Comparative Analysis of Expression Profiles in Shoots and Roots of Tomato Systemically Infected by *Tomato spotted wilt virus* Reveals Organ-Specific Transcriptional Responses

Marco Catoni,¹ Laura Miozzi,¹ Valentina Fiorilli,² Luisa Lanfranco,² and Gian Paolo Accotto¹

¹Institute of Plant Virology, CNR, Strada delle Cacce, 73, 10135 Torino, Italy; ²Department of Plant Biology, University of Turin, Viale Mattioli, 10125 Torino, Italy

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Tomato (*Solanum lycopersicon*), a model species for the family Solanaceae, is severely affected by *Tomato spotted wilt virus* (TSWV) worldwide. To elucidate the systemic transcriptional response of plants to TSWV infection, microarray experiments were performed on tomato. Parallel analysis of both shoots and roots revealed organ-specific responses, although the virus was present in similar concentration. In the shoots, genes related to defense and to signal transduction were induced, while there was general repression of genes related to primary and secondary metabolism as well as to amino acid metabolism. In roots, expression of genes involved in primary metabolism and signal transduction appear unaffected by TSWV infection, while those related to the response to biotic stimuli were induced and those associated to the response to abiotic stress were generally repressed or unaltered. Genes related to amino acid metabolism were unaffected, except for those involved in synthesis of secondary compounds, where induction was evident. Differential expression of genes involved in metabolism and response to ethylene and abscisic acid was observed in the two organs. Our results provide new insight into the biology of the economically important interaction between tomato and TSWV.

Viruses are obligate pathogens responsible for many plant diseases leading to huge losses in crop productions and quality worldwide. Establishment of systemic infection is due to a series of virus-induced modifications, including suppression of post-transcriptional gene silencing (Voinnet 2005), alteration of cell-to-cell and long-distance movement processes (Boevink and Oparka 2005), and host biochemical and physiological changes associated with stress responses as well as developmental defects (Whitham et al. 2006). Depending on the disease, such interactions may cause a range of symptoms, including leaf yellowing and distortion, stunting, and other abnormalities. These

symptoms are the final result of a complex process involving host specificity, environmental factors, and developmental plant stages. Despite economic relevance, the molecular basis of plant–virus interaction and a symptom’s emergence are far from clear.

Microarray technology is a sensitive and reliable tool to study transcriptional changes in host plants during compatible and incompatible interactions with a range of pathogenic fungi, bacteria, and viruses (Wise et al. 2007). Using this technique, Golem and Culver (2003) identified 68 genes differentially expressed in *Arabidopsis* leaves either locally or systemically infected by *Tobacco mosaic virus* (TMV), transcription factors, antioxidants, metabolic enzymes, and transporters. These categories reflect the biochemical and physiological changes involved in development of disease. Soybean plants infected by *Soybean mosaic virus* (SMV) showed transcriptional changes involving many genes associated with hormone metabolism, cell-wall biogenesis, chloroplast functions, and photosynthesis. In those plants, delayed induction of defense-related genes has been suggested to be critical for the establishment of SMV systemic infection (Babu et al. 2008).

Comparative expression profiling of three fruit tree viruses, *Plum pox virus* (PPV), *Tomato ring spot virus* (ToRSV), and *Prunus necrotic ring spot virus* (PNRSV) infecting *Nicotiana benthamiana* leaves, found a positive correlation between the number of regulated genes and the severity of symptoms (Dardick 2007). Looking for sets of genes commonly regulated in response to viruses, Whitham and co-workers (Whitham et al. 2003) selected a group of genes in *Arabidopsis*, primarily correlated with biotic and abiotic stress, differentially expressed in response to five positive-sense RNA viruses. Focusing their attention on *N. benthamiana* systemically infected by two negative-sense RNA viruses, *Impatiens necrotic spot virus* (INSV) and *Sonchus yellow net virus* (SYNV), Senthil and co-workers (2005) found that the different biological lifestyle of the viruses was reflected in a different regulation of heat-shock protein and histone genes.

Viral infection certainly induces metabolic changes involving the whole plant and it is well known that viruses accumulate also in the roots (Samuel 1934). Roots can be primary sites of infection not only for soilborne viruses such as *Beet necrotic yellow vein virus* (Rahim et al. 2007) but also for viruses usually infecting through the leaves, when they are present in a hydroponic circulating medium (Park et al. 1999). There are reports on the kinetics of virus accumulation in roots (Valentine et al. 2002; Lunello et al. 2007). However, to date,

Corresponding author: Gian Paolo Accotto; E-mail: g.accotto@ivv.cnr.it

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*The e-Xtra logo stands for “electronic extra” and indicates that six supplementary tables are published online and that Figure 1 appears in color online.

transcriptomic research in plant–virus interactions has focused only on leaves, the first organs to show the disease symptoms and the easiest to study.

The main goal of this work is to analyze the transcriptional response of tomato plants to *Tomato spotted wilt virus* (TSWV) infection. This RNA virus of ambisense polarity belonging to the genus *Tospovirus* is transmitted by thrips and can infect hundreds of plant species, causing huge crop losses. Our experiments allowed us to analyze how systemic virus infection affects tomato gene expression in both shoots and roots. We used the Tom2 12,000-feature oligonucleotide-based tomato array, available from Boyce Thompson Institute for Plant Research (Ithaca, NY, U.S.A.). Our results highlight a complex pattern of responses with important differences between shoots and roots.

RESULTS

Timing of the analysis.

To define the best time to analyze the transcriptional responses to systemic viral infection, a time course experiment was performed. Because an initial systemic symptom of TSWV infection on tomato is growth reduction, the weight of plants was measured at 0, 7, 14, 21, and 28 days postinoculation (dpi). At 14 dpi, the growth increment became significantly lower in infected plants compared with the control plants (Fig. 1A, B, and C). At the same time, fresh weight of roots collected from infected plants was significantly lower than that of roots from the mock-inoculated plants (data not shown); moreover, the beginning of typical systemic symptoms such as

Fig. 1. Growth of tomato plants following inoculation with *Tomato spotted wilt virus* (TSWV). Representative **A**, TSWV-infected and **B**, mock-inoculated plants 14 days postinoculation. **C**, Growth of plants in time, measured as weekly size increments. Error bars represent the 95% confidence intervals. **D**, Dot blot of total RNA extracted from shoots (left) and roots (right) of TSWV-infected (1, 2, and 3) or mock-inoculated (M) plants 14 days postinoculation, hybridized with a TSWV-specific probe. Numbers above the panel indicate the amount (ng) of RNA loaded in each square.

bronzing on leaves and distortion of the plant apex appeared. Therefore, we decided to collect young aerial expanding leaves and apex (“shoots”) and radical terminal portions of roots (“roots”) at 14 dpi. To confirm systemic infection, viral RNA was estimated semiquantitatively and revealed no evident differences in amounts of viral RNA either between shoots and roots or among the plants (Fig. 1D).

Identification of genes differentially expressed in TSWV-infected shoots and roots.

RNAs deriving from shoots or roots of infected plants were hybridized on the arrays with the corresponding RNAs from mock-inoculated plants. To reduce the biological variability, each hybridization was performed separately on three biological replicates consisting on pools of three to five plants for each experimental condition. To avoid potential variations in labeling efficiency of the fluorescent dyes, a technical replicate (dye swap) was performed for each biological replicate, lead-

ing to a total of 12 hybridizations. A gene was considered differentially expressed when i) its expression ratio in TSWV-infected and mock-inoculated control plants was significantly different (false discovery rate [FDR] < 0.05) and ii) its fold change (FC) was ≤ 0.67 or ≥ 1.5 ($\log_2$ ratio ≤ -0.17 or ≥ 0.17).

According to these criteria, in total, 2,344 genes showed differential expression in shoots and, of these, 1,076 were upregulated in infected plants and 1,268 were downregulated. In roots 1,156 genes showed differential expression: 510 were upregulated and 646 were downregulated (Supplementary Table S1). Genes differentially expressed in both shoots and roots or specifically in one of these organs are shown in a Venn diagram and a hierarchical clustering (Fig. 2). A list of genes belonging to each cluster and for their functional characterization can be found in Supplementary Table S5. In spite of similar virus concentration, TSWV induced or repressed in shoots more than twice the genes regulated in roots. A large proportion (approximately 82%) of all differentially expressed genes were specifi-

Fig. 2. Expression profiles of *Solanum lycopersicum* plants systemically infected by *Tomato spotted wilt virus* (TSWV). **A**, Hierarchical clustering of differential expression profiles for 2,962 genes from shoots and roots. The ratio of fluorescent-dye (Cy3 and Cy5) intensity measured for each co-hybridized cRNA on the array in shoots and roots was converted into $\log_2$ ratios for cluster analysis. Genes with increased expression in virus-infected plants are shown in yellow, genes that were downregulated are colored blue, and those showing no change relative to expression in mock-inoculated plants are in black. Color intensity is proportional to differential expression. **B**, Venn diagram showing regulated genes in shoots and roots during TSWV systemic infection. The total number of genes expressed in each condition is given in parentheses. The number of genes commonly expressed in shoots and roots is provided in the overlapping portions of the circles; the value in square brackets indicates the number of genes regulated in the same direction.

cally regulated only in one of the two organs, whereas 538 genes were regulated in both shoots and roots, 455 of them (85%) in the same direction.

Microarray results were validated by quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR) on the three biological replicates employed in hybridization experiments and in a forth independent biological replicate. Ubiquitin (X58253) was selected as reference gene because it was not regulated in our arrays and in qRT-PCR, as confirmed by analysis of variance (not shown). Regulation of all selected genes, with the exception of SGN-U223075, was confirmed. As already reported (Lopez et al. 2005; Gandia et al. 2007; Guether et al. 2009), qRT-PCR fold changes were usually greater than those observed in microarrays (Table 1).

Functional analysis of genes involved in TSWV infection.

The array data were organized in functional categories according to Gene Ontology (GO) guidelines (The Gene Ontology Consortium 2000). Of 2,962 genes differentially regulated, all except 123 showed a GO annotation in at least one of the three principal GO branches: biological process, molecular function, and cellular component. We concentrated our analyses on “biological process” categories overrepresented (details below) in our datasets. Those categories were organized in five groups: response to biotic and abiotic stimuli and defense response (Fig. 3A), photosynthesis and energy-related categories (Fig. 3B), amino acid metabolism (Fig. 3C), secondary metabolism (Fig. 3D), and signal transduction (Fig. 3E). GO enrichment analysis can be found in Supplementary Table S2. Numbers of genes reported below are those belonging to the overrepresented categories.

Response to biotic and abiotic stimuli and defense response. Analysis of overrepresented functional categories showed a significant enrichment of genes involved in response to biotic or abiotic stimuli (Fig. 3A); 80 genes related to stress or defense response were upregulated in infected shoots and 91 were regulated in infected roots (39 up- and 52 downregulated), among them pathogenesis-related (PR) proteins, disease resistance proteins, glutathione-S-transferases, chitinases, auxin-

induced proteins, and heat-shock proteins. On the whole, the response to biotic stress appears activated in both organs: in total, 15 genes were in common. The response to abiotic stress appears generally activated in shoots (responses to toxin and water) and repressed in roots (responses to toxin, water, iron, and hyperosmotic salinity). Finally, categories involving reactive oxygen species (ROS) were overrepresented in roots but not in shoots.

Photosynthesis and energy-related categories. In shoots, there was widespread repression of genes involved in the photosynthesis and related categories as well as in carbohydrate metabolism (173 genes) (Fig. 3B). Among them, most of the genes related to chlorophyll a/b-binding, ribulose biphosphate carboxylase, and starch metabolism were repressed.

Amino acid metabolism. An opposite trend in gene regulation between shoots and roots was observed with respect to GO categories involved in amino acid biosynthesis and metabolism: 82 genes were downregulated in shoots and 18 upregulated in roots (Fig. 3C). Only two categories were overrepresented in both organs, albeit with opposite regulation: “arginine metabolic process” and “proline metabolic process”. It is worth noting that, in roots, we found several upregulated genes not involved in the biosynthesis of proteins (“β-alanine metabolic process” and “nonprotein amino acid metabolic process”), suggesting that the virus can specifically activate production of secondary metabolites.

Secondary metabolism. Genes involved in secondary metabolism (Fig. 3D) were significantly up- or downregulated in roots whereas, in shoots, only downregulation was observed, with categories involved in vitamin, steroid, and spermidine biosynthesis. Upregulation, only in roots, of genes involved in phenylpropanoid biosynthesis suggests an increased production of compounds such as chalcones, cinnamic acids, flavonoids, and lignins. Because chorismate is a precursor of salicylic acid (Metraux 2002), a plant hormone with antiviral activity (Singh et al. 2004), it is worth noting that genes related to chorismate biosynthesis are activated, but only in roots. On the contrary, for genes related to abscisic acid (ABA) and carotenoid metabolism, only downregulation in roots was observed. Finally, the

Table 1. Validation of microarray data^a

SGN ID	Description	qRT-1*	qRT-2*	qRT-3*	qRT-4**	Microarray	Validated
Shoots							
SGN-U230270	Receptor-like protein kinase ark1	4.87	7.00	5.93	5.02	3.50	Yes
SGN-U231884	myb Transcription factor myb117	4.33	5.03	5.17	4.53	3.72	Yes
SGN-U222064	ap2 Domain transcription factor-like	3.73	2.07	2.03	2.71	1.47	Yes
SGN-U219598	Putative similar to receptor protein kinase	4.60	6.27	6.43	6.02	2.67	Yes
SGN-U223075	tcp Family transcription	-0.47	-0.63	1.46	-1.41	-0.64	No
SGN-U215018	Acid phosphatase	-5.00	-5.20	-3.83	-6.93	-3.47	Yes
SGN-U212743	33-kDa precursor protein of oxygen-evolving complex	-6.15	-1.00	-1.07	-1.93	-0.69	Yes
SGN-U212564	Fructose biphosphate aldolase	-1.28	-0.73	-1.29	-1.33	-1.28	Yes
SGN-U213526	Phosphoglycerate kinase	-7.19	-1.88	-1.80	-1.58	-1.54	Yes
SGN-U218904	Chlorophyll a-b binding protein 1B, chloroplast precursor	-2.86	-3.00	-2.73	-2.75	-1.10	Yes
Z24743	s-Adenosyl-l-methionine synthetase	-4.46	-1.16	-1.60	-1.51	-0.75	Yes
SGN-U225521	Ribulose biphosphate carboxylase	-1.10	-0.63	-0.69	-1.87	-1.94	Yes
Roots							
SGN-U231884	myb Transcription factor myb117	6.03	5.00	5.40	3.70	2.93	Yes
SGN-U222064	ap2 Domain transcription factor-like	4.55	1.97	5.35	3.41	1.63	Yes
SGN-U219598	Putative similar to receptor protein kinase	0.97	0.80	1.77	1.25	0.75	Yes
SGN-U214429	Immediate-early salicylate-induced glucosyltransferase	2.06	1.75	1.94	1.71	1.92	Yes
SGN-U212922	pr Protein	6.55	5.13	6.81	2.97	4.48	Yes
SGN-U216827	Cysteine protease 8	5.30	4.19	4.47	4.67	3.74	Yes
SGN-U226141	Heat-shock transcription factor	3.40	1.39	1.75	1.26	1.58	Yes
SGN-U213732	Dicer-like 2	2.44	1.90	1.88	3.07	1.58	Yes
SGN-U217373	Basic helix-loop-helixfamily protein	-1.37	-0.80	-0.70	-1.43	-0.84	Yes
SGN-U226497	Nodulin-like protein	-5.00	-4.09	-3.16	-2.23	-2.62	Yes

^a Quantitative reverse-transcription polymerase chain reaction was performed on the four biological replicates (qRT-1, -2, -3, and -4) in shoots and roots. Expression values are indicated as log ratio. Ubiquitin was used as reference gene; * indicates samples used in the microarray experiment; ** indicates independent biological replicate.

Fig. 3. Over-represented Gene Ontology (GO) categories ($P < 0.001$) in shoots (gray) and roots (black) of *Tomato spotted wilt virus* (TSWV)-infected plants. Bars represent the percentage of regulated genes in addition to those expected by chance. Up- and downregulation is shown to the right and left of the central Y axis, respectively. GO categories were organized in five groups. Numbers of genes associated with each GO are indicated in parentheses. **A**, Response to biotic and abiotic stimuli and defense response; **B**, photosynthesis and energy related categories; **C**, amino acid metabolism; **D**, secondary metabolism; and **E**, signal transduction.

biosynthesis of spermidine, an important factor together with other polyamines in plant response to stress, was activated in roots and repressed in shoots.

Signal transduction. No categories in this group were overrepresented in roots (Fig. 3E). In shoots, five categories were overrepresented, involving 70 genes (51 up- and 19 downregulated). Three categories were related to response to hormone (auxins, ABA, and salicylic acid) stimulus, in agreement with the importance of hormones in plant–pathogen interactions (Bari and Jones 2009). Interestingly, all the genes involved in the “mitogen-activated protein kinase-kinase-kinase (MAPKKK) cascade” were upregulated. The only category overrepresented in this group among downregulated genes was the “transmembrane receptor protein tyrosine kinase signaling pathway”.

DISCUSSION

Activation of common pathways involved in viral infection.

Transcriptomic information on virus-infected plants is limited and concerns the aerial parts but never the roots. On *Tospovirus* spp., only one case can be found: Senthil and co-workers (2005) analyzed the changes in gene expression in systemically infected leaves of an experimental host plant (*N. benthamiana*) infected by INSV, a virus that usually infects ornamental plants, utilizing heterologous (potato) microarrays. Moreover, to our knowledge, there are no reports dealing with a *Tospovirus* sp. infecting a natural host plant like tomato.

We first compared our results on TSWV-infected tomato with those obtained on INSV-infected *N. benthamiana*, a solanaceous plant like tomato (Senthil et al. 2005) (Supplementary Table S6). In both cases, the upper uninoculated leaves were used at a time point when systemic symptoms first appeared. This analysis showed that 1,139 genes were regulated in both systems; among them, many genes involved in photosynthesis and primary metabolism, hormone metabolism, and response to hormone signaling as well as genes related to biotic and abiotic stress responses. A series of genes involved in chromatin organization, DNA packaging, and protein–DNA complex assembly were also differentially expressed in leaves infected by both viruses. This indicates that the two *Tospovirus* spp. induce a similar basal response in the two plant species.

Dardick (2007) analyzed the transcriptional responses of *N. benthamiana* leaves systemically infected by three positive-sense RNA viruses (ToRSV, PPV, and PNRSV). Comparison with our data showed that only a small pool of genes is commonly regulated in response to TSWV and at least one of the three viruses investigated by Dardick. Overlapping pools were rich in genes related to pathogenesis, upregulated upon virus infection, and genes involved in photosynthesis or related processes, commonly downregulated. An interesting case is represented by SGN-U213594 (FC = 0.62), annotated as *dwarf1/diminuto (DWF1)*, downregulated in aerial tissues infected by both TSWV and ToRSV. In *Arabidopsis*, it is involved in cellular elongation and mutants have a dwarf phenotype; therefore, we suggest its involvement in the stunting observed with both virus infections.

Defense-related genes.

Activation of defense-related genes in response to infection induced by positive single-stranded (ss)RNA (Whitham et al. 2003; Carr et al. 2006; Babu et al. 2008; Ventelon-Debout et al. 2008), double-stranded RNA (Shimizu et al. 2007), negative ssRNA viruses (Senthil et al. 2005), and viroids (Itaya et al. 2002) has been observed in several plants. In TSWV-infected tomato PR, glutathione *S*-transferase (GST), and other defense-related proteins were upregulated. Moreover, several genes involved in ROS metabolism and implicated in stress

response such as catalases, superoxide dismutases, and peroxidases were differentially regulated, in agreement with previous reports on other plant–virus combinations. Alteration of some photosynthetic parameters could be associated also with ROS generation. Downregulation of genes involved in energy metabolism in shoots and roots and those involved in photosynthesis, pigment metabolism, and photorespiration in shoots indicate a general suppression of primary metabolism that could be correlated with the development of systemic symptoms (arrest of growth and bronzing on leaves).

Induction of heat-shock proteins (HSP) is a well-known reaction to biotic and abiotic stimuli. Among the HSP represented on the TOM2 array, HSP70 and HSP83 were upregulated in TSWV-infected roots and shoots, as was observed in *Arabidopsis* infected by plus-strand RNA viruses (Whitham et al. 2006) as well as in *N. benthamiana* infected by INSV and SYNIV, two minus-strand RNA viruses (Senthil et al. 2005). In the case of HSP90, two different genes were annotated to it. Both were found to be induced in TSWV-infected tissues; one of them (SGN-U212643, FC = 1.57) was upregulated only in shoots, as already observed by Senthil and colleagues in INSV and SYNIV infections; the other (SGN-U212639, FC = 1.73) was induced in roots. Another case where a different expression in roots and shoots was evident is the GST gene family. Of the 14 regulated genes, 12 were up- or downregulated in either shoots or roots. The regulation of several gene families, according to our analysis criteria, may suggest an organ-specific regulation like that described above for HSP90 and GST, indicating that viral infection could be perceived differently in distinct parts of the plant.

Transcription factors.

During TSWV infection, the regulation of many transcription factors and DNA- or RNA-related genes was observed in both shoots and roots.

WRKY is a recently emerged family of plant transcription factors involved in response to both abiotic and biotic stimuli (Eulgem and Somssich 2007). In particular, WRKY6 is important for its ability to regulate the expression of defense genes, such as PR-1 in *Arabidopsis* and *N. benthamiana* (Robatzek and Somssich 2002; Whitham et al. 2006). In our TSWV-infected tomato plants, 17 WRKY transcription factors were found differentially expressed; among them, 16 were regulated in shoots (15 upregulated and 1 downregulated), including WRKY6, while 7 were regulated in roots (all upregulated).

Other characterized transcription factor families, such as AP2 and MYB, were also differentially expressed during infection, with 14 and 17 regulated genes, respectively. However, only four genes maintained the same up- or downregulation in both organs. In *Arabidopsis*, rice and tomato expression of AP2 transcription factors, and especially ethylene (ET) response factors, are regulated by plant hormones such as jasmonic acid (JA), salicylic acid, and ET as well as by pathogen challenge (Gutterson and Reuber 2004). In *Arabidopsis*, a large number of MYB transcription factors are strictly related to development and stress or hormone treatments (Chen et al. 2006). Moreover, under normal conditions, these genes are usually expressed preferentially in one or a few organs or tissues, including roots (Czechowski et al. 2004).

The large number of up- or downregulated transcription factor genes that we detected, as well as their organ-specific expression patterns, is consistent with the existence of a highly complex regulatory network underlying the physiological response to viral infection. The upregulation of transcription factors, particularly in the WRKY and AP2 families, could be related to expression of TSWV protein NSs, a silencing suppressor. In fact, it is known that the expression of certain viral

suppressors of RNA silencing causes over-accumulation of mitochondrial (mi)RNA targets and that the majority of known plant miRNA targets encode transcription factors or other regulatory proteins (Jones-Rhoades et al. 2006).

Secondary metabolism and hormones.

As illustrated by the functional analysis (Fig. 3D), the secondary metabolism is strongly affected by virus infection.

In TSWV-infected tomato plants, several genes involved in polyamine biosynthetic pathways, small basic molecules involved in several biological processes in higher plants, were differentially regulated (Fig. 4). In shoots, genes coding for arginase, ornithine decarboxylase, spermidine synthase, spermine synthase, and PAO were all downregulated whereas, in roots, the downregulation of arginase and PAO was maintained while spermidine synthase enzymes were all upregulated. A connection between polyamines biosynthesis and the hypersensitive response (HR), as already suggested in the literature (Takahashi et al. 2004; Lazzarato et al. 2009), can be seen in our dataset because over-representation of the category “spermidine biosynthetic process” in shoots (downregulation) and roots (upregulation) was backed by over-representation of categories involved in HR (oxygen and ROS, the first two categories in Fig. 3A) only in roots. Because overexpression of spermidine synthase in *Arabidopsis* induced increased tolerance to various stresses (Kasukabe et al. 2004), we speculated that TSWV succeeds in keeping low levels of polyamines in the systemically infected leaves, thus maintaining a compatible interaction with the host plant and probably also reducing resistance to environmental stresses.

The biosynthetic pathway of polyamines is connected, via *S*-adenosyl-methionine, to the synthesis of ET, a hormone in-

involved in regulating plant defense responses (Bari and Jones 2009). The two key enzymes in its synthesis, 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase, were mostly activated in the shoots (Fig. 4), whereas no particular trend could be observed in the roots, except for a limited down-regulation of ACC oxidase, suggesting an increase in ET content in shoots. In this organ, ET receptor genes as well as genes involved in ET signal transduction (CTR1 and MAPK3) and some ET-responsive element-binding factors were induced. An analogous response, even if less evident, was observed in the roots, where some ET-responsive genes were upregulated.

Previous microarray experiments revealed that expression of JA and ET biosynthesis and JA- and ET-dependent defense-related genes was reduced in ABA-treated plants (Mohr and Cahill 2007). ABA has a key role in developmental processes such as seed germination, growth, and stomatal aperture and in response to abiotic stresses such as drought, low temperature, and salinity; less defined is its role in disease resistance (Mauch-Mani and Mauch 2005; Verslues and Zhu 2007). Tobacco leaves systemically infected by TMV show increase of ABA content (Whenham et al. 1986), and treatment with exogenous ABA can improve virus resistance (Fraser 1982). By contrast, *Arabidopsis* and tomato ABA-deficient mutants show increased resistance to pathogenic fungi (Audenaert et al. 2002; Mohr and Cahill 2003), and ABA-treated plants are more susceptible to avirulent bacteria (Mohr and Cahill 2007).

In our case, differences in regulation of genes involved in ABA metabolism were observed between shoots and roots: several genes involved in response to ABA were upregulated in infected shoots while several genes associated with ABA metabolism were differentially expressed in roots; among them, 9-cis-epoxycarotenoid dioxygenase (SGN-U214605, FC = 3.14), a key enzyme of ABA synthesis (Burbidge et al. 1999), and four genes (SGN-U213423, FC = 0.35; SGN-U232476, FC = 0.47; SGN-U213424, FC = 0.63; and SGN-U222532, FC = 0.30) with high similarity to ABA 8'-hydroxylase CYP707A1 of *Solanum tuberosum*, a key enzyme of ABA catabolism (Krochko et al. 1998; Kushihiro et al. 2004). ABA turnover in plants is rapid even in unstressed plants (Nambara and Marion-Poll 2005; Verslues and Zhu 2007). In the root, the observed induction of genes related to ABA synthesis and the repression of those involved in its catabolism might suggest an increase of the hormone in this organ. Therefore, the greater number of ABA-responsive genes regulated in shoots (24 in shoots against only 11 in roots) lead us to suggest that, during viral infection, ABA could be produced in roots and transported to shoots, where it could promote the expression of ABA-responsive genes. In support of the root-to-shoot transport hypothesis, we observed differential expression, both in shoots and roots, of two glucosyltransferase genes (SGN-U214429, FC_{Shoot} = 7.40 and FC_{Root} = 3.80; and SGN-U216652, FC_{Shoot} = 11.25 and FC_{Root} = 0.45), which show high similarity with ABA-inducible glucosyltransferase (AOG) of adzuki bean. AOG catalyzes the conjugation of ABA with glucose, a process related to long-distance transport of ABA, presumably in a root-to-leaf direction (Nambara and Marion-Poll 2005). Further analysis is required to elucidate the role of ABA in plant-virus interaction and possibly the dynamic of long-distance signaling through the plant in response to pathogen infection.

Conclusions.

This appears to be the first study of transcriptional response of tomato to systemic infection by TSWV, and the parallel analysis of both shoots and roots has highlighted several interesting features. The number of genes regulated in shoots is approximately twice that regulated in roots (a fact that has not been reported

Fig. 4. Differential expression of genes in the polyamine and ethylene biosynthetic pathways. The prevalent regulation is indicated with arrows on the right of shoots (S) and roots (R). A complete list of the genes involved and their fold change is available in Supplementary Table S3. SAM syn, *S*-adenosyl-methionine synthase; SAM dec, *S*-adenosyl-methionine decarboxylase; Spermidine syn, spermidine synthase; Spermine syn, spermine synthase; PAO, polyamine oxidase; Orn dec, ornithine decarboxylase; Arg dec, arginine decarboxylase; ACCO, 1-aminocyclopropane-1-carboxylate synthase; ACCO, 1-aminocyclopropane-1-carboxylate synthase; SAM, *S*-adenosyl-methionine; dec SAM, decarboxylated *S*-adenosyl-methionine; ACC, 1-aminocyclopropane-1-carboxylate.

before) and the overlap between the two groups is limited, with only 15% of genes co-regulated. This indicates a different response of the two plant organs to infection, although the virus is present everywhere in similar concentration.

In shoots, an induction of genes related to defense and to signal transduction was evident, paralleled by a general repression of genes involved in primary and secondary metabolism, as well as in amino acid metabolism. In roots, expression of transcripts related to primary metabolism and signal transduction appeared unaffected by TSWV infection whereas a response to biotic stimuli was induced, although response to abiotic stress was generally repressed or unaltered. Genes involved in the amino acid metabolism were unaffected, except for those involved in synthesis of secondary compounds, where induction was evident.

Our results provide new insight into the biology of *Tospovirus* sp.–plant interactions in the economically important TSWV–tomato combination. Future studies, based on the transcriptomic data obtained, are expected to identify host genes required for successful virus infection and may lead to strategies to control this and related *Tospovirus* spp.

MATERIALS AND METHODS

Biological materials.

S. lycopersicum cv. Moneymaker seeds were surface-sterilized in 70% (vol/vol) ethanol with a few drops of Tween 20 for 3 min, dipped in 5% (vol/vol) sodium hypochlorite for 13 min, rinsed with distilled water, placed in petri dishes with 0.6% (wt/vol) agar, and incubated for 5 days in the dark (25°C) and for 4 days in the light. The seedlings were then transferred to pots containing sterile quartz sand.

Plants were maintained in a growth chamber at 22°C with 14 h of light and 10 h of darkness and watered twice per week, once with water and once with 125 ml of a modified Long-Ashton nutrient solution (Hewitt 1966). Plants were inoculated with a TSWV tomato isolate (T1012, IVV collection) from Italy 28 days after planting (at the four-leaf stage). Inoculum was prepared from systemically infected leaves of TSWV-infected tomato plants. Approximately 1 g of infected leaf tissue was homogenized in 10 ml of inoculation buffer (10 mM sodium diethyldithiocarbamate, 5 mM ET diamine tetracetic acid, and 20 mM sodium sulfite). Inoculum was applied to the upper side of leaves by gentle rubbing with Carborundum. Mock-inoculated plants, used as control, were subjected to the same protocol using noninfected leaf tissue.

Tissue printing.

To check virus infection, the freshly cut petiole of the youngest available leaf of every plant was printed on a positively charged nylon membrane (Roche, Mannheim, Germany); membranes were then hybridized with a digoxigenin-labeled TSWV-specific probe (Vaira et al. 1995).

Dot-blot analysis.

Semiquantitative analysis of virus concentration was performed using dot-blot hybridization. Two mock-inoculated samples were used as control. A starting quantity of 100 ng of total RNA and fivefold serial dilution was blotted onto a positively charged nylon membrane (Roche) using the Minifold system (Schleicher & Schuell, Dassel, Germany). Membranes were then hybridized with a TSWV-specific probe as described above.

RNA extraction.

Fourteen days after inoculation, plants were removed from pots and roots were washed to remove the sand; then, young shoots (including apex and young expanding leaf) and young

roots (the 6- to 7-cm terminal portions) from infected and mock-inoculated plants were harvested and immediately frozen in liquid nitrogen. Total RNA was purified using Trizol (Invitrogen, Carlsbad, CA, U.S.A.) according to the manufacturer's instructions. Quality and quantity of total RNA were checked using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, U.S.A.). For each experimental condition, three biological replicates were formed, each composed of equal amounts of RNA from three to five plants.

cRNA labeling and microarray hybridization.

For each biological replicate, 500 ng of total RNA was transcribed in cRNA and labeled with Cy3 and Cy5 fluorescent dyes using Low RNA Input Linear Amp Kit, (Agilent Technologies) following the manufacturer's protocol. Slides were treated following the pre-hybridization protocol provided by the manufacturer.

Microarray hybridization was performed using the Gene Expression Hybridization kit (Agilent Technologies). Post-hybridization was performed following the manufacturer's instructions with slight modifications. An additional wash step in 0.05× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 5 min and a dip in absolute ethanol were added before the final quick drying centrifugation. Dye-swap was performed for each biological replicate.

Data acquisition and analysis.

Slides were scanned using a two-channel confocal laser microarray scanner (G2565BA; Agilent Technologies) at a resolution of 10 µm and the laser power was set at 90%. Fluorescence data were processed using ImaGene software (version 5.6; BioDiscovery Inc., El Segundo, CA, U.S.A.) using default quality controls and segmentation values with appropriate adjustment according to the signal intensity of each slide. Normalization and analysis of microarray data were performed using Limma package (Smyth et al. 2005). Within and between arrays, normalization was performed (Lowess normalization). Significantly up- or downregulated genes were filtered for an FDR < 0.05 and expression ratio higher or lower than 1.5- or 0.67-fold, respectively. Cluster and TreeView programs (Eisen et al. 1999) were employed for clustering (hierarchical clustering) and visualizing microarray data.

Functional and metabolic analysis.

GO annotation was obtained using Blast2go software (Conesa et al. 2005), with default parameters. The lists of up- or downregulated genes were searched for overrepresented GO terms. *P* values were computed with Fisher's exact test and a *P* value < 10⁻³ was considered statistically significant (Bluthgen et al. 2005). To avoid too generic GO annotations, we considered only the overrepresented GO terms associated with fewer than 300 annotated genes in the whole TOM2 chip. This analysis was performed using a set of Perl and C programs available from the authors upon request.

Real-time qRT-PCR analysis.

Total RNA was treated with DNAase (Ambion, Foster City, CA, U.S.A.) according to the manufacturer's instructions and the RNA was subsequently quantified using a NanoDrop 1000 spectrophotometer. For each sample, 4 µg of total RNA was used to synthesize cDNA using Stratascript reverse transcriptase (Stratagene, La Jolla, CA, U.S.A.) and SUPERaseIn RNase Inhibitor (Ambion) according to the manufacturer's instructions. Primers (Supplementary Table S4) were designed using the Primer 3 software (Rozen and Skaletsky 2000) and selected from every SGN sequence within or close to the unique oligomer probe.

PCR reactions were carried out in 96-well optical plates in the iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, U.S.A.). Cycling parameters were as follows: 1 cycle at 50°C for 3 min (activation of UNG), 1 cycle at 95°C for 5 min (DNA polymerase activation), and 45 cycles each consisting of 15 s at 95°C (denaturation) and 1 min at 60°C (annealing and extension). Reactions were performed in triplicate in 20 ng of template cDNA, 200 nM gene-specific primers, and Platinum qPCR SuperMix UDG (Invitrogen) in a volume of 25 µl. PCR efficiency was determined for each set of primers by using standard curves with tomato DNA. Melting curve analysis was performed after each reaction.

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AUTHOR-RECOMMENDED INTERNET RESOURCES

The Gene Ontology Consortium website: www.geneontology.org
 Solanaceae Genomics Network: www.sgn.cornell.edu
 Tomato Functional Genomics database: ted.bti.cornell.edu
 ImaGene software: biodiscovery.com
 Primer 3 software: frodo.wi.mit.edu/primer3