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Basal differences in the transcriptional profiles of tomato leaves associated with the presence/absence of the resistance gene *Mi-1* and changes in these differences after infestation by the whitefly *Bemisia tabaci*

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Research Paper

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Abstract

The tomato *Mi-1* gene mediates plant resistance to whitefly *Bemisia tabaci*, nematodes, and aphids. Other genes are also required for this resistance, and a model of interaction between the proteins encoded by these genes was proposed. Microarray analyses were used previously to identify genes involved in plant resistance to pests or pathogens, but scarcely in resistance to insects. In the present work, the GeneChip™ Tomato Genome Array (Affymetrix®) was used to compare the transcriptional profiles of Motelle (bearing *Mi-1*) and Moneymaker (lacking *Mi-1*) cultivars, both before and after *B. tabaci* infestation. Ten transcripts were expressed at least twofold in uninfested Motelle than in Moneymaker, while other eight were expressed half or less. After whitefly infestation, differences between cultivars increased to 14 transcripts expressed more in Motelle than in Moneymaker and 14 transcripts less expressed. Half of these transcripts showed no differential expression before infestation. These results show the baseline differences in the tomato transcriptomic profile associated with the presence or absence of the *Mi-1* gene and provide us with valuable information on candidate genes to intervene in either compatible or incompatible tomato–whitefly interactions.

Introduction

Whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a major pest of both greenhouse and open-field horticultural crops worldwide. Severe damages by *B. tabaci* are caused directly through phloem feeding and indirectly by the transmission of a number of different plant viruses to a wide range of plants in tropical, subtropical, and Mediterranean climate conditions. Among horticultural crops affected by *B. tabaci*, tomato (*Solanum lycopersicum*) is the most common host and the second most important vegetable crop next to potato (FAOSTAT, 2001). Besides its agricultural interest, tomato has several advantages as a model plant, such as small genome (950 Mb), a short generation time, availability of transformation protocols and genetic and genomic resources (Pascual *et al.*, 2009), leading to the complete sequencing of tomato genome (Mueller *et al.*, 2005; Tomato Genome Consortium, 2012). Several tomato varieties are resistant to both B and Q biotypes of *B. tabaci* (Nombela *et al.*, 2000, 2001) currently renamed as Middle East-Asia Minor 1 and Mediterranean species, respectively (De Barro *et al.*, 2011). The resistant response to *B. tabaci* is mediated by the major resistance gene (R gene) *Mi-1* (Nombela *et al.*, 2003), introduced into a cultivated tomato from its wild relative, *S. peruvianum* (Smith, 1944). *Mi-1* also confers resistance against other phloem feeders, such as three species of root-knot nematodes (RKN) *Meloidogyne* spp. (Roberts and Thomason, 1986), the potato aphid *Macrosiphum euphorbiae* (Rossi *et al.*, 1998) and the tomato psyllid *Bactericerca cockerelli* (Casteel *et al.*, 2006). *Mi-1* was localized in a 52-Kb region of the short arm of chromosome 6 of tomato and subsequently cloned (Kaloshian *et al.*, 1998; Milligan *et al.*, 1998). This gene codifies for a CC-NB-LRR protein with 1257 amino acids, similar to other R proteins (Milligan *et al.*, 1998; Williamson, 1998; Martin *et al.*, 2003) and it was the first cloned R gene conferring plant resistance to an insect pest. The *Mi-1* gene is constitutively expressed very early in development in every tissue of resistant tomato (Martinez de Ilarduya and Kaloshian, 2001), but the Mi-1 protein is stored in an inactive conformation in the absence of an attacker organism (Hwang and Williamson, 2003). Upon detection of effector molecules from a nematode or an insect, Mi-1 protein experiences a conformational change and activates different signals leading to the resistance response (Williamson and Roberts, 2009). It is well

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known that, apart from an R gene, the presence and function of additional genes in certain signal transduction pathways leading to defense against the attacker organism are necessary for an effective pest resistance (Williamson and Roberts, 2009). In addition to *Mi-1*, other genes have been identified in tomato that are required for the *Mi-1*-mediated resistance, such as *Rme1* against aphids, nematodes, and whiteflies (Martínez de Ilarduya *et al.*, 2001, 2003, 2004) and *Hsp90* and *Sgt1* against nematodes and aphids (Bhattarai *et al.*, 2007). A model of interaction has been proposed between the proteins encoded by these genes with *Mi-1* forming an R-signaling complex with HSP90 and SGT1, and this complex guards RME1 (Bhattarai *et al.*, 2007). Further research on molecular aspects of plant resistance is essential to identify new components of *Mi-1*-mediated resistance, particularly on the mechanisms regulating those processes related to resistance to insects and the genes that control and modulate the resistant response.

Global analysis of gene expression has been widely done by means of high-performance technologies such as microarrays, allowing the detection of changes in the expression of thousands of genes simultaneously (Berrar *et al.*, 2003). The development of this technology for the analysis of expression profiles, along with the availability of databases of genomic sequence and expressed sequence tag from many plants, has allowed the study of transcriptional reprogramming in many different physiological situations (Aharoni and Vorst, 2001; Rensink and Buell, 2005). This included changes in response to the infection with bacterial pathogens (Tao *et al.*, 2003; Balaji *et al.*, 2008), phytopathogenic nematodes (Puthoff *et al.*, 2003; Alkharouf *et al.*, 2006; Barcala *et al.*, 2010; Uehara *et al.*, 2010; Portillo *et al.*, 2013), or insect feeding (Korth, 2003; Thompson and Goggin, 2006). A number of previous studies have used microarray analysis to identify changes in the plant transcriptomic profiles in response to RKN feeding during compatible and/or incompatible interactions with Arabidopsis (Hammes *et al.*, 2005; Jammes *et al.*, 2005; Barcala *et al.*, 2010; Portillo *et al.*, 2013), soybean (Ibrahim *et al.*, 2011), or tomato (Bar-Or *et al.*, 2005; Bhattarai *et al.*, 2008). More specifically, resistance to RKN mediated by the *Mi-1* gene was studied in tomato roots by means of cDNA microarrays (Schaff *et al.*, 2007; Bhattarai *et al.*, 2008, 2010).

Signaling pathways involved in plant–aphid susceptible interactions have been more frequently studied by comparative transcriptome analysis (de Vos *et al.*, 2007; Kuśnierczyk *et al.*, 2007; Li *et al.*, 2008). These studies suggest, broadly speaking, that aphid feeding causes activation of responses different to those caused by chewing herbivores, with changes in the expression of enzymes involved in the synthesis of secondary metabolites, as demonstrated in rice (Zhang *et al.*, 2004; Cho *et al.*, 2005). Additionally, responses induced by aphids in *Arabidopsis*, *Nicotiana attenuata*, certain gramineae, and tomato were different to changes produced by chewing insects, but similar to those triggered by bacterial and fungal pathogens (Kaloshian and Walling, 2005; Thompson and Goggin, 2006). The fact that whiteflies have the same type of piercing–sucking mouthparts like that of aphids initially led to the assumption that changes provoked by aphids should be the same or very similar to those following whitefly feeding. However, a study of Affymetrix microarrays during whitefly feeding on Arabidopsis showed qualitative and quantitative differences with respect to the results obtained with aphids, not only chewing herbivores (Kempema *et al.*, 2007).

Despite all the aforementioned background, studies have been scarce using microarrays to analyze in the leaf tissues the mechanisms that regulate processes related to plant resistance to insect pests. Previous research on wheat resistance to aphids demonstrated a general activation of the oxidative stress pathway, similar to the resistant responses mediated by pathogen-induced R genes (Boyko *et al.*, 2006). Another relevant study used microarrays to compare susceptible and partially resistant lines of barley in response to aphids (Delp *et al.*, 2009). A similar methodology was used to analyze changes of expression in tomato induced by whitefly feeding throughout insect development, but only on susceptible plants (Estrada-Hernández *et al.*, 2009). However, insufficient use had been made so far of microarray technology to study *Mi-1*-mediated resistance to whiteflies in tomato, or to identify new components of this resistance. So, more than 200 genes differentially expressed in different plant organs were obtained by cDNA arrays in cherry tomato at 25 days of infestation with *B. tabaci* but, again, only on susceptible plants (McKenzie *et al.*, 2005).

In the present work, the GeneChip™ Tomato Genome Array (Affymetrix®), with over 9200 transcripts, was used for the first time in an unbiased study to detect basal differences in the global gene expression of tomato associated with the presence/absence of the R gene *Mi-1*. With this goal, uninfested leaf tissues of adult tomato plants of a susceptible cultivar (Moneymaker) and a *Mi-1*-bearing (resistant) cultivar (Motelle) were analyzed and their transcriptional profiles were compared. In a later phase of this study, plants of the same resistant and susceptible cultivars were again compared by microarrays 2 days after being infested with *B. tabaci* adults, to investigate how whitefly infestation modifies the basal differences previously detected in the comparison of the uninfested Motelle and Moneymaker.

Materials and methods

Insects, plant material, and growth conditions

Adult females of the Mediterranean *B. tabaci* were used for plant infestation. A population of these whiteflies, originally collected from cropped tomato, was reared for several generations in our laboratory, free from any plant pathogen, on the susceptible tomato cv. Marmande.

Six uninfested plants of each tomato cultivar Motelle (*Mi-1*/*Mi-1*) and Moneymaker (*mi-1*/*mi-1*) were compared by microarrays. These cultivars are near-isogenic lines (Laterrot, 1987) differing only in the presence of a 650-kb introgressed region from *Lycopersicon peruvianum* (currently *Solanum peruvianum*) containing the *Mi-1* gene, in chromosome 6 of Motelle (Ho *et al.*, 1992).

Tomato seeds were germinated and the plants were raised inside a growth chamber at a constant temperature of 25°C, L16:D8 h photoperiod and 70% r.h. Plants were grown in 1-liter plastic pots filled with autoclaved vermiculite (number 3, Projar, Spain), irrigated every 15 days with a nutritive complex 20-20-20 (Nutrichem 60; Miller Chemical, Hanover, PA, USA) at a concentration of 3 g l^{-1}, and with water when needed in the meantime.

All plants were 8-week-old, with 8–9 true leaves each, at the time of analysis.

Whitefly infestations

Simultaneously to the analysis of the uninfested plants, six Motelle and six Moneymaker whitefly-infested plants of the

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Figure 1. Tomato plant with three modified Falcon tubes used for whitefly infestation.

same age were compared. For plant infestation, 50 ml Falcon tubes were modified from the clip-cage system for whiteflies (Muñiz and Nombela, 2001). Each tube was cut transversally to remove the conical bottom and a very thin polypropylene tissue (anti-thrips mesh) was attached by paraffin wax to the end of the tube. In addition, a lateral hole was drilled in the tube to introduce the insects later. The selected leaflet was inserted through the other end of the tube. Three modified Falcon tubes were used per plant, and each tube was placed in a well-developed leaflet of a leaf located in the middle-high zone of the plant (fig. 1).

Thirty adult females of *B. tabaci* were selected from the whitefly breading population and deposited into each tube through the lateral hole which was closed by a sponge plug. To maintain the same conditions, empty tubes were placed in the non-infested plants. After 2 days, tubes and whiteflies were carefully removed from all plants.

Sample collection

The samples were collected immediately after removing the whiteflies. From each tomato cultivar (Motelle or Moneymaker) and treatment (infested or non-infested), three biological replicates were collected, each consisting of six leaflets, one from each plant. The collected samples were immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction.

Microarray hybridization and analysis

Gene expression of tomato leaves was performed using the Affymetrix GeneChip™ Tomato Genome Array, which contains over 10,000 probe sets to interrogate over 9200 tomato transcripts (http://www.affymetrix.com/products_services/arrays/specific/tomato.affx). Total RNA was isolated from leaves of plants from three independent biological replicates using Trizol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and further purified with RNeasy mini kit ('clean-up' protocol, Qiagen, Hilden, Germany), following the manufacturers' recommendations, and assessed in a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). cDNA was synthesized from 4 µg of total RNA using one-cycle target labeling and control reagents (Affymetrix®, Santa Clara, CA, USA), to produce biotin-labeled cRNA. The cRNA preparation (15 µg) was fragmented at 94°C for 35 min into segments 35–200 bases in length. Labeled cRNAs were hybridized to Affymetrix® arrays in a hybridization solution containing 100 mM 2-(N-morpholino) ethanesulfonic acid, 1 M Na+, and 20 mM EDTA in the presence of 0.01% Tween 20 to a final cRNA concentration of 0.05 µg ml^{-1} for 16 h at 45°C. Each microarray was washed and stained with streptavidin-phycoerythrin in a Fluidics station 450 (Affymetrix®) and scanned at 1.56 µm resolution in a GeneChip Scanner 3000 7 G system (Affymetrix®).

Bioinformatic and statistical data analyses

The GeneChip intensities were background-corrected, normalized, and summarized by the robust multiarray average (RMA) method (Irizarry *et al.*, 2003) using the affy package from Bioconductor (https://www.bioconductor.org/). Differentially expressed transcripts were determined using the moderated *t* test as implemented in the limma package from Bioconductor (Smyth, 2005). Raw *P* values were adjusted for multiple hypotheses testing using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995). Genes with a fold-change in expression ≥ 2 or ≤ -2 and FDR < 0.05 were considered as differentially expressed.

The VENNY program version 2.1 (Oliveros, 2007) was used to compare the lists of previously selected genes and to identify the genes shared in the different gene lists.

Descriptions of the genes and target sequences corresponding to GeneChip probesets were obtained from Affymetrix, Tomato Annotations Release 36 (NetAffx Analysis Center). Target sequences were also used in BLAST searches of their corresponding tomato genes (version SL3.0 and Annotation ITAG3.10) in Sol Genomics database (Fernandez-Pozo *et al.*, 2015).

Validation of microarray data by real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

For qRT-PCR validation, total RNA was extracted as previously detailed and 1 µg was retrotranscribed with the High Capacity Reverse transcription Kit (Thermo Fisher Scientific) using random primers, and then amplified with the primers listed (table 4) using a Hot FIREPol EvaGreen Plus-based system. The relative quantity ($2 - \Delta\Delta Ct$) of each mRNA was calculated after normalization to the housekeeping gene *Ubi3*.

To analyze the correlation between the data obtained by microarray and qRT-PCR, the Pearson correlation coefficient (r) was calculated using the GraphPad Prism program (version 4.00 for Windows, GraphPad Software, San Diego, California, USA, www.graphpad.com). Data obtained by qRT-PCR were transformed to a logarithmic scale since the microarray data were expressed in log2 scale. The r values oscillate between 1 (total positive correlation between both variables) and −1 (total negative correlation). The program also calculates the coefficient of determination (r^2), which establishes a proportion of variability shared or explained for both variables, and the *P*-value to establish whether the correlation between both variables is statistically significant.

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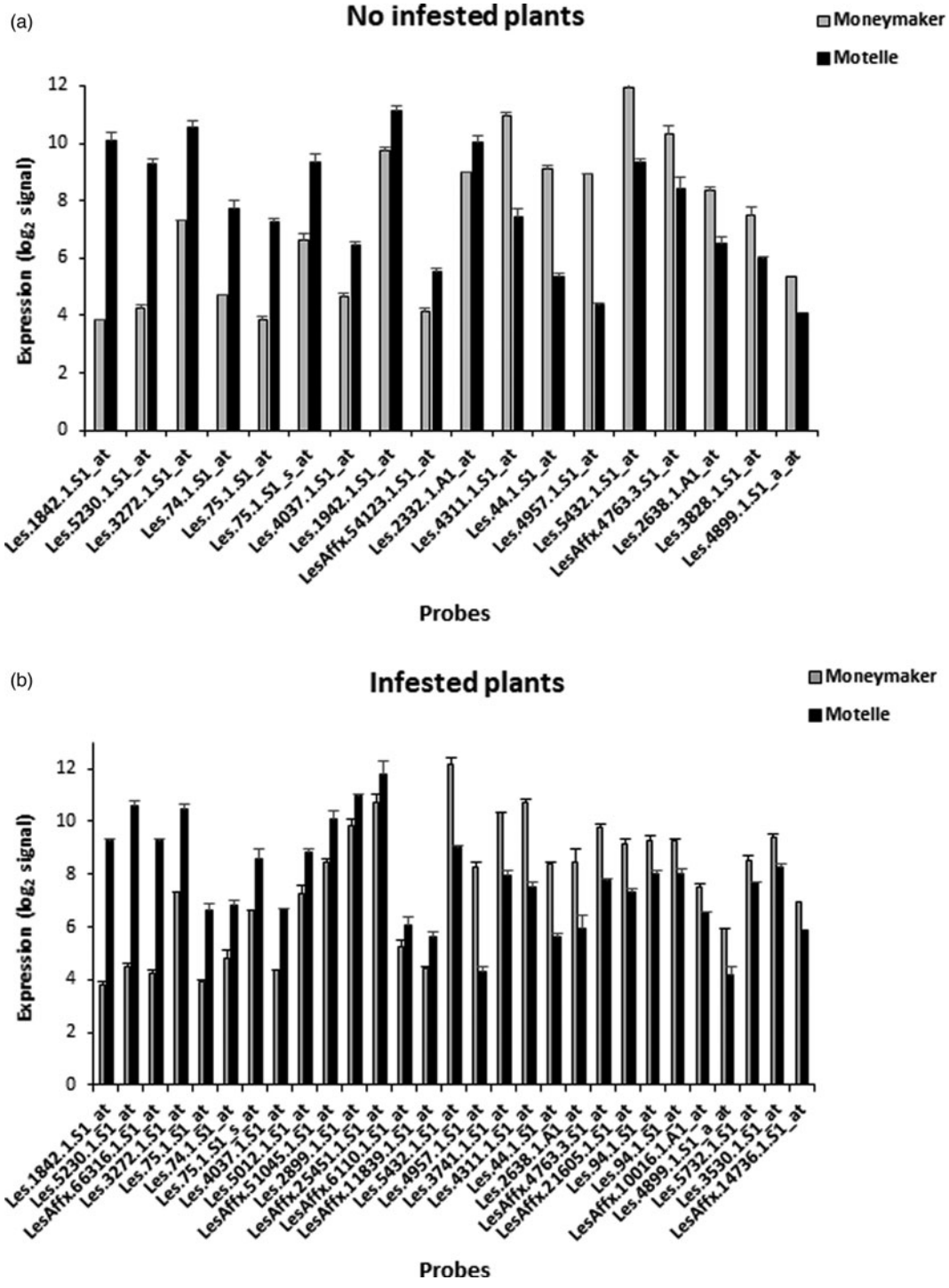

Figure 2. Gene expression levels of the differentially regulated transcripts in each tomato cultivar and treatment. (a) Plants in the absence of infestation. (b) Plants infested by *B. tabaci*. Each bar (gray color for Moneymaker; black color for Motelle) corresponds to the mean signal of three replicates (Log_2 Mean ± SE).

Results

Basal differences between uninfested Moneymaker and Motelle

When comparing Moneymaker and Motelle cultivars in the absence of any infestation, 18 differentially expressed transcripts were obtained (fig. 2a). Of them, ten transcripts were significantly more expressed (up-regulated) in Motelle than in Moneymaker, whereas eight transcripts were less expressed (down-regulated) in Motelle relative to Moneymaker (table 1). Approximately half of these 18 differentially expressed transcripts were expressed more than fivefold in one cultivar than in the other.

Among the ten transcripts more expressed in Motelle than in Moneymker, a transcript stands out (FC = 80.19), with a sequence similar to the *NTGP4* gene of *Nicotiana tabacum*, which

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Table 1. Transcripts up-regulated at least double (fold-change ≥ 2) or down-regulated at least half (fold-change ≤ −2) in leaves of the tomato cv. Motelle compared to cv. Moneymaker, in the absence of infestation and considering only significant values (FDR < 0.05)

ID Affymetrix[a]	GenBank[b]	Description[c]	Locus[d]	ITAG description[e]	Fold-change[f]	FDR[g]
Les.1842.1.S1_at	BT012811	NTGP4 (*Nicotiana tabacum*); protein AIG1-like	Solyc11g028010	GTPase IMAP family member 7; contains Interpro domain IPR006703 AIG1	80.19	1.11×10^{-08}
Les.5230.1.S1_at	BT013535	Elongation factor 1-γ 2-like	Solyc06g011280	Elongation factor 1- γ; contains Interpro domain IPR001662 Translation elongation factor EF1B, γ chain, conserved	28.42	1.01×10^{-08}
Les.3272.1.S1_at	BT012750	Diaminopimelate epimerase, chloroplastic	Solyc09g005700	Diaminopimelate epimerase family protein; contains Interpro domain IPR001653 Diaminopimelate epimerase	11.30	9.57×10^{-08}
Les.74.1.S1_at	AF039682	Root-knot nematode resistance protein(Mi-1.2)	Solyc05g008690	Disease resistance protein RPP13 variant; contains Interpro domain IPR002182 NB-ARC	7.84	5.92×10^{-08}
Les.75.1.S1_at	AF039681	Plant resistance protein /// root-knot nematode resistance protein Mi-1.1 /// Mi-1.2	Solyc05g008690	Disease resistance protein RPP13 variant; contains Interpro domain IPR002182 NB-ARC	6.91	1.01×10^{-08}
Les.75.1.S1_s_at	AF039681	Plant resistance protein /// root-knot nematode resistance protein Mi-1.1 /// Mi-1.2	Solyc05g008690	Disease resistance protein RPP13 variant; contains Interpro domain IPR002182 NB-ARC	6.79	6.49×10^{-08}
Les.4037.1.S1_at	AY178911	Vacuolar H + -ATPase A2 subunit isoform	Solyc06g063330	V-type ATP synthase alpha chain; contains Interpro domain IPR005725 ATPase, V1 complex, subunit A	4.42	2.02×10^{-06}
Les.1942.1.S1_at	AW033120	Mitochondrial outer membrane protein porin of 36 kDa	Solyc01g010760	Porin/voltage-dependent anion-selective channel protein; contains Interpro domain IPR001925 Porin, eukaryotic type	2.50	6.17×10^{-06}
LesAffx.54123.1.S1_at	BE450055	Putative glucuronosyltransferase PGSIP8	Solyc06g060710	Glycogenin 2; contains Interpro domain IPR002495 Glycosyl transferase, family 8	2.08	1.45×10^{-04}
Les.2332.1.A1_at	BG734892	Putative F-box protein	Solyc09g005480	F-box family protein; contains Interpro domain IPR001810 Cyclin-like F-box	2.04	3.01×10^{-02}
Les.4311.1.S1_at	AY269087	DELLA protein GAI (Gibberellic acid-insensitive mutant protein)	Solyc11g011260	GAI	−9.62	4.10×10^{-07}
Les.44.1.S1_at	BF097567	Phytoene synthetase Psy1	Solyc02g079250	3′(2′) 5′-bisphosphate nucleotidase-like protein; contains Interpro domain IPR000760 Inositol monophosphatase	−8.77	1.45×10^{-08}
Les.4957.1.S1_at	BT012983	Adenosine kinase 2-like	Solyc09g007940	Adenosine kinase; contains Interpro domain IPR001805 Adenosine kinase	−8.64	2.34×10^{-02}
Les.5432.1.S1_at	BT013906	selT-like protein	Solyc09g005590	SelT-like protein; contains Interpro domain IPR019389 Selenoprotein T	−5.65	2.89×10^{-09}
LesAffx.4763.3.S1_at	BG123322	Protein MADS AFFECTING FLOWERING 5-like	Solyc12g087830	MADS box transcription factor; contains Interpro domain IPR002100 Transcription factor, MADS-box	−3.15	2.15×10^{-02}
Les.2638.1.A1_at	BG628467	Short-chain dehydrogenase/ reductase 2b	Solyc11g071460	Dehydrogenase/reductase SDR family member 13; contains Interpro domain IPR002347 Glucose/ribitol dehydrogenase	−2.60	2.03×10^{-02}
Les.3828.1.S1_at	X92855	8-hydroxygeraniol dehydrogenase-like	Solyc11g011330	Cinnamyl alcohol dehydrogenase; contains Interpro domain IPR002085 Alcohol dehydro-genase superfamily, zinc-containing	−2.39	3.52×10^{-03}
Les.4899.1.S1_a_at	BT012863	Glutaredoxin, grx, putative (*Ricinus communis*)	Solyc06g008750	Glutaredoxin; contains Interpro domain IPR011905 Glutaredoxin-like, plant II	−2.13	5.14×10^{-03}

[a]Transcript Identifier in the Affymetrix Genechip™.
[b]GenBank (NCBI) Transcript Identifier, provided by Affymetrix (Release 36, January 2017).
[c]Functional description of transcript, provided by Affymetrix (Release 36, January 2017).
[d]Tomato locus, Genome version SL3.0 and Annotation ITAG3.20
[e]Description of the tomato locus (Annotation ITAG3.20)
[f]Relative Expression in Motelle compared to Moneymaker.
[g]FDR value (corrected *P*-value) of the Relative Expression.

participates in different processes of response to biotic stimuli. The next transcript, with 28.42-fold expression greater in Motelle than in Moneymaker, corresponds to a gene with participation in translation elongation. The Les.3272.1.S1_at transcript (FC = 11.30) is similar to an *Arabidopsis thaliana* gene involved in the processes of amino acid biosynthesis. It is also worth noting the overexpression of the gene encoding the H + -ATPase vacuolar subunit that is involved in transport and binding to ATP (FC = 4.42) and the *VDAC* gene related to response to biotic stimuli (FC = 2.50). The final two up-regulated transcripts related to the glycogen glucosyltransferase (FC = 2.08) and to the F-box family protein (FC = 2.04). It is important to mention that three probesets corresponding to the homologous genes *Mi-1.1* and *Mi-1.2* were detected in our analysis, with expression values approximately sevenfold higher in Motelle than in Moneymaker, as expected for the presence of the *Mi-1* locus in Motelle.

Approximately half of the eight transcripts down-regulated in Motelle relative to Moneymaker had highly significant FDR ($P < 0.0001$). The most repressed one (FC = −9.62) encodes the DELLA GAI protein, a negative regulator of gibberellin (GA) signaling. This was followed by the *Pys* gene (FC = −8.77) involved in secondary metabolism, the *ADK* gene (FC = −8.64) which catalyzes AMP synthesis from adenosine and ATP, as well as other gene encoding a selT-like protein (FC = −5.65). Also observed was down-regulation of the gene *MADS-box 15* (FC = −3.15), a transcription factor involved in different plant development processes. The three last genes with lower expression in Motelle than Moneymaker codified a short-chain dehydrogenase/reductase (FC = −2.60), the CAD enzyme (FC = −2.39), key in the synthesis of lignin, and a Glutaredoxin (FC = −2.13) whose function is to protect cells against oxidative stress, thus maintaining cellular homeostasis.

Differences between Moneymaker and Motelle after B. tabaci *infestation*

The analysis of the transcriptomic profiles after 2 days of infestation revealed 28 transcripts with differential expression between Motelle and Moneymaker cultivars. Of them, 14 transcripts were expressed significantly more in Motelle than in Moneymaker, and 14 transcripts were expressed less in Motelle when compared to Moneymaker (fig. 2b). The expression range in up-regulated transcripts was 2–38 times greater in Motelle than in Moneymaker, while the down-regulated transcripts ranged from two to nine times lower in Motelle (table 2).

When these results were compared with those previously obtained from uninfested plants, it was observed that whitely infestation substantially modified the basal differences among Motelle and Moneymaker cultivars (fig. 3). Out of the 18 transcripts differentially expressed in the uninfested plants, 14 were also up-regulated or down-regulated in Motelle regarding Moneymaker after whitefly infestation. Moreover, 14 additional transcripts showed differential expression between cultivars only after infestation with *B. tabaci*.

All differential transcripts common to both analyses (infested and non-infested plants) are listed in table 3 with their corresponding relative expression values before and after whitefly infestation. For most of these transcripts, expression differences between Motelle and Moneymaker were moderately or markedly reduced after infestation. Only in four cases did the differences increase or remain similar to those in uninfested plants.

Seven additional transcripts were up-regulated in infested Motelle with respect to infested Moneymaker (table 2), which had not been previously highlighted in the comparison between cultivars in the absence of infestation. One of them was expressed 12.16-fold higher in Motelle than in Moneymaker, corresponding to a WD-40 repeat family protein. Among the other transcripts, Les.5012.1.S1_at (FC = 3.57) corresponds to the acid phosphatase 1 enzyme which participates in defense response processes, specifically against insects. Moreover, two transcripts correspond to the isoflavone reductase (FC = 3.39) and the methionine sulfoxide reductase (MsrA) (FC = 2.89), which both participate in processes of response to oxidative stress. The sulfate adenyltransferase enzyme (FC = 2.53) participates in sulfur assimilation processes. The transcript LesAffx.6110.1.S1_at (FC = 2.11) corresponds to a pectinesterase, involved in cell wall reorganization processes in response to pathogen attack. Finally, the enzyme NADH dehydrogenase (FC = 2.00) which is the first enzyme in Complex I of the electron transport chain in mitochondria, is correlated with programmed cell death.

Among the seven transcripts down-regulated in Motelle exclusively after whitefly infestation, we can highlight the ELI3 protein related to defense processes (FC = −5.79), a HMG type nucleosome/chromatin assembly factor (FC = −4.03), a signal transduction response regulator (FC = −2.54), and the E3 ubiquitin protein ligase (FC = −2.13). Moreover, the enzymes cytosine-5 DNA methyltransferase (FC = −2.57), aldehyde oxidase (FC = −2.08), and UDP-glucuronate decarboxylase (FC = −2.05), involved in the regulation of gene expression during development, hormone biosynthesis, and membrane-associated metabolic processes, respectively.

Validation of microarray data by qRT-PCR

The relative expression values of the 12 transcripts analyzed by qRT-PCR are shown in table 4. A positive correlation between these data with those previously obtained by microarray analysis was obtained (fig. 4), with a value of the Pearson correlation coefficient (r) of 0.7475, statistically significant ($P < 0.0001$), therefore validating the results obtained by microarray analysis.

Discussion

Considering the literature reviewed so far, this is the first time that transcriptional profiles of non-infested foliar tissues have been compared by microarray from fully developed tomato plants belonging to different genotypes that are differentiated by the presence/absence of the *Mi-1* gene. Motelle and Moneymaker are quasi-isogenic cultivars as they differ only in a 650-kb fragment of chromosome 6 in which *Mi-1* is included (Messeguer *et al.*, 1991; Ho *et al.*, 1992). However, the presence of *Mi-1* also appears to be associated with baseline differences in the expression of other genes not necessarily localized near it. The present study revealed the existence of 18 transcripts differentially expressed in the uninfested leaves, ten of which were expressed at least double in Motelle than in Moneymaker, while the other eight were expressed half or less. In principle, the genes represented by these 18 transcripts could be considered as good candidates to participate in the resistance to piercing–sucking insects mediated by the *Mi-1* gene, although the relevance of each of them must be analyzed individually. Moreover, infestation with whitefly *B. tabaci* produces important changes in the transcriptome of

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Table 2. Transcripts up-regulated at least double (fold-change ≥ 2) or down-regulated at least half (fold-change ≤ −2) in whitefly infested leaves of the tomato cv. Motelle compared to infested leaves of cv. Moneymaker, considering only significant values (FDR < 0.05)

ID Affymetrix[a]	GenBank[b]	Description[c]	Locus[d]	ITAG description[e]	Fold-change[f]	FDR[g]
Les.1842.1.S1_at	BT012811	Protein AIG1-like	Solyc11g028010	GTPase IMAP family member 7; contains Interpro domain IPR006703 AIG1	37.59	3.48×10^{-07}
Les.5230.1.S1_at	BT013535	Elongation factor 1-γ 2-like	Solyc06g011280	Elongation factor 1- γ; contains Interpro domain IPR001662 Translation elongation factor EF1B, γ chain, conserved	26.22	2.45×10^{-08}
LesAffx.66316.1.S1_at	BI921484	E3 ubiquitin-protein ligase TRAF7	Solyc05g013880	WD-40 repeat family protein; contains Interpro domain IPR020472 G-protein beta WD-40 repeat, region	12.16	4.04×10^{-04}
Les.3272.1.S1_at	BT012750	Diaminopimelate epimerase, chloroplastic	Solyc09g005700	Diaminopimelate epimerase family protein; contains Interpro domain IPR001653 Diaminopimelate epimerase	10.49	3.46×10^{-07}
Les.75.1.S1_at	AF039681	Plant resistance protein /// root-knot nematode resistance protein Mi-1.1 /// Mi-1.2	Solyc05g008690	Disease resistance protein RPP13 variant; contains Interpro domain IPR002182 NB-ARC	5.77	5.93×10^{-08}
Les.74.1.S1_at	AF039682	Root-knot nematode resistance protein (Mi-1.2)	Solyc05g008690	Disease resistance protein RPP13 variant; contains Interpro domain IPR002182 NB-ARC	4.89	6.45×10^{-06}
Les.75.1.S1_s_at	AF039681	Plant resistance protein /// root-knot nematode resistance protein Mi-1.1 /// Mi-1.2	Solyc05g008690	Disease resistance protein RPP13 variant; contains Interpro domain IPR002182 NB-ARC	4.39	6.69×10^{-06}
Les.4037.1.S1_at	AY178911	Vacuolar H + -ATPase A2 subunit isoform	Solyc06g063330	V-type ATP synthase alpha chain; contains Interpro domain IPR005725 ATPase, V1 complex, subunit A	3.99	7.90×10^{-06}
Les.5012.1.S1_at	BT013103	Acid phosphatase 1-like	Solyc08g066530	Acid phosphatase-like protein; contains Interpro domain IPR010028 Acid phosphatase, plant	3.57	3.13×10^{-03}
LesAffx.51045.1.S1_at	AI897693	Isoflavone reductase homolog	Solyc10g052510	Isoflavone reductase-like protein 5; contains Interpro domain IPR008030 NmrA-like	3.39	4.02×10^{-03}
Les.2899.1.S1_at	BG628131	Peptide methionine sulfoxide reductase	Solyc03g111720	Peptide methionine sulfoxide reductase msrA; contains Interpro domain IPR002569 Methionine sulphoxide reductase A	2.89	3.13×10^{-02}
LesAffx.25451.1.S1_at	BE450073	ATP sulfurylase 1	Solyc09g082860	Sulfate adenylyltransferase; contains Interpro domain IPR002650 ATP-sulfurylase	2.53	4.84×10^{-02}
LesAffx.6110.1.S1_at	BF098450	21 kDa protein-like	Solyc10g076730	Pectinesterase; contains Interpro domain IPR006501 Pectinesterase inhibitor	2.11	3.08×10^{-02}
LesAffx.11839.1.S1_at	BI208864	NADH dehydrogenase subunit 3 (*Nicotiana tabacum*)	Solyc11g056370	NADH-quinone oxidoreductase subunit; contains Interpro domain IPR000440 NADH:ubiquinone/ plastoquinone oxidoreductase, chain 3	2.00	4.84×10^{-02}
Les.5432.1.S1_at	BT013906	selT-like protein	Solyc09g005590	SelT-like protein; contains Interpro domain IPR019389 Selenoprotein T	−8.58	3.35×10^{-11}
Les.4957.1.S1_at	BT012983	Adenosine kinase 2-like	Solyc09g007940	Adenosine kinase; contains Interpro domain IPR001805 Adenosine kinase	−6.09	4.88×10^{-02}

(*Continued*)

Table 2. (*Continued.*)

ID Affymetrix[a]	GenBank[b]	Description[c]	Locus[d]	ITAG description[e]	Fold-change[f]	FDR[g]
Les.3741.1.S1_at	AF146691	8-hydroxygeraniol dehydrogenase	Solyc11g011340	Tomato locus ELI3; Cinnamyl alcohol dehydrogenase; contains Interpro domain IPR002085 Alcohol dehydrogenase superfamily, zinc-containing	−5.79	1.54×10^{-05}
Les.4311.1.S1_at	AY269087	DELLA protein GAI (Gibberellic acid-insensitive mutant protein)	Solyc11g011260	GAI	−5.74	3.11×10^{-05}
Les.44.1.S1_at	BF097567	Phytoene synthetase Psy1	Solyc02g079250	3′(2′) 5′-bisphosphate nucleotidase-like protein; contains Interpro domain IPR000760 Inositol monophosphatase	−5.47	1.30×10^{-06}
Les.2638.1.A1_at	BG628467	Short-chain dehydrogenase/reductase 2b	Solyc11g071460	Dehydrogenase/reductase SDR family member 13; contains Interpro domain IPR002347 Glucose/ribitol dehydrogenase	−4.83	2.26×10^{-05}
LesAffx.4763.3.S1_at	BG123322	Protein MADS AFFECTING FLOWERING 5-like	Solyc12g087830	MADS box transcription factor; contains Interpro domain IPR002100 Transcription factor, MADS-box	−4.07	1.81×10^{-03}
LesAffx.21605.1.S1_at	AW621713	High mobility group B protein 7	Solyc06g050320	HMG type nucleosome/chromatin assembly factor; contains Interpro domain IPR009071 High mobility group, superfamily	−4.03	7.96×10^{-05}
Les.94.1.S1_at	AJ002140	DNA (cytosine-5)-methyltransferase	Solyc11g030600	cytosine-5 DNA methyltransferase (SMET)	−2.57	7.43×10^{-03}
LesAffx.10016.1.A1_at	CK715733	Two-component response regulator-like APRR1	Solyc06g069690	Pseudo response regulator; contains Interpro domain IPR001789 Signal transduction response regulator, receiver region	−2.54	4.62×10^{-02}
Les.4899.1.S1_a_at	BT012863	Glutaredoxin, grx, putative (*Ricinus communis*)	Solyc06g008750	Glutaredoxin; contains Interpro domain IPR011905 Glutaredoxin-like, plant II	−2.34	1.12×10^{-03}
Les.5732.1.S1_at	BT014465	E3 ubiquitin protein ligase DRIP2-like	Solyc06g008600	Polycomb group ring finger 1; contains Interpro domain IPR018957 Zinc finger, C3HC4 RING-type	−2.13	4.02×10^{-03}
Les.3530.1.S1_at	AF258808	Aldehyde oxidase 1 homolog	Solyc11g071620	Aldehyde oxidase 1	−2.08	2.08×10^{-02}
LesAffx.14736.1.S1_at	AW622136	UDP-glucuronic acid decarboxylase 5	Solyc11g066150	Bifunctional polymyxin resistance protein ArnA; contains Interpro domain IPR016040 NAD(P)-binding domain	−2.05	1.51×10^{-02}

[a]Transcript Identifier in the Affymetrix Genechip™.
[b]GenBank (NCBI) Transcript Identifier, provided by Affymetrix (Release 36, January 2017).
[c]Functional description of transcript, provided by Affymetrix (Release 36, January 2017).
[d]Tomato locus, Genome version SL3.0 and Annotation ITAG3.20
[e]Description of the tomato locus (Annotation ITAG3.20)
[f]Relative Expression in Motelle compared to Moneymaker.
[g]FDR value (corrected *P*-value) of the Relative Expression.

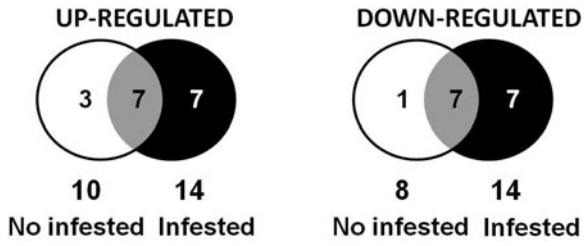

Figure 3. Venn diagrams comparing the number of transcripts with differential expression between tomato cultivars, before (no infested) or after (infested) *B. tabaci* infestation. Up-regulated represent transcripts more expressed in Motelle than in Moneymaker. Down-regulated represent transcripts less expressed in Motelle than in Moneymaker. Only transcripts are included with statistically significant values (FDR < 0.05) of relative expression (fold-change or FC) ≥2 (up) or ≤−2 (down).

tomato leaves, substantially modifying the initial differences between Moneymaker and Motelle.

Expression of Mi-1 *gene*

Three probesets detected as up-regulated in non-infested Motelle corresponded to the homologous genes *Mi-1.1* and *Mi-1.2*. This result, not as expected less interesting, reflects the main difference between both cultivars in the absence of any type of infestation. A similar comparison between Motelle and Moneymaker had been made using the microarray technique in uninfested roots (Schaff *et al.*, 2007), analyzing the expression of 1547 genes, among which the *Mi-1* gene was not included. In contrast, the expression of approximately 9200 genes in foliar tissue was analyzed in the present work to obtain additional information on the basal differences between both cultivars associated with the presence/absence of *Mi-1*. Moreover, the plants analyzed were fully mature, while roots from younger plants (4 weeks old) were used in the previous work by Schaff *et al.* (2007). This is important as *Mi-1*-mediated resistance of tomato to *B. tabaci* is dependent on plant age, and this resistance has very limited effectiveness in 5-month-old or younger plants (Rodríguez-Álvarez *et al.*, 2017). Individual expression of *Mi-1.2* was previously analyzed by RT-PCR in these same tomato genotypes in the absence of any infestation, with expression of this gene in different plant tissues of only Motelle plants (Martínez de Ilarduya and Kaloshian, 2001).

The differential expression of *Mi-1.1*/*Mi.1.2* was also maintained after *B. tabaci* infestation, with expression only slightly lower than that observed prior to infestation. This indicates that whitefly infestation does not cause substantial changes in *Mi-1* expression, which agrees with the results previously obtained by other authors on the attack of nematodes or aphids (Martínez de Ilarduya and Kaloshian, 2001; Goggin *et al.*, 2004). The detection of this fundamental difference between Motelle and Moneymaker in the present survey through global gene expression analysis can be considered as a validity test of this methodology for this purpose. Thus, this finding reinforces the use of DNA microarrays for the identification of differentially expressed genes.

Other baseline differences and changes after whitefly infestation

Leaving aside the *Mi-1* gene, the transcript with the highest difference in expression between non-infested cultivars (more than 80-fold higher in Motelle than in Moneymaker) encoded the NtGP4 (*N. tabacum* geranylgeranylated 4) protein. This basal difference was considerably reduced to 37.59-fold after infestation with *B. tabaci*. Expression of *NtGP4* gene can be involved in the processes of response to biotic stimuli as it was previously demonstrated to be induced in the roots of Moneymaker after nematode infection (Bhattarai *et al.*, 2008). Moreover, *NtGP4* had a higher basal expression in leaves of a tomato cultivar tolerant to saline stress compared to sensitive Moneymaker, although this gene was not related to the salt response in either tomato genotype (Sun *et al.*, 2010). NTGP4 protein is similar to the AIG1 protein from *Arabidopsis* (Biermann *et al.*, 1994; Dykema *et al.*, 1999), which is involved in resistance to pathogenic bacteria in plants containing the resistance gene *RPS2* together with the avirulence gene *avrRpt2* (Reuber and Ausubel, 1996). The AIG1 protein has also been related to the ABA signaling pathway (Kim and Kim, 2006) whose role in the resistance to plant diseases has been reviewed (Ton *et al.*, 2009).

Another transcript that was 28.42-fold more expressed in Motelle than in Moneymaker, represents the translation elongation factor 1-γ. This basal difference was only slightly reduced after infestation with *B. tabaci* (FC = 26.22). Members of the eukaryotic Elongation Factor 1 (eEF1) complex have been implicated in a wide variety of cellular and viral processes (Sasikumar *et al.*, 2012). Upregulation of elongation factor 1-γ-like in leaves has been shown as a first hint at stressful conditions in plants subjected to biotic stress (Weiß and Winkelmann, 2017).

The third gene up-regulated in Motelle regarding Moneymaker (FC = 11.30) encodes the enzyme Diaminopimelate (DAP) epimerase which catalyzes the lysine biosynthesis from aspartate. In addition, it is thought that this enzyme could be used as a component in antimicrobial agents (Hor *et al.*, 2013). The differential expression of this gene between Motelle and Moneymaker remained fairly stable after infestation by *B. tabaci* (FC = 10.49).

Also a gene encoding the vacuolar H + -ATPase A2 subunit showed more than fourfold greater expression in Motelle leaves than in Moneymaker's, and subsequent infestation with *B. tabaci* almost did not alter that difference. The activity of this subunit was described in resistance mediated by *Cf-9* gene to the pathogen *Cladosporium fulvum* expressing the *Avr9* avirulence gene (Piedras *et al.*, 1998). The changes in the permeability of the plasma membrane are of the first events that occur in the defensive responses of the plants after the recognition of pathogens or elicitors. These changes produce a depolarization due to the entry of Ca^{+2} and H^{+} and to the exit of K^{+} and Cl^{-} (Scheel, 1998). These fluxes appear to be necessary for the induction of expression of defensive genes against pathogen attack or wounds (Fukuda, 1996; Jabs *et al.*, 1997; Schaller and Oecking, 1999; Schaller and Frasson, 2001).

In the absence of infestation, another gene up-regulated in Motelle with respect to Moneymaker (FC = 2.50) encodes selective channels for voltage dependent ions (VDACs), or pores formed from transmembrane channel proteins (porins) present in the outer membrane of the mitochondria. These channels were better studied in animal cells than in plants but in both cases they are involved in apoptosis (Voehringer *et al.*, 2000; Okada *et al.*, 2004; Veenman *et al.*, 2008; Kusano *et al.*, 2009; Tateda *et al.*, 2011). It has been demonstrated that VDAC protein is necessary for normal plant growth and for defense in Arabidopsis, regulating the generation of hydrogen peroxide (Tateda *et al.*, 2011). The involvement of hydrogen peroxide in the VDAC pathway was previously observed in the non-specific resistance of *Nicotiana*

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Table 3. Transcripts with differential expression between tomato cultivars detected in the analysis of both uninfested and whitefly-infested plants.

ID Affymetrix[a]	Description[b]	Fold-change[c]	
		Uninfested	Infested
Les.1842.1.S1_at	Solyc11g028010, GTPase IMAP family member 7	80.19	37.59 ↓↓
Les.5230.1.S1_at	Solyc06g011280, Elongation factor 1-γ	28.42	26.22 ↓
Les.3272.1.S1_at	Solyc09g005700, diaminopimelate epimerase,	11.30	10.49 ↓
Les.74.1.S1_at	Solyc05g008690, Root-knot nematode resistance protein Mi-1.2	7.84	4.89 ↓
Les.75.1.S1_at	Solyc05g008690, Root-knot nematode resistance protein Mi-1.1	6.91	5.77 ↓
Les.75.1.S1_s_at	Solyc05g008690, Root-knot nematode resistance protein Mi-1.1 /// Mi-1.2	6.79	4.39 ↓
Les.4037.1.S1_at	Solyc06g063330, vacuolar H + -ATPase A2 subunit	4.42	3.99 ↓
Les.4311.1.S1_at	Solyc11g011260, DELLA protein GAI	−9.62	−5.74 ↓↓
Les.44.1.S1_at	Solyc02g079250, phytoene synthetase Psy1	−8.77	−5.47 ↓↓
Les.4957.1.S1_at	Solyc09g007940, adenosine kinase 2-like	−8.64	−6.09 ↓
Les.5432.1.S1_at	Solyc09g005590, selT-like protein	−5.65	−8.58 ↑↑
LesAffx.4763.3.S1_at	Solyc12g087830, protein MADS AFFECTING FLOWERING 5-like	−3.15	−4.07 ↑
Les.2638.1.A1_at	Solyc11g071460, short-chain dehydrogenase/reductase 2b	−2.60	−4.83 ↑
Les.4899.1.S1_a_at	Solyc06g008750, Glutaredoxin	−2.13	−2.34

[a]Transcript Identifier in the Affymetrix Genechip™.
[b]Tomato locus and description.
[c]Relative Expression in Motelle compared to Moneymaker. ↓↓ and ↑↑ represent marked decreases and increases, respectively, in the expression differences between cultivars. ↓ and ↑ represent moderate decreases and increases.

benthamiana to *Pseudomonas cichorii* (Tateda *et al.*, 2009). VDAC was used as a marker in Arabidopsis of the hypersensitive response (HR) to *Xanthomonas campestris* (Lacomme and Roby, 1999) or plant programmed cell death (Swidzinski *et al.*, 2004). However, the differential expression of this gene between Motelle and Moneymaker was not observed after infestation with *B. tabaci*, suggesting that the attack of this insect does not promote HR in tomato leaves. These data agree with previously obtained results in Arabidopsis with *B. tabaci* where cytological analysis of the leaves showed that no HR was produced after feeding of the whitefly nymphs (Kempema *et al.*, 2007). Similarly, HR was not observed in tomato during the compatible and incompatible interactions with aphids (Martínez de Ilarduya *et al.*, 2003).

Other two transcripts that were expressed approximately double in Motelle than in Moneymaker prior to infestation were not differentially expressed after infestation with *B. tabaci*. The sequence of one of them (FC = 2.08) corresponds to a gene encoding a glycogen glycosyltransferase which was detected in a previous study of microarrays in non-infested roots of Motelle and Moneymaker (Schaff *et al.*, 2007). In the same study, induction of this gene was also detected during nematode incompatible interaction, demonstrating its participation in *Mi-1*-mediated resistance to nematodes. Other studies suggested the involvement of glycosyltransferases in processes of biotic and abiotic stress responses (Vogt and Jones, 2000; Dixon, 2001; Mazel and Levine, 2002; Langlois-Meurinne *et al.*, 2005; Qi *et al.*, 2005; Meissner *et al.*, 2008; von Saint Paul *et al.*, 2011) and synthesis of the cell wall (Lao *et al.*, 2003; Egelund *et al.*, 2004; Baumann *et al.*, 2007). The second transcript (FC = 2.04) is related to an F-box protein. Many proteins in this family are involved in plant vegetative and reproduction growth and development, as abscisic acid (ABA) signaling to affect the seed germination of Arabidopsis (Peng *et al.*, 2012) or regulation of cell death and defense after pathogen recognition in tobacco and tomato (Van Den Burg *et al.*, 2008). To analyze the possible participation of these and other genes in whitefly resistance, it would be necessary to perform complementary studies to obtain their expression differences between infested and non-infested plants.

Among the genes down-regulated in uninfested Motelle compared to uninfested Moneymaker, the largest difference was in the gene encoding the GAI protein (FC = −9.6) that belongs to the GRAS family; these proteins fulfill regulatory functions in different aspects of signaling and plant development (Bolle, 2004; Achard *et al.*, 2006). The GAI protein contains an N-terminal domain DELLA (Silverstone *et al.*, 1998), and proteins sharing this motif are also known as DELLA proteins (Eckardt, 2003). GAI was the second protein that was cloned from this family (Peng *et al.*, 1997) after cloning the SCR protein (Di Laurenzio *et al.*, 1996). DELLAs restrict plant growth by suppressing the action of GAs (Bolle, 2004). Reciprocally, GAs regulate growth through the degradation of DELLA proteins (Harberd, 2003; Jiang and Fu 2007; Wang *et al.*, 2009). In *Arabidopsis* and tomato, these proteins control plant defense by modulating the responses dependent on SA and JA (Navarro *et al.*, 2008; Bari and Jones, 2009; Ding *et al.*, 2013). The fact that lower expression of the GAI protein was obtained in Motelle than in Moneymaker could be associated with greater growth of plants containing the *Mi-1* gene. However, no obvious differences were observed in this study between plants of both genotypes, thus suggesting that the lower expression in Motelle would not affect the development of these plants. Subsequent infestation with *B. tabaci* reduced the difference between Motelle and Moneymaker (FC = −5.74). This reduction could be explained by a lower GAI expression in Moneymaker that would result in plant growth promotion, although this fact was not observed during our work. Alternatively, the reduction in the difference between Motelle

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Table 4. Analysis of relative expression by qRT-PCR

ID Affymetrix[a]	Gene	Oligos	Fold-change[b]	
			Uninfested	Infested
Les.1842.1.S1_at	Solyc11g028010	F-CTCGGTTGAAGGCTGAACTAA R-CCTTTGAGCTCTCTCCAGATATTC	11510.5	1273.6
Les.5230.1.S1_at	Solyc11g028100	F-GGAAAGGAAATTCCCATGTTTGT R-GCTCCTTCTGAGCTTCATCAT	12.07	9.46
Les.3272.1.S1_at	Solyc09g005700	F-GGTGGACCACTTGACATTGA R-AGGAGCTGACCCGTAGAAA	2.47	847.07
Les.4037.1.S1_at	Solyc06g063330	F-ACCTTGAGGATGAAACTCGATAAG R-AGTGTGGACAACAGCACATAA	21.44	12.04
Les.4311.1.S1_at	Solyc11g011260	F-GGTTCGATCCGGTTCATCTG R-TTTCTTCCACCCTGTAACCATC	−1.92	−1.92
Les.44.1.S1_at		F-GTTGTTGTATTGGGCCCTTAAT R-ACAGAATAGGGTTTCCCATAGC	2.13	3.34
Les.4957.1.S1_at	Solyc09g007940	F-GCATCTGGACACAAGAGGATTA R-GGTATAACAGGGAACAGCTTCA	−526.32	−25
Les.5432.1.S1_at	Solyc09g005590	F-TGGAGTCATTGGCCTTGTAAT R-CTGTTCGCACGTAACTGATAGA	−4.55	−5.56
LesAffx.4763.3.S1_at	Solyc12g087830	F-TATTCTCTGCGATGTCGATGTT R-GCATCGCTGTCATACTGTTATTG	−4.35	−4.17
Les.2638.1.A1_at	Solyc11g071460	F-TGTTTCAGTGTCTCTTGGAATTTG R-CAGTTTGCTTCCCTCATTGTTT	2.98	1.75
Les.4899.1.S1_a_at	Solyc06g008750	F-AAAGCACACGCCTGAAATG R-CGAGTGCTCCAATCGAAAGA	−1562.5	−42.19
Les.74.1.S1_at	Mi-1/Mi-1	F-CTTGCGTCTACCGACTCTTTC R-GGTGGAATCTCCTCAAGCTTAC	3.65	28.95
Ubi3	Solyc01g056940	F-GGGCTCACCTACGTTTACAA R-CTCTAAATTACCGTTCATTCGACAA	1	1

[a]Transcript Identifier in the Affymetrix Genechip™.
[b]Relative Expression in Motelle compared to Moneymaker according to qRT-PCR.

and Moneymaker after whitefly infestation can be also explained as an increase of GAI expression in Motelle that would lead to a decrease in GA. This might suggest that DELLAs can be important in the *Mi-1*-mediated resistance against whiteflies. In *Arabidopsis*, DELLAs repress SA signaling pathway during *P. syringae* infection (Navarro *et al.*, 2008). However, SA plays an important role during the *Mi-1*-mediated resistance in tomato against *B. tabaci* (Rodríguez-Álvarez *et al.*, 2015). The role of the GA signaling pathway in plant defense is ambiguous as antagonistic effects have been observed in several studies (De Bruyne *et al.*, 2014). It would be interesting to later complement the present microarray analysis with more specific studies to confirm the role of GA/DELLAs in the *Mi-1*-mediated resistance in tomato against *B. tabaci*.

Another gene down-regulated in Motelle compared with Moneymaker (FC = −8.77) encodes the enzyme Pys1 (phytoene synthase 1) involved in secondary metabolism and related to fruit ripening (Gady *et al.*, 2012). The difference in gene expression between these two cultivars decreased (FC = −5.47) after infestation with *B. tabaci*. Phytoene synthase catalyzes carotenoid biosynthesis (von Lintig *et al.*, 1997; Toledo-Ortiz *et al.*, 2010) and its coding gene is induced in tomato in response to saline stress (Zhou *et al.*, 2007), as well as in banana under abiotic stresses (Kaur *et al.*, 2017), but not in compatible or incompatible interactions to nematodes (Bhattarai *et al.*, 2008). Neither Pys1 has shown differential expression in response of *Arabidopsis* to whiteflies (Kempema *et al.*, 2007).

The expression of the enzyme ADK (adenosine kinase) that catalyzes the synthesis of AMP from adenosine and ATP was more than eight times lower in Motelle than in Moneymaker in the absence of infestation. This difference was slightly reduced to FC = −6.09 after infestation of both cultivars with *B. tabaci*. It was previously known that this gene is induced in *N. benthamiana* after virus infection (Wang *et al.*, 2003) and also by salt stress in *Beta vulgaris* and *Spinacia oleracea*, but not in other related plant species such as *N. tabacum* and *Brassica napus* (Weretilnyk *et al.*, 2001). A more recent study has shown that ADK plays a role in plant development and defense (Liu *et al.*, 2016). Additional analyses would be necessary to determine with certainty if this enzyme plays any role in the *Mi-1*-mediated resistance to whiteflies.

Another gene with lower expression in uninfested Motelle than in Moneymaker (FC = −5.65) encoded a selT-like protein. This difference between cultivars increased to FC = −8.58 after whitefly infestation. SelT-like protein precursors have been related to selenite resistance, as a SELT gene was more induced in a selenite-resistant accession of Arabidopsis than in a selenite-sensitive accession after selenite treatment (Tamaoki *et al.*, 2008).

MADS-box transcription factors are involved in the regulation of different processes of plant development including flowering,

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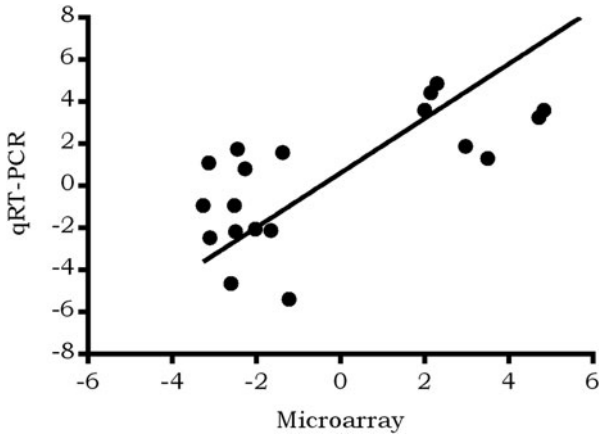

Figure 4. Correlation between gene expression values obtained from the microarray analysis (axis *X*) and from qRT-PCR (axis *Y*), with a statistically significant ($P < 0.0001$) value of the Pearson correlation coefficient ($r = 0.7475$).

fruit development, and embryogenesis (Busi *et al.*, 2003). In the present study, the expression of MADS-box 15 in Motelle was found to be repressed by comparison with Moneymaker in the absence of whiteflies (FC = −3.15) and this difference was maintained and even slightly increased (FC = −4.07) after infestation with *B. tabaci*. The expression of MADS-box 15 had been increased in both compatible and incompatible interactions of tomato with nematodes (Bhattarai *et al.*, 2008). In addition, several tomato MADS-box genes were induced during incompatible interactions with *X. campestris* pv. *Vesicatoria* (Bonshtien *et al.*, 2005) or in response to saline stress (Zhou *et al.*, 2007). Further analysis of the expression of these transcription factors during the compatible and incompatible tomato–whitefly interactions would make it possible to define their role in a possible negative regulation of the development of the plant in favor of defensive processes.

Among the repressed transcripts in Motelle relative to Moneymaker was a gene encoding a short-chain dehydrogenase/reductase, with FC = −2.60 in uninfested plants and FC = −4.83 in whitefly infested plants. The short-chain dehydrogenases/reductases (SDR) constitute one of the largest enzyme superfamilies with over 46,000 members (Persson *et al.*, 2009). More specifically, the transcript Solyc11g071460.1.1 was recently involved in compatible plant–microbe interactions, as it was identified among differential expressed genes in tomato leaf tissue, down-regulated at 24 h post-inoculation with *Bacillus cinerea* (Rezzonico *et al.*, 2017).

The expression of a cinnamyl alcohol dehydrogenase (CAD) was 2.39 times lower in uninfested Motelle than in uninfested Moneymaker, but following infestation with *B. tabaci*, this differential expression was no longer detected. However, ELI3 protein, which is also a type of CAD protein (Logemann *et al.*, 1997), was expressed 5.9 times less in Motelle than in Moneymaker, both infested. CADs are key enzymes of lignin synthesis and they catalyze the reversible conversion of cinnamyl aldehyde to the monolignols that will give rise to lignin, which is why CAD activity is correlated with lignification in tomato (Roth *et al.*, 1997). In addition to its role in lignification, the increase in the expression of some genes encoding CAD enzymes has been associated with a number of defensive responses to pathogens in compatible and incompatible interactions (Kiedrowski *et al.*, 1992; Mitchell *et al.*, 1994; Coelho *et al.*, 2006). Thus, in the interaction, Arabidopsis–whiteflies, the expression of two CAD isoforms increased (Kempema *et al.*, 2007). CAD levels were also overexpressed after infection of parsley with fungi and bacteria (Schmelzer *et al.*, 1989; Somssich *et al.*, 1989; Van Gijsegem *et al.*, 1995; Logemann *et al.*, 1997). More recently, CAD has been shown to be important for the resistance against *Rhizoctonia cerealis* in wheat (Rong *et al.*, 2016). Similarly, during the interaction of tomato with *Xanthomonas axonopodis* pv. *vesicatoria*, the level of plant resistance to the pathogen positively correlated with the levels of CAD enzyme (Umesha and Kavitha, 2011).

In the absence of infestation, a glutarredoxin was expressed 2.13 times less in Motelle than in Moneymaker, and a similar differential expression was maintained after the infestation with *B. tabaci* (FC = −2.34). Glutarredoxins are antioxidant enzymes that play an important role in the control of oxidative stress (Kalinina *et al.*, 2008; Meyer *et al.*, 2008).

Differential genes detected only after infestation with B. tabaci

Among the genes only up-regulated in Motelle compared to Moneymaker when plants were infested by *B. tabaci*, it is a remarkable one (FC = 12.16) corresponding to the E3 ubiquitin-protein ligase TRAF7 which belongs to the WD-40 repeat protein family. In eukaryotes, proteins of this family are involved in a variety of functions such as signal transduction, cell division, cytoskeleton assembly, chemotaxis, RNA processing, and apoptosis (Xu *et al.*, 2004; Stirnimann *et al.*, 2010). The N termini of TRAFs 2–7 render them genuine E3 ubiquitin ligases which are required in the process of protein ubiquitination and determine the substrate specificity (Huang *et al.*, 2016). Interestingly, SCF-TRAFasome formation mediated by TRAF proteins may represent a method used by plants to assemble SCF complexes upon pathogen infection (Huang *et al.*, 2016).

An enzyme similar to acid phosphatase 1 (Aps-1) stands out among the transcripts that were more expressed in Motelle than in Moneymaker only after whitefly infestation (FC = 3.57). Although the function of acid phosphatases is not well known, tomato Aps-1 could participate in response to invader organisms, as its enzyme activity increased in the roots of both susceptible and resistant tomato plants after infection with RKN (Williamson and Colwell, 1991). This was later confirmed in microarray studies (Bhattarai *et al.*, 2008). Moreover, the *Aps-1* gene, closely linked to *Mi-1*, was cloned and has been employed as a molecular marker for the presence of *Mi-1* (Aarts *et al.*, 1991; Williamson and Colwell, 1991).

Three other genes more expressed in Motelle than in Moneymaker, only after infestation with *B. tabaci*, were related to protection against oxidative stress: Firstly, the gene that encodes the enzyme isoflavone reductase (FC = 3.39) is one of the key enzymes in the isoflavonoid biosynthesis and whose antioxidant function has been observed in *Arabidopsis* (Babiychuk *et al.*, 1995) and rice (Kim *et al.*, 2010). The gene encoding the peptide methionine sulfoxide reductase (PMSR) enzyme (FC = 2.89), which may play an important role in cell protection against oxidative stress, as it has been observed with PMSR2 in *Arabidopsis* (Bechtold *et al.*, 2004). Also remarkable is a gene encoding the subunit 3 of the enzyme NADH dehydrogenase (nad3) (FC = 2.00), a subunit present in Complex I of the electron transport chain in mitochondria. Complex I acts as a proton pump toward the intermembrane space of the mitochondria, thus avoiding acidification of the matrix that can lead to oxidative

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stress (reviewed by Subrahmanian *et al.*, 2016) and, ultimately, a cellular damage manifested in an HR. The fact that these three genes were more expressed in Motelle than in Moneymaker after the infestation with *B. tabaci* aligns with results from a previous study where HR was not observed in the *Mi-1*-mediated response of Motelle after aphid attack (Martínez de Ilarduya *et al.*, 2003). This HR was also absent in *Arabidopsis* after whitefly infestation (Kempema *et al.*, 2007). All these data indicate that whitefly infestation does not provoke HR in bearing-*Mi-1* tomato leaves, unlike what happens when roots are attacked by nematodes (Dropkin, 1969).

Two other transcripts over-expressed in Motelle compared to Moneymaker only after infestation with *B. tabaci* were identified. One of them (FC = 2.11) corresponded to a 21 kDa pectinesterase; these enzymes are involved in cell wall reorganization processes as well as in plant response to pathogen attack (McMillan *et al.*, 1993; Wiethölter *et al.*, 2003; Raiola *et al.*, 2011). The second gene encodes the enzyme ATP sulforylase 1 (FC = 2.53) belonging to the family of sulfate adenylyltransferase enzymes. These enzymes are involved in the sulfate assimilation pathway by catalyzing the activation of sulfate ions by ATP to form adenosine-5′-phosphosulfate (APS) and pyrophosphate (Marzluf, 1997). This reaction is the first enzymatic step in the use of sulfate upon its uptake.

Among the genes that were less expressed in Motelle than in Moneymaker after infestation with *B. tabaci*, but without differential expression in non-infested plants, the ELI3 encoding enzyme (FC = −5.79) is a CAD protein (Logemann *et al.*, 1997) which has been discussed above. Also a methyl transferase (FC = −2.57) is involved in different cellular processes among which is the regulation of gene expression during development (Finnegan *et al.*, 1996).

Included in this group are three proteins involved in transcription processes. Transcript LesAffx.21605.1.S1_at (FC = −4.03) corresponds to the high mobility group B protein. Differential expression of this HMG type nucleosome/chromatin assembly factor has been associated with plant leaf development (Rantong *et al.*, 2016) and more recently with thermotolerance in perennial grass (Xu and Huang, 2018). LesAffx.10016.1.A1_at (FC = −2.54) represents a gene of the pseudo response regulator (PRR) family, which are sequentially expressed over the course of the day. More specifically, this locus Solyc06g069690 has been identified in maize with the timing of cab expression1 (*TOC1*) gene (Bendix, 2015), one of the main contributors to the plant clock system (Farré and Liu, 2013). The product of Les.5732.1.S1_at (FC = −2.13) is similar to E3 ubiquitin protein ligase DRIP2 that acts as a negative regulator of the response to water stress in Arabidopsis (Qin *et al.*, 2008).

The last two enzymes only differentially expressed in infested plants are an aldehyde oxidase (AO1) (FC = −2.08) and the UDP-glucuronate decarboxylase 2 (FC = −2.05). AO1 was identified in tomato along with other enzymes of the same family by Min *et al.* (2000) who suggested that each AO could play a different role in the growth and development of this solanaceae. AOs are involved in hormone biosynthesis processes, in particular catalyze the last step of ABA biosynthesis (Min *et al.*, 2000). The activity of the enzyme UDP-glucuronate decarboxylase, involved in membrane-associated metabolic processes, has been detected in several plants and the expression of genes encoding these enzymes in barley has been studied (Zhang *et al.*, 2005). This enzyme has an important role in cell wall biosynthesis (Seifert, 2004).

Conclusions

Genes highlighted in the first phase of this study represent the baseline differences between the transcriptomic profiles of the Motelle and Moneymaker tomato cultivars, associated with the presence of the *Mi-1* gene in the first of them. The observed changes in the relative expression of these genes following whitefly infestation, as well as the emergence of other genes with differential expression, illustrate how the baseline differences between Motelle and Moneymaker are substantially altered by this insect. Taken together, these results provide us with valuable information on candidate genes to intervene in one way or another in the tomato resistance mediated by the *Mi-1* gene to *B. tabaci*. However, to analyze the actual participation of these genes in such a resistance, it would be necessary to perform complementary studies to obtain expression differences between infested and non-infested plants of the same cultivar. Based on the results of the present study, further analyses are currently underway in our laboratory to define the role of these and other genes during the compatible and incompatible interactions of adult tomato plants with the whitefly *B. tabaci*.

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