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ORIGINAL PAPER

Identification of genes differentially expressed in husk tomato (*Physalis philadelphica*) in response to whitefly (*Trialeurodes vaporariorum*) infestation

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Abstract Plants respond to phloem-feeding whiteflies by extensive changes in gene expression. To identify differentially expressed genes in husk tomato plants (*Physalis philadelphica*) infested with *Trialeurodes vaporariorum*, young plants were challenged with adult whiteflies, and forward and reverse subtractive libraries were constructed from infested leaves at 5 and 15 days after infestation. Several genes were identified as up-regulated; these included a diversity of genes involved in plant defense responses, protein synthesis or degradation, and cell wall fortification or modification. Genes required for amino acid biosynthesis, lipid metabolism and synthesis, including cell surface components such as suberin, responses to stress, photosynthesis and other functions, were similarly induced. Down-regulated genes were also identified, most prominently kinases and aquaporin genes. Similarities in defense responses between tomato and *P. philadelphica* were noted regarding the expression of certain genes in response to nematode, aphid, or whitefly. A role for abscisic acid, brassinosteroids, and cytokinins in the regulated response to whitefly infestation in *P. philadelphica* was also implied by the expression pattern of phytohormone-associated genes, including genes coding for proteins containing F-box motifs. Differential expression of selected genes was validated by quantitative real-time PCR. The possible role

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played by some of these genes during whitefly infestation is discussed.

Keywords Greenhouse whitefly · Insect herbivory · *Physalis* · Plant defense · Suppression subtractive hybridization

Introduction

Plants respond to insect herbivory through a complex and diverse defense system, involving physical barriers, toxic chemicals, proteins, and/or recruitment of natural enemies (Howe and Jander 2008; War et al. 2012). Defensive traits either produced constitutively or in response to plant damage affect insect preference (e.g., host plant selection) or performance (e.g., growth rate and development) (Karban and Baldwin 1997). After herbivore recognition, several endogenous signal molecules including jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), salicylic acid (SA), and reactive oxygen species are produced to regulate signal transduction cascades in plant cells, leading to the activation and modulation of defense-related genes (Walling 2000; Rojo et al. 2003; Zhu-Salzman et al. 2005). These pathways are interconnected and can act antagonistically, additively, or synergistically (Mur et al. 2006). Cross-talk between signaling pathways is thought to play an important role in the regulation and fine-tuning of the defense responses, allowing effective local and systemic defense (Zarate et al. 2007; Koornneef and Pieterse 2008). Plant utilize defense mechanisms that are dependent on the attacker; thus, responses modulated after insect herbivory can be correlated with the mode of feeding and/or the amount of tissue damage occurring at the feeding site (Walling 2000, 2008; Heidel and Baldwin 2004; Thompson and Goggin 2006; Kempema et al. 2007; Estrada-Hernández et al. 2009). Chewing herbivores mainly activate JA-mediated defense responses, whereas phloem-feeding insects, in resemblance to plant responses to biotrophic pathogens, activate SA-mediated responses (Moran and Thompson 2001; Kempema et al. 2007; Zarate et al. 2007).

The greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) is an economically important field and greenhouse pest of horticultural and ornamental crops (Inbar and Gerling 2008). Whiteflies are generalist and highly polyphagous insect pests that cause considerable damage to crops by reducing plant vigor, provoking plant stunting, foliage deformation, discoloration and/or defoliation (Berlinger 1986), or by transmission of many plant viruses (Jones 2003). Reduced photosynthesis can also be caused by light blockage resulting from mold growth on honeydew excretion (Byrne and Miller 1990). Compared to chewing caterpillars, phloem feeders cause limited tissue damage (Walling 2000). Plant responses to whitefly infestation result in increased SA-regulated defenses concomitant with a decline in JA-regulated responses, and changes in redox balance and in nitrogen and carbon metabolism (Mayer et al. 2002; Antony and Palaniswami 2006; Gorovits et al. 2007; Kempema et al. 2007; Puthoff et al. 2010; Zhang et al. 2013). However, in *Arabidopsis thaliana*, the suppressed JA-regulated defense pathway controls resistance traits that retard whitefly nymph development (Zarate et al. 2007; Zhang et al. 2013). Additional studies have shown that whitefly infestation in tomato alters gene expression associated predominantly with photosynthesis, senescence, secondary metabolism, and (a)biotic stress depending on the whiteflýs developmental stage (Estrada-Hernández et al. 2009).

Physalis philadelphica Lam (Solanales: Solanaceae) commonly known as husk tomato, is grown as a food crop. Its fruits are important component in the diet and folk medicine of Mexican and Central American populations. This prominence is mostly due to its high nutritional value in terms of vitamins, minerals, and antioxidants, as well as for its potential curative properties (Bock et al. 1995; Maldonado et al. 2011). In Mexico, it has been domesticated and cultivated for centuries, a process that exploited its high morphological variability in terms of vegetative and reproductive traits (Zamora-Tavares et al. 2014). In Mexico, the importance of this crop is still reflected nowadays in the 44,000 ha of cultivated area, producing ca. 588,000 t (SIAP 2013). In 2013, exports to the USA comprised 8 % of domestic production with a market value close to 56 million USD (INEGI 2014). Once exclusively grown in Latin America and the Caribbean isles, *P. philadelphica* is now cultivated in a number of other countries in all continents (Calyecac-Cortero et al. 2007). Conversely, the cultivation of *P. peruviana*, a close relative of *P. philadelphica*, is concentrated predominantly in Colombia and South Africa, and is exported mostly to Europe, Asia, and the United States (Fischer et al. 2014).

Recently, genomic and transcriptomic information has been obtained in *P. peruviana* and *P. alkekengi* species (Simbaqueba et al. 2011; Garzón-Martínez et al. 2012; Wang et al. 2012; Wei et al. 2012; Enciso-Rodríguez et al. 2013; Bolger 2014; Wang et al. 2014), and will be further complemented by the planned genomic sequencing of the above three species as part of the SOL-100 project (http://solgenomics.net). However, information regarding *P. philadelphica* defense response against insect pests is scarce. This situation persists despite the high susceptibility of *Physalis* to several insect herbivores of diverse feeding guilds, from the highly specialized *Heliothis subflexa* (Lepidoptera: Noctuidae), which is an exclusive

insect pest of the *Physalis* genus whose damage is known to cause fruit abscission (Petzold et al. 2009), to numerous soil, leaf, and fruit pests including lepidopteran larvae, leaf miners, aphids, trips, and whiteflies, some of which are vectors of viruses that seriously affect the *Physalis* production (De la Torre-Almaráz et al. 2003; Fischer et al. 2014; Muniz et al. 2014). No data are available regarding the level of damage caused by *T. vaporariorum* infestation on cultivated *Physalis*, although these effects can be similarly devastating in other related crops, such as potato and tomato (Ramos et al. 2002; Morales 2003). Thus, to improve our understanding of the mechanisms underlying insect herbivory response in this species, suppression subtractive hybridization (SSH) libraries were generated from plants infested for 5 or 15 days with *T. vaporariorum*. The objective was to obtain a first description of the gene expression changes induced in *P. philadelphica* during feeding by phloem-feeding whiteflies. A comparison with responses produced by leaf-chewing *Manduca sexta* larvae was also performed. The expression changes were validated through quantitative real-time PCR (qPCR) analysis. The functional significance of a selection of ESTs in relation to whitefly herbivory response and their specificity to certain insect feeding guilds is discussed.

Materials and methods

Plants and insects

Physalis philadelphica cultivar Morada R was grown in 4″ pots using a rich soil mixture [1 part Sunshine Mix 3™ (SunGro Horticulture, Bellevue, WA, USA), 1 part loam and 1 part vermiculite (SunGro Horticulture)], were watered every 2 days and fertilized weekly with a 20–20–20 (N–P–K) soil drench solution (Peters Professional; Scotts-Sierra Horticultural Products, Marysville, OH, USA). Plants were maintained in a growth chamber under a photoperiod of 16 h light at 28 °C and 8 h darkness at 16 °C. Four-week-old plants having 4–5 expanded leaves were used in all the experiments.

Virus-free whiteflies (*T. vaporariorum*) were obtained from a laboratory colony maintained on tomato (*Solanum lycopersicum* L. cv. Rio Fuego) (Solanaceae) plants under controlled conditions of light ($\approx$300 µmol m^{-2} - s^{-1}), temperature (27 °C) and photoperiod (16 h light/8 h dark). All experiments were conducted using 1- to 5-day-old adult whiteflies. Larvae of *Manduca sexta* L. (Lepidoptera: Sphingidae) were provided by the insectarium at Cinvestav, Unidad Irapuato. They were reared on a nutritive gel prepared as described by Yamamoto (1964).

Herbivory assays

For *T. vaporariorum* infestations, plants were confined inside the colony-harboring cages for 5 or 15 days where adults were allowed to oviposit and feed freely. Approximately, 300 whiteflies were counted on each plant; however, no evident signs of damage, such as chlorotic or necrotic lesions were observed. For the SSH assays, foliar tissue from six plants was pooled and flash frozen with liquid nitrogen and stored at −80 °C until needed for analysis. Biological replicate samples destined for qPCR were sampled individually ($n = 3$ plants per time point). For *M. sexta* herbivory, two larvae (3rd instar) were placed per plant ($n = 3$) and allowed to feed freely for 6 h. After this time, larvae were removed and leaves of each plant were collected 18 h later. All experiments included the same number of undamaged, developmentally matching plants, as controls.

RNA isolation

Total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) as instructed. About 1 mg of total RNA was used to isolate the poly (A) mRNA fraction using the PolyATtract mRNA Isolation Systems (Promega, Madison, WI, USA) following the manufactureŕs protocol. For qPCR assays, RNA isolation was performed using RNeasy columns (Qiagen, Valencia, CA, USA) and samples were treated with DNAse (RNase-Free DNase Set, Qiagen). In all cases, RNA integrity was assessed by denaturing electrophoresis on 1 % agarose gels and quantified by a ND-1000 NanoDrop spectrophotometer (NanoDrop Products, Wilmington, DE, USA).

Suppression subtractive hybridization

The cDNA subtraction was performed using the PCR-Select cDNA subtraction kit (Clontech, Palo Alto, CA) according to the manufacturés recommendations. Forward and reverse subtractive hybridizations were performed at 5 and 15 days post infestation (dpi), using cDNAs from infested plants as ‘testeŕ and from control non-infested plants and adult whiteflies as ‘driveŕ in forward subtraction, and vice versa, for reverse subtraction. Subtracted cDNAs were subjected to two rounds of PCR amplification to normalize cDNA populations. The PCR products were cloned into pJET 1.2 vectors (Fermentas, Glen Burnie, MD, USA) which were subsequently used to transform *Escherichia coli* One-Shot Top10 cells (Invitrogen). To assess the insert sizes, colony PCR was performed using Taq DNA polymerase (Fermentas) and nested 1 and 2R primers provided in the subtraction kit. Bacterial clones harboring cDNA inserts were stored at −80 °C in 50 %

(v/v) glycerol/LB broth spiked with carbenicillin (100 mg L^{-1}) in 96-well micro-plates. The cloned fragments obtained were subsequently sequenced.

Sequence analysis

The selected positive clones were sequenced using an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Poor quality sequences and fragments shorter than 100 bp were discarded and those remaining were then edited to remove the adaptor-primer and vector DNA sequences using SeqClean (http://www.tigr.org/tdb/tgi/sotfware/) and Lucy2 (Chou and Holmes 2001). After edition, sequences were used for contig assembly using the CAP3 software (http://seq.cs.iastate.edu/; Huang and Madan 1999). ESTs were compared with non-redundant public databases, using BLASTx algorithms of The Arabidopsis Information Resource (TAIR) (http://arabidopsis.org/index.jsp), National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), and SOL Genomics Network (http://solgenomics.net) (Table S1). Homologies with *E* values lower than e^{-5} were considered to have significant homology and were categorized according to their function. The putative physiological function of these ESTs was classified according to the functional categories of *A. thaliana* proteins (http://mips.gsf.de).

qPCR analysis

After RNA extraction, 2 µg was reverse transcribed with QuantiTect Reverse Transcription kit (Qiagen). qPCR amplifications were performed using SYBR Green detection chemistry in a LightCycler 1.5 System (Roche Diagnostics, Mannheim, Germany). Reactions were prepared in a total reaction volume of 10 µl containing: 2.5 µl of cDNA diluted 1:100 (40 ng) or 1:10, 2 µl each of forward and reverse primers (0.125 µM), 2 µl of LightCycler FastStart DNA MasterPLUS SYBR Green I (Roche Applied Science) and 3.5 µl of sterile deionized distilled water. Gene-specific primers were designed for a number of the differentially up-regulated cDNA clones (Table 1) using Primer Express Software v3.0.1 (Applied Biosystems Life Technologies). *S. tuberosum* L. actin gene (Reca et al. 2008) was employed as the reference gene. The cycling conditions were set as follows: initial denaturation step at 95 °C for 10 min followed by 45 cycles of denaturation at 95 °C for 10 s and annealing at the established temperature for each gene (Table 1) for 7 s. Following the PCR amplification, a melting curve analysis was immediately performed to confirm the specificity of the reactions. Baseline and threshold cycles (Ct) were automatically determined using qPCR System software. Efficiencies for all genes tested were greater than 95 %. Three biological replicates and two technical replicates were analyzed. Relative expression was calculated using the comparative cycle threshold method (Livak and Schmittgen 2001), where delta (Δ) cycle threshold of cDNA from controls was defined as 100 % transcript presence. Transcript abundance data were normalized against the average transcript abundance of the actin gene determined during the assays. The fold change in expression of the target genes in each treatment was calculated using the following equation: $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct\ target\ gene - average\ Ct\ reference\ gene)_{treatment} - (Ct\ target\ gene - average\ Ct\ reference\ gene)_{control}$. The mean expression levels in infested and un-infested (control plants) were compared by *t* tests. Prior to the *t* tests, a Box-cox transformation (Sakia 1992) was performed to meet the assumptions of normality. Comparisons between infestation days were performed via Mann–Whitney tests and comparisons between *T. vaporariorum* and *M. sexta* herbivory were analyzed by *t* tests. Statistical analyses were performed using Mini-Tab 15 and Statistica 8 software.

Results

Sequence data analysis

Two subtractive cDNA libraries enriched in genes responsive to whitefly infestation at 5 and 15 dpi were constructed. A total of 768 differentially expressed clones were randomly selected and sequenced from both libraries. After removing low quality and short length sequences, 349 high-quality ESTs were obtained and clustered into 14 contigs (7 for each library) and 268 singletons (151 and 117 for the 5 and 15 dpi libraries, respectively). The cDNA fragments ranged from 71 to 805 bp, with an average length of 337 bp (Table S2). Sequence redundancy, calculated according to Da et al. (2012), was 18.9 and 19.4 % for 5 and 15 dpi libraries, respectively. BLASTx sequence comparisons obtained from both libraries revealed that 79.8 % of the sequences showed a significant homology (*E* value $< e^{-5}$) with known proteins from other organisms (Tables 2, 3), whereas no hits or non-significant homologies were obtained for the remaining sequences (Table S1 and S2). Due to limitations in the Genbank submission policy, only 251 processed ESTs were deposited in the NCBI (Tables 2, 3).

Functional classification

Putative functional categories were assigned using FunCat version 2.1 (http://mips.gsf.de). ESTs were grouped into

Table 1 Primers used for gene expression analysis by qRT-PCR

Gene symbol	Description	Primer sequence forward/reverse	Amplicon length (bp)	Annealing temperature (°C)
LP	Lesion-inducing protein	TCCTCTTTGCTTCCGTTTTCA/ CCATCTTCCCCAAATTCATTGA	64	61
AQP	Aquaporin-like protein	CACTTGGGATTTGCTCATGAGA/ GGGTCTACTGGGCTGGTCC	96	63
LEA	Late embryogenesis abundant	GTTGGATGTGCCAGTGAAGGT/ CTCCACCAATGTCCCTAACCA	65	64
BGL	β-1, 3-glucanase	TTATTCGATGCTATGTTGGATTCC/ TACCGATCCCCCACCTGAT	63	63
WR	Wound-responsive protein related	GGAGTGAATCATCGGCTTGTTT/ GGCGGGTTCATCGGAAGT	62	61
OSM	Osmotin	GTTGGGCCAAAAGACATAGGG/ CACCAAACACCTTAGCCGAATAC	106	65
SOD	Chloroplast superoxide dismutase	CAATCGACCACCAGCGTTT/ GGAAAGGGTGGCCATGAAC	54	59
CDSP	Chloroplast drought-induced stress protein	GAAGACCACAAAATCGATCAAAAA/ TGGTCCGCAATGCTTCAA	63	59
HSP	Heat-shock protein	TACGGAGGGTGAGGTGGAGTT/ TCATTGTTTAGAGGAGCCATTCC	66	66
GTX	Glutaredoxin family protein-like	TCCATAAACCCAGCGTCCAT/ TCTCCGTAGCTTCCGTGTTTC	63	62
SCP	Serine carboxypeptidase	GTGGCTTATGGAGCTGCTGAA/ CGCCAAGTTGTTCCAAGAGTAAG	96	66
MDHAR	Monodehydroascorbate reductase	GCGGGACACCTGAAGAGAAC/ GCTAGCAACGGGTTGAACTTTT	62	63
KOR	β-1, 4-glucanase (Korrigan)	GTTTCCATGATGTCCGTTCTAATTA/ CAACCAAACCAGCATTTCCA	69	63
ACTIN	Reference gene	GGGATGATATGGAGAAGATA/ AGTACAGCCTGAATAGCAAC	180	57

bp base pairs

ten groups, namely, metabolism, secondary metabolism, energy and photosynthesis, (a)biotic stress, signal transduction, protein synthesis and metabolism, transport, other functions, unknown functions and no hit. The overall functional classification of ESTs between the two libraries was similar in most categories, with (a)biotic stress (21 %), protein synthesis and metabolism (18 %) and metabolism (14 %) representing the major biological processes represented. Up to 20 % ESTs were included in transport, signal transduction, or other function categories. Potential new genes with no similarity to sequences in the public databases and proteins with no defined biologic process constituted 18 and 9 % of the total EST population, respectively (Tables 2, 3, and S3). The functional classification of the 5 dpi forward-subtracted library showed an increase in the protein synthesis and secondary metabolism categories. Conversely, in the reverse-subtracted library, an increase of the general metabolism and photosynthesis categories was observed (Tables 2 and S3). Several genes associated with (a)biotic stress were found to be up-regulated at 5 dpi, including those coding for members of the trypsin inhibitor family of proteinase inhibitors, serine hydroxymethyltransferase, plant aspartic and cysteine proteases, osmotin, and a pathogenesis-related (PR) protein family (Table 2). For the 15 dpi forward-subtracted library, the (a)biotic stress and protein synthesis and metabolism categories where highly represented (Tables 3 and S3). Altered expression of genes required for ordered cellulose–hemicellulose network such as an endo β-1, 4-glucanase Korrigan and a xyloglucan: xyloglucosyl transferase, was also observed. Genes involved in the phenyl propanoid metabolism and in cuticular wax production were up-regulated as well (Table 3).

Table 2 Details of up and down-regulated genes identified in *Physalis philadelphica* plants subjected to a 5 day whitefly (*Trialeurodes vaporariorum*) infestation

EST no. (accession no.)	Putative identity	*E* value	Source
Up-regulated			
Metabolism			
JZ476552	Phosphoribosylformylglycinamidine synthase	$3.00E^{-46}$	*Solanum lycopersicum*
JZ476556	Phosphoenolpyruvate carboxylase 4-like	$5.00E^{-75}$	*S. lycopersicum*
JZ476516	Phosphomannomutase/phosphoglucomutase-like	$2.00E^{-166}$	*S. lycopersicum*
JZ476656	Cytosolic NADP-malic enzyme	$3.64E^{-94}$	*Nicotiana tabacum*
Pp02d05m_E02	Phosphomannomutase/phosphoglucomutase-like	$4.00E^{-69}$	*S. lycopersicum*
Secondary metabolism			
JZ476646	Geranylgeranyl diphosphate reductase, chloroplastic	$3.59E^{-15}$	*N. tabacum*
JZ476655	Trans-resveratrol di-O-methyltransferase-like	$2.00E^{-85}$	*S. lycopersicum*
JZ476658	Methenyltetrahydrofolate synthase domain-containing protein-like	$3.00E^{-103}$	*S. lycopersicum*
Pp02d05m_G07	Hyoscyamine 6-dioxygenase-like	$8.00E^{-11}$	*S. lycopersicum*
Pp01d05m_B05	S-Adenosylmethionine synthase 1	$6.20E^{-142}$	*S. lycopersicum*
Energy and photosynthesis			
JZ476551	Photosystem II core complex proteins psbY	$4.00E^{-53}$	*S. lycopersicum*
JZ476669	Chlorophyll a/b binding protein	$1.07E^{-60}$	*S. tuberosum*
JZ476653	Chlorophyll a/b binding protein 6A, chloroplast	$6.00E^{-35}$	*S. lycopersicum*
Protein synthesis and metabolism			
JZ476667	60S ribosomal protein L23	$7.00E^{-36}$	*Arabidopsis thaliana*
JZ476539	60S ribosomal protein L27a-2	$1.00E^{-27}$	*A. thaliana*
JZ476538	elongation factor 1B beta	$2.00E^{-21}$	*A. thaliana*
JZ476666	60S ribosomal protein L36-3	$9.00E^{-11}$	*A. thaliana*
JZ476651	60S ribosomal protein L23a-2	$5.00E^{-33}$	*A. thaliana*
JZ476506	Ubiquitin 6	$1.00E^{-06}$	*A. thaliana*
JZ476534	Ribosomal protein S24e family protein	$3.00E^{-12}$	*A. thaliana*
JZ476652	Ubiquitin-protein ligase RMA1H1	$4.00E^{-22}$	*C. annuum*
JZ476644	CPR2-like cysteine proteinase	$3.21E^{-71}$	*N. tabacum*
JZ476542	Ly200 protein	$2.21E^{-54}$	*Capsicum annuum*
JZ476545	40S ribosomal protein S17-4	$6.00E^{-33}$	*A. thaliana*
JZ476662	Aspartic proteinase-like protein 2-like	$3.00E^{-63}$	*S. lycopersicum*
JZ476540	Protein translation factor SUI1 homolog isoform 1	$3.00E^{-73}$	*S. lycopersicum*
JZ476515	Translation elongation factor 1 alpha	$2.00E^{-71}$	*N. benthamiana*
JZ476547	Putative ADP-ribosylation factor protein	$8.00E^{-72}$	*Elaeis guineensis*
JZ476530	Leucine-rich repeat receptor-like serine/threonine-protein kinase BAM1-like	$5.00E^{-52}$	*S. lycopersicum*
JZ476668	Constitutive photomorphogenesis protein 10-like	$3.00E^{-48}$	*S. lycopersicum*
Pp01d05m_B08	UDP-glycosyltransferase 85A1-like	$5.00E^{-18}$	*S. lycopersicum*
Pp02d05m_F12	60S ribosomal protein L13-1	$7.00E^{-34}$	*A. thaliana*
Pp01d05m_G09	40S ribosomal protein S11	$2.00E^{-45}$	*S. tuberosum*
Pp02d05m_B08	Oligouridylate binding protein-like protein	$2.54E^{-129}$	*S. tuberosum*
Pp01d05m_H04	Uncharacterized RING finger protein C548.05c-like	$1.00E^{-55}$	*S. lycopersicum*
Transport			
JZ476649	Microsomal glutathione S-transferase 3-like isoform 1	$5.00E^{-12}$	*S. lycopersicum*
JZ476533	Sodium-coupled neutral amino acid transporter 3-like	$1.00E^{-55}$	*S. lycopersicum*
JZ476505	Putative calcium-transporting ATPase 13, plasma membrane-type-like	$2.00E^{-10}$	*S. lycopersicum*

Table 2 continued

EST no. (accession no.)	Putative identity	*E* value	Source
Biotic and abiotic stress			
JZ476504	Wound-responsive protein-related **(WR)**[a]	$3.00E^{-16}$	*S. lycopersicum*
JZ476519	Class II chitinase	$9.91E^{-42}$	*S. tuberosum*
JZ476518	LEA1-like protein **(LEA)**	$3.48E^{-66}$	*C. annuum*
JZ476528	Osmotin-like protein **(OSM)**	$4.60E^{-37}$	*N. tabacum*
JZ476535	protein GIGANTEA-like	$2.00E^{-85}$	*S. lycopersicum*
JZ476537	Putative lesion-inducing protein **(LP)**	$8.07E^{-27}$	*C. annuum*
JZ476544	Aquaporin-like **(AQP)**	$8.23E^{-91}$	*S. lycopersicum*
JZ476543	Metallothionein	$3.80E^{-28}$	*S. nigrum*
JZ476553	Thiazole biosynthetic enzyme	$6.00E^{-11}$	*N. benthamiana*
JZ476647	Chaperonin CPN60-2, mitochondrial-like	$3.00E^{-45}$	*S. lycopersicum*
JZ476659	Serine hydroxymethyltransferase 1	$5.00E^{-13}$	*S. lycopersicum*
Pp02d05m_C04	Stromal 70 kDa heat shock-related protein, chloroplastic-like	$9.00E^{-44}$	*S. lycopersicum*
Pp02d05m_D07	Prepro-beta-1,3-glucanase precursor, partial **(BGL)**	$4.00E^{-69}$	*N. tabacum*
Pp02d05m_E04	HR-like lesion-inducing protein-related isoform 1	$3.00E^{-06}$	*Theobroma cacao*
Pp02d05m_G08	Trypsin inhibitor A-like	$3.00E^{-10}$	*S. lycopersicum*
Signal transduction			
JZ476520	F-box protein PP2-A12-like	$2.00E^{-05}$	*S. lycopersicum*
JZ476522	Calcium-dependent protein kinase	$1.54E^{-20}$	*S. lycopersicum*
Other functions			
JZ476523	Gamma-interferon-inducible lysosomal thiol reductase-like isoform 1	$1.00E^{-20}$	*S. lycopersicum*
JZ476525	Ribonuclease P protein subunit p25-like protein-like	$4.00E^{-43}$	*S. lycopersicum*
JZ476524	Kiwellin-like	$5.00E^{-30}$	*S. lycopersicum*
JZ476555	Putative RNA-binding protein Luc7-like 1-like	$2.00E^{-42}$	*S. lycopersicum*
JZ476549	Auxin repressed/dormancy associated protein	$7.80E^{-31}$	*S. lycopersicum*
JZ476645	Cleavage and polyadenylation specificity factor subunit 5-like	$8.00E^{-10}$	*S. lycopersicum*
JZ476536	Chloroplast protein 12	$1.50E^{-43}$	*N. tabacum*
JZ476548	GDT1-like protein 1, chloroplastic-like	$9.00E^{-42}$	*S. lycopersicum*
Unknown functions			
JZ476554	Uncharacterized protein LOC101266024	$1.00E^{-15}$	*S. lycopersicum*
JZ476511	Uncharacterized protein LOC101255248	$3.00E^{-20}$	*S. lycopersicum*
JZ476531	Uncharacterized protein LOC101250594	$2.00E^{-22}$	*S. lycopersicum*
JZ476657	Uncharacterized protein LOC101264952	$1.00E^{-48}$	*S. lycopersicum*
Pp02d05m_D08	Uncharacterized protein LOC101248693	$1.00E^{-17}$	*S. lycopersicum*
Down-regulated			
Metabolism			
JZ476717	Glycerate dehydrogenase HPR, peroxisomal	$9.00E^{-60}$	*S. lycopersicum*
JZ476592	Anthranilate phosphoribosyltransferase, chloroplastic	$7.42E^{-55}$	*Vitis vinifera*
JZ476607	Putative 3-isopropylmalate dehydrogenase large subunit	$1.31E^{-153}$	*C. annuum*
JZ476584	Cristal-Glass1 protein	$6.64E^{-34}$	*C. annuum*
JZ476587	Lipoyl synthase 2, mitochondrial-like	$6.50E^{-33}$	*G. max*
JZ476614	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic	$1.56E^{-61}$	*N. tabacum*
JZ476705	Granule-bound starch synthase 1, chloroplastic/amyloplastic-like	$5.00E^{-12}$	*S. lycopersicum*
JZ476708	Fructose-bisphosphate aldolase, chloroplast precursor, putative, expressed	$9.00E^{-23}$	*O. sativa*
JZ476619	Phosphoglycolate phosphatase-like	$5.00E^{-77}$	*S. lycopersicum*

Table 2 continued

EST no. (accession no.)	Putative identity	*E* value	Source
JZ476713	Glyceraldehyde-3-phosphate dehydrogenase	$3.00E^{-55}$	*C. annuum*
Pp02r05m_B07	Carbonic anhydrase, partial	$6.00E^{-65}$	*S. tuberosum*
Pp02r05m_F08	Transketolase, chloroplastic	$7.00E^{-49}$	*S. tuberosum*
Energy and photosynthesis			
JZ476608	Thioredoxin H4-like isoform 2	$2.00E^{-15}$	*S. lycopersicum*
JZ476502	Chloroplast oxygen-evolving protein 16 kDa subunit	$7.72E^{-53}$	*N. tabacum*
JZ476718	Ferredoxin I	$3.00E^{-62}$	*S. tuberosum*
JZ476603	Chlorophyllide a oxygenase, chloroplastic-like	$1.00E^{-122}$	*S. lycopersicum*
JZ476605	Plastocyanin, chloroplastic; Flags: Precursor	$9.26E^{-83}$	*S. lycopersicum*
Pp02r05m_F04	Ferredoxin I	$4.00E^{-67}$	*S. lycopersicum*
Protein synthesis and metabolism			
JZ476697	Mediator of RNA polymerase II transcription subunit 33A-like	$7.00E^{-33}$	*S. lycopersicum*
JZ476715	Putative mTERF domain containing protein, identical	$2.00E^{-59}$	*S. demissum*
JZ476593	BTB/POZ domain-containing protein At2g30600	$4.60E^{-78}$	*V. vinifera*
JZ476720	F-box/LRR-repeat protein At3g03360-like	$6.00E^{-37}$	*S. lycopersicum*
Pp02r05m_D08	Ribosomal protein S6-like protein	$2.18E^{-20}$	*S. tuberosum*
Transport			
JZ476696	Adenosine nucleotide translocator	$1.00E^{-42}$	*Brassica oleracea*
JZ476704	Probable peptide/nitrate transporter At5g62680-like	$1.00E^{-101}$	*S. lycopersicum*
Biotic and abiotic stress			
JZ476722	Aquaporin-like protein	$2.00E^{-50}$	*Petunia x hybrida*
JZ476600	Fruit-specific protein-like	$9.00E^{-05}$	*S. lycopersicum*
JZ476711	Skp1	$2.00E^{-34}$	*C. annuum*
JZ476598	3-Ketoacyl-CoA synthase 6-like	$5.00E^{-96}$	*S. lycopersicum*
JZ476588	Heat shock protein binding protein	$2.00E^{-08}$	*S. lycopersicum*
JZ476589	Putative PIP-type aquaporin	$7.54E^{-97}$	*S. lycopersicum*
JZ476590	Signal recognition particle 43 kDa protein, chloroplastic-like	$3.00E^{-69}$	*S. lycopersicum*
JZ476596	Heat shock protein 60	$2.07E^{-44}$	*Ageratina adenophora*
JZ476597	Putative chloroplast thiazole biosynthetic protein	$1.27E^{-39}$	*N. tabacum*
JZ476706	Aquaporin-like protein	$8.70E^{-49}$	*Petunia x hybrida*
JZ476709	Aqp2 protein	$2.00E^{-70}$	*S. lycopersicum*
Pp02r05m_F11	UDP-glycosyltransferase 73C4-like	$2.00E^{-12}$	*S. lycopersicum*
Pp02r05m_B08	Defensin-like protein P322-like	$2.00E^{-26}$	*S. lycopersicum*
Pp01r05m_G04	PIP2	$2.00E^{-12}$	*Karelinia caspia*
Signal transduction			
JZ476610	Mitogen-activated protein kinase	$1.18E^{-20}$	*N. tabacum*
JZ476585	Nucleoside diphosphate kinase	$8.50E^{-59}$	*C. annuum*
JZ476616	Dual specificity protein kinase pyk1 isoform 1	$3.75E^{-92}$	*V. vinifera*
Other functions			
JZ476601	Pentatricopeptide repeat-containing protein At3g49240-like	$4.17E^{-21}$	*G. max*
JZ476611	ATP-dependent RNA helicase DBP2	$5.00E^{-57}$	*S. lycopersicum*
JZ476595	H2A histone	$3.00E^{-43}$	*N. tabacum*
Unknown functions			
JZ476594	Uncharacterized protein LOC101263228	$6.00E^{-27}$	*S. lycopersicum*
JZ476615	Uncharacterized protein LOC101262906	$2.00E^{-92}$	*S. lycopersicum*
JZ476618	Uncharacterized protein LOC101248056	$7.00E^{-28}$	*S. lycopersicum*
JZ476701	Hypothetical protein	$3.00E^{-13}$	*V. vinifera*

Table 2 continued

EST no. (accession no.)	Putative identity	*E* value	Source
JZ476703	Uncharacterized protein LOC101253866	$7.00E^{-10}$	*S. lycopersicum*
JZ476602	Uncharacterized protein LOC101253367	$5.00E^{-34}$	*S. lycopersicum*
JZ476712	Uncharacterized protein At5g03900, chloroplastic-like	$9.00E^{-49}$	*S. lycopersicum*
JZ476719	Uncharacterized protein	$1.00E^{-11}$	*Populus trichocarpa*
Pp01r05m_F03	Uncharacterized protein LOC101262737	$9.00E^{-116}$	*S. lycopersicum*
Pp02r05m_C02	Uncharacterized protein	$5.00E^{-20}$	*S. tuberosum*

[a] Genes in bold-text and underlined were analyzed by qRT-PCR

Confirmation of differentially expressed ESTs by qPCR

To further verify the differential expression of genes in the SSH libraries, a qPCR validation was performed using specific primers (Table 1) for 13 ESTs from the forward-subtracted libraries (6 and 7 from the 5 or 15 dpi sampling time points, respectively). Although there was a particular interest for the analysis of defense-related genes; ESTs were also selected on the basis of length (>100 bp) and e-value (>1e − 6). At 5 dpi, the expression of a wound-responsive protein related (*WR*) and osmotin (*OSM*) was induced. The transcript accumulation levels observed for the *WR* and *OSM* genes were 4.2- and 11.6-fold higher than controls, respectively. Conversely, the genes coding for late embryogenesis abundant *(LEA)*, a lesion-inducing (*LP*), aquaporin-like (*AQP*), and β-1, 3-glucanase (*BGL*) proteins showed no change in expression levels (Fig. 1). The expression of the stress-related chloroplast superoxide dismutase (*SOD*), chloroplast drought-induced stress protein (*CDSP*), heat-shock protein (*HSP*), and glutaredoxin family protein-like (*GTX*) genes was up-regulated within a 3.6- to 5.8-fold range at 15 dpi, whereas serine carboxypeptidase (*SCP*), monodehydroascorbate reductase (*MDHAR*), and β-1, 4-glucanase Korrigan (*KOR*) gene transcripts accumulated to even higher levels, reaching more than a ninefold increase.

To obtain a more detailed temporal expression pattern of those genes whose differential expression was confirmed after SSH experiments, transcript accumulation of specific ESTs (*LEA*, *BGL*, *WR,* and *OSM*) from the 5 dpi forward-subtracted library was measured in plants infested for 15 days and vice versa for 15 dpi-specific ESTs (*SOD*, *CDSP*, *HSP*, *GTX*, *SCP*, *MDHAR*, and *KOR*). In agreement with the 15 dpi subtracted library, 5 genes (*SOD*, *CDSP*, *HSP*, *MDHAR*, and *KOR*) showed higher transcript levels in plants infested for 15 days than in 5 days infested plants. Conversely, specific 5 dpi transcripts were greatly increased in plants infested for 15 days, with a 20.7-, 8.7-, and 700-fold increase for *LEA*, *OSM*, and *BGL* genes, respectively (Fig. 2). Stress-related gene expression of *LEA*, *OSM*, *SOD*, and *SCP* was also measured in plants exposed to *M. sexta* larval feeding, to compare *P. philadelphica* response to other insect feeding guilds, such as leaf-chewing insects, which most frequently produce faster and stronger responses than phloem feeders. As shown above, *OSM* and *SCP* levels were induced after whitefly infestation at 5 dpi, but only *OSM* expression levels were significantly higher than those produced 24 h after *M. sexta* herbivory (Fig. 3). In contrast, *SOD* transcripts were significantly increased in response to *M. sexta* (i.e., 4-fold increase) compared to the expression levels produced in response to *T. vaporariorum* at 5 dpi, whereas *LEA* expression was induced to similar levels in response to both herbivores at the respective time points sampled (Fig. 3).

Discussion

The amount of genomic and transcriptomic data for members of the *Physalis* genus has greatly increased in recent years (see above). One of the benefits of these efforts has been the generation of relevant information regarding defense responses in *P. peruviana*, particularly against *Fusarium* vascular wilt. In addition, gene families and SSR markers in contigs associated with plant immunity, programmed cell death, callose deposition, JA and ET-dependent defense responses, and resistance against bacterial, viral and fungal pathogens have been detected (Simbaqueba et al. 2011; Garzón-Martínez et al. 2012; Enciso-Rodríguez et al. 2013). In this context, the herewith study represents the first attempt to determine the transcriptomic response to whitefly herbivory in *P. philadelphica* plants, which has risen as a leading Solanaceous horticultural crop, but is also used as a plant model for ecology, evolution, and development (Wang et al. 2014). The functional classification of *P. philadelphica* genes isolated by SSH in plants subjected to whitefly infestation,

Table 3 Details of up and down-regulated genes identified from *Physalis philadelphica* plants subjected to 15 day whitefly (*Trialeurodes vaporariorum*) infestation

EST no. (accession no.)	Putative identity	*E* value	Source
Up-regulated			
Metabolism			
JZ476683	Phosphoglycerate kinase, chloroplastic	$6.24E^{-131}$	*Nicotiana tabacum*
JZ476670	1,4-Beta-glucanase, Korrigan **(KOR)**[a]	$4.77E^{-45}$	*Solanum lycopersicum*
JZ476581	GDSL esterase/lipase APG-like	$2.00E^{-25}$	*S. lycopersicum*
JZ476694	GDSL esterase/lipase APG-like	$8.00E^{-53}$	*S. lycopersicum*
Pp02d15m_A02	Glutamate-glyoxylate aminotransferase 2-like	$4.00E^{-05}$	*S. lycopersicum*
Pp02d15m_A04	Biotin carboxylase subunit	$1.24E^{-10}$	*N. tabacum*
Pp02d15m_D05	Monodehydroascorbate reductase **(MDHAR)**	$9.82E^{-79}$	*S. lycopersicum*
Secondary metabolism			
JZ476678	Feruloyl CoA ortho-hydroxylase 2-like	$1.00E^{-08}$	*S. lycopersicum*
Pp02d15m_C12	Glutamyl tRNA Reductase, partial	$4.89E^{-116}$	*N. tabacum*
Energy and photosynthesis			
JZ476690	Glutaredoxin family protein-like **(GTX)**	$5.00E^{-54}$	*S. lycopersicum*
JZ476507	Photosystem II reaction center W protein, chloroplastic-like	$1.00E^{-18}$	*S. lycopersicum*
JZ476563	Oxygen-evolving enhancer protein 2, chloroplastic	$9.00E^{-46}$	*S. tuberosum*
JZ476679	Photosystem I subunit XI	$6.80E^{-39}$	*N. attenuata*
Pp01d15m_F06	photosystem I reaction center subunit X psaK	$6.69E^{-59}$	*N. tabacum*
Pp01d15m_H02	Cytochrome b6-f complex iron-sulfur subunit 1, chloroplastic	$3.95E^{-14}$	*N. tabacum*
Protein synthesis and metabolism			
JZ476560	50S ribosomal protein L27, chloroplastic-like	$8.00E^{-22}$	*S. lycopersicum*
JZ476674	Prolyl 4-hydroxylase subunit alpha-1-like	$1.00E^{-133}$	*S. lycopersicum*
JZ476675	60S ribosomal protein L8-3	$9.00E^{-40}$	*A. thaliana*
JZ476692	Serine carboxypeptidase **(SCP)**	$2.00E^{-58}$	*S. lycopersicum*
JZ476568	Chaperone protein DnaJ-like isoform 2	$2.00E^{-18}$	*S. lycopersicum*
JZ476578	60S ribosomal protein L13a-1	$8.00E^{-09}$	*A. thaliana*
JZ476687	UBX domain-containing protein 6-like	$7.00E^{-32}$	*S. lycopersicum*
JZ476559	D1 protease precursor	$4.00E^{-18}$	*N. plumbaginifolia*
Pp01d15m_B11	Probable E3 ubiquitin-protein ligase HERC2-like	$1.00E^{-37}$	*S. lycopersicum*
Pp01d15m_D01	F-box protein FBW2 isoform 1	$2.55E^{-65}$	*Vitis vinifera*
Pp02d15m_A10	50S ribosomal protein L21, chloroplastic-like	$7.00E^{-20}$	*S. lycopersicum*
Transport			
JZ476574	Triose phosphate/phosphate translocator, chloroplastic	$3.00E^{-23}$	*S. tuberosum*
JZ476671	Putative xanthine/uracil permease	$5.00E^{-47}$	*Plantago major*
JZ476684	Copper transporter 6-like	$4.00E^{-07}$	*S. lycopersicum*
Biotic and abiotic stress			
JZ476583	Uncharacterized protein At3g27210-like	$3.00E^{-22}$	*S. lycopersicum*
JZ476582	MLP-like protein 34-like	$8.00E^{-14}$	*S. lycopersicum*
JZ476558	Heat shock protein **(HSP)**	$2.00E^{-42}$	*Ricinus communis*
JZ476566	Histone deacetylase 19-like isoform 2	$5.00E^{-43}$	*S. lycopersicum*
JZ476695	Plastid thioredoxin M precursor	$3.89E^{-30}$	*N. tabacum*
JZ476573	Ferredoxin-thioredoxin-reductase catalytic subunit B	$1.08E^{-21}$	*S. tuberosum*
Pp01d15m_C09	Chloroplast [Cu–Zn] superoxide dismutase **(SOD)**	$1.37E^{-87}$	*S. nigrum*
Pp01d15m_D02	Chloroplast Drought-induced Stress Protein **(CDSP)**	$1.31E^{-69}$	*S. tuberosum*
Pp01d15m_F12	Ankyrin repeat domain-containing protein 2-like	$1.00E^{-30}$	*S. lycopersicum*
Pp01d15m_C02	Cytochrome P450 NADPH-reductase	$8.00E^{-30}$	*Petunia x hybrida*

Table 3 continued

EST no. (accession no.)	Putative identity	*E* value	Source
Signal transduction			
Pp02d15m_F08	Protein thylakoid formation1, chloroplastic	$1.08E^{-12}$	*S. tuberosum*
Pp01d15m_C10	C2 domain-containing family protein	$2.00E^{-07}$	*Populus trichocarpa*
Other functions			
JZ476576	Protein TIC 62, chloroplastic-like	$5.00E^{-60}$	*S. lycopersicum*
JZ476580	Polyprotein, 3′-partial, putative	$3.15E^{-15}$	*S. demissum*
Unknown functions			
JZ476567	Hypothetical protein	$1.00E^{-22}$	*S. tuberosum*
JZ476570	Uncharacterized protein LOC101259555	$7.00E^{-17}$	*S. lycopersicum*
JZ476682	Uncharacterized protein LOC101264895	$4.00E^{-47}$	*S. lycopersicum*
JZ476689	Uncharacterized protein LOC101266602	$8.00E^{-36}$	*S. lycopersicum*
Pp02d15m_G03	Uncharacterized protein LOC101244590	$6.00E^{-40}$	*S. lycopersicum*
Pp01d15m_F03	Uncharacterized protein LOC101264041	$1.00E^{-100}$	*S. lycopersicum*
Pp02d15m_H08	Uncharacterized protein LOC101248178	$6.00E^{-15}$	*S. lycopersicum*
Down-regulated			
Metabolism			
JZ476625	Alpha-glucan phosphorylase, H isozyme	$3.93E^{-09}$	*S. tuberosum*
JZ476642	Xyloglucan endotransglucosylase-hydrolase XTH7	$2.21E^{-18}$	*S. lycopersicum*
JZ476727	Fructose-bisphosphate aldolase 1	$3.00E^{-61}$	*Theobroma cacao*
JZ476733	Triose phosphate isomerase	$2.08E^{-13}$	*Rosa gymnocarpa*
JZ476624	Non-symbiotic hemoglobin	$4.45E^{-49}$	*S. lycopersicum*
Pp01r15m_B03	Amino-acid acetyltransferase-like	$1.00E^{-57}$	*S. lycopersicum*
Secondary metabolism			
JZ476732	Homogentisate phytyltransferase 2, chloroplastic-like	$6.00E^{-07}$	*S. lycopersicum*
JZ476735	Caffeic acid 3-O-methyltransferase	$4.63E^{-32}$	*C. annuum*
JZ476748	Probable glutathione S-transferase parC-like isoform 2	$5.00E^{-09}$	*Vitis vinifera*
Pp01r15m_E12	Methylecgonone reductase-like	$5.00E^{-32}$	*Cicer arietinum*
Energy and photosynthesis			
JZ476726	UPF0603 protein At1g54780, chloroplastic-like	$7.00E^{-56}$	*S. lycopersicum*
JZ476635	Chloroplast ferredoxin-NADP+ oxidoreductase precursor	$5.99E^{-59}$	*C. annuum*
JZ476622	Chloroplast pigment-binding protein CP26	$5.00E^{-12}$	*C. annuum*
JZ476746	Chlorophyll a/b-binding protein PS II-Type I	$7.00E^{-07}$	*S. tuberosum*
JZ476632	Chloroplast thylakoid lumenal 17.4 kDa protein	$1.00E^{-10}$	*S. tuberosum*
JZ476637	Chlorophyll a/b binding protein 13, chloroplastic	$6.52E^{-39}$	*S. lycopersicum*
Pp02r15m_C05	ATP synthase subunit O, mitochondrial-like	$2.00E^{-62}$	*S. lycopersicum*
Protein synthesis and metabolism			
JZ476747	ATP-dependent zinc metalloprotease FtsH-like	$4.00E^{-68}$	*S. lycopersicum*
JZ476631	Peptidyl-prolyl cis–trans isomerase FKBP53-like	$2.00E^{-36}$	*S. lycopersicum*
JZ476636	Methionine sulfoxide reductase A4	$1.68E^{-73}$	*S. lycopersicum*
JZ476728	MAR-binding protein MFP1 homolog	$7.14E^{-49}$	*N. tabacum*
JZ476627	Proline synthase co-transcribed bacterial homolog protein-like	$1.00E^{-63}$	*S. lycopersicum*
JZ476734	Tripeptidyl-peptidase 2-like	$5.00E^{-37}$	*Fragaria vesca*
Pp02r15m_H04	30S ribosomal protein S6 alpha, chloroplastic-like	$6.00E^{-17}$	*S. lycopersicum*
Pp01r15m_H11	Plastid ribosomal protein l11	$1.63E^{-75}$	*A. thaliana*
Pp02r15m_G04	Metallothionein-like protein type 2	$1.54E^{-16}$	*S. lycopersicum*
Pp02r15m_G05	Peptidyl-prolyl cis–trans isomerase FKBP17-2, chloroplastic-like	$7.00E^{-16}$	*S. lycopersicum*

Table 3 continued

EST no. (accession no.)	Putative identity	*E* value	Source
Transport			
JZ476739	Protein SRG1-like	$9.00E^{-18}$	*S. lycopersicum*
JZ476621	Outer envelope pore protein 16-3, chloroplastic/mitochondrial-like	$3.00E^{-22}$	*S. lycopersicum*
Pp01r15m_B07	Lipid transfer protein	$8.00E^{-16}$	*N. tabacum*
Biotic and abiotic stress			
JZ476745	Aquaporin PIP-type pTOM75	$6.00E^{-07}$	*S. lycopersicum*
Signal transduction			
JZ476640	Putative ADP-ribolylation factor	$3.00E^{-16}$	*A. thaliana*
Other functions			
JZ476639	Rhodanese-like domain-containing protein 14, chloroplastic-like	$9.00E^{-32}$	*S. lycopersicum*
JZ476623	Tubulin beta-1 chain-like	$4.65E^{-41}$	*Brachypodium distachyon*
JZ476736	LisH domain and HEAT repeat-containing protein KIAA1468 homolog	$1.00E^{-67}$	*S. lycopersicum*
JZ476730	DNA binding protein, putative	$2.00E^{-73}$	*Ricinus communis*
JZ476742	Uncharacterized protein LOC101245846	$5.00E^{-28}$	*S. lycopersicum*
Pp02r15m_E05	Tubulin alpha-6 chain	$6.00E_{-67}$	*A. thaliana*
Pp01r15m_B04	Protein CbbY-like	$4.00E^{-136}$	*S. lycopersicum*
Pp02r15m_F10	DEAD-box ATP-dependent RNA helicase 35-like	$2.00E^{-38}$	*S. lycopersicum*
Pp02r15m_G10	Pistil-specific extensin-like protein	$2.00E^{-22}$	*S. nigrum*
Unknown functions			
JZ476633	Uncharacterized protein LOC101253079	$2.00E^{-34}$	*S. lycopersicum*
JZ476628	Uncharacterized protein LOC101259526	$2.00E^{-06}$	*S. lycopersicum*
JZ476626	Predicted protein	$4.74E^{-16}$	*Populus trichocarpa*
Pp01r15m_F04	Hypothetical protein	$6.00E^{-05}$	*Prunus persica*
Pp02r15m_A11	Uncharacterized protein LOC101257692	$1.00E^{-29}$	*S. lycopersicum*

[a] Genes in bold text and underlined were analyzed by qRT-PCR

was found to share similarities with a previous small microarray study performed in whitefly-infested tomato plants sampled at different stages of the insecťs nymphal development (Estrada-Hernández et al. 2009). This finding was in agreement with a study reporting that *Physalis* and tomato had similarity at both molecular marker and DNA sequence levels (Wei et al. 2012).

P. philadelphica plants were found to respond to whitefly infestation, as manifested by relatively abundant changes in gene expression (Tables 2, 3). Several genes associated with biotic and abiotic stress were found to be up-regulated; many of these, have been previously related to damage responses, not only caused by phloem-feeding whiteflies and aphids, but also by pathogens and insects belonging to other feeding guilds. Representative examples were genes coding trypsin proteinase inhibitors and proteolytic enzymes (Estrada-Hernández et al. 2009; Mondego et al. 2011; Dubey et al. 2013). The induction of plant proteases was expected, considering the central role they play in defense responses against pathogens and insects (van der Hoorn and Jones 2004; Khajuria et al. 2013). Similarly relevant was the induction of a serine hydroxymethyltransferase gene, coding for a photorespiratory enzyme whose activity is needed to restrict pathogen-induced cell death by mitigating oxidative damage at the chloroplast (Moreno et al. 2005). The modified expression levels of ribosomal and related genes is also a common phenomenon occurring during plant defense responses (Maleck et al. 2000; Schenk et al. 2000); whereas other genes, such as one coding for a C3HC4-type RING zinc finger ring/U-box superfamily protein, induced in *P. philadelphica* at 5 dpi, might function in specific protein degradation processes, similarly to an analogous pepper protein believed to act as an E3 ligase for polyubiquitination of target PR proteins (Hong et al. 2007). The finding of genes involved in thiamine metabolism was also in agreement with data revealed by the *P. peruviana* leaf transcriptome (Garzón-Martínez et al. 2012). The relevance of this metabolic pathway stems from thiaminés role in the induction of plant defense responses through SA and Ca^{2+}-related signaling pathways (Ahn et al. 2005; Goyer 2010), and from the recently reported, but not fully elucidated, aphid antibiosis and antixenosis effects produced by the thiamine treatment of barley and pea plants (Hamada

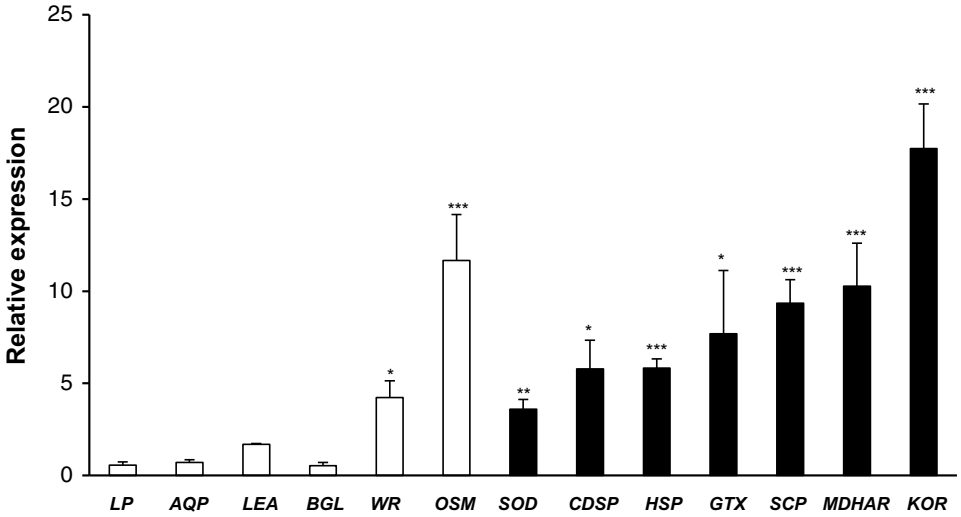

Fig. 1 Validation of SSH sequencing data by qPCR of selected up-regulated genes from *Physalis philadelphica* plants subjected to whitefly (*Trialeurodes vaporariorum*) infestation. The *bars* indicate relative mRNA abundance; expression values in non-infested plants were set to 1. All data were normalized to the actin expression levels. *Asterisks* indicate statistical significant induction (*$P \leq 0.05$; **$P \leq 0.001$; ***$P \leq 0.0001$). *White bars* represent genes isolated at 5 days post infestation (dpi), whereas *black bars* represent genes isolated at 15 dpi. Refer to Table 1 for description of the gene codes employed

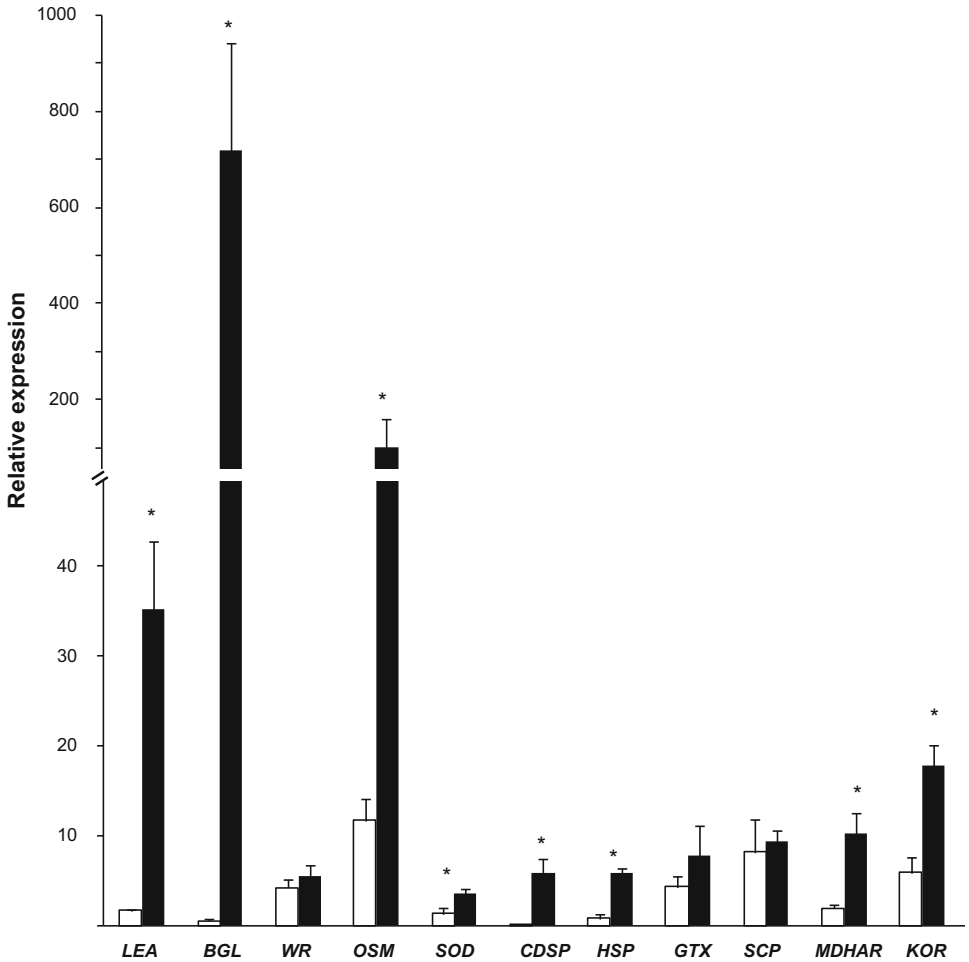

Fig. 2 Expression levels, determined by qPCR, of selected up-regulated genes from suppression subtractive hybridization cDNA libraries of *Physalis philadelphica* plants subjected to whitefly (*Trialeurodes vaporariorum*) infestation at 5 (*white bars*) and 15 (*black bars*) days post infestation. The *bars* indicate relative mRNA abundance; expression values in non-infested plants were set to 1. All data were normalized to the actin expression levels. Significant differences between infestation times are indicated by *asterisks* ($P \leq 0.01$). Refer to Table 1 for description of the gene codes employed

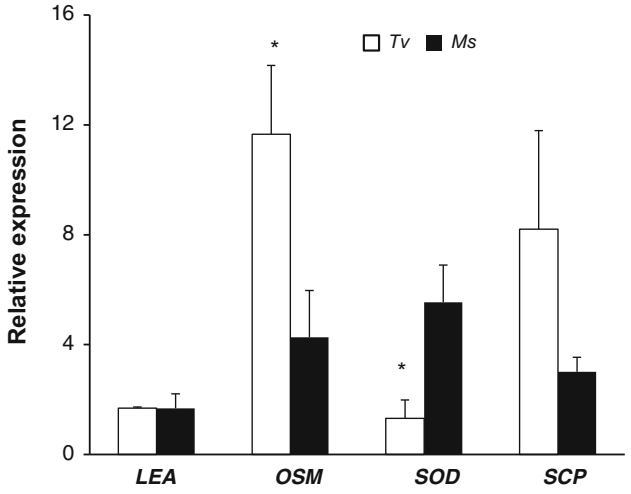

Fig. 3 Comparison of expression levels, determined by qPCR, of selected defense-related genes obtained from suppression subtractive hybridization libraries on *Physalis philadelphica* plants subjected to herbivory by phloem-feeding *Trialeurodes vaporariorum* (*Tv*) whiteflies (*white bars*; 5-day infestation) or *Manduca sexta* (*Ms*) chewing larvae (*black bars*; sampled 18 h after a 6-h feeding period). The *bars* indicate relative mRNA abundance; expression values in non-infested plants were set to 1. All data were normalized to the actin expression levels. Significant differences between expression levels induced by contrasting insect feeding guilds are indicated by *asterisks* ($P \leq 0.01$). Refer to Table 1 for description of the gene codes employed

and Jonsson 2013). Conversely, the strong negative influence that *Trialeurodes* herbivory had on photosynthesis-related gene expression, including genes involved in carbon fixation in *P. philadelphica*, was not surprising considering the widespread down-regulation of photosynthetic genes in response to whitefly infestation and other biotic stresses reported in several other plant species (Bilgin et al. 2010). Additionally, the altered expression levels of several genes considered to be involved in protection against oxidative stress, such as those coding for several thioredoxin superfamily proteins, including the drought-induced CDSP 32 protein, (Broin et al. 2000), two glutathione S-transferases, a metallothionein-like protein, a superoxide dismutase, and a 2-oxoglutarate-dependent dioxygenase (Singh et al. 2008), was expected when placed in the context of the general plant defense responses triggered by diverse insect herbivores (Zhu-Salzman et al. 2008).

The altered expression of endo-1, 4-beta-glucanase Korrigan and xyloglucan: xyloglucosyl transferase genes could have influenced the degree of cell wall reinforcement and, therefore, *P. philadelphica* resistance against whitefly infestation. Endo-1, 4-beta-glucanase Korrigan, required for synthesis of an ordered cellulose–hemicellulose network (Maloney et al. 2012), was induced in *P. philadelphica* at 15 dpi. At the same time, the xyloglucan: xyloglucosyl transferase gene, involved in other processes of cell wall biogenesis, was down-regulated. These findings, related to genes involved in structural carbohydrate modification or synthesis, coincide with the above description of a SSR-containing marker contig associated with callose deposition in *P. peruviana* (Simbaqueba et al. 2011). *P. philadelphica* plants also showed the up-regulation of various genes encoding lipases, lipid transfer proteins, and other down-stream lipid-catabolic enzymes (i.e., two proteins coding for GDSL-motif lipase/hydrolase family proteins). This was consistent with the observation that Hessian fly-resistant wheat plants have a faster mobilization and re-utilization of resources, particularly lipids (Khajuria et al. 2013). Moreover, recent studies in rice have determined that several of these proteins can be important in the plant's defense response against biotic stressors (Chepyshko et al. 2012). Known molecular determinants of whitefly and aphid resistance were also detected in the early stages of whitefly infestation. These included a basic endochitinase, and β-1, 3 glucanase; these genes were also detected by Puthoff et al. (2010) in tomato plants infested both by *Bemisia tabaci* and *T. vaporariorum*. A related study also found that *B. tabaci* herbivory in pepper plants led to the induction of PR protein genes (Yang et al. 2011). Other PR protein genes detected in whitefly-infested *P. philadelphica* plants were osmotin and a PR protein family found to be induced in barley in response to fungal infection (both expressed at 5 dpi) and a putative major latex protein (MLP; expressed at 15 dpi). MLPs have an unknown function; however, it was recently found that some MLPs are related to the Bet v1 protein superfamily that includes cytokinin-specific binding proteins and PR-10 proteins, the latter of which are expressed in response to pathogen attack and/or environmental stresses such as drought, wounding, ultraviolet radiation, and oxidative stress (Lytle et al. 2009). Interestingly, it was recently proposed that cytokinins may be involved in defense responses to aphids and whiteflies (Dubey et al. 2013). In addition, the induction of a xanthine/uracil permease family protein gene, observed at 15 dpi, lends support to the suggested role for cytokinins in the regulation of whitefly–plant interactions.

Additionally, our results further suggest a defensive role for abscisic acid (ABA) and brassinosteroids in *P. philadelphica* plants subjected to whitefly infestation. This is supported by the induction of two different F-box protein genes at early and late phases of the infestation. Thus, the expression of an F-Box family protein PP2 at 5 dpi, resembled the 24-epibrassinosteroid (EBR)-induced up-regulation, of the Cs594 protein in cucumber plants, identified as a phloem defense-related protein 2 that contains an F-box domain (Li et al. 2013 and references therein). The second F-box gene found to be induced at 15 dpi, encodes a protein recognized as a novel negative

regulator of Argonaute1 protein levels that may play a role in ABA signaling and/or response (Earley et al. 2010). The up-regulation of a gene coding for a late embryogenesis desiccation protectant protein Lea14 homolog, at 15 dpi, is also consistent with the proposed role of ABA in the *P. philadelphica*–whitefly interaction, considering that most genes encoding LEA proteins are known to have ABA response elements in their promoters (Hundertmark and Hincha 2008). A correspondence with the enhanced tolerance to biotic stress observed in transgenic tobacco plants overexpressing a novel maize group 3 *LEA* gene is also possible (Liu et al. 2013). This is further supported by the major role played by ABA in signaling and adaptation to abiotic stresses such as water, drought, and salt stress (Leung and Giraudat 1998), which coincides with the strong and stable induction of a salt-tolerance protein homolog in whitefly-infested tomato plants (Estrada-Hernández et al. 2009). In this respect, the recently found function of the GIGANTEA (GI) protein, a flowering time regulator, in the adaptation to salt stress in Arabidopsis (Kim et al. 2013) is in agreement with the induction of the *GI* gene detected at 5 dpi in whitefly-infested plants. Also to be considered is the known cross-talk existing between drought and salt stress and pathogen and/or wound defense pathways (Achuo et al. 2006; Dombrowski 2003). This correspondence might explain the induction, in whitefly-infested *P. philadelphica* plants, of a gene coding for a wound-responsive protein having a closer association with insect feeding guilds other than phloem-feeding whiteflies and aphids, which are known to induce wound-related genes through the JA pathway. A putative ankyrin-repeat protein, induced at 15 dpi, shares a resemblance with HBP1, an ankyrin repeat domain-containing family protein 2 identified in *Nicotiana tabacum* (Fridborg et al. 2003). The capacity of this protein to interact with β-1, 3-glucanase suggests that the ankyrin-repeat protein detected in *P. philadelphica* may be able to regulate callose deposition during whitefly infestation. Such regulation may be relevant for defense, considering that the deposition of callose plugs designed for aphid and whitefly feeding resistance was recently reported in Arabidopsis (Lü et al. 2011) and in cotton (Dubey et al. 2013). These considerations coincide once again with the SSR-containing marker contig associated with callose deposition described in *P. peruviana* (Simbaqueba et al. 2011).

Similar to what was observed in whitefly and aphid-infested cotton (Dubey et al. 2013), whitefly infestation in *P. philadelphica* might have shifted the sucrose and amino acid mobilization patterns by changing the expression profiles of genes related to amino acid and carbohydrate metabolism. These could be represented by the up-regulated expression, at 5 dpi, of genes coding for PEP carboxylase, NADP malic enzyme (ME), two phosphoglucomutases, glutamate dehydrogenase, and an amino acid transporter, followed by the subsequent induction, at 15 dpi, of a glutamate: glyoxylate aminotransferase that can also act as an alanine aminotransferase. In addition, NADP-ME has been found to serve as a pH-stat in a combination with phosphoenolpyruvate carboxylase and to function in plant defense responses induced by UV-B radiation, viral infection or by treatments with fungal elicitors, cellulase, or JA (Doubnerová et al. 2007). A fact to be also considered is that transgenic potato plants overexpressing a bacterial phosphoglucomutase had a dramatically reduced amino acid content that could negatively affect phloem feeders (Lytovchenko et al. 2005). Amino acid content could also depend on the incorporation of ammonium into glutamine by the concerted action of mitochondrial glutamate dehydrogenase and cytosolic glutamine synthetase under particular physiological conditions, including senescence, oxidative damage, stress hormone activation, and plant–pathogen interactions (Miflin and Habash 2002; Pageau et al. 2006). On the other hand, alanine aminotransferase and transmembrane amino acid transporter transcripts were among those found to accumulate in wheat plants under a compatible interaction with virulent Hessian fly larvae, characterized by the insecťs manipulation of the free amino acid content (Saltzmann et al. 2008).

Defense-associated genes, such as an ADP-ribosylating factor (*ARF*), previously reported as part of the molecular interactions occurring between aphids and resistant and susceptible apple tree cultivars, were also identified in *P. philadelphica*. *ARFs* mediate retrograde vesicle trafficking from the Golgi to the endoplasmic reticulum (Kirchhausen 2000), and are components of various plant defense signaling pathways involved in the induction of expression of a subset of PR protein genes or other biological functions associated with defense (Qubbaj et al. 2005; Coemans et al. 2008). Part of the above results additionally supports the notion that a conserved molecular strategy for confronting whitefly, aphid, and nematode challenge appears to be present in both tomato and *P. philadelphica* plants. The induction of a phosphoribosylformylglycinamidine synthase gene, whose role in defense is unknown, but that has been detected in response to nematode feeding in soybean (Vaghchhipawala et al. 2004), and to *Pythium* infection in ginger plants (Kavitha and Thomas 2008), is in support of this possibility. In addition, a receptor-like protein gene, with structural motifs similar to those present in the Mi-1.2 receptor, was detected in whitefly-infested *P. philadelphica* plants at 5 dpi. This finding was in agreement with one of the above reports describing the use of the *P. peruviana* leaf transcriptome for the detection of defense-related genes that could be employed for the control of the highly damaging *Fusarium v*ascular wilt (Enciso-Rodríguez et al.

2013). The several plant immunity genes reported in *P. peruviana* shared the typical architecture of plant resistance (*R*) gene families, including the presence of receptor-like kinase (*RLK*), *TIR-NBS-LRR* and *CC-NBS-LRR* motifs. The richness of *R* genes present in *P. peruviana* and, most probably in *P. philadelphica* too, suggests that several *R* genes and their homologues, in addition to the *Mi-1.2* gene found in *P. philadelphica*, could be associated with plant resistance against whiteflies, similar to what has been reported for aphids (Smith and Boyko 2007). Finally, the down-regulated expression of protein kinase genes, the usual key regulators of plant resistance responses, observed at 5 dpi, suggests the possibility that whiteflies could possess the ability to suppress plant resistance to facilitate infestation. Likewise, the up-regulation of a calcium: sodium antiporter may have been an additional strategy to reduce Ca^{2+} levels in the phloem sap in order to prevent Ca^{2+}-dependent plugging of the sieve tubes. It could also be argued that the down-regulation of a calcium-binding EF hand family protein may have represented a counter-attacking effort on the plant's side, designed to increase the Ca^{2+} levels in the phloem. It must be admitted, however, that these arguments are speculative and require additional extensive experimentation to be validated.

The results of an additional experiment performed to compare the patterns of expression of four defense-related genes produced by phloem-feeding whiteflies and leaf-chewing larvae (Fig. 3) were not in agreement with the results expected based on the published data. The latter indicate that *OSM* and *SCP* genes are usually expressed in conditions associated with mechanical wounding, exogenously applied ET and JA, or leaf-chewing herbivory (Xu et al. 1994; Ryan 2000; Kessler and Baldwin 2002); therefore, high expression levels of the *OSM* and *SCP* genes in *T. vaporariorum*-infested plants were not expected. Nevertheless, increased transcript levels of an *SCP* gene in rice (i.e. *OsBISCPL1*) have been reported after BTH or SA treatments (Liu et al. 2008). Moreover, the overexpression of *OsBISCPL1* showed enhanced disease resistance against *P. syringae* pv. *tomato*, a biotrophic pathogen which is controlled through SA-dependent signaling pathways. Likewise, the overexpression of a pepper osmotin-like protein 1 (CaOSM1) in Arabidopsis suppressed the proliferation of *P. syringae* pv. *tomato* due to enhanced cell death and H_2O_2 accumulation (Choi et al. 2013). Considering the above, it is valid to propose that higher expression of these two genes in whitefly-infested *Physalis* plants could contribute to their defense responses against phloem-feeding herbivores, regulated through the SA signaling pathway. Perhaps, the different time points used to sample the leaf tissue from *T. vaporariorum* and *M. sexta* herbivory-damaged plants did not allow realistic comparisons to be made, and further experimentation will be needed to clarify these results.

In conclusion, the transcriptomic information reported herewith broadens our understanding of the induced defense responses, operating against whitefly infestation in *P. philadelphica* and related species. These plants have gradually acquired agricultural importance due to their widespread cultivation and consumption. The information provided may also help establish more efficient strategies for whitefly control and other similar damaging insect pests in husk tomato.

Author contribution statement MQC, CGM and VCR carried out the experimental work. LMM, RRR and EIL contributed to data analysis. JDF and CSH wrote the manuscript. CSH conceived and designed the experiments.

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