LUCILENE SILVA DE OLIVEIRA

POSTHARVEST ROLE OF JASMONIC ACID AND WOUNDING ON EXPRESSION OF DEFENSE RELATED METABOLISM IN SUGAR BEET ROOTS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, para obtenção do título de Doctor Scientiae.

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APROVADA: 18 de março de 2016.

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Ana Maria Mapeli	Adriano Nunes Nesi
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Dimas Mendes Ribeiro	Karen klotz Fugate

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Fernando Luiz Finger
(Orientador)

Aos meus queridos pais, Fátima e Vicente.

Aos meus irmãos, Ivan e Mislene.

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BIOGRAPHY

LUCILENE SILVA DE OLIVEIRA, daughter of Maria de Fátima Soares Silva de Oliveira and Vicente de Paula Fialho de Oliveira, was born on January 12th, 1987, in Viçosa, Minas Gerais, Brazil.

In 2005, she entered Federal University of Viçosa, graduating in Agronomy Engineering on January 2010.

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RESUMO

OLIVEIRA, Lucilene Silva de, D.Sc., Universidade Federal de Viçosa, março de 2016. **Rota pós-colheita do ácido jasmônico e do ferimento na expressão do metabolismo de defesa em raízes de beterraba açucareira**. Orientador: Fernando Luiz Finger.

Jasmonatos (JA) podem atuar como um indutor da expressão de genes contra estresse biótico e abiótico através de um processo denominado "prime state". Aplicação de JA tem mostrado reduzir o apodrecimento de tecidos e controlar doenças pós-colheita em raízes de beterraba açucareira. Entretanto, os mecanismos envolvidos na indução de defesa na pós-colheita de raízes de beterraba açucareira através da aplicação do JA são desconhecidos. Consequentemente, foi investigado os mecanismos induzidos pelo JA, ao qual protege as raízes durante o armazenamento, identificando e caracterizando genes que são alterados pelo tratamento com JA. O tratamento de raízes de beterraba com 10 µM de JA resultou na alteração significativa da expressão de unigenes. O sequenciamento do mRNA revelou que 30 e 49 genes com potencial de defesa foram upregulated para 2 e 60 dias, respectivamente, após o tratamento com JA. Após 2 dias de tratamento, os níveis de peroxidase, cinamato-4-hidroxilase, quitinase ácida, lacase, proteína de resistência nbs-Irr, proteína relacionada a patogênese da família da taumatina, inibidor de protease e β-glucosidase (variação de 1.8 a 8.3 vezes) apresentaram maior expressão em raízes de beterraba açucareira tratadas que as do controle. Peroxidase, cinnamato-4- hidroxilase e cc-nbs-Irr resistant protein também foram up-regulated 60 dias após a aplicação de JA. Os unigenes upregulated, em ambos os dias avaliados, são relacionados a rotas de metabólicas de biossíntese de compostos secundários, aumento da resistência da parede celular, assim como proteínas relacionadas à interação planta-patógeno. Assim, o presente trabalho sugere que o tratamento com JA pode induzir o "prime state" em beterraba açucareira, induzido uma série de genes relacionados a defesa de plantas. Incluindo enzimas relacionadas a biossíntese de metabólicos secundários e proteínas relacionas a patogêneses. O JA também aumentou a habilidade de células reconhecer patógenos , qual pode resultar em rápida ativação de respostas imune e reduzir a susceptibilidade das raízes a doenças pós-colheita. Em adição as perdas devido ao apodrecimento no armazenamento das raízes, é de extrema importância evitar as perdas de açúcares durante o crescimento. O ataque por larvas de mosca de raízes de beterraba açucareira é um dos danos que reduz

significativamente a produção de raízes e o conteúdo de sacarose, podendo devastar campos de produção. Assim, o estudo de mecanismos de resistência é essencial para prover novas estratégias de controle e reuduzir a aplicação de inseticidas. Neste trabalho foi investigado se a resistência de alguns genótipos é alcançada através da ativação da quitinase, peroxidase e polifenoloxidase. Raízes de nove genótipos de beterraba açucareira, susceptível ou resistente à larva de mosca de raízes de beterraba açucareira, foram feridas quatro semanas após o plantio para mimetizar o ataque por estas larvas. Os resultados mostraram a atividade das enzimas antioxidantes, peroxidase e polifenoloxidase, não está correlacionada a resistência de beterraba açucareira a larva de mosca de raízes de beterraba açucareira. A atividade da quitinase foi signicativamente reduzida em alguns genótipos após o ferimento, entretanto não foi encontrado diferença significativa entre genótipos resistente e susceptível de beterraba açucareira.

ABSTRACT

OLIVEIRA, Lucilene Silva de, D.Sc., Universidade Federal de Viçosa, March, 2016. **Postharvest role of jasmonic acid and wounding on expression of defense related metabolism in sugar beet roots**. Adviser: Fernando Luiz Finger.

Jasmonate (JA) can act as an inducer expression of defense genes against biotic and abiotic stress by process of priming plant. Exogenous application of JA has been shown to reduce rotted tissue and control postharvest pathogen in sugarbeet roots. However, the mechanism involved in the postharvest induction of defense by JA in sugarbeet roots is unknown. Consequently, we investigated the JA-induced mechanisms which protect roots from storage pathogens by identifying and characterizing genes that are altered by JA treatment. JA (10 µM) treatment to sugarbeet roots resulted alteration significant of unigenes expression. RNA-Seq data showed that 30 and 49 putative defense genes were upregulated at 2 and 60 days after JA-treatment, respectively. In sugarbeet roots, peroxidases, cinnamate-4-hydroxilase, chitinase acid, laccases, nbs-Irr resistant, pathogen-related thaumatin family protein, proteinase inhibitor and β-glucosidase were found at higher levels (fold change ranged from 1.8 to 8.3) in treated than control roots at 2 d subsequent to JA-application. At 60 days after JA treatment observed that peroxidase, chitinase, cinnamate-4 hydroxilase and cc-nbs-Irr resistant protein were also up-regulated. These upregulated unigenes are related with biosynthesis of secondary metabolites, cell wall reinforcement, as well as for plant-pathogen interaction. Thus, the present study suggests that JA treatment could prime sugarbeet inducting a series of defense genes, including defense-related proteins and key enzymes related secondary metabolites. JA also increased the ability of sugarbeet cells to recognize pathogen which may result faster activation of immune response and then reduction of infection and susceptibility. In addition to rotted losses in rot storage it is extremely importance to avoid losses during sugarbeet growth. Insect attack by sugarbeet root magoot is one of damage that significantly reduces root yield and sucrose content and can devastate individual fields. Thus, study of resistance mechanism is essential to provide new control strategies and reduce insecticides spray. We investigated if resistance of some genotypes is achieved through activation of chitinase, peroxidase and polyphenoloxidase. Root of nine genotypes sugarbeet, susceptible and resistant to maggot fly, were wounding 4 weeks after planting to mimic maggot attack. The results showed neither peroxidase nor polyphenol oxidase activity is correlated to

maggot fly resistant in sugarbeet roots. We observed that chitinase activity was significantly reduced for some genotypes after wounding, although no significance difference was found between resistant and susceptible sugarbeet genotypes.

INTRODUCTION

Sugarbeet **(**Beta vulgari) is an important crop for refined sucrose production and typically contains 15-20% sucrose based on fresh weight. Therefore, sugarbeet is used as fodder and its leaves can be used as fertilizer (Jaggard & Qi, 2006, UK Agriculture, 2016). Sugarbeet roots are largely produced in France, the United States, Germany, Russia, and Ukraine (FAOSTAT, 2011). Sugarbeet provides approximately 22% of the world's sugar (Südzucker, 2013). In the United States, sugarbeet has represented 55 % of total sugar production and sugarcane for about 45 % (McConnell, 2015).

Sugarbeet grows in a wide variety of temperate climatic conditions, most successfully in northern latitudes and it is planted annually (McConnell, 2015). The crop requires vernalization and long-day conditions to induce flowering and seed production (Lewellen et al., 2009; Milford, 2006). The largest region in the USA for sugarbeet production is in or close to the Red River Valley of western Minnesota and eastern North Dakota (McConnell, 2015). In these regions roots are harvested in late autumn, before the first frost, and storage in outdoor piles for up to 200 days before sugar processing (Tungland et al., 1998). One of the major concerns of sugarbeet producers is the roots storage. Several factors result in sucrose loss during root storage including respiration, rotted and conversion of sucrose to others carbohydrates. Respiration represents 60 to 80% of sucrose loss during storage (Wyse & Dexter, 1971). In relation to storage rot it augments root respiration rate, resulting in storage losses and heat productionin storage piles (Mumford & Wyse, 1976). As storage piles warm, the respiration of both healthy and diseased roots and the incidence and severity of storage rots increase (Campbell & Klotz, 2006).

Controlling of rotted is done by pile management (Campbell and Klotz, 2006), which removes hotspots in the piles. Although pile management efficiency is dependent of weather conditions, hence is necessary to find new alternatives. Fungicides could be used to control postharvest disease but it has deleterious effects on sugarbeet roots characters in absence of disease (Miles et al., 1977; Akeson et al., 1979). Fugate et al. (2012) observed that exogenous application of jasmonate acid (JA) reduces rot tissues in sugarbeet for Botrytis cinerea, Phoma betae and Penicilillium claviforme.

Jasmonates (JA) are lipid-derived molecules that affect various process such as fruit ripening, trendily coiling, production of viable pollen, and response to abiotic and

biotic stress (Creelman & Mullet, 1997, Devoto & Turner, 2003, Robert-Seilaniantz et al., 2011). Jasmonic acid has been proven to be a plant signal molecule that activated defense response (Creeman and Mullet, 1997). Exogenous application of JA has shown to protect various horticultural crops by systemically inducing the same plant defense responses that are activated by endogenous jasmonates (Darras et al., 2011, Tripathi &Dubey, 2004 and Pozo et al., 2005). JA-treatment stimulates the enhance expression of pathogenesis-related protein, heat shock protein, and biosynthesis of many secondary metabolites (Ding et al., 2002, Wang et al., 2014 and Creeman & Mullet, 1997). In previous studies, jasmonic acid treatment at 10 µmol L^{-1} has been revealed effective inhibiting postharvest diseases on sugarbeet against Botrytis cinerea, Phoma betae and Penicillium claviforme (Fugate et al, 2012). However the mechanisms responsible for induction of plant resistance by jasmonate are not well established.

In addition to sucrose losses, in root storage is extremely important to avoid losses during sugarbeet growth. Insect attack by sugarbeet root magoot (SBRM) is one of damage that significantly reduces root yield and sucrose content and can devastate individual fields (Harveson et al., 2009). Root yield losses oscillate from 10 to 100% in severe surgabeet root maggot infestations (Campbell et al., 1998; Cooke, 1993; Dregseth, et al. 2003).

Sugarbeet root maggot (SBRM) is mainly control by application of a granular insecticide that reduces larval populations in sugarbeet fields (Campbell et al., 2008). Those insecticides may be no longer available in the market due concerns about environment impacts (Campbell et al., 2008). Campbell (2005) suggested the development of sugarbeet root maggot resistant hybrids as potencial solution to these concerns.

Two root maggot resistant germplasm lines were released, F1015 (PI605413) and F1016 (PI608437) (Campbell et al., 2000). These lines showed less damage than commercial hybrids and unselected populations (Campbell et al., 2008), although the resistant mechanisms are unknown. Puthoff and Smigocki (2007) identified the expression changes for a large number of genes that were induced by maggot feeding in genotypes that are moderately resistant, and susceptible to the SBRM. In the moderately resistant genotype, many defense genes were different expressed by maggot feeding, among them five polyphenoloxidase (PPO), three chitinase and one peroxidase.

The study of resistance mechanism is essential to provide new control strategies and reduce the use of pesticides. Thus the objective of this study was to investigate JA-

induced mechanisms which protect roots from storage pathogens and resistance mechanisms of some genotypes to SBRM attack.

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CHAPTER I

Postharvest Jasmonic Acid Treatment Causes Long-Term Alterations to the Transcriptome and the Expression of Defense Genes in Sugarbeet Roots

ABSTRACT

Jasmonate (JA) can act as an inducer expression of defense genes against biotic and abiotic stress by process of priming plant. Exogenous application of JA has been shown to reduce rotted tissue and control postharvest pathogen in sugarbeet roots. However, the mechanism involved in the postharvest induction of defense by JA in sugarbeet roots is unknown. Consequently, we investigated the JA-induced mechanisms which protect roots from storage pathogens by identifying and characterizing genes that are altered by JA treatment. JA (10 µM) treatment to sugarbeet roots resulted in significant alteration of unigenes expression. RNA-Seq data showed that 30 and 49 putative defense genes were upregulated at 2 and 60 days after JA-treatment, respectively. In sugarbeet roots, peroxidases, cinnamate-4-hydroxilase, chitinase acid, laccases, nbs-Irr resistant, pathogen-related thaumatin family protein, proteinase inhibitor and β-glucosidase were found at higher levels (fold change ranged from 1.8 to 8.3) in treated compared to control roots at 2 d of JA-application. At 60 days after JA, peroxidase, chitinase, cinnamate-4 hydroxilase and cc-nbs-Irr resistant protein were also up-regulated. These upregulated unigenes are related with biosynthesis of secondary metabolites, cell wall reinforcement, as well as plant-pathogen interaction. Thus, the present study suggests that JA treatment could prime sugarbeet inducting a series of defense genes, including defense-related proteins and key enzymes related to the secondary metabolism. JA also increased the ability of sugarbeet cells to recognize pathogen which may result in faster activation of immune response and reduction of infection and susceptibility.

1. INTRODUCTION

Jasmonates (JA) are naturally occurring lipid-derived compound that acts as phytohormone. JA has many functions in plants, although considerable attention has focused on its role in triggering plant defense mechanisms (Campos et al., 2014; Creeman and Mullet, 1997). It is well established that JA is a signal molecule that activates plant defenses especially against neucrothophic pathogens and herbivores (Glazebrook, 2005). The mechanisms by which JA protects plants is related to inhibition of pathogen growth, or induction of a primed state that allows plants to respond more rapidly to pathogen attack for increased transcript levels on a set of intra- and extra-cellular pathogenesis related genes (Thaler et al., 2002; Ton et al., 2006; Desmond et al., 2006; Chen et al., 2011). The regulatory role of JA in plant resistance to pathogen infections is possibly reached by activate the expression of defense-related genes (Liu et al., 2010). JA stimulates the enhanced expression of pathogenesis-related (PR) proteins such as chitinases and glucanases, antioxidant enzymes and accumulation of host-synthesized phytoalexins or other antifungal compounds (Ding et al., 2002; Wang et al., 2014).

Exogenous application of JA has shown to protect several horticultural crops by systemically inducing the same plant defense responses that are activated by endogenous jasmonates (Darras et al., 2011, Tripathi &Dubey, 2004 and Pozo et al., 2005).When exogenously applied to postharvest products, JA has been shown to result in a lower decay incidence and improved postharvest disease resistance in fruits and vegetables (Creelman & Mullet, 1997). Recent postharvest studies showed that treatment with MeJA triggers a priming mechanism of defense, in Chinese bayberry, sweet cherry, tomato, and grape berries (Wang et al., 2014, Wang et al., 2015a, Thaler et al., 2002, Wang et al., 2015b). In Chinese bayberry, JA enhanced protein levels of phenylalanine ammonia-lyase, and chitinase (Wang et al., 2014). Moreover, JA resulted in accumulation of phenolic compounds, lignin, and phytoalexin (Wang et al., 2014). In tomato fruit MeJA substantially increased the mRNA levels that encoding intracellular and extracellular β-1,3-glucanase and intracellular chitinase (Thaler et al., 2002).

In previous studies, jasmonic acid treatment at 10 µmol L^{-1} revealed effective inhibiting postharvest diseases on sugarbeet against Botrytis cinerea,Phoma betae and Penicillium claviforme (Fugate et al, 2012). However, the specific defense mechanisms involved in JA-induced resistance in sugarbeet still unknown. The objective of this

study was to investigate JA-induced mechanisms that protects roots from storage pathogens by identifying and characterizing genes and gene products altered by JA.

2. MATERIAL 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, BC, 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 0 or 10 μM JA (Cayman Chemical, Ann Arbor, MI) for 1 h at room temperature, then incubated at 20 °C and 90 % relative humidity for up to 60 d in a controlled environment chamber (Conviron, model MTR30, Winnipeg, MB, Canada). Root samples were collected 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 4 replicates per treatment per time point. Experiment was repeated twice**.**

2.2. RNA isolation

Replicates for each time point and treatment within an experiment were pooled using an equal weight of tissue from each root. Repetitions of the experiment were used to make replicates. Total RNA was extracted from lyophilized tissue (20 mg) using a RNeasy Plant Mini Kit (Qiagen, Valencia, CA) with an on-column DNase digestion. RNA quality was determined using an Agilent Technologies (Pal Alsto, CA) 2100 Bioanalyzer.

2.3. RNA sequencing

RNA from roots treated with 0 and 10 μM JA and stored for 2 and 60 d were sequenced. Oligo(dT) magnetic beads were used to isolate mRNA from total RNA. mRNA was fragmented into fragments of approximately 200 bp which were used for first strand cDNA synthesis using random hexamer primers and reverse transcriptase.

Second strand cDNA was synthesized with dNTPs, RNase H, and DNA polymerase I. cDNA was purified with a QiaQuick PCR Extraction Kit (Qiagen). Fragment ends were repaired with T4 DNA polymerase and Klenow DNA polymerase, adenylated at the 3' end, and ligated to sequencing adaptors. Fragments were purified and size selected by agarose gel electrophoresis and amplified by PCR. Amplified products were sequenced by BGI Americas (Cambridge, MA, USA) using an Illumina, Inc. (San Diego, CA, USA) HiSeq 2000 system.

2.4. Bioinformatics analysis

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 was determined using RobiNA software (Lohse et al., 2012), and 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 GO identifiers. The Search Pathway module of KEGG Mapper was used to assign differentially expressed genes to KEGG pathways (Kanehisa et al., 2006).

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

cDNA was synthesized from total RNA using oligo(dT) primers, dNTPs, and SuperScript III (Invitrogen, Foster, CA, USA) reverse transcriptase. Primer pairs for select genes were designed with Primer3Plus (Untergasser et al., 2007) and are listed in Table 1. qRT-PCR was performed on a MJ Research (Watertown, MA, USA) PTC-200 thermal cycler, equipped with a Chromo 4 realtime detector (Bio-Rad Life Science 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 using 40 cycles of 15 s at 95°C and 60 s at 60°C. Three replicates were analyzed for each gene. Melting curves were used to confirm that single products were amplified. PCR products were quantified by themethod of Pfaffl, using β-actin as a reference gene (Pfaffl, 2001).

2.6. Protein extraction and activity assays

Laccase, peroxidase, and cinnamate 4-hydroxylase (C4H) activities were determined by the methods of deMarco and Roubelakis-Angelakis (1997), Fugate et al. (2016), and Bi et al. (2007), respectively. Protein extracts were prepared by adding 5 volumes (w/v) of an extraction buffer to freeze-dried tissue. Extraction buffers were for laccase: 0.1 M sodium acetate, pH 5.0, 1% polyvinylpyrrolidone-40 (PVP-40); POD: 0.1 M potassium phosphate buffer, pH 7.0, 10 mM sodium bisulfite, and 0.5 M NaCl; for C4H: 100 mM potassium phosphate, pH 7.5 and 2 mM 2-mercaptoethanol. Suspensions of tissue and extraction buffer were sonicated for 15 minutes at 4 ^{o}C, filtered over Miracloth (EMD Millipore, Billerica, MA, USA), and centrifuged at 20,000 g for 20 minutes at 4 ^{o}C. Supernatants were used for laccase, POD and total soluble protein assays. For C4H assays, supernatants were passed through Sephadex G-25 columns equilibrated with 100 mM potassium phosphate, pH 7.5.

Enzyme activities were measured spectrophotometrically using a Shimadzu model UV-1601 dual beam spectrophotometer (Kyota, Japan). Laccase activity assays contained protein extract, 0.05 M sodium acetate buffer, 0.03% ABST (w/v) in buffer, 100µM tropolone, 60 unit/ml catalase and activity determined at 25 °C and absorbance at 420 nm . POD activity assays contained protein extract, 0.1 M potassium phosphate buffer, 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 with 6 M HCl (to a certain pH 1.0). The pH was adjusted to 11 with 6 M NaOH, and absorbance at 340 nm was determined. Activity was determined by the change in absorbance relative to controls that lacked trans-cinnamic acid using a p-coumaric standard curve or extinction coefficient. Total soluble protein concentrations were determined using Bio-Rad Protein Assay Reagent (Hercules, CA, USA) with bovine serum albumin as a standard.

3. RESULTS AND DISCUSSION

3.1. Differential expression of sugarbeet root unigenes

Postharvest treatment with 10 µM JA caused significant short-term and long-term alterations to the sugarbeet root transcriptome. A total of 283 differentially expressed unigenes were identified in JA-treated roots 2 d after treatment (Table 2). A total of 326 differentially expressed unigenes were identified in JA-treated roots 60 d after treatment. 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. Of the differentially expressed unigenes, approximately 65% were up-regulated and 35% were down-regulated by JA at both 2 and 60 d. The expression changes observed at both 2 and 60 d after JA treatment suggest that JA has both short-term and long-term effects on sugarbeet roots. Transcriptome changes during the first 7 d after JA or methyl jasmonate (JA) treatment have been reported previously for a variety of plant species (Sasaki-Sekimoto et al., 2005; Gális et al, 2006; Babst et al, 2009; Sun et al, 2013; Hao et al., 2015; Oliveira et al., 2015; Ramírez-Estrada et al., 2016). To our knowledge, however, this is the first report of long-term effects of JA on the transcriptome.

Transcriptome changes that occurred at 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 stored at 2 and 60 d indicates that long-term JA effects were substantially different from short-term effects, and were not simply a continuation of early JA-induced changes.

The effect of JA on changes in gene expression varied from a logarithm (base 2) of the fold change in expression or log(FC) of -13 to +11 (Fig. 2), indicating a range of expression changes of 0.0001 to 2050-fold. The majority of differentially expressed genes were moderately altered in expression, with nearly 80% of differentially

expressed unigenes having log(FC) values of -5 to +5. Differentially expressed unigenes, with annotations and expression level changes, are available in Supplementary Table S1 (2 d) and Supplementary Table S2 (60 d).

3.2. Functional classification of differentially expressed unigenes

3.2.1. Gene ontology classification

A total of 61 and 72 differentially expressed unigenes from roots 2 and 60 d after JA treatment, respectively, had associated gene ontology (GO) terms, and these were used to categorize unigenes into the biological process or molecular function to which they contribute and 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 is comprised of genes that function in the detection of and response to internal and external stimuli. This class 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. Another 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. Jasmonic acid treatment is often associated with increases in antioxidant defenses. However, only 4 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. Pathways affect by JA

A total of 67 and 86 differentially expressed unigenes from roots 2 and 60 d after JA treatment, respectively, had associated Kyoto Encyclopedia of Genes and Genomes (KEGG) 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.

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.

Among pathways modulated by JA, in both time evaluated, biosynthesis secondary metabolites was found to be maximally represented after metabolic pathways. Several studies have showed that exogenous application of JA signaling compounds to the plant cell culture or intact plant stimulates biosynthesis of secondary metabolites, resulting in the expression of a set of defense genes and inducing resistance of host against pathogens. (Farmer et al., 2003, Gundlach et al., 1992, Mueller et al., 1993, Tamogami et al., 1997 and Kozlowski et al., 1999). Secondary metabolites play important mechanism in the plant defense against herbivores and pathogens. These compounds may prevent pathogen infection by limit the pathogen activity, growth, and spread (Daayf et al., 2003 and van Loon et al., 2006). Secondary compounds also may defense plant by reinforce plant cell walls and neutralizing reactive oxygen species and thus prevent the plant from suffering molecular damage caused by microorganisms, insects and herbivores (Heather et al., 2012 and Hassanpour et al., 2011). Similar pathways among secondary metabolic processes was affect by JA during storage, such as phenylpropanoids biosynthesis (5 unigenes at 2 d; 9 unigenes at 60 d), flavonoids biosynthesis (1 unigenes at 2 d; 10 unigenes at 60 d), ubiquinone and other terpenoid-quinone biosynthesis (2 unigenes at 2 d; 4 unigenes at 60 d) stilbenoid, and diarylheptanoid and gingerol biosynthesis (1 unigenes at 2 d; 8 unigenes at 60 d), and

phenylalanine metabolism (4 unigenes at 2 d; 9 unigenes at 60 d). Beyond of secondary metabolism, other important pathway, plant-pathogen interaction, was modified by JA (8 unigenes at 2 d; 7 unigenes at 60 d). The interations between a plant and its pathogens involve ability of a plant to recognize and defend itself against a potential pathogen landing on its surface (Boyd et. al, 2013). The alteration of this pathway by JA can improve capacity of sugarbeet root to recognize pathogen and trigger defense responses.

Interestingly fatty acid degradation/biosynthesis and peroxisome pathway were differently expressed 2 days after JA-treatment (table 3) due 3-fold up-regulation of long-chain acyl-CoA synthetase (ACS) (Suplementary 1). This enzyme is involved several process, such as, formation phospholipids, triacylglycerol and jasmonate (Watkins and Ellis, 2012). According Watkins and Ellis (2012) peroxissomal ACS enzymes seems required to formation of JA in Arabidopsis. Thus, ACS may relate jasmonate biosynthesis in sugarbeet and a possible feedback due JA-treatment. Although a little is known about it and important enzymes related jasmonate biosynthesis were not up-regulated neither 2 days nor 60 days post-treatment.

Plant growth and response to enviromental cues are regulated by phytohormones wich synergistically and/or antagonistically work in a complex network (Denancé et al., 2013; Jaillais and Chory, 2010). Recentetly, auxin, abscic acid, cytokinins, gibberellins and brassinosteroids have been also described to be key regulators of plant immune (Denancé et al., 2013). In sugarbeet roots was observerd that JA modified expression of genes involved in plant hormone signal transduction pathway at both 2 and 60d (table 2 and 3). These results suggest an interaction between JA-signaling and others phytohormones which may be essencial for immune response.

3.2.3. Putative defense-related unigenes

JA stimulates several genes include those involved in jasmonate biosynthesis, secondary metabolism, cell-wall formation, and those encoding stress protective and pathogenesis-related (PR) proteins (Cheong and Choi, 2003; Ding et al., 2002; Wang et al., 2014). In agreement with the literature sugarbeet RNA-Seq data showed that JA treatment induced many genes for the biosynthesis of secondary metabolites, cell-wall formation as well as genes for plant-pathogen interaction. Of the differentially expressed genes 2 d post-treatment, 30 up-regulated and 8 down-regulated putative

defense genes were identified (Table 5). At 60 d post-treatment, 49 up-regulated and 4 down-regulated putative defense genes were identified (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 trifoliate CTV.20, a gene involved in plant response to the Citrus tristeza virus (CTV). Indicating that JA induces different defense responses during the storage wich may result in different level of resistance. Thus roots JA-trated may have diverse levels of resistance if they are infected in different storage time.

JA is involved in induction of several defense genes. However, there are others hormones such as salicylic acid which also induces (Antico, 2012). JA reduced expression of some defense genes in sugarbeet (table 5 and 6) it may happen because this phytohormone can act antagonist to others hormones which may be involved induction of those defense genes.

Peroxidases, cinnamate-4-hydroxilase, chitinase acid, laccases, nbs-Irr resistant, pathogen-related thaumatin family protein, proteinase inhibitor and β-glucosidase were found at higher levels (fold change ranged from 1.8 to 8.3) in treated than control roots at 2 d subsequent to JA-application (Table 5). At 60 d post-treatment observed that peroxidase, chitinase and cinnamate-4 hydroxilase were also up-regulated. Beyond that, retrotransposon protein (9.3), AP2/ERF domain-containing transcription factor (7.1) and leucine-rich repeat (7.1) were greatest expressed among the putative genes defense (Table 6). O-methyltransferase (4.3), ribosome-inactivating protein (4.1), glycine-rich protein (3.5), glutathione S-transferase (3.5), cytochrome P450 (3.5), pathogenesis-related protein (3.3), chalcone isomerase (2.3) and WRKY (2.2) were also up-regulated (Table 6).

The synergist cross-talk between JA and ethylene (ET) is identified to happen generally for the response to necrotrophic pathogen (Pieterse et al., 2012). Induction of AP2/ERF, ethylene-responsive transcription factor and ACC oxidase expression observed 60d (Table 6 and supplementary) suggests cross-talk between JA/ET, activate by JA exogenous in sugarbeet roots. According to Lorenzo et al. (2003), ERF may provide JA and ethylene signaling pathways in Arabidopsis. Interestingly, AP2/ERF is related to activation of defense-related genes (Lorenzo et al., 2003) wich may increase sugarbeet resistance to necrotrophic pathogen during storage.

3.3. Temporal expression analysis of prominent defense unigenes

RNA-sequencing showed that important defense genes in surgarbeet were up-related by JA, such as acid chitin, peroxidase, nbs-Irr resistant protein, pathogenesis-related thaumatin family protein and cinnamate-4-hydroxilase. To confirm these results of the transcriptome sequencing analysis and study the different expression during storage qRT-PCR was performed on 11 selected defense genes.

JA induced resistance in pathogen-infected in sugarbeet was found to involve activation of related pathogen protein (PR) gene expression and stimulation of genes involved the phenylpropanoid pathway. Transcripts level of PR include acid chitinase (CHI), pathogenesis-related thaumatin family protein (PRT) and Nbs-Lrr resistance like protein (Nbs-Lrr) were significantly enhanced in sugarbeet roots by JA. CHI, wich is involved in hydrolyzing polymers of fungal cell walls (Wally et al., 2009), showed expression gene reduction 3-fold after JA application, increasing the expression to 4 and 11-fold 2d and 60 d soon after treatment, respectively (Figure 4). Whereas transcripts level of PRT, protein related rupture the fungal membrane by pore formation (Roberts and Selitrennikoff, 1990), were augmented up to 3-fold at 3 days after treatment (Figure 4). In relation to the three Nbs-Lrr analyzed, they had different expression behave. ccNBS-Lrr was highly expressed, 812-fold, 3d after treatment and this gene was meaningfully up-regulated 2d until 60d after JA application. Transcripts level of Nbs-Lrr I and Nbs-Lrr II were transiently increased, except at 0 and 3d after treatment for Nbs-Lrr II (Figure 4). Interestingly, Nbs-Lrr represents abundant group of proteins involved in the detection of diverse pathogens (McHale et al., 2006). These receptors proteins recognize effectors produced by specific pathogen races and then trigger immune responses (DeYoung and Innes, 2006).

Peroxidase (POD) and cinnamate-4-hydroxylase (C4H), key enzymes of phenylpropanoid pathway, both genes had increased expression at 2d and 3d post-treatment. The peroxidase genes also were induced by JA treatment in potato, arabidopsi, Stylosanthus humilis and rice (Sorokan et al., 2014, Traw et al., 2003, Curtis et al., 1997 and Hiraga et al., 2000). As well as transcripts of C4H were up-regulate in rice, Salvia miltiorrhiza (Bi et al., 2007 and Huang et al., 2008).

In relation to three different laccases, they were immediately up-related after JA-treatment (0d), with maximum expression at 3 days. In this time was observed fold-change of 12-fold for laccase I, and 7-fold for laccase II and III (Figure 5). Plant laccases are involved in monolignols oxidation in the early stages of lignification (Bao et al.,1993). Besides lignification, plant laccases have been shown to be involved wound healing and in the mechanism of defense against external conditions (deMarco and Roubelakis-Angelakis, 1997; Dwivedi et al., 2011). Moreover, recent study with a laccase gene in Arabidopsis showed to be involved in the formation of proanthocyanidin or tannin from the precursor epicatechin (Pourcel et al., 2005). Thus, the increase of genes expression, such as laccase, POD and C4H, related in the strengthening of plant cell walls and the production of toxic radicals could provide pathogen infection reduction in surgarbeet root.

JA has been reported to induce salicylic acid carboxyl methyltransferase, enzyme which converts salicylic acid (SA) into inactive methyl salicylic (MeSA) (Chen et al., 2003 and Koo et al., 2007). Similar effect was observed in sugarbeet roots. JA substantially increased the accumulation of salicylic acid carboxyl methyltransferase transcripts. The enhanced was of 9-fold at 2 days after JA-treatment, reducing approximately to 2-fold in the last days (30 and 60d) (Figure 5). These results demonstrate the antagonistic cross talk between SA and JA. Although both phytohormones are involved in the activation defensives genes against pathogen, they increase resistance to different pathogen. Some researches propose that SA defense against pathogen biotrophs, and JA and/or ethylene responses acting against necrotrophs (McDowell and Dangl, 2000). This may be an advantage for the sugarbeet defense against postharvest pathogens such as Botrytis cinere, Phoma betea and Penicilium claviforme (necrotrophs fungi).

3.4 Enzymes activities for JA-induced defense genes

Peroxidase activity in roots treated was similar to control, indicating that JA application induce expression of peroxidase but not affected its activity (5 and 6). Although cinnamate 4-hydroxylase (C4H) was up-related at 2, 3 and 60 d (Figure 5), its activity was not affect by application of 10 µM jasmonate acid (Figure 6). Previous study has shown that 10 µM jasmonate acid do not affect others enzymes activity in

sugarbeet roots, such as β-1,3-glucanase (Fugate et al, 2012). Therefore, **Wang et al (2015) observed that 10 µM JA applied on grape berries do not inducing the expression of VvNPR1.1 gene and accumulation of the phytoalexins. However, inoculating the berries with B. cinerea after 10 µM JA treatment whereas the 50 or 100 µM JA treatment directly induced these defense responses. Thus, 10 µM JA may trigger priming state on sugarbeet, but it is necessary the presence of the pathogen to activate defense process.

4. CONCLUSION

In conclusion, the present study suggests that JA treatment could prime sugarbeet to induct a series of defense genes, including defense-related proteins and key enzymes related to secondary metabolism.

JA also increased the ability of sugarbeet cells to recognize pathogen which may result faster activation of immune response and then reduction of infection and susceptibility.

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5. FIGURES AND TABLES

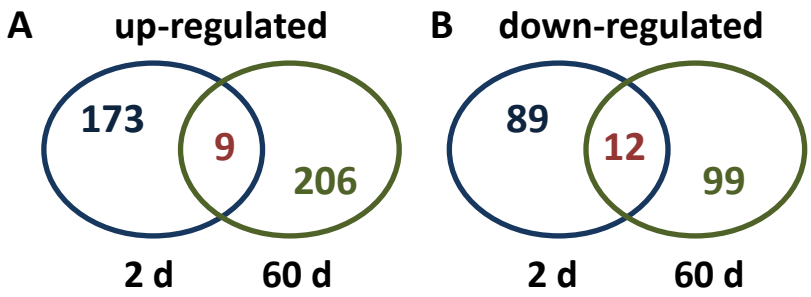

Figure 1- Heterogeneity of differentially expressed unigenes in sugarbeet roots treated with jasmonic acid (JA) and stored for 2 or 60 d. Venn diagrams display the diversity in **(A)** up-regulated unigenes from roots 2 d and 60 d after JA treatment and **(B)** down-regulated unigenes from roots 2 d and 60 d after JA treatment. Roots were treated with 0 or 10 µM JA 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.

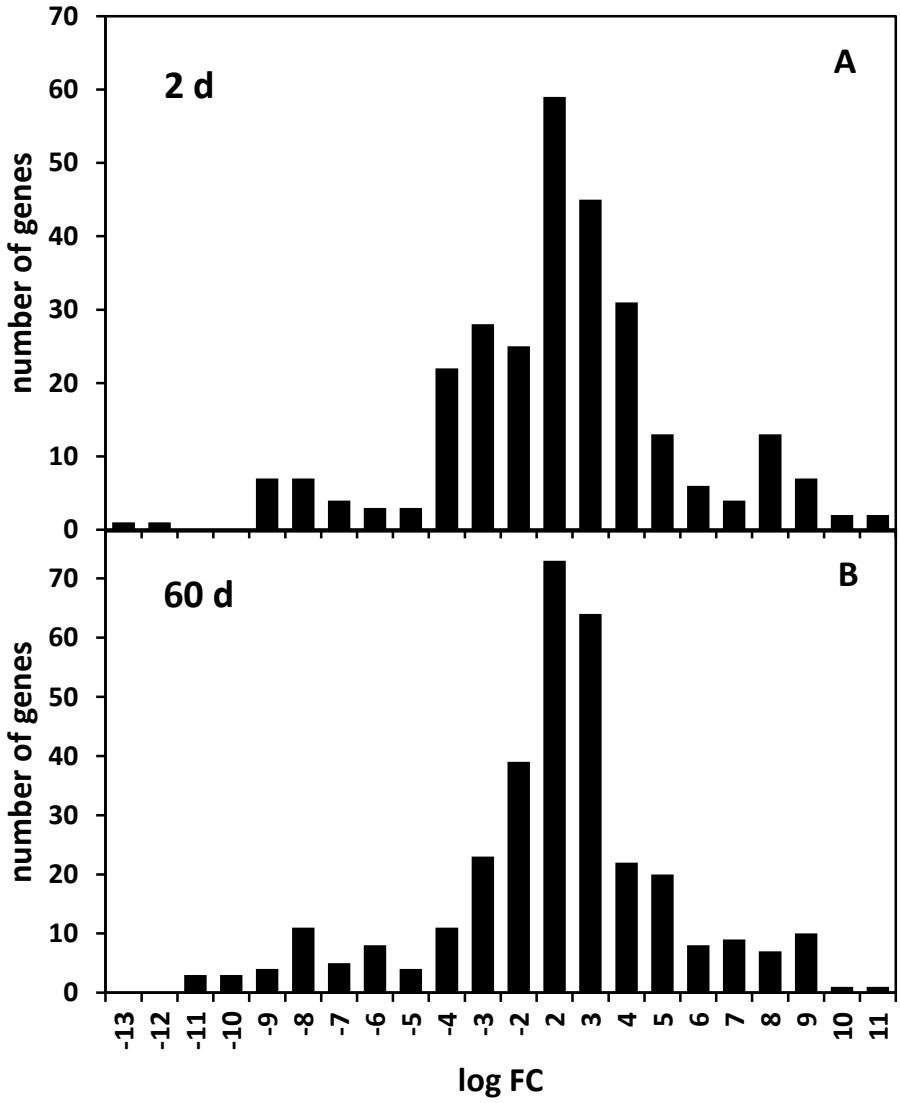

Figure 2. Distribution in the magnitude of gene expression alterations for differentially expressed unigenes from sugarbeet roots 2 and 60 d after jasmonic acid (JA) treatment. The magnitude alteration in gene expression was determined as the $\log_2$(fold change) in the number of RNAs sequenced from JA-treated roots relative to water-treated roots for each unigene. Roots were treated with 0 or 10 μM JA following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d.

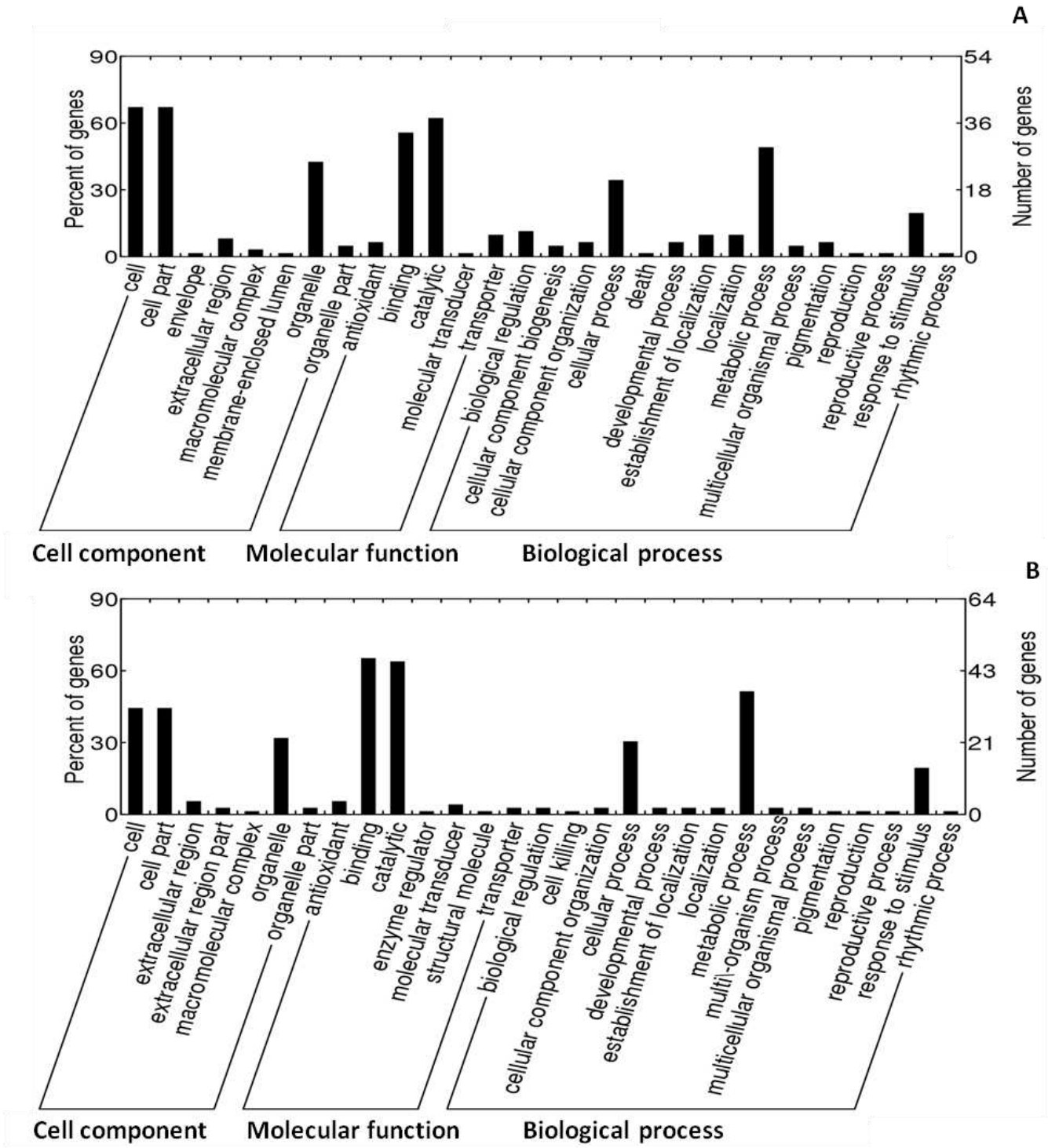

Figure 3. Classification of differentially expressed sugarbeet unigenes due to jasmonic acid (JA) treatment using gene ontology (GO) terms. (A) 2 d after JA treatment. (B) 60 d after JA treatment. Roots were treated with 0 or 10 μM JA following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d.

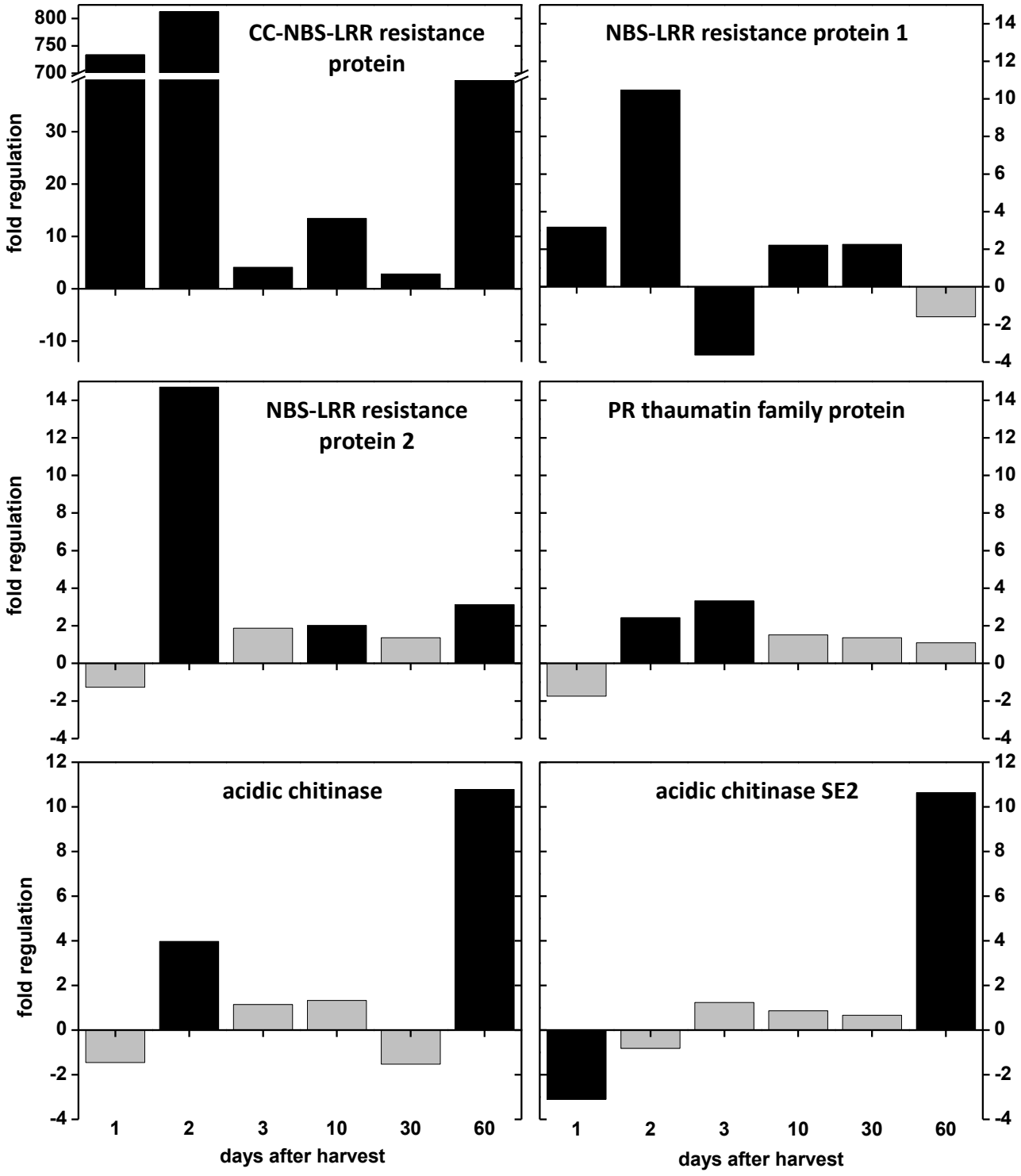

Fig. 4. Effect of jasmonic acid (JA) on expression of defense-related unigenes with homology to 3 putative regulatory proteins and 3 pathogenesis-related (PR) proteins in the 60 d following treatment. Differential expression was determined for unigenes encoding a coiled-coil (CC)-nucleotide-binding site (NBS)-leucine-rich repeat (LRR) resistance protein (unigene 9115), two NBS-LRR resistance proteins (unigenes 77250; 29351), a PR thaumatin family protein (unigene 81205), and two acidic chitinases (unigenes 71046; 53522, 66180, and 70896). Roots were treated with 10 μM 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 greater than |2.0| were considered significant and are indicated by black bars**.**

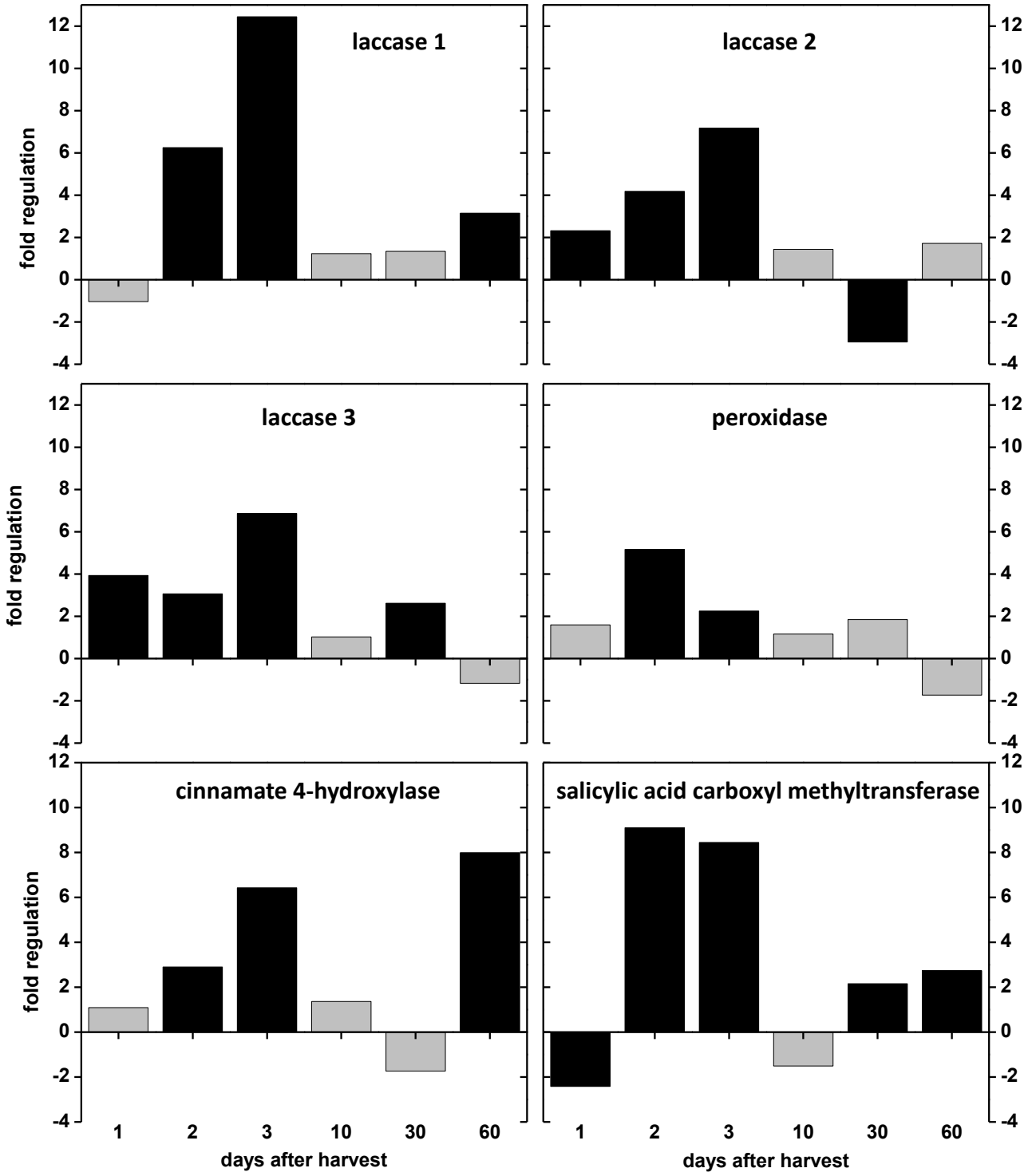

Fig. 5. Effect of jasmonic acid (JA) on expression of defense-related unigenes with homology to 4 oxidase-encoding unigenes and 2 unigenes with prominent roles in secondary metabolism biosynthesis. Differential expression was determined for unigenes encoding 3 putative laccase proteins (unigenes 11281; 51666, 67039, and 68954; 78937), a peroxidase protein (unigene 42562), a cinnamate 4-hydroxylase (unigenes 30677 and 55958), and a salicylic acid carboxyl methyltransferase (unigene 29506). Roots were treated with 10 μM 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 greater than |2.0| were considered significant and are indicated by black bars.

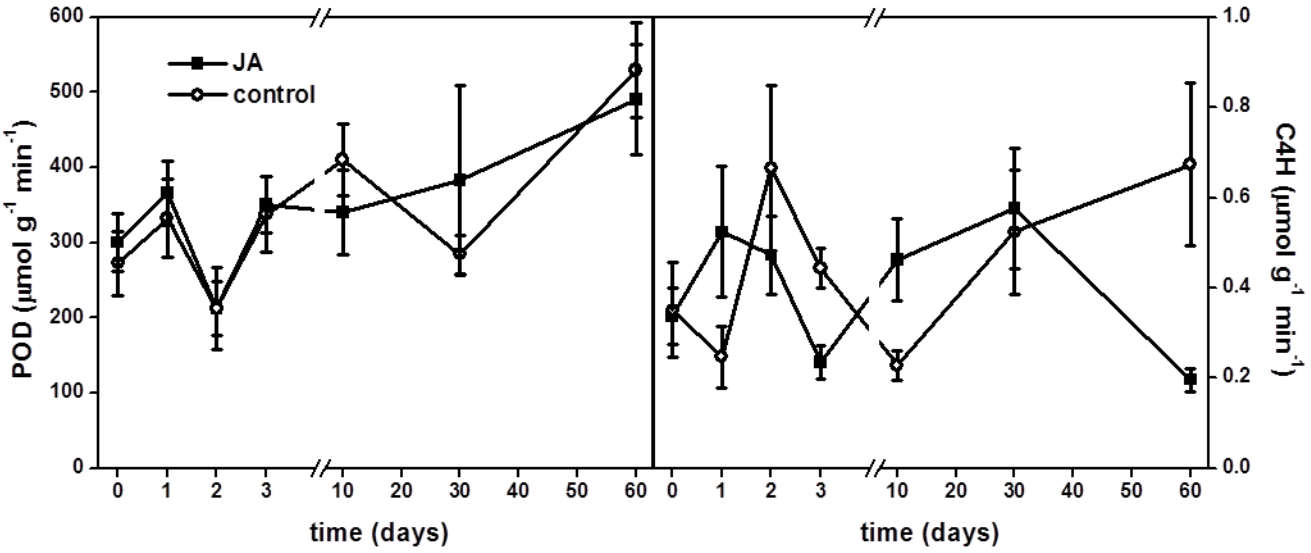

Figure 6. Peroxidase (POD) and cinnamante-4-hydroxylase (C4H) activities after jasmonic acid (JA) treatment of sugarbeet roots. Roots were treated with 10 µM JA or water (control) following harvest and incubated at 20 °C and 90% relative humidity for up to 60 d. Enzyme activities are expressed as a function of total soluble protein concentration.

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., 2013).

Gene	Accession/Unigene number	Forward primer	Reverse primer
Actin	DQ866829	GATTTGGCACCACACCTTCT	TCTTTTCCCTGTTTGCCTTG
acid chitinase	71046	CTGGATTCGGGGTAACCATA	ACGCTGCTAGCTAGGCTGAA
cc-nbs-lrr resistance protein	9115	TGGGCTACTGGCTACTGCTT	GCTTGGATCATCTCCATGCT
cinnamate 4-hydroxylase	30677, 55958	GGTTGGCTAACAACCCTGAA	CTCCTCCTTCCAACACCAAA
laccase I	11281	CCAAGCCACACCACTTACCT	CGTTTATGGAAGGGAGACGA
laccase II	51666, 67039, 68954	AAGGACAGGGTGGTCAGATG	CCAAGTTTGGGCAAGATGAC
laccase III	78937	CCATCATATTTGGGGAGTGG	CAAACCTTTAGCTCCCATGC
nbs-lrr resistant protein 1	77250	GATTGGGAGAAGGCGTACAA	ATCAATCCCTCAGCAATCCA
nbs-lrr resistant protein 2	29351	GGGTAAGAGAGTTGCCAAGC	TCCACAAGTGCAGAAGTTCG
pathogenesis-related thaumatin family protein	81205	GCCGGTGTCTGTCTTAGTCC	CCACCTCCATCCTTGACTTG
peroxidase	42562, 66692	CTGCTCAAGCAACCAAAACC	TAGGCGAGCAGGGACTTTAG
salicylic acid carboxyl methyltransferase	29506	GAGCTGTGGTTGAGCCCTTA	TTCGTCAGCCTCGACTTCTT

Table 2. Occurrence of differentially expressed unigenes in sugarbeet roots 2 and 60 d after jasmonic acid (JA) treatment. Roots were treated with water or 10 µM JA 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 BLAST 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)
putative defense gene	29	48
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)
putative defense gene	8	4

Table 3. Kyota Encyclopedia of Genes and Genomes (KEGG) pathways to which unigenes that were differentially expressed in sugarbeet roots 2 d after acid jasmonate (JA) treatment were assigned. Roots were treated with 10 µM JA 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 ID)
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)
	K05350	bglB; beta-glucosidase (55314)
2-Oxocarboxylic acid metabolism	K00826	branched-chain amino acid aminotransferase (29499)
	K11821	desulfoglucosinolate sulfotransferase A/B/C (66725)
Ubiquinone and other terpenoid-quinone biosynthesis	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. Kyota Encyclopedia of Genes and Genomes (KEGG) pathways to which unigenes that were differentially expressed in sugarbeet roots 60 d after acid jasmonate (JA) treatment were assigned. Roots were treated with 10 µM JA 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 ID)
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)
Biosynthesis of secondary metabolites	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)
	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)
Flavonoid biosynthesis	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)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
Plant-pathogen interaction	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)
	K13457	disease resistance protein RPM1 (10601)
Phenylalanine metabolism	K00430	peroxidase (6736, 51945, 59365, 67714)
	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K00815	tyrosine aminotransferase (66299)
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)
Phenylpropanoid biosynthesis	K00430	E1.11.1.7; peroxidase (6736, 51945,59365,67714)
	K00487	trans-cinnamate 4-monooxygenase (30677, 55958, 77129)
	K00588	caffeoyl-CoA O-methyltransferase (58834)
	K13065	shikimate O-hydroxycinnamoyltransferase (78279)
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. Unigenes that were differentially expressed in sugarbeet roots 2 d after jasmonic acid (JA) treatment that have a putative role in plant defense. Roots were treated with 10 µM JA or distilled 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 (logFC) ≥ 1 and a false detectection rate (FDR) ≤ 0.001, and exhibited significant alterations in expression in both experimental repetitions. Annotations were obtained by a BLAST search of unigene sequence against GenBank's nonredundant (Nr) protein database. Unigenes sequences selected for quantitative PCR analysis are underlined.

Unigene ID	logFC	Reads				E value	Nr-annotation	Nr-ID
		JA_1	$control_1$	JA_2	$control_2$			
10601	8.3	1	-	35	-	9E-25	nbs-lrr resistance protein [Populus trichocarpa]	gi|224096480|ref|XP_002334697.1|
9115	6.0	77	2	237	3	8E-6	cc-nbs-lrr resistance protein [Populus trichocarpa]	gi|224092698|ref|XP_002309702.1|
11281	4.7	5	-	76	3	0	laccase [Liriodendron tulipifera]	gi|1621467|gb|AAB17194.1|
81205	4.5	2	-	45	2	2E-79	pathogenesis-related thaumatin family protein [Arabidopsis thaliana]	gi|15242552|ref|NP_198818.1|
42562	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|
71046	3.8	64	5	24	1	1E-30	acidic chitinase [Elaeagnus umbellata]	gi|3126963|gb|AAC16010.1|
66692	3.8	7	2	178	12	1E-58	peroxidase [Spinacia oleracea]	gi|2956707|emb|CAA76376.1|
29506	3.6	11	2	27	1	2E-11	salicylic acid carboxyl methyltransferase [Mikania micrantha]	gi|227278441|gb|ACP20216.1|
51666	3.5	5	2	40	2	3E-32	laccase 1a [Populus trichocarpa]	gi|224139024|ref|XP_002322961.1|
77250	3.4	56	7	113	9	9E-35	nbs-lrr resistance protein [Populus trichocarpa]	gi|224096480|ref|XP_002334697.1|
29351	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|
67039	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|
68954	2.9	11	6	90	8	6E-59	laccase 1a [Populus trichocarpa]	gi|224139024|ref|XP_002322961.1|
30677	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|
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 [Oryza sativa Japonica Group]	gi|115456593|ref|NP_001051897.1|
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. Unigenes that were differentially expressed in sugarbeet roots 60 d after jasmonic acid (JA) treatment that have a putative role in plant defense. Roots were treated with 10 µM JA or distilled 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 of the fold change in expression (logFC) ≥ 1 and a false detectection rate (FDR) ≤ 0.001, and exhibited significant alterations in expression in both experimental repetitions. Annotations were obtained by a BLAST search of unigene sequence against GenBank's nonredundant (Nr) protein database. Unigenes sequences selected for quantitative PCR analysis 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|
55958	5.1	2	1	68	1	6E-13	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi|4206116|gb|AAD11427.1|
30677	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|
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|
53522	2.5	310	113	392	20	1E-11	endochitinase SE2; Flags: Precursor [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; Flags: Precursor [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	RecName: Full=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	RecName: Full=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 [Fallopia multiflora]	gi|300885273|gb|ADK45325.1|
31086	2.0	175	110	312	15	2E-08	Chain A, Resolution Of The Structure Of The 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 [Nicotiana tabacum]	gi|85068596|gb|ABC69378.1|
70896	1.8	446	195	464	88	4E-07	RecName: Full=Acidic endochitinase SE2; Flags: Precursor [Beta vulgaris]	gi|544000|sp|P36910.1|CHIE_BETVU
6804	1.7	172	132	363	39	1E-06	RecName: Full=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

6. SUPPLEMENTARY

Supplementary 1. Unigenes that were differentially expressed in sugarbeet roots 2 d after jasmonic acid (JA) treatment. Roots were treated with 10 µM JA or distilled 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 (logFC) ≥ 1 and a false detectection rate (FDR) ≤ 0.001, and exhibited significant alterations in expression in both experimental repetitions. Annotations were obtained by a BLAST search of unigene sequence against GenBank's nonredundant (Nr) protein database.

GeneID	logFC	Direction	PValue	Nr-annotation	Nr-ID
Unigene59489	11,2	up	3,4E-32	--	--
Unigene63223	10,5	up	1,2E-25	--	--
Unigene30914	9,8	up	1,0E-19	homeodomain protein GL2-like 1 [Gossypium hirsutum]	gi|177667009|gb|ACB73218.1|
Unigene9468	9,5	up	1,8E-17	--	--
Unigene14056	9,4	up	8,7E-17	--	--
Unigene11899	9,4	up	1,0E-16	--	--
Unigene11557	9,3	up	6,1E-16	--	--
Unigene10113	9,3	up	9,1E-16	--	--
Unigene11714	9,0	up	7,6E-14	Os02g0674800 [Oryza sativa Japonica Group]	gi|115447877|ref|NP_001047718.1|
Unigene17809	8,9	up	4,0E-13	--	--
Unigene72228	8,8	up	5,6E-13	CXE carboxylesterase [Malus pumila]	gi|82697951|gb|ABB89010.1|
Unigene68984	8,3	up	3,3E-10	LOC100281145 [Zea mays]	gi|226504460|ref|NP_001147536.1|
Unigene10601	8,3	up	4,9E-10	nbs-lrr resistance protein [Populus trichocarpa]	gi|224096480|ref|XP_002334697.1|
Unigene29433	8,2	up	7,9E-10	--	--
Unigene76243	8,1	up	2,9E-09	cyclic nucleotide and calmodulin-regulated ion channel-like protein [Arabidopsis thaliana]	gi|7269936|emb|CAB81029.1|
Unigene9056	8,1	up	2,1E-44	--	--
Unigene44886	8,1	up	5,2E-09	--	--
Unigene9554	8,0	up	5,7E-09	--	--
Unigene16364	8,0	up	6,0E-09	--	--
Unigene37275	7,9	up	1,5E-08	--	--
Unigene19671	7,8	up	5,6E-08	plus agglutinin [Chlamydomonas incerta]	gi|60678627|gb|AAX33674.1|
Unigene79514	7,8	up	8,2E-08	Os03g0291200 [Oryza sativa Japonica Group]	gi|115452395|ref|NP_001049798.1|
Unigene26409	7,6	up	3,8E-07	--	--
Unigene25269	7,5	up	4,5E-07	--	--
Unigene38334	7,5	up	7,5E-07	--	--
Unigene13658	7,4	up	1,9E-06	--	--
Unigene14086	7,3	up	2,1E-06	--	--
Unigene23677	6,8	up	7,3E-34	--	--
Unigene79315	6,4	up	7,9E-27	--	--
Unigene4213	6,0	up	1,3E-13	--	--
Unigene9115	6,0	up	1,6E-27	cc-nbs-lrr resistance protein [Populus trichocarpa]	gi|224092698|ref|XP_002309702.1|
Unigene28490	5,9	up	3,1E-13	--	--
Unigene56344	5,8	up	2,2E-12	orf147a [Beta vulgaris subsp. vulgaris]	gi|54606709|dbj|BAD66732.1|

Unigene9968	5,7	up	4,5E-12	retrotransposon protein [Cucumis melo subsp. melo]	gi|307135889|gb|ADN33754.1|
Unigene73703	5,5	up	2,0E-18	--	--
Unigene24061	4,9	up	8,5E-08	--	--
Unigene55419	4,8	up	2,2E-21	--	--
Unigene2809	4,8	up	1,1E-10	--	--
Unigene11281	4,7	up	4,7E-13	laccase [Liriodendron tulipifera]	gi|1621467|gb|AAB17194.1|
Unigene52384	4,7	up	3,9E-07	--	--
Unigene54851	4,7	up	4,3E-07	--	--
Unigene18449	4,6	up	2,6E-19	--	--
Unigene11868	4,6	up	1,3E-06	--	--
Unigene28718	4,5	up	8,0E-12	--	--
Unigene41959	4,5	up	2,1E-06	glyceraldehyde-3-phosphate dehydogenase [Beta vulgaris]	gi|125662890|gb|ABN50381.1|
Unigene29966	4,5	up	2,4E-06	--	--
Unigene81205	4,5	up	2,6E-09	pathogenesis-related thaumatin family protein [Arabidopsis thaliana]	gi|15242552|ref|NP_198818.1|
Unigene25607	4,5	up	2,6E-06	Os04g0410600 [Oryza sativa Japonica Group]	gi|115458260|ref|NP_001052730.1|
Unigene10063	4,5	up	8,8E-18	--	--
Unigene21756	4,4	up	5,6E-11	ESTs gb|AA042581 and gb|H36253 come from this gene [Arabidopsis thaliana]	gi|5668815|gb|AAD46041.1|AC007519_26
Unigene67385	4,3	up	2,2E-10	--	--
Unigene365	4,2	up	2,8E-11	AT3G14850 [Arabidopsis thaliana]	gi|222424963|dbj|BAH20432.1|
Unigene50143	4,2	up	1,2E-07	--	--
Unigene42562	4,2	up	2,1E-14	peroxidase [Spinacia oleracea]	gi|2956705|emb|CAA76377.1|
Unigene65362	4,1	up	2,3E-07	retrotransposon protein, putative, unclassified [Oryza sativa Japonica Group]	gi|108707503|gb|ABF95298.1|
Unigene21264	4,0	up	2,4E-10	cellulose synthase [Populus trichocarpa]	gi|224065557|ref|XP_002301856.1|
Unigene51875	4,0	up	8,2E-15	octicosapeptide/Phox/Bem1p (PB1) domain-containing protein [Arabidopsis thaliana]	gi|15231568|ref|NP_189282.1|
Unigene58265	3,9	up	4,8E-11	--	--
Unigene29022	3,9	up	1,8E-08	Os07g0575900 [Oryza sativa Japonica Group]	gi|115472967|ref|NP_001060082.1|
Unigene28653	3,8	up	1,9E-10	NAC domain protein, IPR003441 [Populus trichocarpa]	gi|224136718|ref|XP_002322398.1|
Unigene71046	3,8	up	2,7E-11	acidic chitinase [Elaeagnus umbellata]	gi|3126963|gb|AAC16010.1|
Unigene34661	3,8	up	2,0E-15	peroxidase [Spinacia oleracea]	gi|2956707|emb|CAA76376.1|
Unigene70517	3,8	up	3,3E-06	SULTR3_5 [Arabidopsis lyrata subsp. lyrata]	gi|297812143|ref|XP_002873955.1|
Unigene73556	3,8	up	3,4E-06	Os07g0572400 [Oryza sativa Japonica Group]	gi|115472919|ref|NP_001060058.1|
Unigene77616	3,8	up	3,6E-18	alpha galactosidase precursor [Coffea arabica]	gi|158934007|emb|CAJ40777.1|
Unigene66692	3,8	up	2,4E-15	peroxidase [Spinacia oleracea]	gi|2956707|emb|CAA76376.1|
Unigene30836	3,7	up	1,6E-07	acyl-CoA synthetase [Beta vulgaris]	gi|111154054|dbj|BAF02671.1|
Unigene80593	3,7	up	1,6E-07	expansin [Glycine max]	gi|27464177|gb|AAO15998.1|AF516879_1
Unigene82314	3,7	up	1,6E-13	cellulose synthase 3 [Eucalyptus grandis]	gi|67003911|gb|AAY60845.1|
Unigene20004	3,6	up	3,5E-08	type-a response regulator [Populus trichocarpa]	gi|224066817|ref|XP_002302230.1|
Unigene29506	3,6	up	5,6E-07	salicylic acid carboxyl methyltransferase [Mikania micrantha]	gi|227278441|gb|ACP20216.1|
Unigene71855	3,6	up	2,6E-13	AT3G26510 [Arabidopsis thaliana]	gi|222423545|dbj|BAH19742.1|
Unigene43079	3,6	up	6,8E-08	germin-like protein [Beta vulgaris]	gi|99646728|emb|CAK22420.1|
Unigene79829	3,6	up	1,0E-06	integral membrane protein like [Zea mays]	gi|195653459|gb|ACG46197.1|
Unigene66914	3,6	up	4,0E-12	--	--
Unigene56055	3,5	up	7,3E-20	--	--
Unigene7343	3,5	up	8,0E-12	cellulose synthase catalytic subunit 12 [Zea mays]	gi|162461937|ref|NP_001105532.1|

Unigene79797	3,5	up	1,5E-08	At1g09610 [Arabidopsis thaliana]	gi|38454180|gb|AAR20784.1|
Unigene40701	3,5	up	3,8E-15	MLP34 (MLP-LIKE PROTEIN 34) [Arabidopsis thaliana]	gi|30698759|ref|NP_565003.3|
Unigene51666	3,5	up	1,9E-07	laccase 1a [Populus trichocarpa]	gi|224139024|ref|XP_002322961.1|
Unigene22446	3,4	up	1,1E-09	--	--
Unigene576	3,4	up	3,4E-10	ATCNGC17 [Arabidopsis lyrata subsp. lyrata]	gi|297798938|ref|XP_002867353.1|
Unigene80721	3,4	up	4,1E-10	sulfate transporter [Populus tremula x Populus alba]	gi|117557144|gb|ABK35749.1|
Unigene4780	3,4	up	1,7E-09	cupin family protein [Arabidopsis thaliana]	gi|15240338|ref|NP_200983.1|
Unigene77250	3,4	up	3,0E-13	nbs-lrr resistance protein [Populus trichocarpa]	gi|224096480|ref|XP_002334697.1|
Unigene59220	3,4	up	4,9E-11	germin-like protein 5 [Glycine max]	gi|196122014|gb|ACG69481.1|
Unigene21306	3,4	up	1,3E-08	TED7 (TRACHEARY ELEMENT DIFFERENTIATION-RELATED 7) [Arabidopsis thaliana]	gi|15239746|ref|NP_199703.1|
Unigene12054	3,4	up	4,5E-17	cellulose synthase [Betula luminifera]	gi|212960417|gb|ACJ38666.1|
Unigene74239	3,3	up	3,9E-09	UPA16 [Capsicum annuum]	gi|257831431|gb|ACV71016.1|
Unigene14068	3,3	up	1,4E-07	AT5g60720/mup24_130 [Arabidopsis thaliana]	gi|15983811|gb|AAL10502.1|
Unigene19343	3,2	up	8,2E-12	--	--
Unigene18548	3,2	up	1,5E-08	methyladenine glycosylase family protein [Arabidopsis lyrata subsp. lyrata]	gi|297844266|ref|XP_002890014.1|
Unigene29351	3,2	up	1,7E-14	nbs-lrr resistance protein [Populus trichocarpa]	gi|224075299|ref|XP_002304589.1|
Unigene77135	3,2	up	3,3E-06	LAC11 (laccase 11); laccase [Arabidopsis thaliana]	gi|22326581|ref|NP_195946.2|
Unigene4405	3,2	up	3,4E-06	zinc finger family protein [Arabidopsis lyrata subsp. lyrata]	gi|297801900|ref|XP_002868834.1|
Unigene30311	3,1	up	1,4E-06	expressed protein [Arabidopsis thaliana]	gi|20197184|gb|AAC16076.2|
Unigene59366	3,1	up	3,9E-07	halo-acid dehalogenase-like hydrolase (ISS) [Ostreococcus tauri]	gi|308806411|ref|XP_003080517.1|
Unigene66324	3,0	up	1,8E-07	--	--
Unigene78120	3,0	up	1,8E-10	Mannan endo-1,4-beta-mannosidase 6	gi|75264487|sp|Q9LZV3.1|MAN6_ARATH
Unigene71638	3,0	up	3,3E-06	homogentisate geranylgeranyl transferase [Hevea brasiliensis]	gi|219842170|dbj|BAH10642.1|
Unigene11112	3,0	up	2,9E-08	--	--
Unigene67039	3,0	up	1,1E-11	laccase 1a [Populus trichocarpa]	gi|224139024|ref|XP_002322961.1|
Unigene29708	3,0	up	1,3E-06	laccase 3 [Populus trichocarpa]	gi|224112579|ref|XP_002316233.1|
Unigene46078	3,0	up	2,1E-13	cysteine proteinase [Petunia x hybrida]	gi|52546920|gb|AAU81593.1|
Unigene43381	2,9	up	3,8E-10	--	--
Unigene56767	2,9	up	1,1E-10	--	--
Unigene68954	2,9	up	4,6E-09	laccase 1a [Populus trichocarpa]	gi|224139024|ref|XP_002322961.1|
Unigene78625	2,8	up	4,3E-09	tracheary element differentiation-related 7B [Zinnia violacea]	gi|229002017|dbj|BAH57855.1|
Unigene52307	2,8	up	2,5E-07	Os11g0522900 [Oryza sativa Japonica Group]	gi|297728403|ref|NP_001176565.1|
Unigene68697	2,8	up	1,3E-07	--	--
Unigene1282	2,7	up	2,0E-10	FAD-binding domain-containing protein [Arabidopsis lyrata subsp. lyrata]	gi|297846030|ref|XP_002890896.1|
Unigene30775	2,7	up	6,2E-09	IRX6 [Arabidopsis thaliana]	gi|30685446|ref|NP_197067.2|
Unigene28401	2,7	up	4,0E-11	vacuolar citrate/H+ symporter [Citrus sinensis]	gi|116804319|gb|ABK27327.1|
Unigene22143	2,7	up	1,1E-07	--	--
Unigene62942	2,7	up	3,4E-06	fasciclin-like AGP 11 [Populus tremula x Populus alba]	gi|47717925|gb|AAT37954.1|
Unigene30677	2,7	up	9,0E-07	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi|4206116|gb|AAD11427.1|
Unigene78937	2,7	up	2,1E-08	laccase [Liriodendron tulipifera]	gi|1621463|gb|AAB17192.1|
Unigene16917	2,6	up	2,6E-09	glucosyltransferase [Phytolacca americana]	gi|204022238|dbj|BAG71127.1|
Unigene23362	2,6	up	1,6E-07	--	--
Unigene66725	2,6	up	9,8E-09	sulfotransferase family protein [Arabidopsis thaliana]	gi|15230602|ref|NP_190093.1|
Unigene43963	2,6	up	2,0E-06	--	--

Unigene80875	2,6	up	2,2E-06	pectinesterase family protein [Arabidopsis lyrata subsp. lyrata]	gi|297823481|ref|XP_002879623.1|
Unigene26919	2,5	up	2,1E-10	glucosyltransferase [Phytolacca americana]	gi|204022238|dbj|BAG71127.1|
Unigene55314	2,5	up	3,2E-06	beta-glucosidase [Arabidopsis lyrata subsp. lyrata]	gi|297830448|ref|XP_002883106.1|
Unigene80067	2,5	up	1,4E-11	serine carboxypeptidase II [Platanus x acerifolia]	gi|94442908|emb|CAJ91147.1|
Unigene19499	2,5	up	3,6E-07	XSP1 (xylem serine peptidase 1); identical protein binding / serine-type endopeptidase [Arabidopsis thaliana]	gi|18411254|ref|NP_567155.1|
Unigene64730	2,5	up	2,1E-06	--	--
Unigene62192	2,4	up	2,4E-10	Os11g0543300 [Oryza sativa Japonica Group]	gi|115485799|ref|NP_001068043.1|
Unigene37107	2,4	up	1,3E-06	--	--
Unigene81639	2,4	up	1,3E-06	cellulose synthase-like protein CslG [Nicotiana tabacum]	gi|73624747|gb|AAZ79231.1|
Unigene38313	2,4	up	1,6E-08	carboxypeptidase type III [Theobroma cacao]	gi|21901929|emb|CAC86383.1|
Unigene62437	2,4	up	1,4E-06	Mannan endo-1,4-beta-mannosidase 6	gi|75264487|sp|Q9LZV3.1|MAN6_ARATH
Unigene17234	2,4	up	7,0E-08	--	--
Unigene78540	2,4	up	2,3E-08	lysine/histidine transporter [Populus trichocarpa]	gi|224111726|ref|XP_002315954.1|
Unigene78451	2,3	up	6,9E-08	germin-like protein 2 [Vitis vinifera]	gi|111379986|gb|ABH09468.1|
Unigene73340	2,3	up	4,0E-09	At3g44710 [Arabidopsis thaliana]	gi|30725352|gb|AAP37698.1|
Unigene58358	2,3	up	1,0E-07	--	--
Unigene56199	2,3	up	7,4E-09	--	--
Unigene66478	2,3	up	1,1E-06	--	--
Unigene75893	2,3	up	8,6E-07	glucose-methanol-choline (GMC) oxidoreductase family protein [Arabidopsis thaliana]	gi|15223677|ref|NP_172871.1|
Unigene32378	2,3	up	6,0E-07	--	--
Unigene30930	2,3	up	8,6E-09	copalyl diphosphate synthase [Populus trichocarpa]	gi|224082644|ref|XP_002306777.1|
Unigene78489	2,3	up	1,8E-08	berberine bridge enzyme-like protein [Arabidopsis thaliana]	gi|16648925|gb|AAL24314.1|
Unigene25408	2,3	up	1,5E-06	wound-inducible carboxypeptidase [Solanum lycopersicum]	gi|7271957|gb|AAF44708.1|AF242849_1
Unigene56439	2,2	up	2,4E-06	carboxypeptidase type III [Theobroma cacao]	gi|21901929|emb|CAC86383.1|
Unigene76951	2,2	up	1,7E-09	--	--
Unigene37099	2,2	up	4,2E-07	extensin-like protein [Citrus junos]	gi|21360370|gb|AAM47507.1|
Unigene54605	2,2	up	9,6E-09	predicted protein [Populus trichocarpa]	gi|224111964|ref|XP_002316037.1|
Unigene31655	2,2	up	2,4E-06	--	--
Unigene65412	2,2	up	9,0E-07	--	--
Unigene61227	2,2	up	3,7E-08	Os11g0522900 [Oryza sativa Japonica Group]	gi|297728403|ref|NP_001176565.1|
Unigene71094	2,2	up	1,5E-08	Os08g0356800 [Oryza sativa Japonica Group]	gi|115476054|ref|NP_001061623.1|
Unigene27189	2,2	up	6,7E-07	Mannan endo-1,4-beta-mannosidase 6	gi|75264487|sp|Q9LZV3.1|MAN6_ARATH
Unigene9061	2,2	up	6,3E-07	--	--
Unigene6570	2,2	up	2,3E-06	PUB26 (PLANT U-BOX 26); binding / ubiquitin-protein ligase [Arabidopsis thaliana]	gi|15222819|ref|NP_175400.1|
Unigene34958	2,1	up	1,1E-07	--	--
Unigene44602	2,1	up	1,2E-06	--	--
Unigene7601	2,1	up	8,7E-07	Os03g0849500 [Oryza sativa Japonica Group]	gi|115456593|ref|NP_001051897.1|
Unigene75317	2,1	up	3,6E-07	Os01g0553400 [Oryza sativa Japonica Group]	gi|115437518|ref|NP_001043315.1|
Unigene25049	2,1	up	7,8E-07	--	--
Unigene6688	2,1	up	1,2E-07	ATNAP8; ATPase, coupled to transmembrane movement of substances / transporter [Arabidopsis thaliana]	gi|145334131|ref|NP_001078446.1|
Unigene76480	2,0	up	2,0E-06	ribonuclease [Pyrus pyrifolia]	gi|1526417|dbj|BAA08475.1|
Unigene9632	2,0	up	7,9E-08	aspartic proteinase 1 [Castanea mollissima]	gi|261264941|gb|ACX55829.1|

Unigene11382	2,0	up	1,7E-06	Os03g0215700 [Oryza sativa Japonica Group]	gi|115451555|ref|NP_001049378.1|
Unigene80285	2,0	up	2,2E-06	wound-inducible carboxypeptidase [Solanum lycopersicum]	gi|7271957|gb|AAF44708.1|AF242849_1
Unigene54921	1,9	up	1,4E-06	--	--
Unigene25940	1,9	up	1,1E-07	--	--
Unigene73083	1,9	up	9,1E-07	--	--
Unigene32676	1,9	up	7,9E-07	amino acid binding protein, putative [Ricinus communis]	gi|255540149|ref|XP_002511139.1|
Unigene68309	1,9	Up	3,3E-06	--	--
Unigene22064	1,8	Up	3,7E-07	ABC transporter family protein [Arabidopsis thaliana]	gi|18398110|ref|NP_564383.1|
Unigene67654	1,8	Up	3,4E-07	proteinase inhibitor [Jatropha curcas]	gi|284433788|gb|ADB85100.1|
Unigene78801	1,8	Up	1,1E-07	white-brown-complex ABC transporter family [Populus trichocarpa]	gi|224073568|ref|XP_002304113.1|
Unigene69921	1,8	Up	2,8E-06	peptide/glutathione transporter OPT1 [Vitis vinifera]	gi|29465762|gb|AAM14671.1|
Unigene70380	1,8	Up	9,5E-07	--	--
Unigene29499	1,8	Up	1,9E-06	4-amino-4-deoxychorismate lyase [Solanum lycopersicum]	gi|50345543|gb|AAT74744.1|
Unigene25150	1,8	Up	3,0E-06	tau class glutathione transferase GSTU45 [Populus trichocarpa]	gi|283135872|gb|ADB11327.1|
Unigene77401	1,7	Up	9,7E-07	vacuolar malate transmembrane transporter [Malus x domestica]	gi|308756027|gb|ADO51068.1|
Unigene80055	1,7	Up	3,0E-06	--	--
Unigene81041	1,7	Up	1,6E-06	abc transporter family protein [Arabidopsis lyrata subsp. lyrata]	gi|297851608|ref|XP_002893685.1|
Unigene82285	1,7	Up	1,0E-06	DNA topoisomerase II [Nicotiana tabacum]	gi|26984133|gb|AAN85207.1|
Unigene78196	1,6	Up	2,8E-06	ribosome-inactivating protein [Beta vulgaris]	gi|99646716|emb|CAK22417.1|
Unigene14728	-1,7	down	1,8E-06	cysteine-rich protein [Glycine max]	gi|223452302|gb|ACM89479.1|
Unigene80850	-1,8	down	3,0E-06	At2g30820 [Arabidopsis thaliana]	gi|44917443|gb|AAS49046.1|
Unigene22684	-1,9	down	2,6E-06	DNA binding [Arabidopsis thaliana]	gi|145336703|ref|NP_175754.2|
Unigene71596	-1,9	down	4,4E-07	--	--
Unigene70675	-1,9	down	7,6E-08	--	--
Unigene57553	-1,9	down	8,5E-07	--	--
Unigene70323	-2,0	down	2,0E-08	--	--
Unigene6185	-2,0	down	3,4E-06	Os12g0477100 [Oryza sativa Japonica Group]	gi|115488538|ref|NP_001066756.1|
Unigene75561	-2,0	down	5,2E-07	--	--
Unigene14821	-2,0	down	2,9E-07	transposon-like protein [Beta vulgaris]	gi|121501704|gb|ABM55245.1|
Unigene24149	-2,0	down	5,8E-07	predicted protein [Populus trichocarpa]	gi|224087451|ref|XP_002308172.1|
Unigene15067	-2,0	down	9,2E-07	--	--
Unigene31403	-2,0	down	4,1E-09	--	--
Unigene28998	-2,0	down	2,4E-06	--	--
Unigene30582	-2,1	down	1,4E-06	--	--
Unigene61759	-2,1	down	4,4E-09	--	--
Unigene69191	-2,1	down	2,1E-06	--	--
Unigene8677	-2,1	down	1,2E-07	--	--
Unigene23616	-2,2	down	9,0E-08	--	--
Unigene49998	-2,3	down	2,6E-10	--	--
Unigene78070	-2,3	down	8,4E-08	Expressed protein [Arabidopsis thaliana]	gi|20198188|gb|AAM15449.1|
Unigene11901	-2,4	down	7,2E-10	Putative gag-pol polyprotein, identical [Solanum demissum]	gi|47824985|gb|AAT38758.1|
Unigene8913	-2,4	down	1,0E-08	--	--
Unigene70657	-2,4	down	1,4E-09	--	--
Unigene59575	-2,4	down	1,4E-07	--	--

Unigene73032	-2,6	down	3,1E-09	--	--
Unigene23606	-2,6	down	2,5E-07	--	--
Unigene68313	-2,6	down	1,2E-07	--	--
Unigene24	-2,6	down	1,2E-12	Os04g0120000 [Oryza sativa Japonica Group]	gi|297602081|ref|NP_001052068.2|
Unigene30280	-2,7	down	7,7E-10	NAC domain protein, IPR003441 [Populus trichocarpa]	gi|224104873|ref|XP_002313601.1|
Unigene10383	-2,7	down	5,6E-09	Os06g0104000 [Oryza sativa Japonica Group]	gi|297605027|ref|NP_001056547.2|
Unigene37217	-2,8	down	1,4E-07	--	--
Unigene24580	-2,8	down	1,2E-08	--	--
Unigene69765	-2,8	down	7,1E-15	--	--
Unigene64880	-2,9	down	3,5E-10	--	--
Unigene17222	-3,0	down	1,2E-06	--	--
Unigene23945	-3,0	down	3,1E-15	--	--
Unigene75619	-3,1	down	4,6E-08	polypeptide with a gag-like domain [Petunia x hybrida]	gi|66841323|dbj|BAD99219.1|
Unigene77229	-3,1	down	8,6E-10	--	--
Unigene60453	-3,1	down	3,4E-07	hypothetical protein OsI_15342 [Oryza sativa Indica Group]	gi|218194582|gb|EEC77009.1|
Unigene27839	-3,1	down	5,1E-08	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene54116	-3,2	down	3,4E-06	--	--
Unigene22134	-3,2	down	1,7E-12	--	--
Unigene33174	-3,2	down	3,4E-07	--	--
Unigene50923	-3,2	down	1,7E-10	--	--
Unigene66460	-3,3	down	2,4E-09	contains similarity to transposase [Arabidopsis thaliana]	gi|4325363|gb|AAD17360.1|
Unigene64406	-3,3	down	4,4E-10	--	--
Unigene27587	-3,3	down	1,1E-09	--	--
Unigene77328	-3,4	down	1,4E-14	Os02g0788800 [Oryza sativa Japonica Group]	gi|115449141|ref|NP_001048350.1|
Unigene16954	-3,4	down	1,8E-15	predicted protein [Micromonas sp. RCC299]	gi|255083164|ref|XP_002504568.1|
Unigene13172	-3,4	down	3,5E-08	--	--
Unigene54043	-3,4	down	2,8E-06	--	--
Unigene52425	-3,5	down	2,7E-06	--	--
Unigene8875	-3,5	down	2,9E-13	--	--
Unigene67659	-3,5	down	1,8E-11	--	--
Unigene32086	-3,6	down	4,6E-08	--	--
Unigene12160	-3,6	down	1,8E-12	--	--
Unigene29784	-3,7	down	1,5E-19	peptidyl-prolyl cis-trans isomerase FKBP-type family protein [Arabidopsis thaliana]	gi|15217972|ref|NP_176141.1|
Unigene55595	-3,7	down	8,3E-16	--	--
Unigene58577	-3,7	down	3,2E-13	--	--
Unigene60498	-3,7	down	2,0E-07	Os01g0290300 [Oryza sativa Japonica Group]	gi|297596578|ref|NP_001042787.2|
Unigene28841	-3,7	down	1,1E-18	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene16171	-3,8	down	4,9E-18	--	--
Unigene68231	-3,8	down	4,1E-11	--	--
Unigene77786	-3,8	down	1,1E-19	--	--
Unigene65035	-3,8	down	2,5E-06	--	--
Unigene7491	-3,8	down	2,4E-06	--	--
Unigene75989	-3,8	down	5,6E-08	--	--
Unigene41825	-3,9	down	1,5E-06	--	--

Unigene76221	-4,0	down	2,9E-10	At2g46910/F14M4.26 [Arabidopsis thaliana]	gi|16604537|gb|AAL24274.1|
Unigene11321	-4,1	down	3,0E-07	--	--
Unigene53228	-4,1	down	3,5E-12	--	--
Unigene39745	-4,1	down	1,4E-07	--	--
Unigene69707	-4,3	down	2,9E-13	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene10646	-4,4	down	1,5E-17	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene57464	-4,6	down	1,6E-06	Homeodomain-like [Medicago truncatula]	gi|124359538|gb|ABN05957.1|
Unigene39504	-4,9	down	7,5E-08	--	--
Unigene72723	-5,3	down	9,9E-10	--	--
Unigene47398	-5,5	down	5,2E-11	--	--
Unigene9625	-5,6	down	3,9E-16	--	--
Unigene71904	-5,9	down	7,0E-22	--	--
Unigene77351	-6,5	down	5,6E-28	CTV.20 [Citrus trifoliata]	gi|24461860|gb|AAN62347.1|AF506028_14
Unigene51970	-7,3	down	1,3E-23	--	--
Unigene55616	-7,4	down	1,9E-06	--	--
Unigene52689	-7,5	down	6,7E-07	--	--
Unigene58664	-7,7	down	1,4E-07	--	--
Unigene43649	-7,7	down	9,4E-08	--	--
Unigene14827	-7,7	down	9,2E-08	--	--
Unigene5228	-7,8	down	5,2E-08	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene56043	-7,9	down	2,1E-08	--	--
Unigene52052	-7,9	down	1,9E-08	--	--
Unigene9165	-8,4	down	1,4E-10	--	--
Unigene51766	-8,6	down	2,0E-11	--	--
Unigene19096	-8,8	down	7,9E-13	--	--
Unigene22590	-8,9	down	1,9E-13	--	--
Unigene7635	-8,9	down	1,3E-13	--	--
Unigene70731	-9,1	down	2,3E-14	--	--
Unigene44384	-9,1	down	1,2E-14	--	--
Unigene9812	-9,2	down	1,7E-15	--	--
Unigene68664	-11,8	down	3,6E-38	--	--
Unigene75487	-12,7	down	7,5E-48	--	--

Supplementary 2. Unigenes that were differentially expressed in sugarbeet roots 60 d after jasmonic acid (JA) treatment. Roots were treated with 10 µM JA or distilled 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 of the fold change in expression (logFC) ≥ 1 and a false detectection rate (FDR) ≤ 0.001, and exhibited significant alterations in expression in both experimental repetitions. Annotations were obtained by a BLAST search of unigene sequence against GenBank's nonredundant (Nr) protein database.

GeneID	logFC	Direction	PValue	Nr-annotation	Nr-ID
Unigene70731	10,7	up	4,3E-29	--	--
Unigene28303	9,7	up	4,5E-20	--	--
Unigene49517	9,5	up	1,1E-18	--	--
Unigene9968	9,3	up	1,4E-17	retrotransposon protein [Cucumis melo subsp. melo]	gi|307135889|gb|ADN33754.1|
Unigene9812	9,0	up	1,7E-15	--	--
Unigene21470	9,0	up	3,9E-15	--	--
Unigene42250	8,9	up	1,2E-14	--	--
Unigene35948	8,9	up	2,2E-14	--	--
Unigene10601	8,8	up	3,3E-14	nbs-lrr resistance protein [Populus trichocarpa]	gi|224096480|ref|XP_002334697.1|
Unigene37058	8,6	up	8,5E-13	ACC oxidase ACCO2 [Manihot esculenta]	gi|62526579|gb|AAX84675.1|
Unigene29680	8,5	up	3,0E-12	--	--
Unigene62575	8,5	up	3,4E-12	--	--
Unigene37233	8,3	up	7,4E-11	--	--
Unigene41641	8,2	up	1,9E-10	orf764 [Beta vulgaris subsp. vulgaris]	gi|54606728|dbj|BAD66751.1|
Unigene37599	8,1	up	2,8E-10	--	--
Unigene62002	8,1	up	6,8E-10	--	--
Unigene18735	7,9	up	2,7E-09	--	--
Unigene66042	7,9	up	2,7E-09	stigma-specific Stig1 family protein [Arabidopsis thaliana]	gi|116830031|gb|ABK27973.1|
Unigene78279	7,5	up	1,0E-07	benzoyl CoA benzoic acid benzoyltransferase [Verbena x hybrida]	gi|84578877|dbj|BAE72881.1|
Unigene23519	7,4	up	2,5E-07	At3g61920 [Arabidopsis thaliana]	gi|30102914|gb|AAP21375.1|
Unigene78854	7,3	up	7,3E-07	--	--
Unigene6478	7,3	up	7,5E-07	--	--
Unigene14056	7,3	up	7,8E-07	--	--
Unigene59377	7,2	up	1,3E-06	--	--
Unigene31815	7,1	up	2,2E-06	--	--
Unigene65993	7,1	up	2,3E-06	AP2/ERF domain-containing transcription factor [Populus trichocarpa]	gi|224083841|ref|XP_002307142.1|
Unigene70406	7,1	up	2,3E-06	Leucine-rich repeat, plant specific [Medicago truncatula]	gi|124360665|gb|ABN08654.1|
Unigene51447	7,1	up	4,0E-06	PIF-like transposase [Daucus carota]	gi|82570160|gb|ABB83644.1|
Unigene26324	6,4	up	7,2E-17	--	--
Unigene40365	6,4	up	2,2E-22	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|8777581|dbj|BAA97099.1|
Unigene22263	6,3	up	3,8E-40	--	--
Unigene62828	6,2	up	1,5E-27	--	--
Unigene60640	5,9	up	4,1E-13	--	--
Unigene10055	5,6	up	1,9E-22	--	--
Unigene61402	5,6	up	2,7E-11	--	--

Unigene76104	5,6	up	7,8E-30	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|9759493|dbj|BAB10743.1|
Unigene1219	5,3	up	1,8E-14	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|8777581|dbj|BAA97099.1|
Unigene72919	5,3	up	3,0E-17	orf315 [Beta vulgaris subsp. vulgaris]	gi|9838367|ref|NP_063979.1|
Unigene10343	5,3	up	5,8E-29	At2g23330 [Arabidopsis thaliana]	gi|38603804|gb|AAR24647.1|
Unigene357	5,2	up	2,3E-18	orf764 [Beta vulgaris subsp. vulgaris]	gi|9838386|ref|NP_063998.1|
Unigene28794	5,2	up	1,5E-21	orf764 [Beta vulgaris subsp. vulgaris]	gi|54606728|dbj|BAD66751.1|
Unigene40247	5,1	up	4,8E-09	orf764 [Beta vulgaris subsp. vulgaris]	gi|54606728|dbj|BAD66751.1|
Unigene55958	5,1	up	6,1E-13	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi|4206116|gb|AAD11427.1|
Unigene50911	5,1	up	7,0E-09	--	--
Unigene8260	5,1	up	5,4E-21	unknown [Silene latifolia]	gi|68685651|gb|AAY99341.1|
Unigene29485	5,0	up	3,9E-15	unknown [Silene latifolia]	gi|68685651|gb|AAY99341.1|
Unigene80130	5,0	up	3,9E-12	root nodule extensin [Pisum sativum]	gi|15021744|gb|AAK77899.1|AF397027_1
Unigene18654	4,9	up	3,0E-14	orf764 [Beta vulgaris subsp. vulgaris]	gi|54606728|dbj|BAD66751.1|
Unigene55733	4,9	up	2,2E-11	--	--
Unigene23402	4,9	up	4,3E-21	--	--
Unigene36726	4,8	up	1,7E-07	--	--
Unigene30677	4,7	up	1,1E-14	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi|4206116|gb|AAD11427.1|
Unigene15306	4,7	up	3,6E-10	--	--
Unigene31012	4,6	up	7,8E-10	--	--
Unigene67111	4,6	up	5,9E-12	pol-polyprotein [Silene latifolia]	gi|68685649|gb|AAY99339.1|
Unigene28563	4,5	up	1,6E-06	PIF-like transposase [Daucus carota]	gi|82570160|gb|ABB83644.1|
Unigene996	4,4	up	4,7E-09	pol-polyprotein [Silene latifolia]	gi|68685649|gb|AAY99339.1|
Unigene20294	4,3	up	1,4E-13	O-methyltransferase [Vitis vinifera]	gi|300077149|gb|ADJ66851.1|
Unigene77129	4,3	up	1,5E-15	cinnamate 4-hydroxylase [Mesembryanthemum crystallinum]	gi|4206116|gb|AAD11427.1|
Unigene8462	4,2	up	7,0E-08	polygalacturonase 3 [Solanum lycopersicum]	gi|12656894|gb|AAC28902.2|
Unigene62603	4,1	up	2,6E-14	late embryogenesis abundant protein-related / LEA protein-related [Arabidopsis thaliana]	gi|30685319|ref|NP_188574.2|
Unigene235	4,1	up	2,3E-25	ribosome-inactivating protein [Beta vulgaris]	gi|99646720|emb|CAK22418.1|
Unigene63017	4,1	up	1,6E-07	--	--
Unigene72241	4,1	up	3,2E-13	late embryogenesis abundant protein-related / LEA protein-related [Arabidopsis thaliana]	gi|30685319|ref|NP_188574.2|
Unigene41643	4,1	up	1,7E-07	--	--
Unigene759	4,1	up	1,7E-07	protein kinase-coding resistance protein [Nicotiana repanda]	gi|225735188|gb|ACO25571.1|
Unigene62484	4,1	up	7,9E-11	late embryogenesis abundant protein-related / LEA protein-related [Arabidopsis thaliana]	gi|30685319|ref|NP_188574.2|
Unigene65143	4,1	up	1,1E-25	ribosome-inactivating protein [Beta vulgaris]	gi|99646720|emb|CAK22418.1|
Unigene71420	4,1	up	2,7E-07	annexin [Fragaria x ananassa]	gi|643076|gb|AAA79922.1|
Unigene43439	4,0	up	1,6E-12	--	--
Unigene1168	4,0	up	5,2E-10	glycine-rich protein [Arabidopsis thaliana]	gi|18397934|ref|NP_565380.1|
Unigene19566	3,9	up	2,0E-09	pherophorin-dz1 protein [Volvox carteri f. nagariensis]	gi|21322711|emb|CAD22154.1|
Unigene62904	3,8	up	4,0E-10	unknown [Picea sitchensis]	gi|116782430|gb|ABK22503.1|
Unigene78655	3,8	up	3,5E-06	--	--
Unigene62754	3,6	up	3,3E-12	--	--
Unigene29657	3,6	up	2,5E-18	--	--
Unigene50108	3,5	up	1,1E-07	glycine-rich protein [Arabidopsis thaliana]	gi|18397934|ref|NP_565380.1|
Unigene76805	3,5	up	1,7E-08	--	--
Unigene26578	3,5	up	1,9E-12	polygalacturonase [Prunus domestica subsp. insititia]	gi|87242603|gb|ABD33834.1|

Unigene79709	3,5	up	7,3E-10	glutathione S-transferase [Salicornia brachiata]	gi|124507403|gb|ABN13680.1|
Unigene77961	3,5	up	4,2E-13	cytochrome P450 [Populus trichocarpa]	gi|224063931|ref|XP_002301307.1|
Unigene74120	3,4	up	1,0E-09	--	--
Unigene189	3,4	up	1,5E-14	--	--
Unigene66478	3,4	up	3,3E-06	--	--
Unigene232	3,4	up	6,4E-07	polyprotein [Oryza australiensis]	gi|86371679|gb|ABC94893.1|
Unigene80006	3,3	up	1,1E-09	Fgenesh protein 60 [Beta vulgaris]	gi|89953439|gb|ABD83313.1|
Unigene19968	3,3	up	4,4E-14	--	--
Unigene20462	3,3	up	1,5E-09	pathogenesis-related protein 10a [Rheum australe]	gi|197312889|gb|ACH63225.1|
Unigene21644	3,3	up	7,4E-11	Fgenesh protein 50 [Beta vulgaris]	gi|89953436|gb|ABD83311.1|
Unigene7786	3,3	up	1,4E-13	--	--
Unigene67367	3,3	up	1,4E-10	Peroxidase 4; Flags: Precursor	gi|223635590|sp|A7NY33.1|PER4_VITVI
Unigene74869	3,2	up	1,6E-07	oxalate oxidase-like germin 171 [Beta vulgaris]	gi|11496133|gb|AAG36666.1|
Unigene66215	3,2	up	1,6E-07	--	--
Unigene25549	3,2	up	3,6E-12	--	--
Unigene16505	3,1	up	4,4E-11	multidrug resistance protein ABC transporter family [Populus trichocarpa]	gi|224110774|ref|XP_002315633.1|
Unigene15140	3,1	up	6,3E-10	--	--
Unigene78883	3,0	up	2,2E-12	geraniol 10-hydroxylase [Picrorhiza kurrooa]	gi|300193870|gb|ADJ68324.1|
Unigene5082	3,0	up	2,1E-12	Defensin-like protein AX2; AltName: Full=Antifungal protein AX2	gi|7993732|sp|P82010.1|DFAX2_BETVU
Unigene58268	3,0	up	1,2E-11	--	--
Unigene22953	3,0	up	1,5E-06	PREDICTED: ripening-related protein-like [Vitis vinifera]	gi|225466053|ref|XP_002263278.1|
Unigene41637	2,9	up	1,6E-06	Os02g0236500 [Oryza sativa Japonica Group]	gi|297598893|ref|NP_001046391.2|
Unigene29030	2,9	up	7,2E-08	--	--
Unigene30886	2,9	up	1,6E-10	gag-pol polyprotein [Phaseolus vulgaris]	gi|38194929|gb|AAR13317.1|
Unigene42008	2,9	up	9,3E-07	pectinesterase family protein [Arabidopsis thaliana]	gi|15225308|ref|NP_180212.1|
Unigene51945	2,9	up	3,2E-08	peroxidase [Populus trichocarpa]	gi|225626263|gb|ACN97181.1|
Unigene76335	2,9	up	7,7E-13	AAA-type ATPase family protein [Arabidopsis thaliana]	gi|15233037|ref|NP_189499.1|
Unigene55419	2,9	up	5,1E-09	--	--
Unigene20311	2,9	up	1,1E-09	--	--
Unigene29055	2,9	up	4,0E-06	cc-nbs-lrr resistance protein [Populus trichocarpa]	gi|224102623|ref|XP_002334156.1|
Unigene39524	2,8	up	6,0E-07	unknown protein [Arabidopsis thaliana]	gi|15233699|ref|NP_194142.1|
Unigene57430	2,8	up	3,5E-08	--	--
Unigene18928	2,8	up	3,6E-07	--	--
Unigene49959	2,8	up	1,2E-09	glycine-rich protein [Arabidopsis thaliana]	gi|18397934|ref|NP_565380.1|
Unigene26231	2,8	up	1,4E-09	Kunitz-type trypsin inhibitor A chain, ACTI-A [Acacia confusa, seeds, Peptide, 136 aa]	gi|299509|gb|AAB26177.1|
Unigene67857	2,8	up	3,1E-06	zeatin O-glucosyltransferase [Glycine max]	gi|28302070|gb|AAM09514.2|AF489874_1
Unigene62726	2,8	up	5,7E-07	PREDICTED: similar to seed specific protein Bn15D18B [Vitis vinifera]	gi|225449909|ref|XP_002267909.1|
Unigene75464	2,8	up	4,8E-14	blight-associated protein P12 [Citrus trifoliata]	gi|160690672|gb|ABX46166.1|
Unigene48882	2,7	up	1,9E-06	unnamed protein product [Blastocystis hominis]	gi|300175335|emb|CBK20646.2|
Unigene19827	2,7	up	2,3E-06	--	--
Unigene24111	2,7	up	1,2E-07	--	--
Unigene4468	2,7	up	1,8E-10	--	--
Unigene69313	2,7	up	1,2E-09	desacetoxyvindoline 4-hydroxylase [Catharanthus roseus]	gi|1916643|gb|AAC49826.1|
Unigene79768	2,7	up	5,9E-07	conserved hypothetical protein [Ricinus communis]	gi|255552702|ref|XP_002517394.1|

Unigene57889	2,7	up	3,2E-10	Os08g0485800 [Oryza sativa Japonica Group]	gi|297726545|ref|NP_001175636.1|
Unigene80014	2,7	up	4,2E-14	glucan endo-1,3-beta-D-glucosidase [Beta vulgaris subsp. vulgaris]	gi|4584556|emb|CAA53545.1|
Unigene3502	2,7	up	6,3E-09	LTRGag-pol-polymerase 3 [Arabidopsis lyrata subsp. lyrata]	gi|158828152|gb|ABW81031.1|
Unigene50153	2,7	up	1,5E-12	--	--
Unigene28357	2,7	up	2,7E-11	AATP1 (AAA-ATPase 1); ATP binding / ATPase/ nucleoside-triphosphatase/ nucleotide binding [Arabidopsis thaliana]	gi|15242536|ref|NP_198817.1|
Unigene77564	2,7	up	1,7E-06	Os01g0957100 [Oryza sativa Japonica Group]	gi|115442331|ref|NP_001045445.1|
Unigene73809	2,7	up	4,9E-10	1-aminocyclopropane-1-carboxylate oxidase homolog; AltName: Full=Protein E8	gi|119640|sp|P10967.1|ACCH3_SOLLC
Unigene15911	2,7	up	1,9E-06	--	--
Unigene80473	2,7	up	2,8E-10	LOC100284967 [Zea mays]	gi|226496057|ref|NP_001151334.1|
Unigene57895	2,7	up	9,6E-07	--	--
Unigene11899	2,6	up	7,1E-09	--	--
Unigene7863	2,6	up	2,2E-13	--	--
Unigene29068	2,6	up	2,2E-06	--	--
Unigene19460	2,6	up	8,3E-08	chitinase-B, PLC-B [Phytolacca americana=pokeweed, leaves, Peptide, 274 aa]	gi|998516|gb|AAB34670.1|
Unigene48415	2,6	up	3,4E-13	pathogenesis-related protein [Tamarix hispida]	gi|217331222|gb|ACK38253.1|
Unigene66442	2,6	up	3,3E-12	beta-1,3-glucanase [Vitis riparia]	gi|37992763|gb|AAR06588.1|
Unigene69978	2,6	up	2,3E-06	epsin N-terminal homology (ENTH) domain-containing protein / clathrin assembly protein-related [Arabidopsis thaliana]	gi|15231451|ref|NP_190238.1|
Unigene13236	2,6	up	5,7E-09	--	--
Unigene63637	2,5	up	3,2E-06	--	--
Unigene13125	2,5	up	1,3E-09	Protein kinase; Type I EGF [Medicago truncatula]	gi|87162753|gb|ABD28548.1|
Unigene51546	2,5	up	3,3E-07	--	--
Unigene53522	2,5	up	1,2E-11	RecName: Full=Acidic endochitinase SE2; Flags: Precursor	gi|544000|sp|P36910.1|CHIE_BETVU
Unigene69899	2,5	up	3,3E-07	LOC100284967 [Zea mays]	gi|226496057|ref|NP_001151334.1|
Unigene63292	2,5	up	8,1E-07	kinase family protein [Arabidopsis lyrata subsp. lyrata]	gi|297814900|ref|XP_002875333.1|
Unigene62919	2,5	up	4,2E-08	histone 2 [Populus trichocarpa]	gi|224087383|ref|XP_002308145.1|
Unigene59365	2,5	up	6,5E-07	peroxidase [Mirabilis jalapa]	gi|46949194|gb|AAT07453.1|
Unigene42388	2,4	up	2,1E-07	flavonoid 3'-hydroxylase [Ipomoea coccinea]	gi|219551881|gb|ACL26685.1|
Unigene19666	2,4	up	2,3E-08	chitinase [Chenopodium amaranticolor]	gi|2570162|dbj|BAA22966.1|
Unigene70380	2,4	up	1,1E-10	--	--
Unigene18805	2,4	up	2,1E-07	pathogenesis-related protein 1a [Beta vulgaris]	gi|205271005|emb|CAP66260.1|
Unigene66180	2,4	up	7,6E-11	RecName: Full=Acidic endochitinase SE2; Flags: Precursor	gi|544000|sp|P36910.1|CHIE_BETVU
Unigene8943	2,4	up	6,1E-08	Protein kinase; Peptidoglycan-binding LysM [Medicago truncatula]	gi|87162779|gb|ABD28574.1|
Unigene76071	2,3	up	3,1E-08	WRKY50; transcription factor [Arabidopsis thaliana]	gi|22327079|ref|NP_197989.2|
Unigene75121	2,3	up	2,6E-07	Os07g0656900 [Oryza sativa Japonica Group]	gi|115473819|ref|NP_001060508.1|
Unigene10098	2,3	up	4,8E-08	--	--
Unigene23533	2,3	up	3,2E-06	F12K11.6 [Arabidopsis thaliana]	gi|6692693|gb|AAF24827.1|AC007592_20
Unigene57365	2,3	up	2,1E-10	--	--
Unigene67438	2,3	up	3,7E-06	--	--
Unigene22942	2,3	up	4,2E-08	Glutathione S-transferase; class-phi	gi|417093|sp|Q04522.3|GSTF_SILCU
Unigene81280	2,3	up	1,6E-09	Chalcone--flavonone isomerase	gi|122233481|sp|Q45QI7.2|CFI_CAMSI
Unigene56574	2,3	up	7,1E-08	--	--
Unigene53192	2,2	up	3,1E-06	--	--
Unigene70397	2,2	up	2,0E-09	chitinase [Beta vulgaris subsp. vulgaris]	gi|4584552|emb|CAA53544.1|

Unigene11500	2,2	up	9,6E-08	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene27612	2,2	up	3,8E-07	WRKY transcription factor [Artemisia annua]	gi|298108803|gb|ADI56655.1|
Unigene62435	2,2	up	7,4E-10	thaumatin-like protein [Mirabilis jalapa]	gi|46949200|gb|AAT07456.1|
Unigene26313	2,1	up	4,1E-09	Putative 22 kDa kafirin cluster; Ty3-Gypsy type [Oryza sativa]	gi|18767374|gb|AAL79340.1|AC099402_4
Unigene80215	2,1	up	3,5E-08	--	--
Unigene72126	2,1	up	1,1E-09	osmotin-like protein [Atriplex nummularia]	gi|166940|gb|AAA32908.1|
Unigene80552	2,1	up	2,5E-09	retrotransposon protein [Beta vulgaris]	gi|121501699|gb|ABM55240.1|
Unigene63276	2,1	up	7,1E-07	--	--
Unigene25992	2,1	up	9,7E-08	PKS [Fallopia multiflora]	gi|300885273|gb|ADK45325.1|
Unigene16319	2,1	up	5,8E-07	hypothetical protein VOLCADRAFT_117048 [Volvox carteri f. nagariensis]	gi|302835353|ref|XP_002949238.1|
Unigene82285	2,1	up	1,3E-09	DNA topoisomerase II [Nicotiana tabacum]	gi|26984133|gb|AAN85207.1|
Unigene42798	2,0	up	2,4E-07	pathogenesis-related protein 4A [Pisum sativum]	gi|7381203|gb|AAF61434.1|AF137351_1
Unigene31086	2,0	up	2,1E-08	Chain A, Resolution Of The Structure Of The Allergenic And Antifungal Banana Fruit Thaumatin-Like Protein At 1.7a	gi|88191901|pdb|1Z3Q|A
Unigene70653	2,0	up	8,6E-09	chitinase [Beta vulgaris subsp. vulgaris]	gi|4584552|emb|CAA53544.1|
Unigene79221	2,0	up	4,6E-07	Pectinesterase; Pectinesterase inhibitor [Medicago truncatula]	gi|124360335|gb|ABN08348.1|
Unigene54837	2,0	up	1,0E-06	--	--
Unigene39535	2,0	up	2,3E-07	formamidase [Lupinus albus]	gi|222840535|gb|ACM68705.1|
Unigene1130	2,0	up	9,2E-08	pol protein [Cucumis melo subsp. melo]	gi|28558781|gb|AAO45752.1|
Unigene41811	2,0	up	3,3E-06	chitinase-B, PLC-B [Phytolacca americana=pokeweed, leaves, Peptide, 274 aa]	gi|998516|gb|AAB34670.1|
Unigene79381	2,0	up	1,4E-07	chalcone isomerase [Garcinia mangostana]	gi|222478417|gb|ACM62743.1|
Unigene67714	2,0	up	2,0E-08	peroxidase [Tamarix hispida]	gi|224612179|gb|ACN60161.1|
Unigene16954	1,9	up	6,6E-07	predicted protein [Micromonas sp. RCC299]	gi|255083164|ref|XP_002504568.1|
Unigene7964	1,9	up	1,5E-07	Retrotransposon gag protein [Solanum demissum]	gi|113205348|gb|ABI34354.1|
Unigene38961	1,9	up	1,3E-07	At2g23330 [Arabidopsis thaliana]	gi|38603804|gb|AAR24647.1|
Unigene26504	1,9	up	6,9E-08	DNA topoisomerase II [Nicotiana tabacum]	gi|26984133|gb|AAN85207.1|
Unigene24889	1,9	up	5,9E-08	phenylpropanoid:glucosyltransferase 1 [Nicotiana tabacum]	gi|13492674|gb|AAK28303.1|AF346431_1
Unigene31995	1,9	up	9,6E-08	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|9759493|dbj|BAB10743.1|
Unigene20593	1,9	up	9,5E-07	COL1 [Beta vulgaris subsp. vulgaris]	gi|186911828|gb|ACC95129.1|
Unigene29471	1,9	up	1,0E-07	tRNA-splicing endonuclease positive effector-related [Arabidopsis thaliana]	gi|15218807|ref|NP_176757.1|
Unigene82057	1,9	up	1,6E-07	F1E22.16 [Arabidopsis thaliana]	gi|6686402|gb|AAF23836.1|AC007234_8
Unigene40318	1,8	up	4,3E-07	Os01g0731100 [Oryza sativa Japonica Group]	gi|115439737|ref|NP_001044148.1|
Unigene103	1,8	up	2,4E-07	LOC100284129 [Zea mays]	gi|226494043|ref|NP_001150498.1|
Unigene61328	1,8	up	6,8E-07	--	--
Unigene31473	1,8	up	3,7E-06	FAD linked oxidase, N-terminal [Medicago truncatula]	gi|87240745|gb|ABD32603.1|
Unigene35063	1,8	up	2,5E-06	--	--
Unigene1078	1,8	up	5,8E-07	CYP81B2v1 [Nicotiana tabacum]	gi|85068596|gb|ABC69378.1|
Unigene8561	1,8	up	2,3E-06	carbohydrate oxidase [Lactuca sativa]	gi|18652398|gb|AAL77102.1|AF472608_1
Unigene32409	1,8	up	3,6E-06	formamidase [Lupinus albus]	gi|222840535|gb|ACM68705.1|
Unigene71716	1,8	up	1,7E-06	phi-1 [Nicotiana tabacum]	gi|3759184|dbj|BAA33810.1|
Unigene70896	1,8	up	4,4E-07	Acidic endochitinase SE2; Flags: Precursor	gi|544000|sp|P36910.1|CHIE_BETVU
Unigene66299	1,7	up	3,9E-06	aminotransferase family protein [Populus trichocarpa]	gi|224133454|ref|XP_002328046.1|
Unigene23724	1,7	up	7,7E-07	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|8777581|dbj|BAA97099.1|
Unigene6804	1,7	up	1,2E-06	Glutathione S-transferase; class-phi	gi|417093|sp|Q04522.3|GSTF_SILCU
Unigene80332	1,7	up	9,6E-07	cytochrome P450 [Populus trichocarpa]	gi|224067244|ref|XP_002302427.1|

Unigene30512	1,7	up	3,4E-06	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|8777581|dbj|BAA97099.1|
Unigene17858	1,7	up	2,7E-06	F18O14.11 [Arabidopsis thaliana]	gi|8778443|gb|AAF79451.1|AC025808_33
Unigene72859	1,7	up	1,5E-06	C2H2L domain class transcription factor [Malus x domestica]	gi|302398685|gb|ADL36637.1|
Unigene58834	1,6	up	2,9E-06	anthocyanin-O-methyltransferase [Vitis vinifera]	gi|226374634|gb|ACO52469.1|
Unigene23944	1,6	up	1,9E-06	salt responsive protein 2 [Solanum lycopersicum]	gi|195549553|gb|ACG50004.1|
Unigene41745	-1,6	down	3,6E-06	--	--
Unigene44285	-1,7	down	9,5E-07	--	--
Unigene77992	-1,7	down	1,0E-06	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|10177143|dbj|BAB10503.1|
Unigene30589	-1,7	down	3,5E-06	SWITCH1 splice variant S [Arabidopsis thaliana]	gi|16033414|gb|AAL13233.1|
Unigene72546	-1,8	down	1,6E-06	Flotillin-like protein 3	gi|300680953|sp|D2XNR0.1|FLOT3_MEDTR
Unigene79986	-1,8	down	5,1E-07	--	--
Unigene5595	-1,8	down	2,3E-07	predicted protein [Populus trichocarpa]	gi|224128810|ref|XP_002320427.1|
Unigene4641	-1,9	down	1,7E-06	retroelement pol polyprotein-like [Arabidopsis thaliana]	gi|8843739|dbj|BAA97287.1|
Unigene69518	-1,9	down	1,8E-06	IQ-domain 24 [Arabidopsis lyrata subsp. lyrata]	gi|297806753|ref|XP_002871260.1|
Unigene11249	-1,9	down	2,5E-06	--	--
Unigene28998	-1,9	down	4,3E-07	--	--
Unigene77724	-1,9	down	3,5E-06	polypeptide with a gag-like domain [Petunia x hybrida]	gi|66841323|dbj|BAD99219.1|
Unigene30454	-2,0	down	4,9E-07	RPS9 (RIBOSOMAL PROTEIN S9); structural constituent of ribosome [Arabidopsis thaliana]	gi|15222063|ref|NP_177635.1|
Unigene12193	-2,0	down	8,3E-07	--	--
Unigene24790	-2,0	down	4,4E-07	--	--
Unigene17421	-2,0	down	2,6E-06	--	--
Unigene59374	-2,1	down	3,7E-07	predicted protein [Populus trichocarpa]	gi|224118476|ref|XP_002317828.1|
Unigene15532	-2,1	down	2,3E-06	--	--
Unigene36589	-2,1	down	9,9E-08	--	--
Unigene71080	-2,1	down	2,9E-08	--	--
Unigene59792	-2,1	down	3,4E-07	--	--
Unigene21988	-2,2	down	3,6E-06	--	--
Unigene15445	-2,2	down	4,4E-07	nodulin MtN3 family protein [Arabidopsis lyrata subsp. lyrata]	gi|297809311|ref|XP_002872539.1|
Unigene64507	-2,2	down	6,9E-07	cellulose synthase [Leucaena leucocephala]	gi|307557871|gb|ACU87559.2|
Unigene10578	-2,2	down	1,0E-07	S-locus lectin protein kinase family protein [Arabidopsis thaliana]	gi|15220348|ref|NP_172600.1|
Unigene76471	-2,3	down	3,4E-07	transcription factor bZIP113 [Glycine max]	gi|145652367|gb|ABP88238.1|
Unigene32901	-2,3	down	6,9E-08	--	--
Unigene25429	-2,3	down	3,0E-09	--	--
Unigene18394	-2,3	down	2,8E-06	--	--
Unigene76413	-2,3	down	5,6E-09	--	--
Unigene71630	-2,3	down	1,4E-08	--	--
Unigene34253	-2,3	down	3,0E-08	--	--
Unigene81891	-2,3	down	2,1E-06	AT1G42550 [Arabidopsis thaliana]	gi|227202588|dbj|BAH56767.1|
Unigene61796	-2,4	down	3,0E-08	Os01g0605700 [Oryza sativa Japonica Group]	gi|115438366|ref|NP_001043522.1|
Unigene57204	-2,4	down	1,9E-07	--	--
Unigene59048	-2,4	down	2,4E-06	--	--
Unigene24149	-2,5	down	3,5E-11	predicted protein [Populus trichocarpa]	gi|224087451|ref|XP_002308172.1|
Unigene58710	-2,5	down	1,2E-08	polypepetide with reverse transcriptase and RNaseH domains [Petunia x hybrida]	gi|66841325|dbj|BAD99221.1|
Unigene30081	-2,5	down	5,1E-08	leucine-rich repeat receptor-like protein kinase [Arabidopsis thaliana]	gi|224589491|gb|ACN59279.1|

Unigene69822	-2,5	down	2,5E-10	ascorbate peroxidase [Citrus maxima]	gi|221327587|gb|ACM17463.1|
Unigene5192	-2,5	down	9,9E-08	60S acidic ribosomal protein P1 [Zea mays]	gi|195618310|gb|ACG30985.1|
Unigene12799	-2,5	down	1,8E-07	--	--
Unigene44561	-2,6	down	2,1E-06	--	--
Unigene24976	-2,6	down	9,4E-07	--	--
Unigene1607	-2,7	down	1,7E-06	--	--
Unigene18794	-2,7	down	1,5E-12	xyloglucan endo-transglycosylase [Carica papaya]	gi|14029149|gb|AAK51119.1|
Unigene55182	-2,7	down	1,9E-07	--	--
Unigene25667	-2,7	down	1,5E-06	--	--
Unigene27525	-2,8	down	5,9E-08	--	--
Unigene22684	-2,8	down	4,7E-12	DNA binding [Arabidopsis thaliana]	gi|145336703|ref|NP_175754.2|
Unigene15955	-2,9	down	2,2E-06	--	--
Unigene79554	-2,9	down	7,1E-16	xyloglucan endo-transglycosylase [Carica papaya]	gi|14029149|gb|AAK51119.1|
Unigene52968	-2,9	down	1,6E-06	Os01g0252600 [Oryza sativa Japonica Group]	gi|297596453|ref|NP_001042605.2|
Unigene8821	-3,0	down	2,7E-09	--	--
Unigene68723	-3,0	down	8,6E-07	--	--
Unigene67309	-3,0	down	2,5E-13	galactosyltransferase [Arabidopsis lyrata subsp. lyrata]	gi|297798750|ref|XP_002867259.1|
Unigene9954	-3,1	down	1,2E-06	--	--
Unigene2512	-3,1	down	1,6E-09	--	--
Unigene29814	-3,3	down	1,2E-06	RNA-directed DNA polymerase (Reverse transcriptase) [Medicago truncatula]	gi|124360741|gb|ABN08718.1|
Unigene67883	-3,3	down	3,5E-09	--	--
Unigene59967	-3,4	down	1,8E-19	--	--
Unigene59227	-3,4	down	4,2E-09	--	--
Unigene74415	-3,6	down	3,5E-21	salt-induced protein [Atriplex nummularia]	gi|31879434|dbj|BAC77695.1|
Unigene7710	-3,6	down	3,5E-07	--	--
Unigene67844	-3,7	down	1,1E-09	--	--
Unigene16197	-3,7	down	1,8E-07	--	--
Unigene78524	-3,8	down	6,3E-08	--	--
Unigene3074	-3,9	down	1,8E-18	--	--
Unigene12160	-4,1	down	2,5E-07	--	--
Unigene5517	-4,1	down	1,4E-15	--	--
Unigene75619	-4,1	down	2,1E-12	polypeptide with a gag-like domain [Petunia x hybrida]	gi|66841323|dbj|BAD99219.1|
Unigene9729	-4,3	down	1,6E-10	--	--
Unigene27305	-4,5	down	1,2E-11	--	--
Unigene1062	-4,6	down	1,1E-06	--	--
Unigene76395	-4,6	down	4,4E-10	--	--
Unigene9468	-5,0	down	1,2E-08	--	--
Unigene63223	-5,2	down	9,0E-14	--	--
Unigene37188	-5,5	down	1,0E-15	hypothetical protein [Beta vulgaris]	gi|261865347|gb|ACY01928.1|
Unigene971	-5,6	down	9,7E-12	--	--
Unigene72723	-5,8	down	1,1E-12	--	--
Unigene70996	-6,0	down	1,0E-13	--	--
Unigene20927	-6,0	down	3,2E-14	Os09g0268600 [Oryza sativa Japonica Group]	gi|115478262|ref|NP_001062726.1|
Unigene9056	-6,2	down	6,8E-30	--	--

Unigene77351	-6,2	down	2,6E-25	CTV.20 [Citrus trifoliata]	gi|24461860|gb|AAN62347.1|AF506028_14
Unigene9369	-6,3	down	5,4E-22	--	--
Unigene34462	-7,3	down	6,3E-07	--	--
Unigene49318	-7,3	down	6,3E-07	--	--
Unigene41065	-7,4	down	3,8E-07	polypeptide with a gag-like domain [Petunia x hybrida]	gi|66841323|dbj|BAD99219.1|
Unigene16171	-7,4	down	3,6E-25	--	--
Unigene55072	-7,5	down	2,4E-07	--	--
Unigene26255	-7,5	down	1,5E-07	--	--
Unigene22847	-7,5	down	1,5E-07	--	--
Unigene54490	-7,6	down	9,1E-08	--	--
Unigene23677	-7,7	down	3,0E-27	--	--
Unigene66911	-7,8	down	1,4E-08	PREDICTED: hypothetical protein [Vitis vinifera]	gi|225430788|ref|XP_002267433.1|
Unigene43649	-7,9	down	6,0E-09	--	--
Unigene64502	-8,0	down	1,6E-09	--	--
Unigene11557	-8,2	down	3,1E-10	--	--
Unigene67302	-8,2	down	2,1E-10	H0702G05.2 [Oryza sativa Indica Group]	gi|90265070|emb|CAH67743.1|
Unigene63392	-8,2	down	2,1E-10	--	--
Unigene46892	-8,3	down	9,4E-11	--	--
Unigene68231	-8,8	down	1,0E-13	--	--
Unigene59909	-9,1	down	1,0E-15	--	--
Unigene73904	-9,2	down	7,5E-16	--	--
Unigene59489	-9,2	down	4,4E-16	--	--
Unigene55595	-9,9	down	1,3E-21	--	--
Unigene60726	-10,0	down	2,1E-22	--	--
Unigene79315	-10,3	down	1,2E-24	--	--
Unigene71555	-10,7	down	1,3E-28	--	--
Unigene75908	-10,9	down	5,0E-30	--	--
Unigene77786	-10,9	down	3,9E-30	--	--

CHAPTER II

Involvement of antioxidant enzymes and chitinase in sugarbeet defense against fly maggot by wounding

ABSTRACT

Sugarbeet is an alternative crop for the production of refined sucrose where weather conditions do not allow sugarcane growth. During sugarbeet production, many factors reduce root yield and sucrose content, including insect damage. Thus, study of resistance mechanism is essential to provide new control strategies and reduce insecticides spray. We investigated if resistance of some genotypes is achieved through activation of chitinase, peroxidase and polyphenoloxidase. Nine genotypes sugarbeet roots, susceptible and resistant to maggot fly, were wounding 4 weeks after planting to mimic maggot attack. The results showed neither peroxidase nor polyphenol oxidase activity is correlated to maggot fly resistant in sugarbeet roots. We observed that chitinase activity was significantly reduced for some genotypes after wounding, although no significance difference was found between resistant and susceptible sugarbeet genotypes.

1. INTRODUCTION

The sugarbeet root maggot (SBRM), Tetanops myopaeformis von Röder, has been recognized the most destructive sugarbeet insect in various regions of the United States and in Alberta, Canada (Yun, 1986 and Campbell et al., 2011). Root yield losses oscillate from 10 to 100 % in severe surgabeet root maggot infestations (Campbell et al., 1998; Cooke, 1993; Dregseth, et al. 2003). According Campbell et al., (1998), absence of control in regions, such as, Minnesota and eastern North Dakota would result yield losses of 40 %. Adults female emerge to recently planted sugarbeet field in later spring or early summer and pupate in the soil close to the seedlings (Campbell et al., 2011). Developing larvae scrap the surface of the beet, resulting in blackening roots at the wound and plant wilt (Michael, 1995).

SBRM is mainly control by application of a granular insecticide that reduces larval populations in sugarbeet fields (Campbell et al., 2008). Counter (terbufos) and Lorsban (chlorpyrifos) are two organophosphate insecticides used extensively on the field (Dexter, et al., 1998). Those insecticides may be no longer available in the market because of concerns about environment impacts (Campbell et al., 2008). Campbell (2005) suggested the development of sugarbeet root maggot resistant hybrids as potencial solution to these concerns.

Two root maggot resistant germplasm lines, F1015 (PI605413) and F1016 (PI608437), have been publicly released (Campbell et al., 2000). These lines show less damage than commercial hybrids and unselected populations (Campbell et al., 2008), although the resistant mechanisms are unknown. Puthoff and Smigocki (2007) investigated sugarbeet root responses to SBRM larvae infection in lines moderately resistant and susceptible to the SBRM. They identified expression changes for a large number of genes that were induced by maggot feeding in genotypes that are moderately resistant, and susceptible to the SBRM. In the moderately resistant genotype many defense genes were different expressed by maggot feeding, among them five polyphenol oxidase (PPO), in which one of them was 7 times identified.

PPO has been reported to be induced by biotic and abiotic stress such as fungus, insects and wounding in several plants. PPO is antioxidant enzyme that catalyze the oxidation of monophenols and o-diphenols to o-quinones. O-quinones are highly reactive intermediate compounds that readily polymerize forming melanin which increase cell wall resistance to insects and pathogens (Zhao et al., 2009). In addiction derivates of PPO reaction can covalently link to nucleophilic side chain of amino acids reducing the nutritive value of

proteins and the plant digestibility. Thereby this enzyme produce more toxic compound than the original phenol (Bhonwong et al., 2009).

Puthoff and Smigocki (2007) also found three chitinase and one peroxidase (POD) different expressed by maggot feeding in moderately resistant sugarbeet genotype. Many others plants have been shown to induce PODs in response to insect attack (He et al., Stout et al., 2009; Gulsen et al., 2010). POD, similarly to PPO, is involved reinforcement cell wall and produces toxins that reduce the plant digestibility, in which results in drastic effects on insect growth and development (Birecka and Miller, 1974; Chen et al., 2009). Likewise PPO and POD, chitinase may be induced by fungal infection, insect infestation and mechanical wounding. Plant chitinases have an important role in plant defense which it hydrolyses chitin molecules which are the main structural component in fungal cell wall and insect's skeleton (Spanò et al., 2015; Sietsma and Wessels, 1979).

The objectives of this study were to investigate whether resistance of some genotypes is achieved through activation of those enzymes mentioned. The study of the source of genetic resistance will potentially provide genetic or metabolic markers that could be used to screen for insect resistance or target genetic modifications for improved SBRM resistance.

2. MATERIAL AND METHODS

2.1. Plant materials and treatments

Sugarbeet plants of 9 genotypes with varying susceptibility to the sugarbeet root maggot were greenhouse grown in Sunshine Mix #1 (Sun Gro Horticulture, Vancouver, BC, Canada) in 1-L pots with supplemental light under a 16 h light/8 h dark regime, using 20 plants per genotype. Once mechanical wounding and herbivore may exhibit similar response patterns, sugarbeet roots were wounded to mimic maggot fly feeding.Twenty-eight days after sowing, the taproots of 10 plants of each genotype were wounded. To wound, potting mix from one side of the taproot was removed, and a serrated blade of approximately 1 cm in length, with 4 serrations, was scraped up the length of the taproot once to mimic the injury caused by SBRMs. After wounding, potting mix was replaced around the wounded taproots, and plants were allowed to continue to grow in the greenhouse. All roots (i.e., 10 wounded roots and 10 unwounded roots per genotype) were harvested 48 h after administration of the wound treatment. Leaves and petioles were removed from harvested roots with a knife roots were gently washed to remove potting media. Roots were rapidly cut into approximately 1 cm^3 pieces and flash frozen in liquid nitrogen. Two roots from each treatment and genotype were combined to generate 5 replicates per wound treatment and genotype. Prior to analysis, root samples were freeze-dried, ground to a fine powder, and stored at -80 °C. The genotypes used and their susceptibility to SBRM is listed in Table 1.

Table 1- Sugarbeet genotypes, their susceptibility to sugarbeet root maggot (SBRM) and descriptions of their lineage.

Genotype	Susceptibility	Description
ACH-817	xxxx	commercial hybrid
F1010	xxx	high sugar line selected from world collection
19961009H2	xxx	Cercospora resistant Colorado line
C564aa	xxx	sugarbeet line from California
F1015	xx	F1010 = female parent
F1016	x	F1010 = pollinator parent
F1024	x	selected from F1016 / 19961009H2 cross
14N0026	x	sugarbeet line selected from C564aa/PI 179180 cross
MagRes1	x	F1024 Pollinator susceptible Beta seed female

Susceptibility rating: xxxx= very susceptible; xxx= susceptible; xx= moderate resistant; and x= resistant to SBRM.

2.2. Enzyme activity assays

Activities of peroxidase (POD), polyphenol oxidase (PPO), and chitinase (CHI) were determined using the methods of Ferrareze et al. (2013). Proteins were extracted with 10 volumes (v/w) of an extraction buffer to freeze-dried tissue. Extraction buffers were: POD extraction buffer: 0.1 M potassium phosphate, pH 7.0, and 10 mM sodium bisulfate, and 0.5 M NaCl; PPO extraction buffer: 0.1 M potassium phosphate, pH 6.5, and 1% polyvinylpyrrolidone-40 (PVP-40); CHI extraction buffer: 0.1 M sodium acetate, pH 6.4, and 14 mM β-mercaptoethanol. Suspensions of tissue and extraction buffer were sonicated (Model 4.6, Mettler Electronics, Anaheim, CA, USA) for 15 minutes at 4 ^{o}C, filtered over Miracloth (EMD Millipore, Billerica, MA, USA), and centrifuged at 21,000 x g for 20 minutes at 4 ^{o}C. Supernatants containing soluble proteins were used for enzyme activity and total soluble protein assays.

POD and PPO activities were measured spectroscopically using a Shimadzu model UV-1601 dual-beam spectrophotometer (Kyota, Japan). CHI was measured spectroscopically using a Molecular Devices SpectraMAX Plus microplate reader (Sunnyvale, CA, USA). POD activity assays contained protein extract, 0.1 potassium phosphate, pH 6.5, 15 mM guaiacol, and 5.9 mM hydrogen peroxide. Activity was determined at 25 °C using the maximum change in absorbance at 470 nm during in the first 3 minutes of the reaction. PPO activity assays contained protein extract, 0.1 M potassium phosphate, pH 6.5, and 50 mM catechol. Activity was determined at 25 °C using the maximum change in absorbance at 420 nm. Chitinase activity was determined by absorbance changes at 550 nm and 37 °C in solutions containing 0.5 g L-1 carboxymethyl-chitin-remazol brilliant violet (CM-Chitin-RBV; LOEWE Biochemica GmbH, Sauerlach, Germany) and 50 mM sodium acetate, pH 6.4 using the substrate manufacturer's protocol. All enzymes activities were expressed by absorbance changes as a function of total soluble protein concentration. Total soluble protein concentrations were determined using Bio-Rad Protein Assay Reagent (Hercules, CA, USA) with bovine serum albumin as a standard.

2.3. Statistical analysis

Statistical analyses were conducted with Minitab Statistical Software (ver. 16.2.3, State College, PA, USA). Analysis of variance and Tukey range tests were used to determine significant differences in enzyme activities between genotypes. Two-sample t-tests were used to determine differences in enzyme activities between wounded and unwounded roots for each genotype. For all analyses, $n = 5$. Means were considered significantly different when $P \leq 0.05$.

3. RESULTS AND DISCUSSIONS

3.1. Polyphenol oxidase

According to Bhonwong et. al (2009) cotton plants transformed expressing tomato's PPO, PPO activity related negatively with weight gains and foliar consumption of cotton bollworm, substantiating the defensive role of PPO against this insect, cotton bollworm and beet armyworm. Therefore in many plant PPO activity is correlated in resistance to herbivory, such as, transgenic cotton plants (Helicoverpa armigera and Spodoptera exigua), transgenic populous (Malacosoma disstria), beans (Melanoplus differentialis), and potato (L. decemlineata) (Bhonwong et al 2009; Wang and Constabel, 2004; Alba-Meraz and Choe, 2002; Castañera et al, 1996). To ilucidate wether sugarbeet's PPO is also involved in the defense against herbivory, we evaluated sugarbeet genotypes resistant and susceptible to maggot fly. Aiming to simulate damage and responses caused on roots by maggot fly they were wounded and then of forty eight hours roots were harvested to PPO assays.

PPO activity in MagRes1 wounded roots was more than double in relation unwounded roots (Table 2). Despite of injury have resulted in significant increase on antioxidant activity of PPO in MagRes1, resistant genotype, there was not same effect for others resistant genotypes. Thus, no correlation between resistant/susceptible sugarbeet genotypes evaluated and PPO activity was found. Germplasm lines showed different levels of PPO activity. The highest activity was found in lines moderate resistant (F1015=2.29 $dA.min^{-1}.mg\ pt^{-1}$), while lowest activity in lines resistant (F1024=0.74 $dA.min^{-1}.mg\ pt^{-1}$), regardless roots were wounded or not (Table 2).

These results suggest that PPO activity may not be a strong evidence of their participation in direct defensive mechanisms. Similar results were found in hybrids of coffee plants, susceptible and resistant, where coffee plants do not differ for POD and PPO activity in response to the attack by the leaf miner (Ramiro et al, 2006).

Table 2- Polyphenol oxidase activity to unwounded and wounded sugarbeet genotypes.

Genotype	Susceptibility	Unwounded dA.min^{-1}.mg pt^{-1}		Wounded dA.min^{-1}.mg pt^{-1}		unwounded and wounded dA.min^{-1}.mg pt^{-1}	
ACH-817	Xxxx	2.04	b	1.89	b	1.96	abc
F1010	Xxx	1.19	cd	2.11	ab	1.64	bc
19961009H2	Xxx	1.05	cd	1.79	b	1.42	bc
C564aa	Xxx	1.72	bc	1.58	bc	1.65	bc
F1015	Xx	2.75	a	2.84	a	2.79	a
F1016	X	1.30	cd	1.54	bc	1.42	bc
F1024	X	0.68	d	0.81	c	0.74	c
14N0026	X	2.89	ab	2.05	ab	2.47	ab
MagRes1*	X	1.11	cd	2.25	ab	1.68	bc
Mean		1.74	A	1.54	A		

Susceptibility rating: xxxx= very susceptible; xxx= susceptible; xx= moderate resistant; and x= resistant to SBRM. * indicate significantly difference between unwounded and wounded means at 0.05 level. Difference amog means within a column followed by the same letter are not signicant, according to Tukey range tests ($P \leq 0.05$).

3.2. Peroxidase

Peroxidases (PODs) are an important component of the immediate response of plants to wounding and insect damage. PODs act as a defense mechanism through repair of damaged cell walls and formation of quinones by oxidation of phenols (Birecka and Miller, 1974; Bhonwong et al., 2009).

Similar to PPO results were found for POD activity. POD activity showed no significance changes between unwounded and wound roots, except to 19961009H2 genotype. In this genotype, POD activity was reduced in 56% by wounding (Table 3). No correlation was found between resistant/susceptible to maggot fly sugarbeet genotypes evaluated and POD activity. Among germplasm lines evaluated a large variation to POD activity was observed. In which 19961009H2, susceptible genotype, showed the highest constitutive activity (27.45 $dA.min^{-1}.mg\ pt^{-1}$), while in wounded roots C564aa, F1024, and 14N0026 showed highest activity (21.63, 18.29 and 18.18 $dA.min^{-1}.mg\ pt^{-1}$) (Table 3).

Table 3- Peroxidase activity to unwounded and wounded sugarbeet genotypes.

Genotype	**Susceptibility**	**Unwounded $dA.min^{-1}.mg\ pt^{-1}$**		**Wounded $dA.min^{-1}.mg\ pt^{-1}$**		**unwounded and wounded $dA.min^{-1}.mg\ pt^{-1}$**	
ACH-817	Xxxx	10.09	def	12.01	**bc**	11.14	cde
F1010	Xxx	8.86	ef	12.38	**b**	10.67	de
19961009H2*	Xxx	27.45	a	12.13	**bc**	19.79	ab
C564aa	Xxx	23.64	b	21.63	**a**	22.63	a
F1015	Xx	9.75	def	12.85	**b**	11.30	cde
F1016	X	8.39	ef	9.61	**bc**	9.00	e
F1024	X	12.73	cd	18.29	**a**	15.51	bc
14N0026	X	14.90	c	18.18	**a**	16.54	b
MagRes1	X	6.49	f	7.75	**c**	7.12	e
Mean		14.40	A	13.36	**A**		

Susceptibility rating: xxxx= very susceptible; xxx= susceptible; xx= moderate resistant; and x= resistant to SBRM. * indicate significantly difference between unwounded and wounded means at 0.05 level. Difference amog means within a column followed by the same letter are not signicant, according to Tukey range tests ($P \leq 0.05$).

2.3. Chitinase

Chitinase has been reported trough transgenic plants a resistant plant mechanism to attack by insect and pathogens. Chitinase hydrolyzes the chitin polymer, wich is the principal structural component in fungal cell wall and insect keleton, into to N-acetyl glucosamine (Sietsma and Wessels, 1979; Van Aalten et al., 2000). For instance, tomato plants expressing poplar chitinase inhibits Colorado potato beetle development (Lawrence and Novak 2006). Furthermore, transgenic tobacco plants expressing chitinase have shown increased resistance to Lepidoptera insects (Ding et al. 1998).

Few studies have evaluated the response to biochemical level when comparing resistant and susceptible genotypes to plague. Studies in molecular level are more common, mainly with pathogens. Some sugarbeet genotypes showed variation on chitinase activity in response to wounding. Chitinase activity was significantly reduced in C564aa genotypes (75%), 14N0026 (67%), MagRes1 (73%) and ACH-817 (65%) (Table 4). Contrary to our expectations, the others maggot resistant genotypes had no increased on enzyme activity when mechanically stressed. So, no significance difference was found between resistant and susceptible sugarbeet.

Table 4- Chitinase activity to unwounded and wounded sugarbeet genotypes

Genotype	**Susceptibility**	**Unwounded** $dA.min^{-1}.mg\ pt^{-1}$		**Wounded** $dA.min^{-1}.mg\ pt^{-1}$		**unwounded and wounded** $dA.min^{-1}.mg\ pt^{-1}$	
ACH-817*	Xxxx	1.82	abc	0.64	**c**	1.23	cd
F1010	Xxx	1.96	abc	1.85	**a**	1.91	a
19961009H2	Xxx	1.69	bc	1.72	**a**	1.7	ab
C564aa*	Xxx	1.75	abc	0.43	**c**	1.09	d
F1015	xx	1.56	c	1.7	**ab**	1.63	abc
F1016*	X	1.99	a	1.39	**b**	1.69	ab
F1024	X	1.59	c	1.13	**b**	1.36	bcd
14N0026*	X	1.40	abc	0.46	**c**	0.93	d
MagRes1*	X	1.92	ab	0.52	**c**	1.22	cd
Mean		1.74	A	1.09	B		

Susceptibility rating: xxxx= very susceptible; xxx= susceptible; xx= moderate resistant; and x= resistant to SBRM. * indicate significantly difference between unwounded and wounded means at 0.05 level. Difference amog means within a column followed by the same letter are not signicant, according to Tukey range tests ($P \leq 0.05$).

4. CONCLUSIONS

We can conclude that the highest level in gene expression of chitinase, PPO and POD resistant genotype (F1016) in relation to susceptible (F1010) reported by Puthoff and Smigocki (2007) do not imply in increased activity in any evaluated enzyme**.** Confirming to Stout et al. (1994), the stimulation of enzymes oxidative by insect feeding does not constitute evidence for a defensive role against insects.

Other factor could be explain our results is maggot fly and wounding do not result in same effect, maybe it is necessary some elicitors produced by maggots.

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GENERAL CONCLUSIONS

- In conclusion, the present study suggests that JA treatment could prime sugarbeet to induct a series of defense genes, including defense-related proteins and key enzymes related secondary metabolites.
- JA also increased the ability sugarbeet cells to recognize pathogen which may result faster activation of immune response and then reduction of infection and susceptibility.
- We can conclude that the highest level in gene expression of chitinase, PPO and POD resistant genotype (F1016) in relation to susceptible (F1010) seen by Puthoff and Smigocki (2007) do not imply increased activity in any evaluated enzyme**.** Confirming to Stout et al. (1994), the stimulation of enzymes oxidative by insect feeding does not constitute evidence for a defensive role against insects.
- Other factor could be explain our results is maggot fly and wounding do not result in same effect, maybe is necessary some elicitors produced by maggot.