Gene profiling in partially resistant and susceptible near-isogenic tomatoes in response to late blight in the field

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SUMMARY

In order to better understand resistance to *Phytophthora infestans* in tomato, we compared the global gene expression of the susceptible tomato, M82, with its more resistant near-isogenic line, 6-2 (IL6-2), under field conditions using a microarray with more than 12 800 tomato expressed sequence tags (ESTs). Because variance in the field was a major concern, we investigated the likelihood of false positives or false negatives and demonstrated that either probability was very low. The two isolines had indistinguishable constitutive gene expressions prior to inoculation. However, a few genes were particularly prone to variation in both isolines in the absence of *P. infestans*. Included among these genes were *catalase*, genes coding for pathogenesis-related proteins, *endochitinase* and *cytochrome P450*. In response to inoculation with *P. infestans*, a time course of gene expression identified 1248 transcripts that were similarly induced or repressed in each line, and 991 that were differentially expressed between the two lines. These differences provide hypotheses to explain the difference in resistance between the two isolines. For example, one hypothesis is that genes up-regulated in IL6-2 in response to inoculation with *P. infestans*, but not up-regulated in M82, contribute to the resistance in IL6-2. Using virus-induced gene silencing (VIGS), we were able to partially silence two such genes—one encoded a protein with homology to an *R* gene with the Toll/interleukin-1 receptor–nucleotide-binding site–leucine-rich repeat (TIR-NBS-LRR) motif (37O19) and the other encoded a peroxisomal membrane protein (35P7). Partial silencing of 37O19 reduced the resistance in IL6-2 ($P = 0.001$), but had no effect on the response of M82. Partial silencing of 35P7 reduced the resistance in IL6-2 moderately significantly ($P = 0.067$), but had no effect in M82. We expect that hypotheses developed from this gene expression study performed under field conditions will provide an important avenue to an accurate understanding of the genes involved in resistance.

INTRODUCTION

Late blight caused by *Phytophthora infestans* is one of the most devastating diseases of both tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) worldwide (Fry, 2008). The causal agent of the Irish potato famine in the mid-1800s still threatens global tomato and potato production, causing billions of dollars annually in yield losses and control strategies (Haverkort *et al.*, 2008). Because of the historical significance and higher economic impact of potato late blight, the focus of most of the work has been performed using the *P. infestans*–potato pathosystem as a model. However, tomatoes are often affected—as illustrated by the pandemic in northeastern USA in 2009 (Moskin, 2009).

To understand the molecular mechanisms of late blight resistance, investigations of global gene expression in the host have been performed, and genes differentially regulated between compatible and incompatible interactions, or between compatible and less compatible interactions, have been identified. Avrova *et al.* (2004) used suppression subtractive hybridization (SSH) to identify potato genes up-regulated in *R2*-mediated resistance. They found eight genes that were up-regulated early in an incompatible interaction, but that were not induced or were induced at later time points in a compatible interaction. Ros *et al.* (2004, 2005) used a cDNA macroarray to quantify the differential expression of 182 genes in potato cultivars with different levels of late blight resistance. Wang B.L. *et al.* (2005) monitored gene expression at nine time points in a moderately resistant potato cultivar following late blight infection using a cDNA microarray containing 1009 expressed sequence tags (ESTs) from an SSH library. A study from our laboratory used a cDNA microarray to identify differentially expressed potato genes in a compatible interaction (Restrepo *et al.*, 2005). When a subset of genes was investigated in the incompatible interaction, *plastidic carbonic anhydrase*, which was down-regulated significantly in the compatible interaction, showed a transient increase in expression in the incompatible interaction before being down-regulated. When this gene was silenced in *Nicotiana benthamiana* (as a proxy for *S. tuberosum*), the plant became more susceptible to *P. infestans* (Restrepo *et al.*, 2005).

A common theme in these studies is that the inoculations were conducted in controlled conditions on glasshouse-grown plants

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and/or detached leaflets, and not in the field. These controlled conditions have obvious advantages as they enable one to reduce many sources of environmental variation, such as humidity, light intensity, air temperature and photoperiod, among others. However, these highly controlled conditions may render the plant highly responsive to nontreatment subtle environmental cues (Edwards *et al.*, 2010; Krug and Fink, 1989; Wang *et al.*, 1999).

In contrast with plants grown in a growth chamber, plants in the field are subjected to a dynamic and diverse environment. This environment is characterized by abiotic and biotic changes, including temperature, wind, insects and pathogens. It is therefore well recognized that plants grown in a glasshouse differ from plants grown in the field (i.e. see Dalal *et al.*, 1966). It is also known that genotypic expression is often dependent on the environment, and genetic parameters estimated in glasshouse experiments may differ from those expressed in the field (Falconer and Mackay, 1996; Gillespie and Turelli, 1989). In strawberry, resistance to powdery mildew in a glasshouse test did not correlate well with field evaluation when the infection pressure was low (Nelson *et al.*, 1995). When the goal is to identify genes that confer resistance in the field, it follows that field-grown plant material should be used.

To date, there have been no reports on the global gene expression of susceptible and resistant tomatoes in response to late blight infection under field conditions. Near-isogenic lines should respond similarly to the environment, so that a comparison of near-isogenic plants should not reflect different responses caused by the environment, and differences in response to infection should be specific to the particular interaction. The development of a set of introgression lines (ILs) by Eshed and Zamir (1995), each of which contains a chromosome segment from *Lycopersicon pennellii* in the background of tomato cultivar M82, enabled the demonstration that isoline 6-2 (IL6-2), containing part of chromosome 6 of *L. pennellii*, showed considerable late blight resistance in comparison with the susceptible parent M82 (Smart *et al.*, 2007). The pathogen in IL6-2 grew more slowly and showed less sporulation than in M82. The quantitative trait locus (QTL) associated with the introgression explained 25% of the phenotypic variance in a segregating F2 population of a cross between *L. pennellii* and *L. esculentum* (M82) (Smart *et al.*, 2007). Thus, IL6-2 and M82 constitute near-isogenic lines that differ in late blight resistance.

In this study, the TOM1 tomato cDNA microarray (Alba *et al.*, 2004), containing more than 12 800 EST clones, was used to monitor gene expression in the field in the near-isogenic tomato genotypes M82 and IL6-2, with or without *P. infestans* inoculation. The objectives were as follows: (i) to determine the extent of variation and variance in gene expression among field-grown plants; and (ii) to identify candidate genes for involvement in the resistance in IL6-2. We demonstrated that the variance was sufficiently low to enable us to detect differences in gene expression—

despite the unexpected discovery of a small number of genes that appear to be particularly likely to show variable expression in the absence of attack by *P. infestans*. It was further demonstrated that gene expression in the two genotypes was very similar before inoculation, but differed significantly after inoculation. When two differentially expressed genes (induced in IL6-2, but not in M82) were partially silenced, the resistance of IL6-2 was diminished, but the resistance of M82 was not changed.

RESULTS

Variance in gene expression among field-grown tomato plants

A major concern was that the variance in gene expression of field-grown plants would be so high that it would be difficult to identify treatment differences. This situation would lead to both false negatives (real differences masked) and false positives (high variance leading to incorrect inferences of difference). We therefore assessed the variance in gene expression in the absence of a treatment effect. At 4 weeks after transplantation, tissue was collected individually from six plants of each genotype. Samples from the same genotype were randomly assigned to two groups (A and B) of three plants each, and each plant from group A was compared against a different plant in group B using the TOM1 tomato cDNA microarray (three biological replicates). After filtering out low-quality data points and normalizing the data using MIDAS (see Experimental procedures), data were analysed for those transcripts that were detectable in all three biological replicates.

Within a genotype, the mean expression ratio between the two groups (A and B) was calculated. Because all plants were from the same treatment, there should have been no significant differences between the two groups. For the vast majority of transcripts from either genotype, this expectation was realized. Less than 0.2% of transcripts had a between-group expression ratio larger than 1.5 and no transcript had differences greater than 1.8-fold (Table 1). When a small expression ratio requirement was applied, false positives could be effectively avoided. For example, no transcript passed the $P = 0.01$ and expression ratio ≥ 1.5 criteria. When the data were analysed by significance analysis of microarray (SAM) (Tusher *et al.*, 2001) and the false discovery rate (FDR) was relaxed to 10%, only three transcripts in IL6-2 and two in M82 were identified as having significant differences in between-group expression, and none showed a fold expression ratio greater than 1.6 (Table 1).

Stress-response genes were more variable in the field

To examine whether some genes were more likely than others to be detected as differentially expressed in the absence of a treatment effect, we analysed the variance of the $\log_2$ expression ratio

Table 1 Variation in gene expression between two randomly assigned groups (A and B) of field-grown noninoculated tomato plants.

Genotype	Expression ratio A/B	Number of transcripts	Number of transcripts identified as $\log_2(A/B) > 0$ by <i>t</i> -test with <i>P</i> value			
			0.05	0.01	0.001	0.0001
IL6-2	≥ 1.0	10317	520	114	12	0
	≥ 1.1	1284	172	45	3	0
	≥ 1.2	267	24	7	0	0
	≥ 1.3	107	7	2	0	0
	≥ 1.4	47	2	0	0	0
	≥ 1.5	17	1	0	0	0
	≥ 1.6	1	0	0	0	0
M82	≥ 1.7	0	0	0	0	0
	≥ 1.0	8638	355	57	5	0
	≥ 1.1	1967	151	25	4	0
	≥ 1.2	441	30	3	0	0
	≥ 1.3	109	8	1	0	0
	≥ 1.4	32	3	1	0	0
	≥ 1.5	13	2	0	0	0
	≥ 1.6	4	1	0	0	0
	≥ 1.7	1	0	0	0	0

of the 7294 transcripts detectable in all three biological replicates of healthy plants for each tomato genotype. For any given transcript, the $\log_2$ expression ratio between two plants in the same group has an expected value of zero. Deviation from this expected value represents the random difference between two plants or the measurement error, and thus its variance is a measurement of the variability of the expression of individual transcripts.

We ranked the 7294 transcripts in each genotype based on the observed variance of the $\log_2$ expression ratio, with the transcript showing the largest variance at the top. If transcripts are equally stable, individual transcripts will have equal probability to be at any position in the rank, and the chance that it appears in the top *n* list in both genotypes can be calculated as $(n/7294)^2$.

However, the observed values were much higher, and the ratio between the observed and expected values increased for smaller *n* (Table 2). For example, 34 transcripts were among the top 200 transcripts listed in both genotypes (Table 2). This was more than six times the expected value, indicating that the expression of most of these transcripts was intrinsically more variable than that of other transcripts. Sequences were available for 25 of the 34 transcripts and, for the transcripts whose sequences showed similarity to genes of known function, the majority had predicted functions involved in the stress response, such as catalase, pathogenesis-related (PR) proteins, endochitinase and cytochrome P450, etc. (Table 3).

Differences in constitutive expression between the near-isogenic genotypes

To detect potential differences in constitutive gene expression, three noninoculated IL6-2 plants were compared with three noninoculated M82 plants collected at the same time point (12 h before inoculation). In SAM, even when the FDR was increased to 30%, only seven transcripts appeared to be differentially

Table 2 Comparison between observed and expected number of transcripts shared by M82 and IL6-2 among the top *n* transcripts with highest variance between randomly assigned groups of field-grown noninoculated tomato plants. Only the 7294 transcripts for which data from all three biological replicates were available for both tomato genotypes were included in the analysis.

<i>n</i>	Observed	Expected*	Observed/expected
200	34	5.5	6.2
500	118	34.3	3.4
1000	276	137.1	2.0

*Expected values were computed as $(n/7294) \times (n/7294) \times 7294$.

expressed. The mean expression ratios of six of the seven transcripts were less than 1.3 and the other was less than 1.5. In Student's *t*-test, no transcript surpassed the $P = 0.01$ and expression ratio ≥ 1.5 criteria, and only one surpassed $P = 0.05$ with the same expression ratio requirement. This was well within the variations observed among field-grown plants of the same genotype (Table 1). We therefore found no significant quantitative difference in constitutive expression between these two tomato genotypes.

Analysis of differentially expressed genes in response to inoculation

To determine the difference in gene expression of M82 relative to IL6-2 in response to *P. infestans*, a time course analysis was performed. Plants were inoculated with a tomato specialized isolate (US-17) and tissue was collected at 12, 36 and 60 h after inoculation (hai). In addition, a few plants of each genotype were removed to the glasshouse for observation. By 4 days following inoculation, symptoms were observed on all plants in the glasshouse, and these plants were then destroyed. The microarrays were probed using samples from healthy versus infected tissue for each cultivar at each time point. This design allowed us to identify

Table 3 List of 34 genes in both isolines that were more variable than expected by chance. These genes were likely to be detected as differentially expressed in the absence of a treatment effect.

Array ID	SGN_U	Automatic annotation
1-1-3.1.20.8	SGN-U143191	<i>Catalase isozyme 1</i>
1-1-4.2.9.3	SGN-U143238	<i>Pathogenesis-related (PR) leaf protein 4 precursor (P4)</i>
1-1-5.2.16.3	SGN-U143238	<i>PR leaf protein 4 precursor (P4)</i>
1-1-1.4.19.19	SGN-U143242	<i>PR leaf protein 6 precursor (Ethylene-induced protein P1) (P14) (P14A)</i>
1-1-2.3.14.10	SGN-U143242	<i>PR leaf protein 6 precursor (Ethylene-induced protein P1) (P14) (P14A)</i>
1-1-2.4.16.4	SGN-U143242	<i>PR leaf protein 6 precursor (Ethylene-induced protein P1) (P14) (P14A)</i>
1-1-5.1.9.3	SGN-U143242	<i>PR leaf protein 6 precursor (Ethylene-induced protein P1) (P14) (P14A)</i>
1-1-2.2.15.15	SGN-U143859	<i>Hypothetical protein precursor (clone TPP11)—tomato</i>
1-1-5.4.13.12	SGN-U143859	<i>Hypothetical protein precursor (clone TPP11)—tomato</i>
1-1-7.1.9.2	SGN-U144222	<i>Gonidia forming protein GlsA [Lilium longiflorum]</i>
1-1-1.2.9.4	SGN-U144297	<i>Acidic 26-kDa endochitinase precursor</i>
1-1-6.3.14.8	SGN-U144297	<i>Acidic 26-kDa endochitinase precursor</i>
1-1-8.3.14.8	SGN-U144297	<i>Acidic 26-kDa endochitinase precursor</i>
1-1-5.2.1.16	SGN-U144894	<i>Histone H2A</i>
1-1-3.3.16.10	SGN-U145329	<i>Unknown protein [Oryza sativa (japonica cultivar-group)]</i>
1-1-1.1.1.16	SGN-U146045	<i>Histone H1.41—garden pea</i>
1-1-2.3.16.4		<i>No sequence</i>
1-1-7.1.18.9	SGN-U147888	<i>Putative purple acid phosphatase precursor [Ipomoea batatas]</i>
1-1-7.1.19.5	SGN-U147937	<i>Expressed protein [Arabidopsis thaliana]</i>
1-1-8.3.15.21	SGN-U148300	<i>Expressed protein [Arabidopsis thaliana]</i>
1-1-7.2.19.14	SGN-U148649	<i>Probable α-glucosidase (EC 3.2.1.20)—potato</i>
1-1-5.1.19.15	SGN-U149297	<i>VPS13-like protein [Arabidopsis thaliana]</i>
1-1-2.2.16.10	SGN-U152005	<i>Cytochrome P450, putative [Arabidopsis thaliana]</i>
1-1-7.1.15.17	SGN-U154221	<i>No hits found</i>
1-1-1.3.16.4	SGN-U155534	<i>Ribulose biphosphate carboxylase small chain 3A/3C</i>
1-1-1.1.6.20		<i>No sequence</i>
1-1-1.3.14.10		<i>No sequence</i>
1-1-1.3.19.19		<i>No sequence</i>
1-1-1.4.16.4		<i>No sequence</i>
1-1-2.2.16.4		<i>No sequence</i>
1-1-3.1.7.15		<i>No sequence</i>
1-1-4.1.15.1		<i>No sequence</i>
1-1-7.1.7.5		<i>No sequence</i>
1-1-7.3.12.7		<i>No sequence</i>

those genes that were differentially expressed between the two cultivars throughout the time course, as well as those induced or repressed similarly in both interactions. Three biological repetitions with two technical replicates (dye swaps) were used for each comparison.

Data were analysed using a two-factor (cultivar and time point) analysis of variance (ANOVA) and SAM using stringent criteria. In ANOVA, a gene-shaving step was first applied: only those transcripts that had at least a two-fold change in expression for at least one time point in at least one of the tomato genotypes were retained. Only 3007 transcripts passed this step. Using ANOVA with a $P = 0.001$ threshold, 883 transcripts were found to be differentially expressed between M82 and IL6-2 (Table S1, see Supporting Information). Visual inspection of the data indicated that some transcripts were likely to be differentially expressed between these two genotypes at some time point(s), but were not identified by ANOVA because of missing values at other time point(s). We complemented ANOVA using SAM to analyse data at each time point. In SAM, a two-fold change criterion was applied and FDR was set to zero. SAM identified 727 transcripts differentially expressed between M82 and IL6-2 (Table S1), 108 of which had not been identified by ANOVA. In total, ANOVA and SAM identified 991 differentially expressed transcripts, with 619 transcripts common to both methods. Most transcripts identified by SAM, but not by ANOVA, had one or more missing values. ANOVA was also used to detect transcripts similarly induced or repressed in M82 and IL6-2. In total, 1248 transcripts were identified, with 513 being induced (Table S2, see Supporting Information) and 735 repressed (Table S3, see Supporting Information).

Microarray validation using quantitative real-time polymerase chain reaction (qRT-PCR)

To validate the microarray results, we analysed (using qRT-PCR) the expression profile of five genes that were differentially expressed between M82 and IL6-2 (Fig. S1, see Supporting Information). Most of the genes had similar profiles with both techniques. In general, qRT-PCR proved to be more sensitive than microarray, showing a greater difference (either induction or repression). A discrepancy between qRT-PCR and microarray was observed for *B-glucanase (Glu-B)* in both genotypes at 60 hai, and *glutathione S-transferase (GST)* at 60 hai.

Clustering analysis

The expression profiles of the 991 transcripts identified by ANOVA and SAM were grouped into 10 clusters using the *K*-means clustering method with Pearson-uncentred distance (Fig. 1). Cluster 1 included 135 transcripts that were induced in IL6-2, but showed no significant change in M82. Cluster 2 included 286 transcripts that were repressed in IL6-2, but showed no significant change in

Fig. 1 Cluster analysis of differential gene expression between near-isogenic tomato genotypes M82 and IL6-2 in response to late blight infection in the field, using a *K*-means clustering method with Pearson-unc centred distance. Error bars are the 99% confidence interval of the mean. For a complete list of the genes in each cluster, see Table S3.

M82. Clusters 3 and 4 contained 73 and 165 transcripts, respectively, that were induced or repressed, respectively, in M82, but showed no significant change in IL6-2. Cluster 5 contained 94 transcripts that were induced in IL6-2 and repressed in M82, and cluster 6 contained 69 transcripts that were repressed in IL6-2 and induced in M82. The other four clusters included transcripts that were induced or repressed in both genotypes, but differed in the timing and/or level of differential expression. The transcripts in the 10 clusters are shown in Table S4 (see Supporting Information).

Differences in gene expression between IL6-2 and M82 were identified at each time point. These differences are illustrated using mapman (<http://mapman.gabipd.org>) in Figs 2–4 (an interactive, higher resolution version of these figures generated by mapman is available at: <http://mapman.gabipd.org/web/guest/tomato-time-course>). At 12 hai, expression differences in 11 transcripts were analysed; most transcripts represented genes which are putatively involved in biotic stress (Fig. 2). A receptor-like protein kinase was induced in IL6-2 more than two-fold at 12 hai, whereas expression did not change in M82. Another protein kinase was induced in M82, starting at 12 hai, but not in IL6-2. Two transcripts of 1-aminocyclopropane-1-carboxylate oxidase [ACC oxidase—catalysing the last step of ethylene biosynthesis; Yang and Hoffman, 1984] showed higher expression in M82. Other transcripts differentially expressed at 12 hai encoded a putative disease resistance protein, a proteinase inhibitor, a gene coding for a calmodulin-binding region, an RNA-binding protein and a

stress-induced late-embryogenesis protein. Another interesting difference between the isogenic lines at 12 hai was that IL6-2 transcription factors and signalling pathways were induced, whereas they were repressed slightly in M82. However, the gene coding for GST was first induced in M82, but did not show any change in IL6-2 at this early time point (Fig. 2).

At 36 hai, the differences in gene expression between the two isogenic lines became more pronounced. Signal transduction and transcription factors (ERF, WRKY and MYB) were strongly induced at this time point in IL6-2, but suppressed in M82. In addition, *glucanase* and *mitogen-activated protein kinase* (MAPK)-encoding genes showed a high level of expression in IL6-2, whereas no induction was observed in M82. Heat shock proteins, secondary metabolites, auxins and cell wall-related genes were down-regulated in both isogenic lines (Fig. 3).

Finally, at 60 hai, the differences in gene expression between the isogenic lines were even more pronounced (Fig. 4). Genes associated with abiotic stress, heat shock proteins and brassinosteroids were repressed in IL6-2. In contrast, in M82, many of the genes involved in abiotic stress and heat shock proteins were not down-regulated and the brassinosteroid genes were up-regulated. Genes associated with secondary metabolites and proteolysis were highly induced at this time point in IL6-2, but their expression remained almost unchanged in M82. In IL6-2, *glucanase* expression increased over time, reaching its highest expression at 60 hai; in M82, it was down-regulated (Fig. 4).

Fig. 2 Genes that were differentially expressed between near-isogenic tomato genotypes IL6-2 and M82 at 12 h after infection with *Phytophthora infestans* using mapman (<http://mapman.gabipd.org>); red denotes down-regulated pathways and blue denotes up-regulated pathways. ABA, abscisic acid; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; SA salicylic acid. An interactive high-quality version of these figures, generated by mapman, is available at: <http://mapman.gabipd.org/web/guest/tomato-time-course>.

Fig. 3 Genes that were differentially expressed between near-isogenic tomato genotypes IL6-2 and M82 at 36 h after infection with *Phytophthora infestans* using mapman (<http://mapman.gabipd.org>); red denotes down-regulated pathways and blue denotes up-regulated pathways. ABA, abscisic acid; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; SA salicylic acid. An interactive high-quality version of these figures, generated by mapman, is available at: <http://mapman.gabipd.org/web/guest/tomato-time-course>.

Functional characterization of candidate genes using virus-induced gene silencing (VIGS)

The potential roles of several genes that were expressed differentially between M82 and IL6-2 in response to inoculation with *P. infestans* were investigated using VIGS. We targeted genes that were induced in IL6-2, but were nonresponsive in M82. The expression profiles of two such sequences are illustrated in Fig. 5. In order to predict the timing of silencing, the *phytoene desaturase* (*PDS*) gene was used. Silencing of *PDS* causes the plants to exhibit a photobleached phenotype (*PDS* inhibits carotenoid biosynthesis; Kumagai *et al.*, 1995). In our experiments, when silencing was successful, it was typically successful in about 30% of plants and, for these, each leaf was a mosaic of silenced and nonsilenced tissue; thus, for any particular leaflet, silencing was partial—typical for tomato (Rotenberg *et al.*, 2006). Photobleaching of tomato tissue typically occurred about 20 days after inoculation with *Agrobacterium* carrying pTRV2:*PDS* and pTRV1. Therefore, inoculations of silenced plants with *P. infestans* were performed 21 days after agroinfiltration with the gene of interest.

Thirty-one genes were targeted for silencing (Table S5, see Supporting Information). Before inoculation with *P. infestans* at 21 days after VIGS treatment, three leaflets of each test plant were assayed using semi-quantitative reverse transcriptase-PCR to determine whether the gene of interest had been silenced. To make sure that the difference in amplification was a result of the gene silencing, and not of differences in the amount of RNA used, each gene was normalized with the *actin* transcript (Fig. S2, see Supporting Information). Assays were conducted on treatment and control (empty vector and no vector) plants and assessed after 20, 25 and 30 cycles. We found reduced expression of two of the 31 genes. One gene coded for a Toll/interleukin-1 receptor–nucleotide-binding site–leucine-rich repeat (TIR-NBS-LRR) homologue (37019) and the other for a peroxisomal membrane protein (35P7) (Fig. S2).

Pathogen growth was measured using qRT-PCR analyses of silenced and control plants. The results of these experiments confirmed that *P. infestans* grew more slowly in IL6-2 than in M82 ($P = 0.0044$), as reported previously (Smart *et al.*, 2007) (Fig. 6). In accordance with our hypothesis, *P. infestans* grew more rapidly

Fig. 4 Genes that were differentially expressed between near-isogenic tomato genotypes IL6-2 and M82 at 60 h after infection with *Phytophthora infestans* using mapman (<http://mapman.gabipd.org>); red denotes down-regulated pathways and blue denotes up-regulated pathways. ABA, abscisic acid; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; SA salicylic acid. An interactive high-quality version of these figures, generated by mapman, is available at: <http://mapman.gabipd.org/web/guest/tomato-time-course>.

when the TIR-NBS-LRR homologue (37019) was silenced in IL6-2 ($P = 0.0001$) than in control IL6-2 plants (Fig. 6). IL6-2 plants silenced for the TIR-NBS-LRR homologue (37019) were about as susceptible as M82 plants. The results for silencing the peroxisomal membrane protein (35P7) were similar, in that there was an increased pathogen growth rate in silenced IL6-2 plants ($P = 0.066$) relative to control IL6-2 plants. IL6-2 plants silenced for 35P7 were about as susceptible as M82 plants silenced for 35P7 (Fig. 6). Silencing either gene in M82 did not cause a change in pathogen growth rate.

The sequences that were cloned into TRV2 were used to search the tomato genome database (<http://solgenomics.net>). Clone 35P7 corresponded to unigene SGN-U563130 [peroxisomal membrane protein (PMP36)] and had a single imperfect match on chromosome 5 of the genome assembly. Clone 37019 corresponded to unigene SGN-U564334 [disease resistance protein (TIR-NBS-LRR class)] and had a perfect match on chromosome 12. Additional hits were found on other chromosomes, for example, chromosomes 9 and 11, but with lower sequence identity, and none of these hits had more than 15 continuous identities in nucleotides, which is important for silencing purposes.

DISCUSSION

A crucial question concerning a gene profiling experiment in the field is whether or not the variance associated with field-grown plants precludes the detection of important differences. If different plants of the same treatment show significant variance in gene expression, and the number of biological replicates is not sufficiently large, many differentially expressed genes will not be identified with high confidence (false negatives) and a number of nondifferentially expressed genes may be identified as differentially expressed by chance (false positives). Our assessment of the variation in gene expression among field-grown tomato plants demonstrated that the variance in this type of experiment was not large. When comparing two groups of uninoculated plants, false positives were eliminated with a moderate fold-change requirement. The rather low variance in the field experiment indicates that true positives can be identified with high confidence. This low variance enabled three very important discoveries: (i) that some genes (including several that have been associated with response to particular pathogens) were unexpectedly highly variable in expression; (ii) that gene expression in the two genotypes prior to

Fig. 5 Expression ratios in M82 and IL6-2 on infection by *Phytophthora infestans* of a peroxisomal membrane protein (35P7) (A) and a Toll/interleukin-1 receptor–nucleotide-binding site–leucine-rich repeat (TIR-NBS-LRR) homologue (37019) (B), as determined by microarray analysis. Both genes were up-regulated in IL6-2 relative to M82 in response to *P. infestans* inoculation.

inoculation was remarkably similar; and (iii) that genes that were differentially expressed after inoculation seemed likely to have a role in explaining the difference in resistance between IL6-2 and M82.

The discovery that several genes were particularly variable was unexpected. The fact that these genes were detected as more variable in each tomato isline suggests that this variability is not a false positive. In addition, the variability in these genes is entirely consistent with the fact that these genes have often been identified as differentially expressed in response to pathogen attack. It is difficult to determine the mechanism behind this enhanced variability. One possibility is that there are chance interactions between microbes and some (but not all) plants in the field. These chance interactions may cause enhanced expression of these genes in plants affected by these microbes. Another possibility is that these genes are inherently more responsive to diverse stimuli, such as abiotic fluctuations (temperature, precipitation and relative humidity), nutrient uptake and several biotic stresses. In a recently published article, Richards *et al.* (2012) assessed the genome-wide gene expression patterns under field conditions of *Arabidopsis thaliana* plants, and examined the correlation between gene expression variability and changes in the environment. Their results showed that gene expression fluctuations correlated with precipitation and temperature. Despite the variability of field conditions, genes were organized in a relatively small number of clusters, and most of the genes had been identified previously as responsive to biotic or abiotic stimuli in laboratory conditions. It seems important to determine whether these genes

are generally responsive to diverse perturbations and not specifically to pathogen attack.

A second unexpected result was that we detected no significant quantitative differences in constitutive gene expression between the two isolines before inoculation (at the –12-hai time point). In IL6-2, a section in chromosome 6, delimited by markers TG279 and TG352, was replaced by the homologous region from *L. pennellii* (Eshed and Zamir, 1995). These two markers define a 5.9-Mb region, approximately 12.8% of chromosome 6 and 0.6% of the whole tomato genome (<http://solgenomics.net>). It may well be that the differences between these two isolines are all expressed after inoculation. The differential response to infection could be caused by a difference in the regulatory region in some of the genes located on this segment, or a small sequence difference in coding region that cannot be detected by hybridization. This would be consistent with the fact that these two isolines are so closely related. However, other explanations may also apply. For example, it is possible that some genes that are differentially expressed between the two isolines are not detected because of small differences. Alternatively, some genes not on the TOM1 microarray may have differential constitutive expression. However, the fact that we detected no differences in constitutive gene expression suggests that most of the differences between the two isolines are expressed after inoculation.

In many respects, the two isolines responded similarly to inoculation with *P. infestans*. Many of the genes that were induced on inoculation in both isolines are predicted to have signal transduction or disease resistance function. For example, 42 transcripts of

Fig. 6 Relative amount of *Phytophthora infestans* in plants as determined by quantitative real-time polymerase chain reaction (qRT-PCR) analysis at 4 days after inoculation. Lower thresholds of detection (CT values) indicate greater abundance of *P. infestans*, which is indicative of greater susceptibility. Results for M82 are in columns 1, 3, 5 and 7 (filled columns) and results for IL6-2 are in columns 2, 4, 6 and 8 (open columns). Treatments were: plants silenced for peroxisomal membrane protein 35P7 (columns 1 and 2); plants silenced for Toll/interleukin-1 receptor–nucleotide-binding site–leucine-rich repeat (TIR-NBS-LRR) homologue 37029 (columns 3 and 4); plants subjected to transformation with the empty vector (columns 5 and 6); plants transformed with no vector (columns 7 and 8). IL6-2 plants not silenced for either protein were less susceptible than M82 plants not silenced for either protein, in that the CT values were larger, indicating smaller amounts of *P. infestans* in IL6-2 relative to those in M82 (compare column 8 with column 7 and column 6 with column 5). Silencing of either 35P7 or 37019 eliminated the difference in resistance between IL6-2 and M82 (compare column 1 with column 2 and column 3 with column 4). IL6-2 plants silenced for the peroxisomal membrane protein (35P7) were more susceptible than nonsilenced IL6-2 plants, in that *P. infestans* was detected after fewer PCR cycles ($P = 0.067$, compare column 2 with columns 6 and 8). IL6-2 plants silenced for 37019 were more susceptible to *P. infestans* relative to nonsilenced plants, in that *P. infestans* was detected after fewer PCR cycles (at lower CT values, $P = 0.001$) (compare column 4 with columns 6 and 8).

putative PR genes were induced to similar levels in both cultivars. It seems unlikely that these genes are involved in the greater resistance to late blight in IL6-2 in comparison with M82. Perhaps these genes are involved in basal resistance.

The third important result was that there were many genes which, in response to inoculation, were expressed differentially in IL6-2 relative to M82. It seems possible that some of these genes might be involved in the greater resistance of IL6-2, either singly or in complex association with other genes. Candidates that seem to be particularly interesting are those that were induced in IL6-2, but were either nonresponsive or suppressed in M82. There were many such candidates, including putative *R* genes, *MAPK* genes and genes encoding for transcription factors, such as *ERF*, *WRKY* and *MYB*. Of these genes, we selected 31 (Table S5) that were reliably and most strongly differentially induced to test for functional analysis via VIGS. Although VIGS is one technology for the investigation of gene function, it is not always successful in tomato. Thus, we only succeeded in silencing two of these genes: a TIR-NBS-LRR homologue (37019) and one coding for a peroxisomal membrane protein (35P7). Even when successful in tomato,

silencing via VIGS occurs as a mosaic, so that silencing in an entire leaflet is not complete (Rotenberg *et al.*, 2006). Despite these limitations, silencing of either of these two genes caused IL6-2 to be more susceptible to *P. infestans*, but had no effect on M82.

The roles of these two genes are not definitely known, but some hypotheses are plausible. The TIR-NBS-LRR homologue could possibly contribute to resistance in a very modest manner. Some *R* genes are hypothesized to have a residual effect, and thus contribute to quantitative resistance (Rauscher *et al.*, 2010). Peroxisomes have signal molecule-generating capacity, such as superoxide radicals and nitric oxide, important for cellular metabolism under biotic or abiotic stress (Corpas *et al.*, 2001). Therefore, partial silencing of the peroxisomal membrane protein homologue might have hampered cellular signalling in tomato, rendering it more susceptible to *P. infestans*.

We were unable to locate the putative TIR and peroxisomal membrane proteins in the introgression section of IL6-2. It seems likely that the TIR gene was located in chromosome 12. Our screening revealed that no peroxisomal-related genes have been annotated or reported in this genome to date. However, information on peroxisomal-related genes inferred from evidence on other species and from BLAST results showed a hit against a sequence from chromosome 5. Thus, we could not find evidence of possible off targeting effects. If these genes are indeed not located in the introgressed region, their effect on resistance could be mediated by other factors in this region. Thus, there is also a possibility of a transacting factor in the introgression that is responsible for the differential regulation of the genes. However, all of these inferences remain speculative until more is learned about this system.

Our results using microarray data and qRT-PCR are in agreement with previous reports (Morey *et al.*, 2006; Wang H. *et al.*, 2005). For example, Wang H. *et al.* (2005) conducted a large-scale comparison between Taq-Man qRT-PCR and two microarray platforms, and reported that the majority of genes showed similar trends between the microarray data and the qRT-PCR data. However, there were some differences. They also noted that the microarray platforms usually reported less dramatic changes, and they attributed this to 'fold change compression' in microarray data as a result of factors such as signal saturation, dynamic range and cross-hybridization.

Field experiments are very expensive to conduct, but are justified if they provide a useful perspective and reliable data. The useful perspective is provided by the very diverse and dynamic environment in which field-grown plants exist. Plants in the field are subjected to a variety of diverse stresses (wind, rain, sun, insects, other pathogens) that could and do induce many responses. Therefore, the comparison between the inoculated and noninoculated plants is very important to eliminate the effects of this diverse environment. Our hypothesis is that results with field-grown plants are likely to be less influenced by nontreatment perturbations because of the complexity of the environment in

which they exist. The absence of constitutive differences between IL6-2 and M82 is consistent with the fact that the diverse environment had a similar effect on both genotypes. In addition, the low variance observed in this experiment supports the idea that the dynamic and diverse environment is not an obstacle in such experiments. It was fascinating to learn that some genes commonly found to be responsive to pathogen attack were particularly variable in the absence of inoculation with *P. infestans*. It will be interesting to determine whether these genes also play a role in the resistance of IL6-2 relative to M82. The very large number of genes induced in response to *P. infestans* in IL6-2, but not induced in M82, might be particularly important in deciphering the resistance in IL6-2 relative to M82. Initial results with two of these genes [a TIR-NBS-LRR homologue (37019) and one coding for a peroxisomal membrane protein (35P7)] indicated that they could be involved in the resistance of IL6-2. Obviously, there are many more candidates to evaluate, and such analysis should enhance our understanding of the resistance in IL6-2. Once the roles of such genes are known, those most useful can be targeted for use in breeding programmes.

EXPERIMENTAL PROCEDURES

Plants, inoculation and tissue collection

Three-week old glasshouse-grown M82 and IL6-2 tomato plants were transplanted to the field (Freeville, NY, USA), 4 weeks prior to inoculation, in a randomized complete block design with three blocks (biological replicates). Each plant was at least 1 m away from any other plants to avoid cross-contamination. Half of the plants in each block were sprayed until run-off with a sporangia suspension (20 000 sporangia/mL) of a tomato-specialized isolate US970001 (ATCC # MYA-2350), a member of the US-17 clonal lineage, in the early evening (approximately 19:30 h) when the temperature was about 20 °C. The other plants were mock inoculated with sterile water. Plots were sprinkler irrigated (30 min at a rate of 0.2 cm/h) before inoculation to ensure favourable environmental conditions for infection. Leaf tissue was collected 12 h before inoculation (considered as the zero time point) and at 12, 36 and 60 hai. Unique plants were used for each collection, such that tissue was collected from each plant only once. These collections were 24 h apart and were performed between 07.00 and 08.00 h to avoid differences in gene expression caused by diurnal or circadian expression cycles. To account for possible changes caused by alterations in the environment and plant age, noninoculated controls were included at each time point for each genotype. All tissue samples were flash frozen in liquid nitrogen in the field immediately after collection and stored at –80 °C until RNA extraction. Some plants were not harvested, but instead were observed daily for symptoms. These plants were destroyed immediately on detection of symptoms.

RNA extraction, probe preparation and hybridization on microarrays

RNA was extracted from each of three independent biological replicates and analysed independently. Each biological replicate consisted of two

plants per genotype. Total tomato leaf RNA was extracted using the hot-phenol protocol of Perry and Francki (1992), as modified by Gu *et al.* (2000). mRNA was isolated using a Dynabeads® mRNA Purification Kit (DynaL-Biotech, Carlsbad, CA, USA), following the manufacturer's instructions.

cDNA was synthesized from 0.4–2.0 µg of mRNA by reverse transcriptase and subsequently labelled using a SuperScript™ Indirect cDNA Labelling Core Kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Briefly, after cDNA synthesis and purification, 5 µL of dimethylsulphoxide (DMSO)–dye solution (Cy3™ or Cy5™) was added to the cDNA, and incubated for 1 h in the dark. Once the cDNA was labelled, it was purified to remove any unreacted dye. cDNA probes were dried in a centrifugal evaporator (Jovan RC 10.10, Guelph, Ontario, Canada) at 150 g and 55 °C for 50 min in the dark. Dried labelled cDNA Cy5™ and Cy3™ probes were resuspended in a final volume of 60 µL of Corning Universal hybridization solution (Pronto!™ CORNING®, Corning, NY, USA) and combined into a single tube. To avoid potential dye-related differences in labelling efficiency, the same procedure was followed for the corresponding dye swap.

Microarray analysis

Tomato cDNA was hybridized on a cDNA microarray (TOM1 BTI: <http://www.sgn.cornell.edu>) with approximately 12 000 tomato EST clones representing 8500 independent tomato genes (Alba *et al.*, 2004). To determine the level of variation in gene expression among field plants, and whether the gene expression of some genes was more variable than others, we collected samples from 12 tomato plants (six of M82 and six of IL6-2) 12 h before inoculation. Each sample from each genotype was randomly assigned to either of two groups (A and B) of three plants each, and each plant from group A was compared against a different plant in group B using the TOM1 tomato cDNA microarray (three biological replicates). For the *P. infestans*–tomato interaction time course study, samples from inoculated plants were compared with those from mock-inoculated plants of the same genotype collected at the same time point. This design allowed us to detect transcripts that were differentially expressed between these two genotypes, as well as those similarly induced or repressed in both genotypes. For the transcripts that were differentially expressed between the two genotypes, we were able to determine expression patterns for each genotype.

Slides were scanned using an Axon GenePix 4100 microarray scanner (Axon Instruments Inc., Union City, CA, USA). The photomultiplier settings were adjusted in each channel so that only a few highly expressed transcripts had saturated pixels. The 16-bit scanned TIFF images were analysed using GenePix Pro version 4.1 software (Axon Instruments Inc.). The median pixel intensity of a given spot without background correction was used as the signal intensity (Alba *et al.*, 2004). Data filtering included two steps. In the first step, each image was visually inspected and low-quality spots were excluded from further analysis. In the second step, each spot with an intensity less than the local background plus two standard deviations in either channel, and its combined intensity in Cy5 and Cy3 channels less than two times the combined local background, was excluded from further analysis. Data were normalized using LOWESS (locally weighted scatterplot smoothing) employing the block technique implemented in MIDAS software (The Institute for Genomic Resources, TIGR). After nor-

malization, the log₂ expression ratio of a transcript between the two samples was obtained by averaging the corresponding values from dye-swap technical replicates. If a value was available only from one of the two technical replicates, that value was used.

qRT-PCR to validate the array

In order to validate the microarray results, we performed a qRT-PCR for five genes typically thought to be involved in resistance and known to be differentially expressed between IL6-2 and M82. Total RNA (300 ng) was reverse transcribed and total transcript levels were determined by qRT-PCR using the Eurogentec Reverse Transcriptase qPCR Master MIX kit (Seraing, Belgium) following the manufacturer's protocol. Reverse transcription and PCRs were carried out in triplicate in 96-well plates. Controls lacking reverse transcriptase and lacking template were included. The relative expression of genes between M82 and IL6-2 genotypes was estimated using the $\Delta\Delta C_t$ method (User Bulletin #2; ABI PRISM 7700 Sequence Detection System, Applied Biosystems, Foster City, CA, USA). The genes, primers and probes were as follows: (i) *endochitinase 3 precursor* (FW, AGTGTCGAAGCCAGTGTCATCT; RV, GCTGCTTATGTCTCCACCAGAA; probe, CCGAAACCTCTACCTGGCCC); (ii) *glyceraldehyde 3-phosphate dehydrogenase B* (FW, AGTGGATGTGTGACATTTGTAACAA; RV, ACCTTCAATTGGCAACTGTTC; probe, CTGCTCAACTCACTCCCAAGACCACAGG); (iii) probable *GST* (FW, GCCCTTCCATTTTGCCTAAAG; RV, CTGACCCCTTATCATCGACATATT; probe, TGATCGAGCTTTAGCGCGTTTTGGG); (iv) *carbonic anhydrase, chloroplast precursor* (FW, GAAATGTCAACTGCTTCCATTAACAA; RV, CAACGGGACGAGTAGGTTTCT; probe, TGCCTTACTCTCTCCCTGCTCAAGCT); (v) *basic glucanase* (FW, GATCTGTAGGAATTGTTGTGCAGAGA; RV, CATTGCTTGTGTGGACCAA; probe, TGGATGGCGTCTGCTGGTGC).

Functional analysis of silenced genes

The effect of TIR-NBS-LRR (37019) and the gene coding for the peroxisomal membrane protein (35P7) on pathogen growth was determined after silencing each gene in both M82 and IL6-2. Inoculations of plants were performed with sporangia and zoospores of *P. infestans* isolate US050007, which is a member of the US-11 clonal lineage, highly compatible with tomato. We were unable to use the US-17 clonal lineage isolate employed in the field experiments as this isolate lost its virulence in culture. Sporangia were dislodged from tomato leaflets (cultivar Sunrise) with sporulating late blight lesions in 100 mL of distilled water. The sporangium concentration was determined using a haemocytometer and adjusted to 4000 sporangia/mL. Subsequently, the sporangia were incubated at 4 °C for 1 h to release the zoospores. This mixture of sporangia and zoospores was applied to plants with a hand-held sprayer until run-off. Plants were incubated at 15 °C and 100% relative humidity for the next 7 days, when they were evaluated for disease severity. Data were recorded from those plants in which the gene was demonstrated to be silenced (see below).

VIGS

Thirty-one host genes (Table S5), which were up-regulated in IL6-2, but not in M82, after inoculation, were investigated. Attempts to silence these

genes were performed using a tobacco rattle virus (TRV)-based vector, as described by Liu *et al.* (2002), using the GATEWAY® Technology system (Invitrogen), according to the manufacturer's instructions. Two clones per construct were sequenced to verify the insertion of the gene of interest. pTRV1 and pTRV2 were individually transformed into *Agrobacterium* strain GV3101. *Agrobacterium* carrying pTRV1 was mixed with the *Agrobacterium* carrying pTRV2 in a 1:1 ratio into a final volume of 10 mL. *Agrobacterium* infiltration was performed using a 1-mL needle-less syringe in cotyledons of 10-day-old tomato plants. There were three independent trials of the experiment for each vector, and there were 10 plants of each cultivar per vector and for each of the controls (empty vector and no vector).

After inoculation, plants were kept at 18 °C with a 12-h photoperiod. In each experiment, one treatment consisted of plants inoculated with *Agrobacterium* carrying a vector with the *PDS* gene (pTRV2:PDS). Photobleaching of the plants served as a visual guide to determine when gene silencing was achieved. Generally, silencing was accomplished within 20 days after the inoculation with *Agrobacterium*.

Once silencing had been accomplished as determined by the PDS phenotype (in our case, 4-week-old plants), the three youngest leaflets from each of the 10 plants per treatment were collected and flash frozen in liquid nitrogen. Tissue from each plant was analysed separately for gene silencing. Immediately after collection of the tissue, plants were inoculated with *P. infestans*.

Reverse transcriptase-PCR to confirm gene silencing

To determine the degree of gene silencing, we used reverse transcriptase-PCR. DNase-treated RNA (100 µg/µL) was used for cDNA synthesis and PCR amplification, employing SuperScript® III one step reverse transcriptase-PCR Platinum® Taq HiFi (Invitrogen), following the manufacturer's instructions. PCR was carried out in a 25-µL volume containing 2 µM of each of the primers. PCR conditions consisted of one cycle of 55 °C for 30 min, followed by one cycle of 95 °C for 5 min, followed by 20, 25 or 30 cycles of a three-step procedure (1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C) and a final step of 5 min at 72 °C. As a control, reverse transcriptase-PCR of the tomato *actin* gene was performed with the following primers: FW, 5'-CCAAAGCCCAATCGAGAGAA-3'; RV, 5'-GGTACCACCACTGAGGACGA-3'. PCR conditions for *actin* were the same as for the detection of the gene coding for the peroxisomal membrane protein and the TIR-NBS-LRR homologue (above).

Pathogen quantification in silenced plants

Pathogen growth in plants in which the gene coding for the peroxisomal membrane protein (35P7) or TIR-NBS-LRR homologue (37019) was silenced was compared with pathogen growth in control plants using qRT-PCR. At 4 days after inoculation, three inoculated leaflets from each plant were harvested and the *Phytophthora* internal transcribed spacer (ITS) region was quantified. At the moment of collection, disease symptoms were not detectable on the leaflets. DNA was extracted using 2 × cetyltrimethylammonium bromide (CTAB) buffer with 0.2% β-mercaptoethanol. All samples were diluted to 100 ng/µL, and a total of 300 ng was used per PCR. The qRT-PCR primers and probes, as well as PCR conditions, were those described by Restrepo *et al.* (2005). Total transcript levels were determined by qRT-PCR using the ABI PRISM 7000 sequence

detection system (Applied Biosystems). An ANOVA was used to determine the effect of gene silencing on *P. infestans* growth. Statistical analyses were carried out using MINITAB version 14.

Location of the TIR and peroxisomal membrane genes

Query sequences for the TIR and peroxisomal membrane genes were searched against the tomato genome (*Solanum lycopersicum*) sequenced and assembled by the International Tomato Genome Sequencing Project by the Sol Genomics Network (Bombarely *et al.*, 2011; Mueller *et al.*, 2009). BLAST was performed in three different ways: (i) a local BLAST using the genome chromosomes; (ii) a search performed in the Sol Genomics Tomato BLAST webpage (<http://solgenomics.net/tools/blast/index.pl>); and (iii) a BLAST performed in the National Center for Biotechnology Information (NCBI) BLAST webpage against the nonredundant database and the *Solanum lycopersicum* data contained in NCBI. For each BLAST, conditions were modified, searching for a threshold of $1E-6$ and word size of 20, to perform a most precise BLAST to search against regions of tomato that could cause off targeting effects.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Validation of microarray results using quantitative real-time polymerase chain reaction (qRT-PCR). Expression profiles of genes identified in the arrays after *Phytophthora infestans* inoculation of IL6-2 and M82. Left column, qRT-PCR was used to validate the microarray (right column) results.

Fig. S2 The expression of Toll/interleukin-1 receptor–nucleotide-binding site–leucine-rich repeat (TIR-NBS-LRR) (37O19) and peroxisomal membrane protein (35P7) was reduced in both M82 and IL6-2, as determined by reverse transcription semi-quantitative polymerase chain reaction at 25 cycles of amplification. For TIR-NBS-LRR, lanes 1–4 show the silenced plants (M82, 1–3; IL6-2, 4). The nonsilenced controls are shown in lanes 5–11 (5, 6, M82 no

vector; 7, 8, IL6-2 no vector; 9, 10, M82 empty vector; 11, IL6-2 empty vector). The lower panel depicts the amplification of the tomato *actin* gene as a loading control, amplified at 30 cycles. For the peroxisomal membrane protein, lanes 1–6 are from silenced plants (IL6-2, 1–3; M82, 4–6). Lanes 7–14 are the nonsilenced controls (7, 8, IL6-2 empty vector; 9, 10, M82 empty vector; 11, 12, IL6-2 no vector; 13, 14, M82 no vector). Again the lower panel depicts the amplification of the tomato *actin* gene as a loading control, amplified at 30 cycles. This figure shows the results of one trial, but results from all trials were similar.

Table S1 Differentially expressed genes in M82 and IL6-2 in response to late blight infection in the field.

Table S2 Genes similarly induced in M82 and IL6-2 in response to late blight infection.

Table S3 Genes similarly repressed in M82 and IL6-2 in response to late blight infection.

Table S4 List of genes in 10 clusters that were differentially expressed in M82 and IL6-2 in response to late blight infection.

Table S5 Genes selected for viral-induced gene silencing (VIGS).