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Tomato defense to the powdery mildew fungus: differences in expression of genes in susceptible, monogenic- and polygenic resistance responses are mainly in timing

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Abstract *Oidium neolycopersici* is a causal agent of tomato powdery mildew. In this paper, gene expression profiles were investigated of susceptible, monogenic- and polygenic resistant tomato genotypes in response to *O. neolycopersici* infection by using cDNA-AFLP. Around 30,000 TDFs (Transcript Derived Fragments), representing ~22% of the transcriptome based on in silico estimation, were identified and 887 TDFs were differentially expressed (DE-TDFs) upon inoculation with *O. neolycopersici* spores. Forty-two percent of the identified DE-TDFs were detected in both the compatible and incompatible interactions, a subset of these were studied for their temporal patterns. All of these common induced DE-TDFs displayed an expression peak at 7 days post incoluation in monogenic resistant response but sustained up-regulation in the susceptible and the polygenic resistant response. While more than half of these common DE-TDFs showed earlier timing in incompatible interactions compared to compatible interaction. Only 2% of the identified DE-TDFs were specific to either the monogenic or the polygenic resistant response. By annotation of the 230 sequenced DE-TDFs we found that 34% of the corresponding transcripts were known to be involved in plant defense, whereas the other transcripts played general roles in signal transduction (11%), regulation (24%), protein synthesis and degradation (11%), energy metabolism (12%) including photosynthesis, photorespiration and respiration.

Keywords Basal defense · cDNA-AFLP · Monogenic resistance · *Oidium neolycopersici* · Polygenic resistance · *Solanum lycopersicum*

Abbreviations

DE-TDF	Differentially expressed TDF
DPI	Days post inoculation
HPI	Hours post inoculation
TDF	Transcript derived fragment
HR	Hypersensitive response

Introduction

In nature, plants have to face the attacks from a variety of intruders, such as viruses, bacteria, fungi and insects. Most plants can protect themselves against non-specific pathogens with passive defense mechanisms including cell wall thickness and waxy, anti-microbial components. To protect themselves against attack of specific pathogens and pests, active defense systems are very important whereby resistance genes play pivotal roles. More than 50 plant disease resistance (*R*) genes have been cloned (Coaker et al. 2005), most of which match the corresponding avirulence (*Avr*) genes of pathogens

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according to the well-known gene-for-gene model (Flor 1971). Typically, the race-specific resistance response is associated with Hypersensitive response (HR) microscopically and/or macroscopically. Several race non-specific resistance genes like RPW8, RPG1 and FLS (reviewed by Hammond-Kosack and Parker 2003), have also been cloned. The mechanisms of both race-specific and race non-specific resistance responses are well studied in some well-studied plant-pathogen model systems like the barley—*Blumeria graminis f.sp.hordei (Bgh)* pathosystem (Schulze-Lefert and Vogel 2000) and tomato—*Cladosporium fulvum* pathosystem (Joosten and de Wit 1999). However, far less is known of the mechanisms underlying quantitative resistance governed by a number of genes.

Fungal diseases are widespread and are one of the biggest threats for plant health. Tomato powdery mildew caused by the biotrophic fungus, *O. neolycopersici*, has recently become a very important disease of tomato (*S. lycopersicum*) worldwide. There are two known species of tomato powdery mildew in the *Oidium* genus, *O. lycopersici* occurring in Australia and *O. neolycopersici* occurring in the rest of the world; conidia form mainly a chain for *O. lycopersici* and single spores for *O. neolycopersici* (Jones et al. 2001). The disease has caused large damage in the European tomato production, especially in the glasshouse production. Although the cultivated tomato is susceptible to the fungus, resistance occurs in many wild species of tomato (Lindhout et al. 1994a, b), such as *S. habrochaites (*former *Lycopersicon hirsutum)* and *S. neorickii* (former *L. parviflorum*). Several cultivars that carry monogenic *R* genes are now on the market. The monogenic dominant resistance genes *Ol-1* and *Ol-3* introgressed from *S. habrochaites* G1.1560 and G1.1290 respectively have been fine-mapped on the long arm of Chromosome 6 (Lindhout et al. 1994a, b; Huang et al. 2000a, b; Bai et al. 2005). Three resistance QTLs were introgressed from *S. neorickii* G1.1601 and have been mapped on Chromosomes 6 and 12 (Bai et al. 2003). Both the monogenic *Ol-1* gene and the three *Ol*-QTLs have been introgressed into the tomato cultivar Moneymaker (MM) and the resistance mechanisms have been studied microscopically. Previous studies showed that the resistance response caused by *Ol-1* is strongly associated with HR (Huang et al. 2000a, b; Bai et al. 2005), while the resistance in *S. neorickii* governed by three major resistance QTLs is less associated with HR (Huang et al. 2000a, b).

cDNA-AFLP is a genome-wide expression analysis technology that does not require prior knowledge of gene sequences. This PCR-based technique combines a high sensitivity with a high specificity, allowing detection of rarely expressed genes and distinction between homologous genes (Bachem et al. 1998; Reijans et al. 2003). Since the first introduction of cDNA-AFLP to profile genes involved in potato tuber development (Bachem et al. 1996), more than 50 papers have been published on different biological processes using this platform. Based on these results, cDNA-AFLP is considered as a reliable and available technique for laboratories, especially for organisms with little sequence information.

In this paper, cDNA-AFLP was employed to compare gene expression profiles in the susceptible genotype (Moneymaker), a monogenic resistant line containing *Ol-1* and a *S. neorickii* accession, which is the donor of the *Ol*-QTLs in response to infection with *O. neolycopersici*. The outcome will increase our understanding of the mechanisms of the tomato—*O. neolycopersici* interaction. Our data indicate that a large part of the differences between basal defense in the compatible interaction and *R*-gene (*R*-QTL) mediated responses in the incompatible interactions of tomato and *O. neolycopersici* is due to the timing of the expression of genes involved. Remarkably, the monogenic resistant response results in an expression peak of DE-TDFs at 7 DPI (Days Post Inoculation), while in both the susceptible MM and the polygenic resistant *S. neorickii* accession these DE-TDFs are constantly up-regulated.

Results

Tomato plants grow optimally under natural light conditions in the glass houses, however since the light condition is seasonally and experiments cannot be repeated under identical conditions, we decided to use the climate cell to carry out the inoculation experiments. Four experiments were accomplished to optimize the growth conditions for tomato plants and disease tests in climate cells (Wageningen University). The optimal conditions are described in the materials and methods section. Based on the microscopic observations of the infection process (Huang et al. 1998), macroscopic observation of the disease progress and protein analysis of intercellular fluid (data not shown), time-points for sample collection after spore-suspension and mock inoculation were chosen. For experiment one leaf material was collected from 0 to 72 HPI (Hours Post Inoculation), for experiment two from 0 to 7 DPI for the resistant lines and from 0 to 14 DPI for the susceptible Moneymaker.

Specificity, in silico transcriptome coverage and TDF redundancy of cDNA-AFLP

The experimental design consisted of two randomized blocks. The cDNA AFLP profiles of 8 primer combinations demonstrated that cDNA patterns between similar samples (genotype/treatment/time) were almost identical. Therefore, the samples from one block were used for full scale gene expression profiling and the samples of the other block were stored in the –80°C freezer. Since constitutive TDFs of all samples showed uniform intensity by using 10 random primer combinations, the samples collected at different time-points can be pooled for efficient large-scale cDNA-AFLP screening without causing false differentials. For the pooling, pre-amplification products of all the time-points were bulked per genotype-treatment prior to selective amplification: hereafter referred to as bulk time-point analyses. In experiment one, 72 primer combinations were used to screen the bulks. Since only five weakly differential TDFs were found, it was decided to focus on experiment two, in which samples were collected at later time-points, to obtain DE-TDFs. In total, there are 256 possible primer combinations for *Ase*I + 2/*Taq*I + 2, and 1024 possible primer combinations for *Eco*RI + 3/*Mse*I + 2 (Table 1). In experiment two, totally 768 primer combinations (*Ase*I + 2/*Taq*I + 2 and *Eco*RI + 3/*Mse*I + 2) were used in bulk time-point analyses, and 331 primer combinations resulted in DE-TDFs (Table 1). On average, each primer combination revealed 40 clear bands, so that approximately 30,000 TDFs were surveyed.

Tomato ESTs (average length of ESTs is 450 bp) downloaded from the NCBI database have been assembled into 15,098 contigs (Tentative Concensus, TCs) with a mean length of 900 bp. The computer program RE-Predictor (Jifeng Tang, unpublished program) was written to estimate transcriptome coverage in cDNA AFLP profiling studies. The principle of this program is as following: recognition sites of the restriction enzymes used in cDNA-AFLP were used to search the tomato TC database. The TCs were considered to be covered by the enzyme combination, if they contain both recognition sites of the two enzymes used in cDNA AFLP with a distance ranging from 50 bp to 500 bp, which coincides with the informative fragment range in an actual cDNA-AFLP fingerprint on LICOR gels. The percentage of covered TCs predicts the coverage of the transcriptome of that enzyme combination. By using RE-Predictor and the tomato contig database, transcriptome coverage of *Mse*I/*EcoR*I and that of *Taq*I/*Ase*I in cDNA-AFLP were estimated to be 23% and 18%, When both enzyme combinations are used and the overlap between them is considered, the total coverage is 36% (Table 1), In the cDNA-AFLP screening described in this paper, not all possible selective primer combinations (768 out of 1280) were employed and the proportional coverage of the used primer combinations was 22% (Table 1). The in silico TDF redundancies for *Ase*I + 2/*Taq*I + 2 and for *Eco*RI + 3/*Mse*I + 2, which refer to the number of AFLP fragments per tomato contig estimated by RE-predictor are 1.23 and 1.57 respectively, but the joint in silico redundancy increases to 1.6 (Table 1), since both enzyme combinations have an overlapping coverage.

Differentially expressed TDFs identified in bulk time-point analyses

Among the visualized TDFs, 887 up-regulated DE-TDFs were detected (Table 1) and no obviously down-regulated DE-TDFs were observed. The up-regulated DE-TDFs revealed in bulks showed a number of differential expression patterns (Fig. 1). Generally, the DE-TDFs can be divided into four classes. About 53% of the 887 DE-TDFs displayed induction only in the compatible interaction (class I), while being absent or constitutively expressed in incompatible interactions

Table 1 Overview of cDNA-AFLP analysis in bulk time-point analyses, in silico estimation of transcriptome coverage and predication of TDF redundancy

	PC[a] number		Percentage of PC giving DE-TDF (%)	DE-TDF obtained	Transcriptome coverage of PCs		TDF redundancy[d]
	Total	Used			Total-PC[b] (%)	Used-PC[c] (%)	
*Ase*I + 2/*Taq*I + 2	256	128	31	95	18	9	1.3
*Eco*RI + 3/*Mse*I + 2	1024	640	45	792	23	14	1.5
Total	1280	768	43	887	36	22	1.6

[a]PC: primer combination

[b]The coverage was estimated based on total number of PCs

[c]The coverage was estimated based on the number of used PCs in cDNA-AFLP analysis

[d]Redundancy was estimated based on total PCs using RE-predictor, the redundancy of used PCs was supposed to be the same

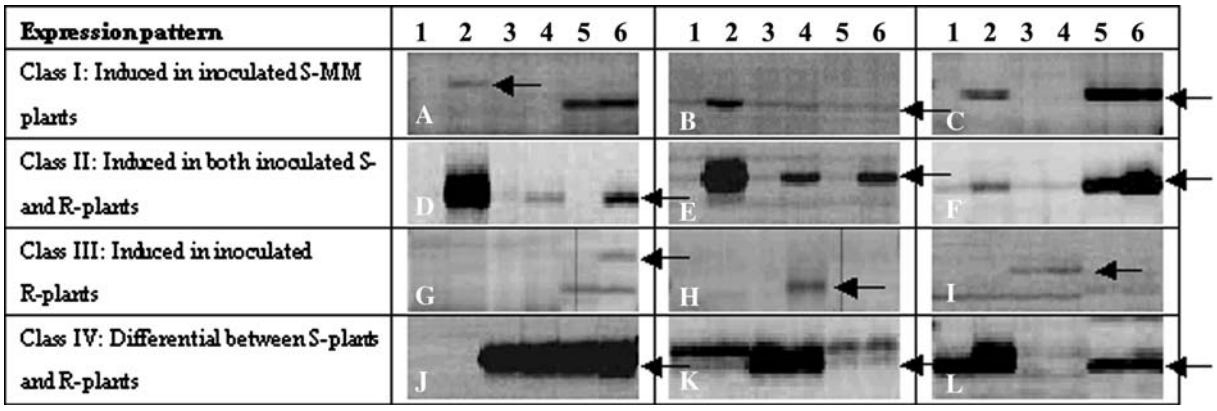

Fig. 1 Sections of cDNA-AFLP images showing 12 representative TDFs that are differentially expressed between genotypes and/or treatments in bulk time-point analyses. Lanes 1–6 represent the pools of all the time-point samples: 1 susceptible genotype MM mock-inoculated with water, 2 susceptible genotype MM inoculated with spore suspension of *O. neolycopersici*, 3 monogenic resistant genotype R-Ol-1 mock inoculated with water, 4 monogenic resistant genotype R-Ol-1 inoculated with spore suspension of *O. neolycopersici*, 5 polygenic resistant genotype R-QTL mock inoculated with water and 6 polygenic resistant genotype R-QTL inoculated with spore suspension of *O. neolycopersici*. Arrows point at different DE-TDFs. Panel A–L represents different sections of cDNA-AFLP images displayed by different or the same primer. combinations

(Fig. 1, panel A–C). The DE-TDFs of class II (42% of the identified DE-TDFs) were induced in inoculated pools of compatible Moneymaker (here after referred to as S-MM), incompatible BC_1S_2 plants homozygous for the resistance gene *Ol-1* (hereafter referred to as R-Ol-1) and *S. neorickii* G1.1601, a wild tomato accession, which harbors three major *Ol*-QTLs (hereafter referred to as R-QTL), or induced in the inoculated pools of S-MM and one of the incompatible pools (R-Ol-1 or R-QTL) (Fig. 1, panel D–F). Very few monogenic resistance-specific (~0.5%) or polygenic resistance-specific (~1.5%) DE-TDFs (class III) were detected (Fig. 1, panel G–I). Class IV consisted of DE-TDFs (~3%) that were not induced by fungi as above three classes, but they may still be associated with resistance because of the differential expression pattern or level between the compatible pools (S-MM) and incompatible pools irrespective of the treatment (Fig. 1, panel J–L).

Time course and pattern of DE-TDFs identified in individual time-point analyses

For each time-point, leaf tissue was collected from one unique tomato plant to avoid that wound responses mask the pathogen-induced responses. The different plants can, also be considered as biological repeats within each genotype. To exclude the DE-TDFs caused by developmental processes from the DE-TDFs caused by pathogen-induced responses, samples from mock-inoculated plants were always compared to leaf samples of inoculated plants in individual time-point analyses (Fig. 2).

One hundred and 10 primer combinations, which identified 248 DE-TDFs in the bulks, were chosen for individual time-point analyses to confirm the identity and display the timing of DE-TDFs. In individual time-point analyses, samples of all time-points of both inoculated and mock-inoculated genotypes, which comprise 46 interactions (genotypes × treatments × time-points), were investigated. All the 248 DE-TDFs found in bulk time-point analyses were identified again in individual time point analyses.

In total, 129 DE-TDFs of class I in the bulks were only induced in inoculated S-MM at seven DPI or later in individual time-point analyses. The DE-TDFs of class II in the bulk time-point analyses were subclassified into class II-1, 2, 3 and 4 in individual time-point analyses. About 60% (52) of the 89 DE-TDFs of class II-1, 2 and 3, started expression earlier or had obviously higher expression level at the starting time-point in the monogenic R-Ol-1 and/or polygenic R-QTL compared to S-MM. The other 40% (37) of the 89 DE-TDFs displayed similar timing in S-MM, R-Ol-1 and/or polygenic R-QTL. In addition, all the DE-TDFs associated with R-Ol-1 showed an expression peak at 7 DPI in R-Ol-1. Twelve class-II-4 DE-TDFs are induced in inoculated S-MM and in the incompatible interaction R-Ol-1 or R-QTL but constitutively expressed in the other incompatible interaction.

DE-TDFs, which belong to class III in the bulk time-point analyses, were displayed as class III-1 and 2 in individual time-point analyses. Four class III-1 DE-TDFs were only induced in inoculated R-Ol-1 plants and two class-III-2 DE-TDFs were induced in inoculated R-QTL. Twelve DE-TDFs of class-IV that were

Class	Expression pattern*	Number of DE-TDF	Further description of expression pattern	Expressional timing of DE-TDFs in different genotypes/treatments
				M-W* M-I* O-W* O-I* P-W* P-I*
				DPI: 0 1 2 3 4 7 9 11 14 0 1 2 3 4 7 9 11 14 0 1 2 3 4 7 9 0 1 2 3 4 7 9 0 1 2 3 4 7 9 0 1 2 3 4 7 9
Class I	MI	129	Only induced in inoculated S-MM	
Class II-1	MIOIPI	64 (38**)	Induced in inoculated S- and R-plants. In R-Ol-1 there is always a high-level expression peak at 7 DPI***	
Class II-2	MIOI	8 (5**)	Induced in inoculated S-MM and R-Ol-1. In R-Ol-1 there is always an expression peak at 7 DPI	
Class II-3	MIPI	17 (9**)	Up-regulated in inoculated S-MM and R-QTL	
Class II-4	MI(OW)OI(PW)PI	12	Induced in inoculated S-MM, constitutively expressed and/or induced in R-Ol-1 or R-QTL	
Class III-1	OI	4	Specific expression in inoculated R-Ol-1	
Class III-2	PI	2	Specific expression in inoculated R-QTL	
Class IV	Constitutively differential	12	Constitutively expressed in S- and R-plants with higher expression level in R-Ol-1 and/or R-QTL or only constitutively expression in R-plants	
Total		248		

Fig. 2 Different classes of the DE-TDFs displayed in individual time-point analyses are classified based on the response specificity, which is illustrated by representative DE-TDFs in cDNA-AFLP image sections * I: inoculated with spore suspension of *O. neolycopersici*, W: mock inoculated with water; M: susceptible genotype MM, O: monogenic resistant genotype R-Ol-1 and P: polygenic resistant genotype R-QTL. **Number in brackets refers to DE-TDFs giving earlier expression in R-Ol-1 and R-QTL. ***Days post inoculation

not associated with inoculation but showed different expression levels or patterns between R-Ol-1 and R-QTL in the bulks, were confirmed as class IV DE-TDFs in individual time-point analyses.

The cDNA-AFLP fingerprints in individual time-point analyses showed that the constitutively expressed TDFs have a very uniform intensity among different inoculated and mock-inoculated genotypes. A semi-quantitative RT-PCR (reverse transcription PCR) of all the samples with actin-derived primer pairs further proved the uniformity of templates (Fig. 3). The identities of eighteen DE-TDFs identified in bulks were confirmed using semi-quantitative RT-PCR with primer pairs designed based on the sequences of nineteen DE-TDFs. Three primer pairs were used in RT-PCR to confirm expression pattern of the DE-TDFs in individual time-point analyses. One of these three primer pairs showed the same temporal pattern between cDNA-AFLP and RT-PCR (Fig. 3) and the other primer pairs resulted in an earlier timing of the target bands in RT-PCR compared to cDNA-AFLP.

Sequence information

Two hundred and thirty DE-TDFs were successfully sequenced and annotated by Blasting against EST database of TIGR and NCBI. Based on the possible origin of the transcripts (plant/pathogen) and the putative function of the transcripts, we divided them into nine groups (Table 2). About 34% (79) of the sequenced DE-TDFs had no match in the databases (group I). One hundred and fifty one of the 230 sequences matched homologous information in the databases. Among the 151 sequenced DE-TDFs with hits in databases, 5 TDFs are likely from pathogen origin (group G) because they have good hits in fungal EST databases but not in tomato EST databases; and 26 TDFs are homologous to sequences with unknown functions (group H). One hundred and twenty of the 151 TDFs showed homology to plant ESTs with known functions and represented transcripts with a role in known defense, which refers to transcripts proved to be involved in defense (group A), or with more general roles. For the latter class, we made a division into transcripts involved in signaling (group B) and regulation (group C) and into transcripts with housekeeping functions, like protein synthesis and degradation (group D) and energy metabolism (group E) and a group with homology to genes that have not been associated with defense before (F). We calculated that about 34% (41) of the 120 function-informative transcripts, which were homologous to sequences with known function from plants, were directly involved in plant defense, while approximately 11% (13), 24%

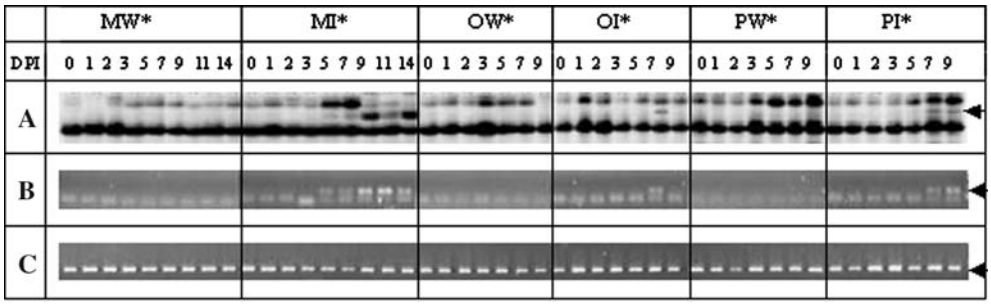

Fig. 3 Comparison of cDNA-AFLP image of a DE-TDF (A) and semi-quantitative RT-PCR with primer pair designed based on the sequence of the DE-TDF (B). Semi-quantitative RT-PCR of actin was used as a constitutive control (C). * I: inoculated with spore suspension of *O. neolycopersici*, W: mock-inoculated with water; M: susceptible genotype MM, O: monogenic resistant genotype R-Ol-1 and P: polygenic resistant genotype R-QTL. Arrows point at the target DE-TDF (A), corresponding band in RT-PCR (B) and actin derived band in RT-PCR (C)

(29), 11% (13) and 12% (14) of these transcripts were associated with signal transduction, regulation, protein synthesis and degradation, energy metabolism including photosynthesis, photorespiration and respiration, respectively (Table 2).

By linking the blast results and functional classification to expression pattern and timing we predicted the general function of different sets of DE-TDFs (Appendix 1). We concluded that about half of the set of function-informative class II DE-TDFs, which are common for both compatible and incompatible interactions, are homologous to transcripts involved in known defense (Appendix 1, No. 2–6 and 16–29). From this functional group of DE-TDFs, 15 out of 20 were earlier induced in incompatible interactions compared to compatible interactions. Four of these class II DE-TDFs represent signaling components and were earlier induced in incompatible interactions (Appendix 1, No. 7–8) or displayed similar timing in both incompatible and compatible interactions (Appendix 1, No. 30–31). Nine of these class II DE-TDFs (Appendix 1, No. 9–11 and 32–37) represent genes with roles in transcription regulation, and 6 of these 9 DE-TDFs (Appendix 1, No. 32–37) were earlier induced in incompatible interactions. Two class III DE-TDFs that were only induced in fungal inoculated monogenic R-Ol-1 (Appendix 1, No. 56–57) are associated with transcription regulation and known defense respectively. However, 3 class III DE-TDFs specifically induced in fungal inoculated *S. neorickii* (polygenic R-QTL) (Appendix 1, No. 58–60) are related to known defense and housekeeping functions but not with transcription regulation. In addition, more than half (7 of 13) of the sequenced class-IV DE-TDFs (Appendix 1, No.

Table 2 Classification of 230 sequenced DE-TDFs based on BLAST results

	Blast results of DE-TDF sequences	Group	Number
Function informative[a]	Known defense responses (secondary metabolate synthesis, cell wall associated and oxidative burst, etc.)	A	41
	Signal transduction (GTP-binding proteins, kinases, etc.)	B	13
	Regulation (transcription factors, heat shock proteins, etc.)	C	29
	Ubiquination pathway and protein synthesis related	D	13
	Photosynthesis, photorespiration and respiration	E	14
	Other[b]	F	10
Subtotal			120
No functional information in plant EST databases	Pathogen derived[c]	G	5
	Unknown[d]	H	26
	No hits[e]	I	79
Subtotal			110
Total			230

[a]With functional information from plant EST databases

[b]Genes that encode proteins with functions not associated with defense before

[c]Good-match found in fungal databases but not in plant databases

[d]Genes that encode proteins of unknown functions

[e]No homologous match in databases

61–73), which displayed a higher expression level in R-Ol-1 and/or R-QTL compared to S-MM, are associated with transcription regulation (Appendix 1, No 63–69).

Discussion

Tomato powdery mildew is a recently appeared fungal disease (Jones et al. 2000). Little is known of transcriptional responses during the interaction of tomato with *O. neolycopersici*. To elucidate the tomato defense responses during the interaction of tomato and *O. neolycopersici*, we carried out a comprehensive study of the fungal-induced changes at the transcriptional level to identify up- or down-regulated genes. cDNA-AFLP was used to detect genes induced in the susceptible interaction, monogenic- (associated with HR) and polygenic resistance (with yet unknown mechanism) responses. Compared with the DNA chips methodology, cDNA-AFLP is an unbiased method, which can be used to reveal altered expression of any gene that carries the suitable restriction site (Durrent et al. 2000). In addition, cDNA-AFLP has a very high reproducibility, which was confirmed using RNA gel blots (Bachem et al. 1996). Amplification of fragments from constitutively expressed genes can provide internal control bands for every primer combination (Durrant et al. 2000). Our results also showed that TDFs from constitutively expressed genes have uniform intensity and serve as internal controls for differentially expressed TDFs. We have sequenced one constitutive TDF and found that it was homologous to a constitutively expressed gene ferredoxin NADP reductase, which is a component of the photosynthesis complex.

Most detected transcriptional responses occur late in the infection process

In experiment one, leaves were sampled at earlier time-points (0–72 HPI) to detect genes involved in early responses of tomato against powdery mildew fungi. Using 72 primer combinations in bulk time-point analyses only 5 weak DE-TDFs were detected. This result may coincide with the fact that the resistance responses of both R-Ol-1 and R-QTL are post-haustorial, and haustorium formation occurs at 24–41 HPI (Huang et al. 1998; Bai et al. 2005). This may also be explained by the fact that powdery mildew fungi interact solely with epidermal cells of tomato where the earlier expression of genes in attacked epidermal cells could be diluted by the uninfected mesophyll cells in the whole-leaf samples. The use of epidermal strips in future gene expression studies may increase the sensitivity to detect earlier interaction transcriptional events.

In experiment two, later time-points were added for sampling and a large-scale cDNA-AFLP screening was conducted to detect DE-TDFs. Almost all DE-TDFs induced in inoculated resistant genotypes were also induced in inoculated S-MM, showing that gene expression changes between compatible and incompatible interaction overlap to a great extent. However, ~60% of these DE-TDFs showed an earlier induction in resistant genotype(s) compared to S-MM (Fig. 2). Apparently, the initiation of defense response in S-MM is too slow to stop the spread of *O. neolycopersici*. Similar results were obtained in gene expression studies in *Arabidopsis* (Maleck et al. 2000). The whole-leaf sampling strategy used in the cDNA-AFLP analysis of the present study may account for the difficulty to get a theoretical ''absent'' or ''present'' expression profiling between compatible and incompatible interaction of tomato and *O. neolycopersici*. In barley, *B. graminis* attack induces indistinguishable expression profiles in both resistant and susceptible whole-leaf samples (Gregersen et al. 1997), while epidermal cells of leaves from susceptible and resistant genotypes show a mosaic of responses with respect to forming effective papillae or allowing pathogen penetration (Gjetting et al. 2004). Similarly, microscopic observations on the interaction between tomato and *O. neolycopersici* indicated that both S-MM and R-Ol-1 leaves display a mosaic of attacked epidermal cells that display a compatible and incompatible interaction with the fungus. However, the proportions of ''resistant'' and ''susceptible'' cells are different between susceptible and resistant plants (data not shown).

The differences in expression timing of DE-TDFs between the compatible and incompatible interaction do not clarify which genes are specific to ''resistant'' or ''susceptible'' leaf cells. A single-cell analysis method has been established to generate transcript profiles from individual epidermal cells in barley and proven useful for analyzing papilla-resistant and successfully infected cells separately (Gjetting et al. 2004). The single-cell analysis method may be helpful to check the specificity of interesting DE-TDFs found in the whole-leaf interaction of tomato and *O. neolycopersici*.

Transcript coverage and number of genes involved in tomato—*O. neolycopersici* interactions

By using the computer program-RE-predictor and the database in which average length of EST-contigs is

900 bp, it was estimated that the in silico redundancy of TDFs surveyed by *Mse*I/*Eco*RI and *Taq*I/*Ase*I is 1.6 (Table 1). In the present study, 887 TDFs are differentially expressed (Table 1), of which 44% (390 TDFs) are associated with incompatible interactions of tomato and *O. neolycopersici*. Taking the redundancy (1.6 times) into account, about 245 non-redundant genes are likely represented by the 390 TDFs. These 245 genes resulted from cDNA-AFLP displayed by 768 *Mse*I + 2/EcroRI + 3 and *Taq*I + 2/*Ase*I + 2 primer combinations, covering ~22% of the transcriptome (Table 1). Thus we concluded that ~1100 (245/22%) non-redundant tomato genes are potentially involved in the resistance responses to *O. neolycopersici*. EST contigs predicted that the tomato genome encodes ~35,000 genes (Van der Hoeven et al. 2002), Hence about 3% (1100/35,000) of all the tomato transcripts are thought to be altered in abundance during the incompatible interaction of tomato and *O. neolycopersici.* This percentage of 3% is in the same order of magnitude as the percentages found in other studies: cDNA-AFLP analysis showed that approximately 1% of tobacco genes are differentially transcribed in Avr9-triggered defense responses in cultured *Cf9*-cells (Durrant et al. 2000); 1.5% of the total *A. thaliana* gene set is co-regulated with SAR and in response to infection of pathogens (Maleck et al. 2000); 2% of the total numbers of genes (35,000) were estimated to be differentially expressed in tomato leaves of RG-PtoR plants four hours after *Pseudomonas* infection in comparison to RG-ptoS/RG-prf3 plants (Mysore et al. 2002).

More genes induced in compatible interaction compared to the incompatible interactions

Interestingly, more DE-TDFs were revealed in the susceptible interaction compared to incompatible interactions of tomato and *O. neolycopersici*: 42% of the 887 DE-TDFs were induced in both interactions, 53% of the 887 DE-TDFs are only associated with the susceptible interaction, while only 2% of the DE-TDFs are specific to resistance responses. From studies on the mechanism of *MLO* in barley, it is assumed that the powdery mildew fungus has evolved means to exploit host defense signaling to its own advantage (Panstruga 2003). There is even evidence that powdery mildew fungi actively suppress host-cell death during compatible interaction, causing the ''green island'' effect' (Schulze-Lefert and Vogel 2000). The ''green island'' effect of a compatible interaction between barley and the powdery mildew fungus (a biotroph) illustrates massive pathogen-induced changes of cell death regulation resulting in cell death suppression in invaded cells and leaf senescence suppression (Hückelhoven et al. 2003). In this study, the tomato powdery mildew fungus used is also a biotroph, not only combating plant defense, but also suppressing plant cell death, which may explain why more than half of the DE-TDFs are only associated with the compatible interaction of tomato and *O. neolycopersici*. The genes specific to the susceptible interaction are induced late, about 98% DE-TDFs of class I (only associated with inoculated S-MM), appeared at or after seven DPI (Fig. 2), suggesting that they may play a role in susceptibility. The genes identified in the compatible and incompatible interactions could be responsible for the basal defense in S-MM, which limits the pathogen infection to some extent. It cannot be excluded that some of the DE-TDFs are of fungal origin.

Expression peak in R-Ol-1 may coincide with formation of HR

An expression peak was detected at 7 DPI in R-Ol-1 for all the 64 class II-1 and eight class II-2 DE-TDFs. This may correspond to the timing and pattern of slow HR in the R-Ol-1, as fungal growth starts to be arrested at seven DPI. It will be interesting to see, whether the expression peak will be earlier in inoculated *Ol-4* lines, since in these lines cell death at primary haustoria is very effective and there is generally no continued hyphae growth after 3 DPI (Bai et al. 2005). In R-QTL, the 64 class-II-1 and 17 class II-3 DE-TDFs showed continuously up-regulated expression comparable to that in S-MM, except that about 55% of these DE-TDFs (Fig. 2) showed earlier expression in inoculated R-QTL compared to inoculated S-MM. Although we did not detect an induction peak for DE-TDFs in inoculated R-QTL, there may be a later expression peak at 9 DPI (9 DPI is not included in the present study). Interestingly, most of the class-II-1 DE-TDFs showed higher expression levels in compatible interactions at 9 DPI compared to incompatible interactions and ongoing up-regulation at 11 and 14 DPI. These two time-points cannot be compared to the resistant genotypes, as these were not evaluated. These class II DE-TDFs that are expressed in both resistant and susceptible interactions are involved in basal defense. That basal defense operates against pathogen attack even in susceptible plants was clearly illustrated by the identification of several super-susceptible mutants (reviewed by Hammond-Kosack and Parker 2003). The observation that the response in S-MM is slow but constantly increases till later time-points can

be explained by the fact that there are much more interaction sites between tomato cells and fungi in inoculated susceptible plants compared to resistant plants. Especially in later time-points infection pressure continues in the susceptible interaction so that continuously more cells are penetrated by haustoria, whereas in this stage, many cells in R-Ol-1 undergo HR and fungal growth ceases, thus the 'defense machinery' slows down.

Expressional timing difference of the overlapping components between the response pathways of compatible and incompatible interaction is crucial

In this study it appears that the genes induced in both compatible and incompatible interactions (class II) with functions in known defense responses (group A) are generally earlier induced in incompatible interactions compared to the compatible interaction. This conclusion is based on the annotation, expression pattern and timing of DE-TDFs of group A (known defense) (Appendix 1, No. 2–6 and 16–29). Since only four DE-TDFs from this class II were involved in signaling (group B, Appendix 1, No. 7–8 and 30–31), we cannot make any hypothesis concerning timing differences between compatible and incompatible interactions. Six of the nine DE-TDFs with group C functions (transcription regulation) are earlier induced in incompatible interactions compared to compatible interaction (Appendix 1, No. 9–11 similar timing; No. 32–37 earlier in incompatible interactions). In contrast, 4 out of 5 group D genes (protein synthesis/degradation) display similar timing in compatible and incompatible interactions (Appendix 1, No. 12–15 and 38). The data also indicated that genes, which displayed constitutively higher expression level in incompatible interaction compared to compatible interaction, are often associated with transcription regulation (Appendix 1, No. 63–69) (Fulop et al. 2005). In general, the data suggest that most of the sequenced function-informative DE-TDFs, which showed earlier timing in incompatible interactions or were resistance specific, are involved in known defense and transcription regulation (Appendix 1). Therefore, we conclude that the quicker or higher-level expression of transcription factors and known defense genes may be crucial for the final fate of the interaction between tomato and *O. neolycopersici*. Hence the difference between in the resistance responses mediated by *Ol-1* and the 3 *Ol*-QTLs on the one hand and basal defense in the compatible interaction on the other hand is quantitative rather than qualitative.

Similarly, for the interaction of *Arabidopsis* and the bacterial pathogen *P. syringae*, a quantitative model was proposed and further discussed to decipher the difference between *R*-gene mediated defense and basal defense in the compatible interaction (Tao et al. 2003; Eulgem 2005). This quantitative model is consistent with the tomato—*O. neolycopersici* system in this study, since expression of genes involved in the compatible and incompatible interactions mainly differed in timing.

Possible resistance mechanisms involved in tomato and *O. neolycopersici* interactions

From the sequence information of many DE-TDFs we conclude that oxidative burst (H_2O_2) and HR play a role in the interaction of tomato and *O. neolycopersici*, since many related genes were induced during the interaction such as Glutathione S-transferase (Appendix 1, No. 20 and 27–28), ascorbate peroxidase (Appendix 1, No. 17), peroxiredoxin 3 (Appendix 1, No. 4), malate oxidoreductase/dehydrogenase (Appendix 1, No. 25/26) and pyruvate dehydrogenase kinase (Appendix 1, No. 43) (Chen et al. 2003). The HR in tomato infected by *O. neolycopersici* is associated with the production of H_2O_2 (unpublished histological data) and HR was proven to be the main response of R-Ol-1 against *O. neolycopersici* (Bai et al. 2005). Meanwhile, the transcript profiling data of fungal inoculated *S. neorickii*, carrying three R-QTLs provided evidence that the resistance mechanism of R-QTL is also associated with oxidative burst and HR similar to that of R-Ol-1, since a similar set of genes was induced during the interaction with the fungus in both genotypes. Even though a former study concluded that resistance in *S. neorickii* (R-QTL genotype) is less associated with HR compared to that of the resistance in R-Ol-1 (Huang et al. 2000a, b), further histological investigation on Near Isogenic Lines carrying individual QTLs and combinations thereof supports this hypothesis (paper in preparation).

The cDNA-AFLP profiles also indicated that SA (salicylic acid) is a signal to mediate the resistance response to the fungus in tomato. First, several genes that are key enzymes of SA synthesis, like shikimate dehydrogenase (Appendix 1, No. 3) and phenylalanine ammonia-lyase (Appendix 1, No. 24) are activated during the interaction; secondly several pathogenesis related (PR) genes, which are normally involved in the SA pathway, such as chitinase (Appendix 1, No.2), P69 (Appendix 1, No. 42) and PR-1 (protein assay, data not shown), are induced during the interaction. Disease tests and gene expression studies on the interaction of

O. neolycopersici and NahG tomatoes, which are deficient in SA mediated responses, will confirm this conclusion.

Conclusion

In the tomato—*O. neolycopersici* interaction, twice as many genes are induced in the compatible interaction as in the incompatible interactions. Genes involved in basal defense of the compatible interaction and *R*-gene mediated response of the incompatible interactions overlap to a great extent. The expression differences of these genes involved in basal defense of compatible interactions, monogenic and polygenic resistance responses are mainly in timing. Oxidative burst and the SA pathway are involved in both the compatible interaction and in monogenic resistant and polygenic resistance mediated interactions of tomato and *O. neolycopersici*.

Materials and methods

Plant materials

Three tomato genotypes were used in the cDNA profiling experiments: *S. lycopersicum* cv. Moneymaker (referred to as S-MM), as susceptible genotype; BC_1S_2 plants homozygous for the resistance gene *Ol-1* (referred to as R-Ol-1), generated by backcrossing MM with a breeding line harboring *Ol-1* introgressed from *S. habrochaites* G1.1560 and being selected using linked molecular markers; *S. neorickii* G1.1601, a wild tomato accession (referred to as R-QTL), which harbors three major *Ol*-QTLs.

Fungal material and inoculum preparation

Oidium neolycopersici was collected from infected tomato plants in the Netherlands (Lindhout et al. 1994a) and is continuously maintained on S-MM plants in growth chambers at 20 ± 2°C, relative humidity (RH) 70% and 16 h day-length. Fresh spores were washed from seriously infected leaves with water to prepare the inoculum (2×10^4 spores/ml). Water was sprayed as mock inoculation.

Experimental set-up of and sampling

All plants were grown in climate cells under optimal temperature, photoperiod and light conditions (20 ± 2°C, 16 h daytime, light intensity 150 μmol/m^2/s). Two independent inoculation experiments were performed as biological controls for cDNA-AFLP analysis. The experimental design consisted of two randomized blocks for both experiments with S-MM as borderlines and controls for spontaneous infection. Four-week-old plants were used for whole-plant inoculation as described by Bai et al. (2003). The second and third true leaves were collected and directly put into liquid N_2 and the remaining plant was kept for macroscopic disease evaluation. For each leaf sample another plant was used. In experiment one, samples were collected from inoculated and mock-inoculated plants of S-MM, R-Ol-1 and R-QTL at 0, 5, 24, 29, 48, 72 HPI. In experiment two, samples were collected at 0, 1, 2, 3, 4, 7 DPI for both resistant genotypes and at the same time points plus 9 and 14 DPI for S-MM.

cDNA-AFLP

RNA isolation and cDNA synthesis were accomplished according to the cDNA-AFLP protocol of Bachem et al. (1998) (also can be found at http://www.dpw.wau.nl/pv/). In brief, the ''hot-phenol'' method was used to isolate RNA. The concentration and integrity of total RNA were measured with the spectrophotometer (Eppendorf, Germany) and checked on 1% agarose gel. For mRNA purification and enrichment, polyA$^+$-RNA was extracted from 20 μg of total RNA using poly-d[T]$_{25}$V oligonucleotides coupled to paramagnetic beads (Dynal A.S. Oslo, Norway). Double-strand cDNA was synthesized using SuperScriptII RNase H$^-$ reverse transcriptase, RNase H and DNA polymerase I (*E. coli*) (all purchased from Invitrogen life technology, USA). Double-strand cDNA was extracted with phenol: chloroform (1:1), ethanol-precipitated and dissolved into a suitable volume sterilized H_2O. The cDNA quality was checked on 1% agarose gel and the concentration was measured by using a spectrophotometer (Eppendorf, Germany). Template preparation followed the standard AFLP protocol (Vos et al. 1995; Bachem et al. 1996). Two restriction enzyme combinations *Ase*I/*Taq*I and *Mse*I/*Eco*RI were used (sequence details of primers and adaptors see Bachem et al. (1996) and Vos et al. (1995)). For the large scale screening, pre-amplification products of all the time-points were bulked per genotype-treatment prior to selective amplification: hereafter referred to as bulk time-point analyses. Primer pairs of *Eco*RI + 3/*Mse*I + 2 and *Ase*I + 2/*Taq*I + 2 were used for selective amplification. Selective amplification was conducted with one of the two primers labeled with IRD700 or IRD800 (LICOR, USA). PCR products were separated on 6% PAGE gel and visualized with a LICOR sequencer (LICOR, USA).

Excision and sequencing of interesting fragments

Interesting DE-TDFs were excised from PAGE gel using the Odyssey machine (LICOR, USA), and then re-amplified with *M13r_M00* (5′-GGATAACAATTTCACACAGGGATGAGTCCTGAGAA) and *M13f_E00* (5′-TTTCCCAGTCACGACGTTGGACTGCGTACCAATTC) or *Ase*I00 (5′-CTCGTAGACTGCGTACCTAAT) and *Taq*I00 (5′-ACGATGAGTCCTGACCGA) and purified over G50 columns (Amersham Bioscience, USA). The PCR products were sequenced directly (Greenomics and Baseclear, The Netherlands).

Sequence analyses, primer designing and RT-PCR

The BLAST results were obtained against TIGR (http://www.tigr.org/tdb/tgi/plant.shtml) tomato/*Arabidopsis* TC databases using BLASTN and TBLASTX. Primers were designed based on the obtained DE-TDF sequences using the program Primer 3 (http://frodo.wi.mit.edu/cgi-bin/ primer3/). The primer sequences of actin were obtained from literature (Ditt et al. 2001). Semi-quantitative RT-PCR was conducted with the designed primers following the PCR program: 94 °C 1 min (min); 94 °C 30 s (s), 60 °C 30 s and 72 °C 1 min for 30 cycles; 72 °C 7 min. The PCR products were displayed on 1.2% agarose gels.

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Appendix 1 List of the DE-TDFs with homologies (e value < 5e–02)

No.	PC-size[a]	Pattern[b]	Class[c]	Timing[d]	e value	Group[e]	Homology annotation[f]
1	M20E58-200	MI	I	NA	1.0e–4	A	Infected *Arabidopsis* Leaf *Arabidopsis thaliana* cDNA, mRNA sequence
2	M12E58-290	MIOIPI	II-1	–	8.9e–44	A	Basic 30 kDa endochitinase precursor (PR-2)
3	M12E62-186	MIOIPI	II-1	–	2.8e–19	A	3-dehydroquinate dehydratase/shikimate dehydrogenase isoform 2
4	M18E41-260	MIOIPI	II-1	–	5.3e–17	A	Peroxiredoxin 3,
5	M22E61-510	MIOIPI	II-1	–	1.1e–34	A	Selenium binding protein
6	M23E55-430	MIOIPI	II-1	–	1.0e–29	A	*N*-hydroxycinnamoyl-CoA:tyramine *N*-hydroxycinnamoyl transferase THT1-3 [*Lycopersicon esculentum*]
7	M12E62-196	MIOIPI	II-1	–	1.3e–17	B	GDP dissociation inhibitor
8	M21E49-265	MIOIPI	II-1	–	1.1e–3	B	Protein kinase-like protein {*Arabidopsis thaliana*}
9	A16T13-262	MIOIPI	II-1	–	4.0e–17	C	Glucose-regulated protein 78
10	M14E47-332	MIOIPI	II-1	–	1.4e–20	C	J8-like protein {*Arabidopsis thaliana*}
11	M21E53-310	MIOIPI	II-1	–	4.2e–4	C	Nucleolin (Protein C23)
12	M13E64-325	MIOIPI	II-1	–	2.1e–47	D	Ribosomal protein L27a
13	M15E34-170	MIOIPI	II-1	–	1.0e–14	D	40S ribosomal protein S4. [Potato] {*Solanum tuberosum*}
14	M16E58-205	MIOIPI	II-1	–	1.0e–7	D	Chloroplast protease {*Capsicum annuum*}, complete
15	M23E55-196	MIOIPI	II-1	–	8.4e–15	D	Tragopogon dubius large subunit 26S ribosomal RNA gene, partial sequence, partial (80%)
16	A13T13-400	MIOIPI	II-1	+	4.9e–17	A	Aspartic proteinase—related
17	A16T13-235	MIOIPI	II-1	+	2.1e–6	A	Ascorbate peroxidase
18	M12E42-265	MIOIPI	II-1	+	2.5e–18	A	Cytochrome P450 76A2 CYPLXXVIA2) (P-450EG7)
19	M13E49-176	MIOIPI	II-1	+	8.1e–10	A	Snakin2 {*Solanum tuberosum*}, complete
20	M13E51-460	MIOIPI	II-1	+	1.3e–71	A	Probable glutathione S-transferase
21	M13E66-330	MIOIPI	II-1	+	2.3e–50	A	Protein disulfide isomerase
22	M14E42-429	MIOIPI	II-1	+	3.1e–23	A	Short-chain acyl-CoA oxidase
23	M15E70-150	MIOIPI	II-1	+	8.3e–12	A	AKIN gamma, partial (82%)
24	M18E43-380	MIOIPI	II-1	+	2.4e–53	A	Phenylalanine ammonia-lyase (PAL)
25	M20E37-365	MIOIPI	II-1	+	4.4e–69	A	Malate oxidoreductase, cytoplasmic
26	M21E34-182	MIOIPI	II-1	+	6.0e–20	A	Malate dehydrogenase mRNA, complete cds; nuclear gene for mitochondrial product
27	M21E49-455	MIOIPI	II-1	+	3.9e–71	A	Probable glutathione S-transferase
28	M21E53-455	MIOIPI	II-1	+	1.1e–64	A	Probable glutathione S-transferase

Appendix 1 continued

No.	PC-size[a]	Pattern[b]	Class[c]	Timing[d]	e value	Group[e]	Homology annotation[f]
29	M21E57-312	MIOIPI	II-1	+	1.0e–41	A	Expressed protein, weakly similar to putative PrMC3
30	M13E49-150	MIOIPI	II-1	+	2.0e–5	B	Putative GTP-binding protein {Oryza sativa (japonica cultivar-group)}
31	M14E67-135	MIOIPI	II-1	+	8.4e–09	B	Serine/threonine protein kinase kkialre-like 1 {*Homo sapiens*}, partial (1%)
32	M12E60-245	MIOIPI	II-1	+	2.2e–22	C	DEAD box RNA helicase (RH26)
33	M13E64-215	MIOIPI	II-1	+	1.5e–25	C	Enolase (2-phosphoglycerate dehydratase)
34	M13E64-315	MIOIPI	II-1	+	6.0e–10	C	myb-related transcription factor TH
35	M14E42-355	MIOIPI	II-1	+	3.3e–21	C	MADS-box transcription factor
36	M15E71-220	MIOIPI	II-1	+	1.1e–27	C	Homeobox, complete
37	M20E37-270	MIOIPI	II-1	+	9.2e–38	C	Storekeeper protein, partial
38	M12E62-800	MIOIPI	II-1	+	4.4e–115	D	Ubiquitin
39	M13E64-370	MIOIPI	II-1	+	7.4e–33	E	UDP-glucoronosyl/UDP-glucosyl transferase family protein contains Pfam profile: PF00201
40	A13T24-226	MIOIPI	II-1	+	2.2e–05	F	Adenylosuccinate synthetase
41	M17E62-160	MIOIPI	II-1	NA	1.4e–2	A	S-adenosyl-l-homocysteine hydrolase
42	M17E49-195	MIOIPI	II-1	NA	3.0e–14	A	P69C protein
43	M22E47-430	MIOIPI	II-1	NA	3.0e–33	A	Pyruvate dehydrogenase kinase {*Arabidopsis thaliana*}
44	M21E47-170	MIOIPI	II-1	NA	2.1e–6	B	Ras-related GTP-binding protein (RAN3) identical to atran3 [*Arabidopsis thaliana*] GI:2058280
45	M21E48-190	MIOIPI	II-1	NA	2.8e–9	C	RNA-binding protein {*Arabidopsis thaliana*}, partial
46	M12E62-180	MIOIPI	II-1	NA	9.0e–20	E	Putative heme A farnesyltransferase homolog {Oryza sativa (japonica cultivar-group)}
47	M21E52-220	MIOIPI	II-1	NA	1.6e–2	F	Oxidoreductase short-chain dehydrogenase/reductase family-like protein {*Arabidopsis thaliana*}
48	M16E75-185	MIOIPI	II-1	NA	3.5e–5	H	Putative protein
49	M21E56-370	MIOIPI	II-1	NA	6.0e–19	H	Unknown protein {*Arabidopsis thaliana*}
50	A13T13-85	MIPI	II-3	–	5.1e–10	D	Yippee like protein
51	M13E48-251	MIPI	II-3	–	3.0e–8	B	Putative GTP-binding protein
52	M13E48-195	MIPI	II-3	–	4.2e–18	D	Hexameric polyubiquitin {*Nicotiana sylvestris*}
53	A13T24-230	MIOWOIPI	II-4	+	1.4e–7	A	Tomato genome clone BH144711.1 homology to Apoptosis inhibitor {*Arabidopsis thaliana*}
54	M15E76-390	MIOWOIPI	II-4	+	8.1e–68	E	60S acidic ribosomal protein
55	M12E58-355	MIOIPWPI	II-4	+	3.2e–56	E	Tragopogon dubius large subunit 26S ribosomal RNA gene, partial sequence
56	A18T23-108	OI	III-1	+	4.9e–4	A	Cytochrome P450 family protein
57	M19E35-205	OI	III-1	+	1.8e–20	C	Arginine/serine-rich protein, a kind of RNA-binding protein contains domain of splicing factor
58	M13E53-319	PI	III-2	+	7.0e–6	A	Putative senescence-associated protein {*Pisum sativum*}
59	M22E55-229	PI	III-2	+	7e–21	A	Putative senescence-associated protein {*Pisum sativum*}
60	M14E47-310	PI	III-2	+	3.5e–16	E	Chlorophyll A-B binding protein 13 chloroplast precursor (LHCII type III CAB-13). [Tomato]
61	M22E35-520	OWOI	IV	+	1.3e–21	A	Tobamovirus multiplication 2B
62	M14E72-209	OWOI	IV	+	4.9e–2	B	GTP-binding protein Rab6 -common tobacco
63	M16E68-255	OWOI	IV	+	1.2e–37	C	RNA helicase (RH16), a kind of translation initiation factor kinase
64	M14E42-465	OWOI	IV	+	1.4e–71	C	Enolase (2-phosphoglycerate dehydratase)
65	M13E40-220	OWOIPWPI	IV	+	3.0e–20	C	Transcription elongation factor
66	M13E40-235	OWOIPWPI	IV	+	3.0e–20	C	Transcription elongation factor
67	M12E34-275	OWOIPWPI	IV	+	2.0e–20	C	Putative RING zinc finger protein {*Arabidopsis thaliana*}
68	M21E57-280	OWOIPWPI	IV	+	6.1e–40	C	Nam-like protein 10, a kind of transcription factor
69	M12E42-225	PWPI	IV	+	1.0e–19	C	Nuclear transport factor 2 (NTF2) family protein/RNA recognition motif (RRM)-containing protein
70	M19E61-189	OWOI	IV	+	6.0e–16	D	60S ribosomal protein L6 (YL16-like)
71	M11E69-195	OWOI	IV	+	3.9e–20	E	Acetolactate synthase II chloroplast precursor (EC 4.1.3.18)

Appendix 1 continued

No.	PC-size[a]	Pattern[b]	Class[c]	Timing[d]	e value	Group[e]	Homology annotation[f]
72	M18E41-220	OWOI	IV	+	2.8e–34	H	Expressed protein, partial (66%)
73	M11E69-190	PWPI	IV	+	4.1e–27	H	Hypothetical protein F22K20.8

[a]DE-TDFs were named after primer combination-fragment size

[b]Abbreviations in this column represent the expression pattern of DE-TDFs, I: inoculated with spore suspension of *O. neolycopersici*, W: mock-inoculated with water; M: susceptible genotype MM, O: monogenic resistant genotype R-Ol-1 and P: polygenic resistant genotype R-QTL

[c]Classes in this table have the same indication as those in Figs. 1 and 2

[d]The ''earlier timing'' refer to whether the DE-TDF were earlier expression in resistant genotypes compared to S-MM; in this column, ''+'' represents that the DE-TDFs showed earlier timing in resistant genotypes or specific to resistance genotypes; ''–'' represents that same temporal pattern of the DE-TDF was displayed between resistant and susceptible genotypes. ''NA'' means that the corresponding DE-TDFs were only identified in bulk time-point analyses but no time-course data available

[e]The functional groups have same interpretation as those in Table 2

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