to categorize unigenes by the biological process or molecular function to which they contribute or the cellular component to which they localize (Fig. 3). Among biological processes, the greatest number of GO-annotated unigenes at both 2 and 60 d after treatment were functionally assigned to metabolic processes (30 unigenes at 2 d; 37 unigenes at 60 d), a functional class that includes genes involved in metabolism at the organismal level. Cellular processes, comprised of genes involved in metabolism at the cellular level, was the second most populated category within biological processes at both time points (21 unigenes at 2 d; 22 unigenes at 60 d). A total of 12 and 14 GO-annotated unigenes at 2 and 60 d after JA treatment were categorized into the response to stimulus classification. This classification contains genes that function in the detection of and response to internal and external stimuli and includes genes involved in plant response to chemicals, hormones, and biotic and abiotic stresses.

Among molecular functions, 62 and 64% of GO-annotated unigenes, 2 and 60 d after JA treatment, respectively, were categorized as having catalytic activity, indicating a large portion

of GO-annotated unigenes were putatively enzymes. Many of these unigenes were also categorized as having binding activity, and 56 and 65% of GO annotated unigenes, 2 and 60 d after JA treatment, respectively, were categorized as having binding activity, a classification that includes genes with regulatory functions as well as some enzymes. Jasmonate treatment is often associated with increases in antioxidant defenses (Jung, 2004; Salimi et al., 2016; Sasaki-Sekimoto et al., 2005). However, only four differentially expressed, GO-annotated unigenes at both 2 and 60 d after JA treatment were classified as having antioxidant activity. Among cellular components, a large number of unigenes were unsurprisingly assigned to the cell (41 unigenes at 2 d; 32 unigenes at 60 d) or cell part (41 unigenes at 2 d; 32 unigenes at 60 d).

3.2.2. Kyoto Encyclopedia of Genes and Genomes pathway mapping

A total of 67 and 86 differentially expressed unigenes from roots at 2 and 60 d after JA treatment, respectively, had associated Kyoto Encyclopedia of Genes and Genomes identifiers. These identifiers were used to map differentially expressed unigenes to KEGG pathways. Differentially expressed unigenes in roots 2 d after JA treatment mapped to 41 KEGG pathways (Table 3). Differentially expressed unigenes in roots 60 d after JA treatment were more diverse and mapped to 50 KEGG pathways (Table 4). At both 2 and 60 d after JA treatment, 28% of the KEGG-annotated, differentially expressed unigenes were functionally assigned to metabolic pathways, a broad classification that includes enzymes involved in primary and secondary metabolism. In roots 2 and 60 d after JA treatment, 18 and 37% of KEGG-annotated,

differentially expressed unigenes, respectively, were assigned to the biosynthesis of secondary metabolites.

Differentially expressed unigenes mapped to several metabolic pathways involved in plant defense. In general, more differentially expressed unigenes from roots 60 d after JA treatment mapped to putative defense-related pathways than unigenes from roots 2 d after JA treatment. Putative defense-related pathways to which more than a single unigene mapped included plant-pathogen interaction (8 unigenes at 2 d; 7 unigenes at 60 d), phenylpropanoid biosynthesis (5 unigenes at 2 d; 9 unigenes at 60 d), phenylalanine metabolism (4 unigenes at 2 d; 9 unigenes at 60 d), flavonoid biosynthesis (1 unigenes at 2 d; 10 unigenes at 60 d), stilbenoid, diarylheptanoid and gingerol biosynthesis (1 unigenes at 2 d; 8 unigenes at 60 d), ubiquinone and other terpenoid-quinone biosynthesis (2 unigenes at 2 d; 4 unigenes at 60 d), glutathione metabolism (4 unigenes at 60 d), flavone and flavonol biosynthesis (3 unigenes at 60 d), and terpenoid backbone biosynthesis (3 unigenes at 60 d).

3.2.3. Putative defense-related unigenes

Using annotations, GO classifications, and KEGG pathway mapping, differentially expressed unigenes with putative roles in plant defense were identified. From the differentially expressed unigenes 2 d post-treatment, 30 up-regulated and 8 down-regulated putative defense unigenes were identified (Table 5), while at 60 d post-treatment, 49 up-regulated and 4 down-regulated putative defense unigenes were found (Table 6). Of these putative defense unigenes, only three were differentially expressed at both time points: an up-regulated unigene

with homology to cinnamate 4-hydroxylase (unigene 30677), a down-regulated unigene with homology to a gene for an *Arabidopsis thaliana* DNA binding protein (unigene 22684), and a down-regulated unigene (unigene 77351) with homology to *Citrus trifoliata* CTV.20, a gene involved in plant response to the *Citrus tristeza virus* (CTV).

At least 25% of unigenes with putative defense roles had annotations indicating involvement in secondary metabolite biosynthesis (6 unigenes at 2d; 17 unigenes at 60 d). Another 24% of defense-related unigenes encoded putative regulatory proteins (9 unigenes at 2d; 12 unigenes at 60 d). These putative regulatory proteins included nucleotide-binding site-leucine-rich repeat (NBS-LRR) and N-terminal coiled-coil (CC) NBS-LRR family proteins which encode plant disease resistance (R) proteins, NAC domain proteins, WRKY transcription factors, ribosome-inactivating proteins, leucine-rich repeat proteins, protein kinase family proteins, and an APETELA2/ethylene responsive factor (AP2/ERF) domain-containing transcription factor. Unigenes that encode pathogenesis-related (PR) proteins accounted for 17% of differentially expressed, defense-related unigenes and were more prevalent at 60 d than at 2 d post-treatment (3 unigenes at 2 d; 12 unigenes at 60 d). Unigenes with putative PR functions encoded thaumatin-like proteins (3 unigenes), chitinases (6 unigenes), β -1,3-glucanases (2 unigenes), a proteinase inhibitor (1 unigene), a PR-10a protein (1 unigene), an oxalate oxidase-like germin (1 unigene), and a PR protein of unknown function (1 unigene). Differentially expressed unigenes annotated as having oxidase activity were also common in roots at both 2 and 60 d after JA treatment and comprised 15% of the unigenes with putative defense-related functions (9 unigenes at 2 d; 4 unigenes at 60 d). Seven laccase and two peroxidase annotated unigenes were up-regulated in roots 2 d after JA treatment; three peroxidase and one oxalate

oxidase annotated unigenes were up-regulated in roots 60 d after JA treatment. Five percent of differentially expressed defense-related unigenes were involved in cell wall modifications (1 unigene at 2 d; 3 unigenes at 60 d). These unigenes were annotated as encoding three pectinesterases and one glycine-rich protein.

3.3. Temporal expression of prominent defense unigenes using qRT-PCR

Changes in gene expression during the 60 d post-JA incubation period were determined for 19 of the upregulated defense-related unigenes identified by RNA sequencing (Tables 5 and 6). Unigenes were selected based on their level of expression, extent of induction by JA, and putative function. Since unigenes are sequence assemblies that are typically a portion of a full length gene, some unigenes mapped to the same gene, and the 19 selected unigenes were homologous to 12 unique genes. Encoded by these 12 genes, were three plant disease R proteins, three putative PR proteins, two enzymes with prominent roles in secondary metabolism, and four oxidase enzymes.

Unigenes 9115, 10601 and 77250, and 29351, which were annotated as CC-NBS-LRR or NBS-LRR proteins, were generally upregulated in the 60 d after JA treatment (Fig. 4A-C). Encoding putative plant disease R proteins that are involved in pathogen detection (Belkhadir et al., 2004; DeYoung and Innes, 2006), the three genes for CC-NBS-LRR and NBS-LRR proteins were most highly expressed 2 d after JA treatment. Elevated expression continued for 60 d for genes encoding the CC-NBS-LRR resistance protein and NBS-LRR resistance protein 2 and continued for 30 d for the gene encoding NBS-LRR resistance protein 1.

Expression profiles of three putative pathogenesis-related genes revealed few similarities in the effect of JA on their expression (Fig. 4D-F). A putative PR thaumatin-family protein, encoded by unigene 81205, was transiently elevated in expression 2 and 3 d after JA treatment and was relatively unchanged in expression 1, 10, 30, and 60 d post-treatment. An acidic chitinase, encoded by unigene 71046, was upregulated 2 and 60 d after JA treatment and was unaltered in expression at all other time points. Another chitinase, acidic chitinase SE2, encoded by unigenes 53522, 66160 and 70896, was down-regulated 1 d after JA treatment, unaltered in expression 2 to 30 d post-treatment, and significantly upregulated 60 d post-treatment.

Four JA-induced oxidases, including three laccase genes and one peroxidase gene, shared similarities in their expression profiles (Fig. 5A-D). All four oxidase genes were maximally upregulated in the first 3 d after JA treatment. The peroxidase encoded by unigenes 42562 and 66692 was most highly upregulated 2 d after JA treatment; the three laccase genes, encoded by unigenes 11281 (laccase 1), 51666, 67039 and 68954 (laccase 2), and 78937 (laccase 3), were most highly upregulated 3 d after treatment. For all four oxidases, up-regulation by JA ceased by 10 d. No significant induction of any of the oxidases was observed with continued incubation time, except for transient increases in laccase 1 expression at 60 d post-treatment and laccase 3 expression at 30 d post-treatment.

Cinnamate 4-hydroxylase (C4H) catalyzes the conversion of *trans*-cinnamic acid to *p*-coumaric acid, a key reaction in the early steps of the phenylpropanoid pathway which leads to the production of lignins, flavonoids, coumarins, isoflavonoids, and a variety of other plant defense compounds (Lu et al., 2006). The JA-induced C4H gene identified by RNA sequencing

(unigenes 30677 and 55958) was up-regulated 2, 3 and 60 d after JA treatment with maximal up-regulation at 60 d (Fig. 5E). Salicylic acid carboxyl methyltransferase (SAMT) catalyzes the methylation of salicylic acid (SA) to methyl salicylate to create a volatile SA metabolite that can decrease endogenous SA levels and serve as an airborne signal to prime SA-induced defenses in adjacent plants (Koo et al. 2007; Shulaev et al., 1997). The JA-induced SAMT gene identified by RNA sequencing (unigene 29506) was down-regulated 1 d after JA treatment, but was up-regulated 2-60 d post-treatment, with maximal expression observed 2-3 d after treatment (Fig. 5F).

3.4. Enzyme activities for JA-induced defense genes

Enzymatic activities of laccase, peroxidase, chitinase, and cinnamate 4-hydroxylase were determined to evaluate the extent that transcriptional changes in the genes encoding for these proteins led to changes in their activities. Despite significant increases in laccase transcripts 1-3 d after JA treatment (Fig. 5A-C), laccase activity was below the level of detection at 1, 2, 3, 10, 30, and 60 d post-treatment (data not shown). POD activity was measured with high salt-containing extracts that were expected to contain both soluble and cell wall peroxidase proteins (Fry, 1988). While POD transcripts increased 2-3 d after JA treatment (Fig. 5D), POD activity was unaltered from that of water-treated controls throughout the 60 d after treatment (Fig. 6A). Chitinase activity in JA-treated roots was measured previously and was unchanged at 1, 2, 3, 10, and 60 d after JA treatment and reduced 30 d after treatment relative to water-treated roots (Ferrareze et al., 2013). In contrast, chitinase gene expression decreased 1 d after

JA treatment and increased 2 and 60 d after JA treatment (Fig. 4E-F). Similar to laccase, POD, and chitinase, JA-induced changes in C4H gene expression did not lead to similar changes in C4H activity. C4H activity was present at low and relatively unchanging levels in both JA and water-treated roots throughout the 60 d after JA treatment (Fig. 6B). However, C4H expression increased 2, 3, and 60 d after treatment in JA-treated roots relative to water-treated controls (Fig. 5E).

4. Discussion

Postharvest treatment with JA induced significant short-term and long-term changes to the transcriptome of sugarbeet roots. Two days after JA treatment, 283 unigenes were identified that were altered in their expression due to JA treatment. Sixty days after treatment, 326 differentially expressed unigenes were identified. Alterations to the transcriptome during the first 7 d after JA or methyl jasmonate (MeJA) treatment have been reported previously for a variety of plant species (Babst et al, 2009; Gális et al, 2006; Hao et al., 2015; Oliveira et al., 2015; Ramírez-Estrada et al., 2016; Salzman et al., 2005; Sasaki-Sekimoto et al., 2005; Sun et al, 2013b). However, to our knowledge, this is the first report of a jasmonate treatment causing long-term transcriptomic changes. Nevertheless, jasmonates are responsible for long-term physiological effects on sugarbeet roots and other harvested plant organs, including improved disease resistance in sugarbeet roots and pears stored for 50 and 60 d, respectively (Fugate et al., 2012; Zhang et al., 2009), and improved resistance to chilling injury in cherry tomato, peach and loquat fruits stored for 21, 35, and 35 d, respectively (Cai et al., 2011; Jin et al., 2009; Zhang

et al., 2012). Long-term, JA-induced changes in the transcriptome may play a role in these long-term, jasmonate-induced physiological effects.

Fifty-four percent of JA-altered unigenes were unannotated or encoded proteins with no known function. The remaining 46% of JA-altered unigenes were annotated and were predominantly involved in plant metabolism. When JA-altered unigenes were classified into the biological processes to which they contribute using GO terms, metabolic processes and cellular processes were the two most highly populated categories. These categories contain genes that are involved in plant metabolism at the organismal and cellular level. Classification of JA-altered unigenes by molecular function based on GO annotations demonstrated a predominance of unigenes with catalytic and binding functions, indicating that JA affected the expression of numerous enzymes and regulatory proteins. KEGG pathway analysis similarly indicated a preponderance of JA-altered unigenes involved in metabolism, especially secondary metabolism, as a large number of unigenes mapped to metabolic pathways, a category that includes enzymes involved in primary and secondary metabolism, and to the biosynthesis of secondary metabolites pathway. Large changes in the transcriptome due to exogenous JA or MeJA treatment have been previously reported for a number of plant species (Babst et al, 2009; Gális et al, 2006; Hao et al., 2015; Lee and Choi, 2013; Oliveira et al., 2015; Ramírez-Estrada et al., 2016; Salzman et al., 2005; Sasaki-Sekimoto et al., 2005; Sun et al, 2013b; Zang et al., 2015). As in sugarbeet root, cellular and molecular processes, and binding and catalytic functions typically dominate GO classifications, and metabolic pathways and biosynthesis of secondary metabolites generally predominate as the KEGG pathways to which JA-altered genes map (Oliveira et al., 2015; Zang et al., 2015).

JA treatment of sugarbeet roots altered the expression of many unigenes involved in plant defense. A total of 88 unigenes, or 15% of all JA-altered unigenes, were assigned putative roles in plant defense, including unigenes that encoded pathogenesis-related proteins, plant disease resistance proteins, enzymes involved in secondary metabolite biosynthesis, oxidases, and plant cell wall-modifying proteins. The variety of JA-induced defense genes in sugarbeet indicates JA's potential to protect roots by multiple mechanisms including production of antifungal and antimicrobial compounds, stimulation of antioxidant defenses, and stiffening and strengthening of cell walls. Elevated expression of genes encoding PR proteins, and enzymes involved in secondary metabolite biosynthesis in response to jasmonate elicitation is common in plants, and reports of JA or MeJA inducing genes for oxidases, cell wall modifying enzymes, and R proteins can be found (Bailey et al., 2005; Ding et al., 2002; Gális et al., 2006; Hao et al., 2015; Oliveira et al., 2015; Salzman et al., 2005; Sun et al., 2013b; Zang et al., 2015). The collection of defense genes induced by a postharvest JA treatment, however, was unique to sugarbeet roots.

JA induced a number of defense-related regulatory proteins, including an APETELA2/ethylene responsive factor (AP2/ERF) domain-containing transcription factor and a gene for salicylic acid carboxyl methyltransferase (SAMT), an enzyme that regulates active SA concentrations and is involved in plant-to-plant SA signaling (Koo et al., 2007; Shulaev et al., 1997). Upregulation of regulatory genes was expected because of JA's well-established role as a signaling compound for plant defenses (Ballaré, 2011; Wasternack and Hause, 2013), while alteration of AP2/ERF and SAMT expression suggests interactions between JA, ethylene, and SA-regulated pathways in sugarbeet root. Cross-talk between jasmonates, ethylene, and

salicylate in the elicitation of plant defenses has been reported in other plant species (Koornneef and Pieterse, 2008; Thaler et al., 2012).

Long-term changes in the sugarbeet transcriptome due to JA treatment were distinctly different from short-term changes. Of the 578 unigenes that were altered in expression, only 21 unigenes, or 3.6% of all unigenes, were commonly altered in expression at both 2 and 60 d. Long-term effects of JA on the sugarbeet root transcriptome, therefore, were not a prolongation of transcriptome changes from 2 d. In general, differentially expressed unigenes were more diverse at 60 d than at 2 d, as JA-altered unigenes mapped to 22% more KEGG pathways at 60 d after treatment than at 2 d after treatment. Additionally, upregulated defense unigenes were more abundant at 60 d post-treatment, and approximately 60% more differentially expressed defense unigenes were identified at 60 d than at 2 d. Unigenes that encode PR proteins and enzymes involved in the biosynthesis of secondary metabolites were induced by JA at both 2 and 60 d after treatment. However, unigenes for PR proteins were 3-fold more abundant at 60 d and unigenes that participate in secondary metabolite biosynthesis were nearly 2-fold more abundant at 60 d. In contrast, unigenes encoding oxidases, especially those for laccases, were more numerous at 2 d after JA treatment than at 60 d. Differentially expressed unigenes for regulatory proteins were approximately evenly distributed between 2 and 60 days post-treatment. However, plant disease resistance proteins were more abundant at 2 d after JA treatment, and WRKY transcription factors, which have been identified as key regulators of plant defense responses to biotic and abiotic stresses (Chen et al., 2012; Pandey and Somssich, 2009), were differentially expressed only at 60 d.

Determination of changes in expression for a number of defense-related genes throughout the 60 d storage period revealed similarities in expression for some defense genes. Most notable were similarities in expression for three putative R protein-encoding genes and three laccase genes. Three genes for R proteins, i.e., CC-NBS-LRR resistance protein, NBS-LRR resistance protein 1, and NBS-LRR resistance protein 2, all exhibited the greatest induction 2 d after JA treatment and were generally up-regulated throughout the 60 d after JA treatment. The three laccase genes, i.e., laccase 1, laccase 2, and laccase 3, were upregulated 2 and 3 d after JA treatment, exhibited the greatest upregulation at 3 d post-treatment, and were no longer upregulated by 10 d post-treatment. Similarities in expression among sugarbeet root defense genes may be coincidental. However, similarities in expression have often been interpreted as signs of co-regulation of genes (Gachon et al., 2005; Yu et al., 2003). In contrast, no similarities in expression were noted for the PR-protein encoding genes for which time-courses were generated, suggesting there was no co-regulation of these genes.

Determination of enzyme activities for four major families of upregulated defense genes indicated that JA-induced alterations in gene expression did not cause similar alterations in protein activity. RNA sequencing identified five laccase genes, five peroxidase genes, three chitinase genes, and one cinnamate 4-hydroxylase gene that were upregulated 2 or 60 d after JA treatment. However, in the 60 d following JA treatment, laccase activity was undetectable, peroxidase and chitinase activities were not elevated over activities of untreated roots, and C4H exhibited minor fluctuations in activity that bore no relationship to changes in C4H gene expression. The discrepancy between gene expression and enzyme activity suggests that JA probably functions as a primer, rather than an inducer, of plant defenses for these four families

of genes (Conrath et al., 2002). As a primer, JA does not directly induce plant defense responses, but alters the plant's molecular machinery to allow for a more rapid and vigorous induction of defenses upon subsequent challenge with a biotic or abiotic stress (Conrath, 2011). Elevation in transcript levels for defense-related genes, as occurred for laccase, peroxidase, chitinase and C4H genes in JA-treated sugarbeet root, is documented as a priming mechanism for plant defense responses (Christou et al., 2013; 2014; Savvides et al., 2016).

Postharvest JA treatment of sugarbeet roots restricts the progression of storage rot caused by *B. cinerea*, *P. claviforme*, and *P. betae* (Fugate et al., 2012). Concurrent with that protection, we report here that JA causes major changes to the sugarbeet root transcriptome and alters the expression of many genes with putative roles in plant defense. The mechanisms by which JA protects postharvest sugarbeet roots against storage rots remains to be established. However, upregulation of genes for PR proteins, enzymes involved in the biosynthesis of secondary metabolites, oxidases, and cell wall-modifying proteins, suggests that JA affords protection by stimulating production of antifungal and antimicrobial compounds, inducing antioxidant defenses, and by stiffening and strengthening cell walls. Upregulation of genes for regulatory proteins, including plant disease resistance genes and genes involved in ethylene and SA signaling and metabolism, indicate that JA potentially protects sugarbeet roots by promoting their ability to recognize and respond to plant pathogens and through interaction with ethylene and SA-induced defense-related pathways. The protection afforded by JA to postharvest sugarbeet roots occurs quickly, is durable for at least 50 d (Fugate et al., 2012), and coincided with both short-term and long-term changes in gene expression. Long-term transcriptome changes, however, were distinctly different from short-term transcriptome

changes, suggesting that the protection afforded by JA shortly after treatment differs from the protection provided months later. Additionally, evidence presented here suggests that JA acts as a primer, rather than an inducer, of plant defenses in sugarbeet root, as changes in gene expression did not result in similar changes in enzyme activity.

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Figure captions

Fig. 1. Heterogeneity of differentially expressed unigenes in sugarbeet roots treated with jasmonic acid (JA) and stored for 2 or 60 d. Venn diagrams display the diversity in **(A)** up-regulated unigenes from roots 2 d and 60 d after JA treatment and **(B)** down-regulated unigenes from roots 2 d and 60 d after JA treatment. Roots were treated with 10 μ M JA or water following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d. Differentially expressed unigenes between JA and water-treated roots were determined by RNA-sequencing.

Fig 2. Distribution in the magnitude of changes in gene expression for differentially expressed unigenes from sugarbeet roots 2 and 60 d after jasmonic acid (JA) treatment. The magnitude change in gene expression was determined as the $\log_2$ (fold change) in the number of RNAs sequenced from JA-treated roots relative to water-treated roots for each unigene. Roots were treated with 10 μ M JA or water following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d.

Fig. 3. Classification of differentially expressed sugarbeet unigenes due to jasmonic acid (JA) treatment using gene ontology (GO) terms **(A)** 2 d after JA treatment and **(B)** 60 d after JA treatment. Roots were treated with 10 μ M JA or water following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d.

Fig. 4. Effect of jasmonic acid (JA) on expression of defense-related unigenes with homology to three putative regulatory proteins and three pathogenesis-related (PR) proteins in the 60 d following treatment. Differential expression was determined for unigenes encoding a coiled-coil (CC)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR) resistance protein (unigene 9115), two NBS-LRR resistance proteins (unigenes 10601 and 77250; 29351), a PR thaumatin family protein (unigene 81205), and two acidic chitinases (unigenes 71046; 53522, 66180, and 70896). Roots were treated with 10 μ M JA or water (control) following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d. Differential expression was determined by qRT-PCR. Alterations in fold regulation less than |2.0| were considered insignificant and are indicated by gray bars (Holstege et al., 1998).

Fig. 5. Effect of jasmonic acid (JA) on expression of defense-related unigenes with homology to four oxidase-encoding unigenes and two unigenes with prominent roles in secondary metabolite biosynthesis. Differential expression was determined for unigenes encoding three putative laccase proteins (unigenes 11281; 51666, 67039, and 68954; 78937), a peroxidase protein (unigenes 42562 and 66692), a cinnamate 4-hydroxylase (unigenes 30677 and 55958), and a salicylic acid carboxyl methyltransferase (unigene 29506). Roots were treated with 10 μ M JA or water (control) following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d. Differential expression was determined by qRT-PCR. Alterations in fold regulation less than |2.0| were considered insignificant and are indicated by gray bars (Holstege et al., 1998).

Fig. 6. Effect of jasmonic acid on (A) peroxidase (POD) and (B) cinnamate 4-hydroxylase (C4H) activities. Roots were treated with 10 μ M JA or water (control) following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d. Activities are expressed as μ mole product formed per g protein per minute. Data are the mean $\pm$ SE of the mean, with n = 8.

Fig. 5

Fig. 6

Table 1

Primer sequences used for qPCR analysis of select sugarbeet genes. Unigene numbers correspond to the unigenes identified in a sugarbeet transcriptome (Fugate, 2013; Fugate et al., 2014). Multiple unigene numbers are presented for unigenes that coalign to different portions of the same gene as determined by alignment of unigenes to the sugarbeet genome (Dohm et al., 2014).

Gene	Accession/Unigene number	Forward primer	Reverse primer
β -actin	DQ866829	GATTTGGCACACACCTTCT	TCTTTCCCTGTTTGCCTTG
acidic chitinase	71046	CTGGATTCGGGGTAACCATA	ACGCTGCTAGCTAGGCTGAA
acidic chitinase SE2	53522, 66180, 70896	CATCAAACATGCCAACAGG	CTGGACTGACCCCAAGATA
CC-NBS-LRR resistance protein	9115	TGGGCTACTGGCTACTGCTT	GCTTGGATCATCTCCATGCT
cinnamate 4-hydroxylase	30677, 55958	GGTTGGCTAACAACCCTGAA	CTCCTCCTCCAACACCAAA
laccase I	11281	CCAAGCCACACCACTTACCT	CGTTTATGGAAGGGAGACGA
laccase II	51666, 67039, 68954	AAGGACAGGGTGGTCAGATG	CCAAGTTTGGGCAAGATGAC
laccase III	78937	CCATCATATTTGGGGAGTGG	CAAACCTTTAGCTCCCATGC
NBS-LRR resistance protein 1	10601, 77250	GATTGGGAGAAGGCGTACAA	ATCAATCCCTCAGCAATCCA
NBS-LRR resistance protein 2	29351	GGGTAAGAGAGTTGCCAAGC	TCCACAAGTGCAGAAGTTCCG
pathogenesis-related thaumatin family protein	81205	GCCGGTGTCTGTCTTAGTCC	CCACCTCCATCCTTGACTTG
peroxidase	42562, 66692	CTGCTCAAGCAACCAAAACC	TAGGCGAGCAGGGACTTTAG
salicylic acid carboxyl methyltransferase	29506	GAGCTGTGGTTAGCCCTTA	TTCGTCAGCCTCGACTTCTT

Table 2

Frequency of differentially expressed unigenes in sugarbeet roots 2 and 60 d after jasmonic acid (JA) treatment. Roots were treated with 10 μ M JA or water and stored at 20 °C for 2 or 60 d after treatment. Differentially expressed unigenes were identified by RNA sequencing. Values in parentheses are the percentage of the total number of differentially expressed unigenes. Unigenes with no BLAST match were those that returned no matches from a BLASTx search of the gene sequence against the GenBank nr database. Unknown function denotes unigenes that matched a gene sequence within GenBank for a protein of unknown function.

	2 d	60 d
total number of unigenes	283	326
up-regulated (%)	182 (64)	215 (66)
no BLAST match	69 (24)	74 (23)
BLAST match	113 (40)	141 (43)
unknown function	10 (4)	15 (5)
down-regulated (%)	101 (36)	111 (34)
no BLAST match	75 (27)	74 (23)
BLAST match	26 (9)	37 (11)
unknown function	9 (3)	10 (3)

Table 3

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to which unigenes that were differentially expressed in sugarbeet roots 2 d after jasmonic acid (JA) treatment were assigned. Roots were treated with 10 μ M JA or water for 1 h and incubated for 2 d at 20 °C. Differential expression of unigenes was determined by RNA sequencing.

Pathway	KEGG ID	KEGG annotation (unigene number)
Metabolic pathways	K00134	glyceraldehyde 3-phosphate dehydrogenase (41959)
	K00423	L-ascorbate oxidase (11281, 29708, 67039, 68954, 77135, 78937)
	K00430	peroxidase (34661, 42562, 66692)
	K00487	trans-cinnamate 4-monooxygenase (30677)
	K00826	branched-chain amino acid aminotransferase (29499)
	K01051	pectinesterase (80875)
	K01113	phoD; alkaline phosphatase D (25607)
	K01897	long-chain acyl-CoA synthetase (30836)
	K04120	ent-copalyl diphosphate synthase (30930)
	K05350	beta-glucosidase (55314)
	K08248	mandelonitrile lyase (75893)
	K09833	homogentisate phytyltransferase / homogentisate geranylgeranyltransferase (71638)
Biosynthesis of secondary metabolites	K00134	glyceraldehyde 3-phosphate dehydrogenase (41959)
	K00430	peroxidase (34661, 42562, 66692)
	K00487	trans-cinnamate 4-monooxygenase (30677)
	K00826	branched-chain amino acid aminotransferase (29499)
	K01183	chitinase (71046)
	K04120	ent-copalyl diphosphate synthase (30930)
	K05350	bglB; beta-glucosidase (55314)
	K08248	mandelonitrile lyase (75893)
	K09833	homogentisate phytyltransferase / homogentisate geranylgeranyltransferase (71638)
	K11821	desulfoglucosinolate sulfotransferase A/B/C (66725)
Plant-pathogen interaction	K05391	cyclic nucleotide gated channel, other eukaryote (576, 76243)
	K13430	serine/threonine-protein kinase PBS1 (14728)
	K13457	disease resistance protein (6185, 7601, 10601, 29351, 77250)
Phenylpropanoid biosynthesis	K00430	peroxidase (34661, 42562, 66692)
	K00487	trans-cinnamate 4-monooxygenase (30677)
	K05350	beta-glucosidase (55314)
Plant hormone signal transduction	K13946	auxin influx carrier (16917, 26919, 78540)
	K14492	two-component response regulator ARR-A family (20004)
	K14493	gibberellin receptor GID1 (72228)
Phenylalanine metabolism	K00430	peroxidase (34661, 42562, 66692)
	K00487	trans-cinnamate 4-monooxygenase (30677)
Starch and sucrose metabolism	K01051	pectinesterase (80875)
2-Oxocarboxylic acid metabolism	K05350	bglB; beta-glucosidase (55314)
	K00826	branched-chain amino acid aminotransferase (29499)
Ubiquinone and other terpenoid-quinone biosynthesis	K11821	desulfoglucosinolate sulfotransferase A/B/C (66725)
	K00487	trans-cinnamate 4-monooxygenase (30677)
	K09833	homogentisate phytyltransferase / homogentisate geranylgeranyltransferase (71638)
Cyanoamino acid metabolism	K05350	beta-glucosidase (55314)
	K08248	mandelonitrile lyase (75893)
Biosynthesis of amino acids	K00134	glyceraldehyde 3-phosphate dehydrogenase (41959)
	K00826	branched-chain amino acid aminotransferase (29499)
ABC transporters	K05658	ATP-binding cassette, subfamily B (MDR/TAP), member 1 (6688)
	K05681	ATP-binding cassette, subfamily G (WHITE), member 2 (78801, 81041)
Folate biosynthesis	K01113	alkaline phosphatase D (25607)

Tryptophan metabolism	K11821	ST5A_B_C; desulfoglucosinolate sulfotransferase A/B/C (66725)
Pentose and glucuronate interconversions	K01051	pectinesterase (80875)
Carbon fixation in photosynthetic organisms	K00134	glyceraldehyde 3-phosphate dehydrogenase (41959)
Pantothenate and CoA biosynthesis	K00826	branched-chain amino acid aminotransferase (29499)
Valine, leucine and isoleucine degradation	K00826	branched-chain amino acid aminotransferase (29499)
Other glycan degradation	K01191	alpha-mannosidase (29022)
Flavonoid biosynthesis	K00487	trans-cinnamate 4-monooxygenase (30677)
Pyruvate metabolism	K00102	D-lactate dehydrogenase (cytochrome) (1282)
Fatty acid degradation	K01897	long-chain acyl-CoA synthetase (30836)
Spliceosome	K12823	ATP-dependent RNA helicase DDX5/DBP2 (19671)
Valine, leucine and isoleucine biosynthesis	K00826	branched-chain amino acid aminotransferase (29499)
Metabolism of xenobiotics by cytochrome P450	K00799	GST; glutathione S-transferase (25150)
Diterpenoid biosynthesis	K04120	ent-copalyl diphosphate synthase (30930)
Glutathione metabolism Fatty acid metabolism	K00799	glutathione S-transferase (25150)
Fatty acid metabolism	K01897	long-chain acyl-CoA synthetase (30836)
Glycolysis / Gluconeogenesis	K00134	glyceraldehyde 3-phosphate dehydrogenase (41959)
Lysosome	K13289	cathepsin A (carboxypeptidase C) (25408, 38313, 52307, 56439, 61227, 80067, 80285)
Fatty acid biosynthesis	K01897	long-chain acyl-CoA synthetase (30836)
Stilbenoid, diarylheptanoid and gingerol biosynthesis	K00487	trans-cinnamate 4-monooxygenase (30677)
Degradation of aromatic compounds	K00487	trans-cinnamate 4-monooxygenase (30677)
Peroxisome	K01897	long-chain acyl-CoA synthetase (30836)
Ascorbate and aldarate metabolism	K00423	L-ascorbate oxidase (11281, 29708, 67039, 68954, 77135, 78937)
Glucosinolate biosynthesis	K11821	desulfoglucosinolate sulfotransferase A/B/C (66725)
Carbon metabolism	K00134	glyceraldehyde 3-phosphate dehydrogenase (41959)
Base excision repair	K01246	DNA-3-methyladenine glycosylase I (18548)
Amino sugar and nucleotide sugar metabolism	K01183	chitinase (71046)
Aminobenzoate degradation	K01113	alkaline phosphatase D (25607)
Two-component system	K01113	alkaline phosphatase D (25607)

Table 4

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways to which unigenes that were differentially expressed in sugarbeet roots 60 d after jasmonic acid (JA) treatment were assigned. Roots were treated with 10 μ M JA or water for 1 h and incubated for 60 d at 20 °C. Differential expression of unigenes was determined by RNA sequencing.

Pathway	KEGG ID	KEGG annotation (unigene number)
Metabolic pathways	K00430	peroxidase (6736, 51945, 59365, 67714)
	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00517	E1.14.-.- (1078, 77961, 80332)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K00660	chalcone synthase (25992)
	K00815	tyrosine aminotransferase (66299)
	K01051	pectinesterase (79221)
	K01115	phospholipase D1/2 (19566, 80130)
	K01184	polygalacturonase (8462, 26578)
	K01859	chalcone isomerase (79381, 81280)
	K05280	flavonoid 3'-monooxygenase (42388, 78883)
	K05933	aminocyclopropanecarboxylate oxidase (37058)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
	K00430	peroxidase (6736, 51945, 59365, 67714)
	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
Biosynthesis of secondary metabolites	K00517	E1.14.-.- (1078, 77961, 80332)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K00660	chalcone synthase (25992)
	K00815	tyrosine aminotransferase (66299)
	K01183	chitinase (19460, 19666, 41811, 53522, 66180, 70397, 70653, 70896)
	K01859	chalcone isomerase (79381, 81280)
	K05280	flavonoid 3'-monooxygenase (42388, 78883)
	K05356	all-trans-nonaprenyl-diphosphate synthase (16505, 31995, 37058, 58710, 76104)
	K05933	aminocyclopropanecarboxylate oxidase (37058)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K00660	chalcone synthase (25992)
	K01859	chalcone isomerase (79381, 81280)
	K05280	flavonoid 3'-monooxygenase (42388, 78883)
Flavonoid biosynthesis	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
	K13420	LRR receptor-like serine/threonine-protein kinase FLS2 (30081)
	K13424	WRKY transcription factor 33 (27612, 76071)
	K13430	serine/threonine-protein kinase PBS1 [EC:2.7.11.1] (17858, 63292)
	K13449	pathogenesis-related protein 1 (18805)
Plant-pathogen interaction	K13457	disease resistance protein RPM1 (10601)
	K00430	peroxidase (6736, 51945, 59365, 67714)
	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K00815	tyrosine aminotransferase (66299)
Phenylalanine metabolism	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00517	E1.14.-.- (1078, 77961, 80332)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
	K00430	E1.11.1.7; peroxidase (6736, 51945, 59365, 67714)
Stilbenoid, diarylheptanoid and gingerol biosynthesis	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00517	E1.14.-.- (1078, 77961, 80332)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
	K00430	E1.11.1.7; peroxidase (6736, 51945, 59365, 67714)
Phenylpropanoid biosynthesis	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
	K00430	E1.11.1.7; peroxidase (6736, 51945, 59365, 67714)
	K01115	phospholipase D1/2 (19566, 80130)
Endocytosis	K01115	phospholipase D1/2 (19566, 80130)

	K03283	heat shock 70kDa protein 1/8 (23724)
	K12471	EPN; epsin (69978)
Spliceosome	K03283	heat shock 70kDa protein 1/8 (23724)
	K12811	ATP-dependent RNA helicase DDX46/PRP5 (21644)
	K12878	THO complex subunit 1 (3502)
Pentose and glucuronate interconversions	K01051	pectinesterase (79221)
	K01184	polygalacturonase (8462, 26578)
Starch and sucrose metabolism	K01051	pectinesterase (79221)
	K01184	polygalacturonase (8462, 26578)
Ubiquinone and other terpenoid-quinone biosynthesis	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00815	tyrosine aminotransferase (66299)
Plant hormone signal transduction	K13415	protein brassinosteroid insensitive 1 (70406)
	K13449	pathogenesis-related protein 1 (18805)
Circadian rhythm - plant	K00660	chalcone synthase (25992)
	K12135	zinc finger protein CONSTANS (20593)
RNA transport	K12878	THO complex subunit 1 (3502)
	K14326	regulator of nonsense transcripts 1 (29471, 82057)
Glutathione metabolism	K00434	L-ascorbate peroxidase (69822)
	K00799	glutathione S-transferase (6804, 22942, 79709)
Flavone and flavonol biosynthesis	K05279	flavonol 3-O-methyltransferase (20294)
	K05280	flavonoid 3'-monooxygenase (42388, 78883)
Cysteine and methionine metabolism	K00815	tyrosine aminotransferase (66299)
	K05933	aminocyclopropanecarboxylate oxidase (37058)
Ribosome	K02942	large subunit ribosomal protein LP1 (5192)
	K02996	small subunit ribosomal protein S9 (30454)
Tropane, piperidine and pyridine alkaloid biosynthesis	K00815	tyrosine aminotransferase (66299)
Isoquinoline alkaloid biosynthesis	K00815	tyrosine aminotransferase (66299)
Apoptosis	K04733	interleukin-1 receptor-associated kinase 4 (759)
cAMP signaling pathway	K01115	phospholipase D1/2 (19566, 80130,)
Metabolism of xenobiotics by cytochrome P450	K00799	glutathione S-transferase (6804, 22942, 79709)
Lysosome	K13289	cathepsin A (carboxypeptidase C) (75121)
MAPK signaling pathway	K03283	heat shock 70kDa protein 1/8 (23724)
Degradation of aromatic compounds	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
Carbon metabolism	K01455	formamidase (32409, 39535)
ABC transporters	K05666	ATP-binding cassette, subfamily C (CFTR/MRP), member 2 (16505)
Benzoxazinoid biosynthesis	K13229	4-dihydroxy-1,4-benzoxazin-3-one-glucoside dioxygenase (69313, 73809)
Phenylalanine, tyrosine and tryptophan biosynthesis	K00815	tyrosine aminotransferase (66299)
Protein processing in endoplasmic reticulum	K03283	heat shock 70kDa protein 1/8 (23724)
Toll-like receptor signaling pathway	K04733	interleukin-1 receptor-associated kinase 4 (759)
Limonene and pinene degradation	K00517	E1.14.-.- (1078, 77961, 80332)
Zeatin biosynthesis	K13495	cis-zeatin O-glucosyltransferase (67857)
mRNA surveillance pathway	K14326	regulator of nonsense transcripts 1 (29471, 82057)
Nitrogen metabolism	K01455	formamidase (32409, 39535)
Aminobenzoate degradation	K00517	E1.14.-.- (1078, 77961, 80332)
RNA degradation	K12619	5'-3' exoribonuclease 2 (1219, 29814)
Tyrosine metabolism	K00815	tyrosine aminotransferase (66299)
Glyoxylate and dicarboxylate metabolism	K01455	formamidase (32409, 39535)
Ascorbate and aldarate metabolism	K00434	L-ascorbate peroxidase (69822)
Amino sugar and nucleotide sugar metabolism	K01183	chitinase (19460, 19666, 41811, 53522, 66180, 70397, 70653, 70896)
Cyanoamino acid metabolism	K01455	formamidase (32409, 39535)
Ribosome biogenesis in eukaryotes	K12619	5'-3' exoribonuclease 2 (1219, 29814)
Biosynthesis of amino acids	K00815	tyrosine aminotransferase (66299)
Glycerophospholipid metabolism	K01115	phospholipase D1/2 (19566, 80130)
Polycyclic aromatic hydrocarbon degradation	K00517	E1.14.-.- (1078, 77961, 80332)
Ether lipid metabolism	K01115	phospholipase D1/2 (19566, 80130)
Terpenoid backbone biosynthesis	K05356	all-trans-nonaprenyl-diphosphate synthase (31995, 58710, 76104)

Table 5

Differentially expressed unigenes with putative roles in plant defense in sugarbeet roots 2 d after treatment with jasmonic acid (JA) or water. Freshly harvested roots were treated with 10 μ M JA or water for 1 h and incubated for 2 d at 20 °C. Gene expression was determined by RNA sequencing. Differentially expressed genes were defined as those with an absolute value of the logarithm of the fold change in expression ($\log_{2}FC$) ≥ 1 , a false detection rate (FDR) ≤ 0.001 , and were significantly altered in expression in both repetitions of the experiment. Raw number of RNA-sequencing reads are listed for both biological repetitions where JA₁ and control₁ are the raw reads for JA and water-treated roots from the 1st biological repetition, and JA₂ and control₂ are the raw reads for JA and water-treated roots from the 2nd biological repetition. Annotations were from a BLASTx search of unigene sequences against GenBank's nonredundant (nr) protein database. Annotations from a BLAST search against the sugarbeet genome are available (Table 3, in Oliveira et al., in press). Unigenes analyzed by quantitative RT-PCR are underlined.

Unigene ID	logFC	Reads				E value	Nr-annotation	Nr-ID
		JA ₁	control ₁	JA ₂	control ₂			
<u>10601</u>	8.3	1	-	35	-	9E-25	NBS-LRR resistance protein [Populus trichocarpa]	gi 224096480 ref XP_002334697.1
<u>9115</u>	6.0	77	2	237	3	8E-6	CC-NBS-LRR resistance protein [Populus trichocarpa]	gi 224092698 ref XP_002309702.1
<u>11281</u>	4.7	5	-	76	3	0	laccase [Liriodendron tulipifera]	gi 1621467 gb AAB17194.1
<u>81205</u>	4.5	2	-	45	2	2E-79	pathogenesis-related thaumatin family protein [Arabidopsis thaliana]	gi 15242552 ref NP_198818.1
<u>42562</u>	4.1	6	1	117	6	1E-24	peroxidase [Spinacia oleracea]	gi 2956705 emb CAA76377.1
65362	4.1	22	1	14	1	2E-6	retrotransposon protein, putative, unclassified [Oryza sativa Japonica Group]	gi 108707503 gb ABF95298.1
28653	3.8	3	2	69	3	5E-57	NAC domain protein, IPR003441 [Populus trichocarpa]	gi 224136718 ref XP_002322398.1
<u>71046</u>	3.8	64	5	24	1	1E-30	acidic chitinase [Elaeagnus umbellata]	gi 3126963 gb AAC16010.1
<u>66692</u>	3.8	7	2	178	12	1E-58	peroxidase [Spinacia oleracea]	gi 2956707 emb CAA76376.1
<u>29506</u>	3.6	11	2	27	1	2E-11	salicylic acid carboxyl methyltransferase [Mikania micrantha]	gi 227278441 gb ACP20216.1
<u>51666</u>	3.5	5	2	40	2	3E-32	laccase 1a [Populus trichocarpa]	gi 224139024 ref XP_002322961.1
<u>77250</u>	3.4	56	7	113	9	9E-35	NBS-LRR resistance protein [Populus trichocarpa]	gi 224096480 ref XP_002334697.1
<u>29351</u>	3.2	101	17	200	16	1E-28	NBS-LRR resistance protein [Populus trichocarpa]	gi 224075299 ref XP_002304589.1
77135	3.2	5	1	32	3	1E-105	LAC11 (laccase 11); laccase [Arabidopsis thaliana]	gi 22326581 ref NP_195946.2
<u>67039</u>	3.0	25	9	160	15	4E-61	laccase 1a [Populus trichocarpa]	gi 224139024 ref XP_002322961.1
29708	3.0	4	1	43	5	1E-125	laccase 3 [Populus trichocarpa]	gi 224112579 ref XP_002316233.1
<u>68954</u>	2.9	11	6	90	8	6E-59	laccase 1a [Populus trichocarpa]	gi 224139024 ref XP_002322961.1
<u>30677</u>	2.6	19	3	44	7	3E-75	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi 4206116 gb AAD11427.1
78937	2.6	21	9	85	8	1E-83	laccase [Liriodendron tulipifera]	gi 1621463 gb AAB17192.1
16917	2.6	31	14	112	9	7E-37	glucosyltransferase [Phytolacca americana]	gi 204022238 dbj BAG71127.1
80875	2.6	5	2	54	8	1E-120	pectinesterase family protein [Arabidopsis lyrata subsp. lyrata]	gi 297823481 ref XP_002879623.1
26919	2.5	34	25	242	24	6E-60	glucosyltransferase [Phytolacca americana]	gi 204022238 dbj BAG71127.1
55314	2.5	10	2	51	9	2E-41	beta-glucosidase [Arabidopsis lyrata subsp. lyrata]	gi 297830448 ref XP_002883106.1
25408	2.3	13	6	77	13	1E-99	wound-inducible carboxypeptidase [Solanum lycopersicum]	gi 7271957 gb AAF44708.1 AF242849_1
7601	2.1	67	13	70	19	1E-13	Os03g0849500 disease resistance RPP8-like protein [Oryza sativa Japonica]	gi 115456593 ref NP_001051897.1

							Group]	
80285	2.0	14	13	128	24	1E-108	wound-inducible carboxypeptidase [Solanum lycopersicum]	gi 7271957 gb AAF44708.1 AF242849_1
22064	1.8	57	44	377	80	6E-23	ABC transporter family protein [Arabidopsis thaliana]	gi 18398110 ref NP_564383.1
67654	1.8	173	78	328	63	4E-08	proteinase inhibitor [Jatropha curcas]	gi 284433788 gb ADB85100.1
81041	1.6	157	142	969	226	1E-115	ABC transporter family protein [Arabidopsis lyrata subsp. lyrata]	gi 297851608 ref XP_002893685.1
78196	1.6	644	297	490	66	3E-6	ribosome-inactivating protein [Beta vulgaris]	gi 99646716 emb CAK22417.1
22684	-1.8	1	98	2	190	1E-108	DNA binding [Arabidopsis thaliana]	gi 145336703 ref NP_175754.2
30280	-2.7	53	98	-	89	1E-82	NAC domain protein, IPR003441 [Populus trichocarpa]	gi 224104873 ref XP_002313601.1
27839	-3.1	22	58	3	102	6E-32	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1
28841	-3.8	6	38	1	24	6E-43	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1
69707	-4.3	30	214	-	174	7E-50	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1
10646	-4.4	5	61	-	37	1E-58	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1
77351	-6.5	8	107	-	66	6E-58	CTV.20 [Citrus trifoliata]	gi 24461860 gb AAN62347.1 AF506028_14
5228	-7.8	-	16	-	10	5E-20	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1

Table 6

Differentially expressed unigenes with putative roles in plant defense in sugarbeet roots 60 d after treatment with jasmonic acid (JA) or water. Freshly harvested roots were treated with 10 μ M JA or water for 1 h and incubated for 60 d at 20 °C. Gene expression was determined by RNA sequencing. Differentially expressed genes were defined as those with an absolute value of the logarithm (base 2) of the fold change in expression ($\log_{2}FC \geq 1$), a false detection rate ($FDR \leq 0.001$), and were significantly altered in expression in both repetitions of the experiment. Raw number of RNA-sequencing reads are listed for both biological repetitions where JA₁ and control₁ are the raw reads for JA and water-treated roots from the 1st biological repetition, and JA₂ and control₂ are the raw reads for JA and water-treated roots from the 2nd biological repetition. Annotations were from a BLASTx search of unigene sequences against GenBank's nonredundant (nr) protein database. Annotations from a BLAST search against the sugarbeet genome are available (Table 4 in Oliveira et al., in press). Unigenes analyzed by quantitative RT-PCR are underlined.

Unigene ID	logFC	Reads				E value	Nr-annotation	Nr-ID
		JA ₁	control ₁	JA ₂	control ₂			
9968	9.3	84	-	1	-	1E-17	retrotransposon protein [Cucumis melo subsp. melo]	gi 307135889 gb ADN33754.1
65993	7.1	2	-	16	-	2E-06	AP2/ERF domain-containing transcription factor [Populus trichocarpa]	gi 224083841 ref XP_002307142.1
70406	7.1	3	-	15	-	2E-06	Leucine-rich repeat, plant specific [Medicago truncatula]	gi 124360665 gb ABN08654.1
<u>55958</u>	5.1	2	1	68	1	6E-13	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi 4206116 gb AAD11427.1
<u>30677</u>	4.7	4	2	96	2	1E-14	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi 4206116 gb AAD11427.1
20294	4.3	8	3	89	2	1E-13	O-methyltransferase [Vitis vinifera]	gi 300077149 gb ADJ66851.1
235	4.1	108	44	949	20	2E-25	ribosome-inactivating protein [Beta vulgaris]	gi 99646720 emb CAK22418.1
65143	4.1	125	72	1237	13	1E-25	ribosome-inactivating protein [Beta vulgaris]	gi 99646720 emb CAK22418.1
50108	3.5	6	4	39	-	1E-07	glycine-rich protein [Arabidopsis thaliana]	gi 18397934 ref NP_565380.1
79709	3.5	20	7	55	-	7E-10	glutathione S-transferase [Salicornia brachiata]	gi 124507403 gb ABN13680.1
77961	3.5	10	9	137	5	4E-13	cytochrome P450 [Populus trichocarpa]	gi 224063931 ref XP_002301307.1
20462	3.3	25	5	52	3	1E-09	pathogenesis-related protein 10a [Rheum australe]	gi 197312889 gb ACH63225.1
67367	3.3	66	10	36	1	1.E-10	peroxidase 4	gi 223635590 sp A7NY33.1 PER4_VITVI
74869	3.2	12	6	40	-	2E-07	oxalate oxidase-like germin 171 [Beta vulgaris]	gi 11496133 gb AAG36666.1
42008	2.9	10	4	40	3	9E-07	pectinesterase family protein [Arabidopsis thaliana]	gi 15225308 ref NP_180212.1
51945	2.9	11	6	66	5	3E-08	peroxidase [Populus trichocarpa]	gi 225626263 gb ACN97181.1
29055	2.9	4	1	38	5	4E-06	CC-NBS-LRR resistance protein [Populus trichocarpa]	gi 224102623 ref XP_002334156.1
75464	2.8	640	132	288	11	5E-14	blight-associated protein P12 [Citrus trifoliata]	gi 160690672 gb ABX46166.1
69313	2.7	31	14	111	9	1E-09	desacetoxyvindoline 4-hydroxylase [Catharanthus roseus]	gi 1916643 gb AAC49826.1
80014	2.7	397	307	1596	23	4E-14	glucan endo-1,3-beta-D-glucosidase [Beta vulgaris subsp. vulgaris]	gi 4584556 emb CAA53545.1
77564	2.7	25	3	30	6	2E-06	Os01g0957100 [Oryza sativa Japonica Group]	gi 115442331 ref NP_001045445.1
19460	2.6	34	11	54	4	8E-08	chitinase-B, PLC-B [Phytolacca americana=pokeweed, leaves, Peptide, 274 aa]	gi 998516 gb AAB34670.1
48415	2.6	398	180	986	62	3E-13	pathogenesis-related protein [Tamarix hispida]	gi 217331222 gb ACK38253.1
66442	2.6	121	108	506	4	3E-12	beta-1,3-glucanase [Vitis riparia]	gi 37992763 gb AAR06588.1
13125	2.5	122	40	91	-	1E-09	Protein kinase; Type I EGF [Medicago truncatula]	gi 87162753 gb ABD28548.1
<u>53522</u>	2.5	310	113	392	20	1E-11	acidic endochitinase SE2 [Beta vulgaris]	gi 544000 sp P36910.1 CHIE_BETVU
63292	2.5	21	13	53	1	8E-07	kinase family protein [Arabidopsis lyrata subsp. lyrata]	gi 297814900 ref XP_002875333.1
59365	2.5	9	6	69	9	6E-07	peroxidase [Mirabilis jalapa]	gi 46949194 gb AAT07453.1

66180	2.4	263	92	333	28	8E-11	acidic endochitinase SE2 [Beta vulgaris]	gi 544000 sp P36910.1 CHIE_BETVU
8943	2.4	51	26	81	1	6E-08	protein kinase; peptidoglycan-binding LysM [Medicago truncatula]	gi 87162779 gb ABD28574.1
76071	2.3	81	23	74	9	3E-08	WRKY50; transcription factor [Arabidopsis thaliana]	gi 22327079 ref NP_197989.2
22942	2.3	57	28	102	6	4E-08	glutathione S-transferase; AltName: Full=GST class-phi [Silene vulgaris]	gi 417093 sp Q04522.3 GSTF_SILCU
81280	2.3	130	75	265	12	2E-09	chalcone--flavonone isomerase; Short=Chalcone isomerase [Camellia sinensis]	gi 122233481 sp Q45QI7.2 CFI_CAMSI
11500	2.2	14	9	151	29	1E-07	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1
27612	2.2	105	29	23	-	4E-07	WRKY transcription factor [Artemisia annua]	gi 298108803 gb ADI56655.1
62435	2.2	428	239	667	18	7E-10	thaumatin-like protein [Mirabilis jalapa]	gi 46949200 gb AAT07456.1
72126	2.1	700	359	1029	62	1E-09	osmotin-like protein [Atriplex nummularia]	gi 166940 gb AAA32908.1
80552	2.1	112	63	719	141	2E-09	retrotransposon protein [Beta vulgaris]	gi 121501699 gb ABM55240.1
25992	2.1	59	33	160	21	1E-07	PKS, homology to chalcone synthase [Fallopia multiflora]	gi 300885273 gb ADK45325.1
31086	2.0	175	110	312	15	2E-08	Chain A, allergenic and antifungal banana fruit thaumatin-like protein At 1.7a	gi 88191901 pdb 1Z3Q A
79221	2.0	53	32	122	13	5E-07	pectinesterase; pectinesterase inhibitor [Medicago truncatula]	gi 124360335 gb ABN08348.1
41811	2.0	50	27	71	5	3E-06	chitinase-B, PLC-B [Phytolacca americana=pokeweed, leaves, Peptide, 274 aa]	gi 998516 gb AAB34670.1
79381	2.0	115	76	227	17	1E-07	chalcone isomerase [Garcinia mangostana]	gi 222478417 gb ACM62743.1
24889	1.9	336	201	883	150	6E-08	phenylpropanoid:glucosyltransferase 1 [Nicotiana tabacum]	gi 13492674 gb AAK28303.1 AF346431_1
1078	1.8	246	140	353	45	6E-07	CYP81B2v1, homology to cytochrome P450 [Nicotiana tabacum]	gi 85068596 gb ABC69378.1
70896	1.8	446	195	464	88	4E-07	acidic endochitinase SE2; Flags: Precursor [Beta vulgaris]	gi 544000 sp P36910.1 CHIE_BETVU
6804	1.7	172	132	363	39	1E-06	glutathione S-transferase class-phi [Silene vulgaris]	gi 417093 sp Q04522.3 GSTF_SILCU
80332	1.7	308	205	544	71	1E-06	cytochrome P450 [Populus trichocarpa]	gi 224067244 ref XP_002302427.1
58834	1.6	272	193	434	47	3E-06	anthocyanin-O-methyltransferase [Vitis vinifera]	gi 226374634 gb ACO52469.1
69822	-2.5	1	201	2	47	5E-43	ascorbate peroxidase [Citrus maxima]	gi 221327587 gb ACM17463.1
30081	-2.5	38	224	1	63	1E-129	leucine-rich repeat receptor-like protein kinase [Arabidopsis thaliana]	gi 224589491 gb ACN59279.1
22684	-2.8	13	28	37	271	1E-108	DNA binding [Arabidopsis thaliana]	gi 145336703 ref NP_175754.2
77351	-6.2	9	17	11	102	6E-58	CTV.20 [Citrus trifoliata]	gi 24461860 gb AAN62347.1 AF506028_14