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Overlapping sets of transcripts from host and non-host interactions of tomato are expressed early during non-host resistance

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Abstract

Natural immunity present in all the plants against most of the pathogens is called as non-host resistance (NHR). Although NHR is most durable form of resistance, it was less studied compared to other forms of resistance. We compared transcriptional changes in tomato during non-host (*Magnaporthe grisea*) and compatible (*Alternaria alternata* f. sp. *lycopersici*) interactions using Agilent microarray GeneChip containing ~44,000 probe sets. The experiment was designed to understand the early and late responses of tomato leaves inoculated with non-host and compatible pathogens. Microarray data revealed that the expression profiles in the non-host and compatible interactions at 6 h post inoculation (hpi) and 24 hpi largely overlapped indicating that a set of genes are activated during plant-pathogen interaction. However, these genes were expressed much earlier in NHR compared to a compatible interaction. NHR is, therefore, an accelerated and amplified basal defense response. Transcripts involved in energy production (carbohydrate metabolism and photosynthesis) were down-regulated, whereas transcripts associated with catabolic processes (starch and sucrose hydrolysis) were up-regulated in both the interactions at 6 and 24 hpi. We have also identified that the pathway involved in synthesis of volatile compounds like 2-phenylethanol was induced during NHR in tomato. This is the first report of transcriptome profile in tomato during non-host interactions against *M. grisea*.

Keywords: *Alternaria alternata*, *Magnaporthe grisea*, Microarray, Non-host resistance, Tomato.

Abbreviations: NHR_non-host resistance; HXT_hexose transporters; PAL_phenylalanine ammonia lyase; 4CL_4-coumarate-CoA ligase; LOX_lipoxygenase; AADC_aromatic amino acid decarboxylase; CT_*P*-coumaroyl tyramine; FT_feruloyl –CoA-tyramine.

Introduction

Plants are constantly exposed to several pathogenic micro-organisms, but, disease is rare due to innate resistance present in all plants. The native resistance of most plant species against a wide variety of pathogens is known as non-host resistance (NHR), which confers durable protection to plant species (Uma et al., 2011). A plant species that does not succumb to disease, when infected by a pathogen, is referred to as a non-host plant for that pathogen and the interactions as non-host interactions. NHR is genetically complex and involves several components of constitutive and inducible plant defenses. Penetration of a non-adapted pathogen on non-host plant is restricted by the structural barriers like deposition of callose at cell walls and lignin formation, as a first line of defense. Upon breaching of the structural barriers by pathogen, inducible defense response like accumulation of reactive oxygen species (ROS) that lead to hypersensitive cell death.

A plant is resistant or susceptible to a specific pathogen depending on the speed and rate at which the same host defense molecules are produced, suggesting that the resistance is based on quantitative rather than qualitative differences (Tao et al., 2003). Total transcriptome analysis of *Arabidopsis* and barley, during non-host and compatible interactions, did not show significant host- or non-host specific expression (Tao et al., 2003; Lee et al., 2004; Zimmerli et al., 2004; Eichmann et al., 2006; Stein et al., 2006). Down-regulation of house-keeping or development-related genes during non-host interactions of *Arabidopsis* with powdery mildew represents physiological requirement for allocation of resources to express non-host defense (Zimmerli et al., 2004). Most of the differentially expressed defense-related genes were common to both non-host and compatible interactions indicating that these genes are likely components of basal defense responses (Zimmerli et al., 2004). *Arabidopsis* inoculated with *Blumeria graminis* pv. *hordei* (*Bgh*) (non-host) produced a more dramatic up- or down-transcript response than *Erysiphe cichoracearum* (host), because, *Bgh* cannot suppress host basal defenses, whereas *E. cichoracearum* suppresses the basal defenses (Stein et al., 2006). NHR and basal host defense of barley are functionally related. The NHR to different fungal pathogens is associated with more robust regulation of a complex and largely non-overlapping sets of pathogen-responsive genes involved in similar metabolic or signaling pathways (Zellerhoff et al., 2010).

Magnaporthe grisea is a hemibiotrophic fungus causing blast disease on rice, while *Alternaria alternata* f. sp. *lycopersici* is a necrotrophic fungus causing stem canker on tomato. We obtained the transcriptome profile in tomato (*Lycopersicon esculentum* cv. Money maker), for which the genome sequence was available. We compared the transcript profiles of tomato during non-host interactions with *M. grisea* and compatible interactions with *A. alternata* f. sp. *lycopersici* to know the genes involved in NHR. Here, we report that a set of defense-genes that are commonly expressed in both host and non-host interactions, are expressed early during NHR.

Results and Discussion

Changes in transcriptome of tomato during non-host and compatible interactions

Transcriptome changes were studied using the GeneChip ® Tomato Genome Array (Agilent) to measure and compare the difference in transcript accumulation (44, 000 probes) between pathogen- and mock-inoculated tomato leaves at 6 and 24 hpi. Two independent replications of the experiment were conducted. All GeneChip data were analyzed using GeneSpring GX v11.5 software.

Differentially regulated genes (more than one-fold change with a $P \leq 0.01$) in non-host and compatible interactions of tomato were identified at 6 and 24 hpi using mock-inoculated reference samples. In the compatible interaction, the number of up-regulated genes was 1525, 2815 at 6 and 24 hpi, respectively, while the number of down-regulated genes was 1614 and 3263 at 6 hpi and 24 hpi, respectively (Fig 1A). In non-host interactions of tomato, the number of up-regulated genes was 1713 and 1709 at 6 and 24 hpi, respectively. The number of genes down-regulated was 1856 and 1620 at 6 and 24 hpi, respectively (Fig 1A). The number of transcripts actually responding to pathogen inoculation is less than the number in Fig. 1A, B, because multiple probe sets have the same annotation that represent the same gene. Differentially regulated genes, monitored at different time points, were pooled as one gene set for the compatible and one set for non-host interactions at a single time point. About two-thirds of the regulated genes overlapped between the interactions (Fig 1B).

In the available tomato genome sequence, information on gene annotations was limited and for many genes the function is still unknown. Therefore, we followed an orthology prediction (majorly with *Solanum tuberosum*) for all gene probe sets that can be probed by the GeneChip and assigned gene ontology (GO) annotation to all the genes identified as differentially regulated transcripts and categorized in to nine categories based on their possible role *viz*. carbohydrate metabolism, photosynthesis, transport, transcription, defense, stress, cell wall, lipid metabolism and signal transduction (Fig 2). Most of the transcripts belonged to two categories like "transport" and "transcription". A substantial overlap of the differentially expressed transcripts was in line with similar observations made by Tao et al. (2003) and Thilmony et al. (2006). These overlapping transcripts, however, appeared early during non-host interactions. We discuss the significance of some of these differentially expressed genes in plant-pathogen interactions.

Differential expression of defense-related genes

Several genes annotated as PR proteins, putative *R*-genes and fungal cell wall degrading enzymes were grouped under this category. Transcripts related to different classes of *PR*-genes were abundant in both the interactions (Table 1). No significant quantitative change in expression of *PR*-genes was observed between compatible and non-host defense responses of tomato leaves. Cysteine proteases are key enzymes in the regulation of cell death and cysteine protease inhibitors also exist as counterparts to these enzymes to control cell death. In the present study, cysteine protease was highly up-regulated, whereas cysteine protease inhibitor was highly down-regulated at early hours of non-host interactions (6 hpi) compared to compatible interactions. Solomon et al. (1999) reported that plant cell death can be regulated by

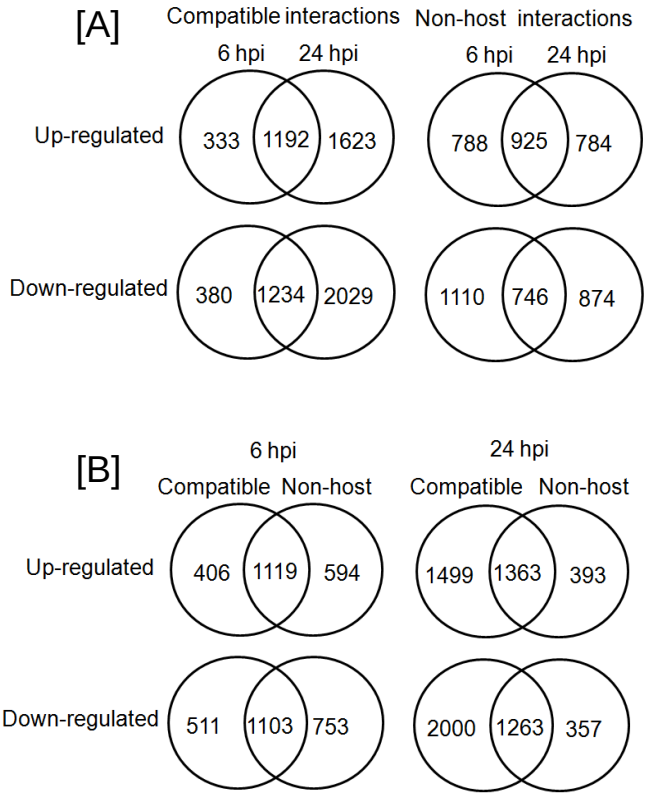

Fig 1. The number of differentially expressed transcripts during non-host and compatible interactions. Venn diagrams showing the number of transcripts that were differentially expressed in tomato leaves during non-host and compatible interactions at a level of $\log_2$ expression value of ≥ 1 and ≤ -1 with $P<0.01$. (A) Number of differentially regulated transcripts during compatible interactions *vs* mock inoculated (left) and non-host interactions *vs* mock-inoculated (right) at 6 and 24 hpi compared. (B) Number of specific and overlapped transcripts between compatible and non-host interactions of tomato at 6 and 24 hpi.

activity poised between the cysteine proteases and the cysteine protease inhibitors.

The transcripts encoding expansins and xyloglucangly-cosylases (XET7, 6 and 4) were down-regulated in tomato during non-host and compatible interactions (Table 2). Among the different cellulose synthases, CesA2 and CesA4 were up-regulated, whereas CesA3 and CesA1 were down-regulated in both the interactions. Loss-of-function or treatment with inhibitors of CESA3, which leads to decrease in the cellulose content of the wall, causes constitutive expression of genes of JA/ET signaling or results in production of lignin in response to pathogen attack or wounding (Caño-Delgado et al., 2003; Ellis et al., 2002). In the present study, the transcript coding for *CESA3* was down-regulated in both non-host and compatible interactions.

Expression of signaling-related genes during tomato defense responses

Several genes involved in signal transduction events were differentially expressed in both the interactions such as receptor kinases, protein kinases and calcium-mediated signal transduction proteins (Table 3). Components of MAP kinase cascades were differentially regulated in line with the

Table 1. Defense-related transcripts that are differentially regulated during compatible and non-host interactions compared to mock-inoculated tomato leaves. hpi-hours post inoculation.

Agilent probe ID	Annotations	*M. grisea*		*A. alternata*	
		6hpi	24hpi	6hpi	24hpi
A_96_P014176	PR5-like protein	13.03	13.32	13.42	12.72
A_96_P000206	Pathogenesis-related protein 1b	8.18	10.96	8.64	11.18
A_96_P089309	β-1,3-glucanase	8.78	8.78	8.88	10.36
A_96_P077909	Pathogenesis-related protein STH-2	7.95	10.08	8.58	12.03
A_96_P078394	Probable glutathione S-transferase	5.92	6.14	7.20	6.27
A_96_P156561	Pathogenesis-related protein PR P23	4.43	5.48	4.71	4.72
A_96_P189314	Pathogenesis-related protein 10	4.40	6.22	4.69	6.60
A_96_P100859	Subtilisin-like protease	4.00	2.56	4.18	4.56
A_96_P152326	Pathogenesis-related protein 1	2.78	4.68	3.41	5.37
A_96_P253872	Acidic endochitinase precursor	2.47	2.21	1.76	3.59
A_96_P095694	Putative thaumatin-like protein	2.87	2.63	3.03	1.54
A_96_P075974	Endochitinase 1	2.97	5.26	3.35	4.87
A_96_P139547	Pathogenesis related protein PR-1	2.68	4.92	1.24	5.18
A_96_P018276	Acidic class II 1,3-beta-glucanase	1.63	2.18	1.40	2.15
A_96_P013446	Proteinase inhibitor type-2	3.90	10.50	6.52	13.34
A_96_P151571	Cationic peroxidase precursor	8.53	8.11	8.63	9.79
A_96_P093534	9-divinyl ether synthase (StDES)	8.23	7.99	8.91	9.07
A_96_P000936	Wound-induced protein WIN2	4.43	6.19	7.20	6.27
A_96_P067211	Wound-induced protein WIN1	4.22	6.02	4.81	5.82
A_96_P100859	Subtilisin-like protease	4.00	2.56	4.18	4.56
A_96_P118227	Putative disease resistance protein	3.52	4.59	4.14	5.37
A_96_P020146	Chymotrypsin inhibitor I, A, B and C subunits	3.39	4.21	3.26	3.50
A_96_P249007	Avr9/Cf-9 rapidly elicited protein 75	2.74	4.37	3.62	4.60
A_96_P030386	CC-NBS-LRR protein	3.20	3.42	3.42	2.52
A_96_P056796	MLO1 protein	3.65	3.09	3.87	3.53
A_96_P006596	CC-NB-LRR protein	2.81	3.47	3.06	3.94
A_96_P073894	CC-NB-LRR protein	2.33	2.26	2.41	2.23
A_96_P015756	Putative disease resistance protein	2.89	2.24	2.50	2.97
A_96_P045356	Cysteine protease	5.25	3.61	4.31	5.07
A_96_P097919	Wounding-induced ribonuclease	1.74	3.05	1.95	3.38
A_96_P014281	ss-galactosidase	1.90	4.63	1.51	6.55
A_96_P091704	Multicystatin	-8.40	-8.15	-6.48	-7.55
A_96_P012921	Endo-β-1,4-glucanase	-2.61	-2.15	-3.51	-4.84
A_96_P098909	Metallocarboxypeptidase inhibitor	-5.90	-4.89	-6.20	-8.48
A_96_P150631	EDS1 protein	-3.37	-4.62	-3.25	-4.87
A_96_P015701	RGC1 (Fragment)	-2.10	-3.02	-2.02	-2.53
A_96_P218389	Resistance gene-like	-2.53	-5.63	-2.23	-8.08
A_96_P072149	Resistance gene-like	-2.45	-5.84	-2.20	-8.55

Fold changes in up- (with no prefix) and down-regulated (with negative mark prefix) between mock- and pathogen (*M. grisea* and *A. alternata* f. sp. *lycopersici)* inoculated tomato leaves are given.

evidence that MAP kinase modules play important roles in plant immunity (Pedley and Martin, 2004). We identified that many transcripts encoding MAP kinase signaling cascade (MPK3, 4, 1, MAP7K, WIPK, and MKK4) were up-regulated in tomato during non-host and compatible interactions (Table 3). The MAP kinase, LeMPK3 was implicated in resistance to *Pseudomonas* and *Xanthomonas* bacterial strains (Ekengren et al., 2003; Mayrose et al., 2004). The orthologues of tobacco SIPK and WIPK, tomato MPK2 and MPK3, were activated in the AvrPto-Pto system tomato (Pedley and Martin, 2004).

In our current study, Ca^{2+}-dependent protein kinases like CDPK4, CDPK and calmodulin 5/6/7/8-like protein were up-regulated in both the interactions. Activation of phospholipases contributes to the production of a potent second messenger phosphatidic acid, which modulates the activity of a variety of proteins involved in defense signaling (Legendre et al., 1993). Transcripts encoding enzymes involved in lipid signaling pathways like phospholipases (PLDa2, PLC3 and PLC2) were up-regulated at early hours of interactions (Table 3).

Regulation of hormone-metabolism related genes

Accumulation of transcripts related to hormone (JA and ET) metabolism was observed after inoculation with either of the pathogens (Table 3). The transcripts related to JA/ET biosynthesis like lipoxygenase (LOX), allene oxide synthase (AOS1 and AOS2), 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase), and ACC synthase were up-regulated in both the interactions (Table 3). The JA/ET hormones are positive regulators of resistance to necrotrophic fungi (Glazebrook, 2001) and may be part of the basal defense response.

The genes involved in degradation of gibberellins to non-active gibberellins, like gibberellin 2-oxidase, gibberellin 7-oxidase, and gibberellin 20-oxidase were down-regulated in both interactions of tomato (Table 3). But Lee et al. (2004) reported that gibberellin 2-oxidase was up-regulated during

Table 2. Cell wall-related transcripts that are differentially regulated during compatible and non-host interactions compared to mock-inoculated tomato leaves. hpi-hours post inoculation.

Agilent probe ID	Annonations	*M. grisea*		*A. alternata*	
		6hpi	24hpi	6hpi	24hpi
A_96_P042366	Cellulose synthase (StCesA4)	7.36	7.38	7.78	7.00
A_96_P100319	Pectin methylesterase 3	6.85	7.39	6.59	7.44
A_96_P099499	Hydroxyproline-rich glycoprotein	6.59	5.86	6.81	6.47
A_96_P058846	Feruloyl transferase	4.41	5.71	5.05	8.19
A_96_P130347	Xyloglycan endo-transglycan	5.22	2.62	5.18	2.85
A_96_P117727	Xyloglucan ndotransglycosylase/hydrolase XTH-6	5.77	7.38	5.45	9.02
A_96_P073939	Xyloglucanendotransglucosylase-hydrolase XTH3	4.42	1.43	3.85	1.31
A_96_P243974	Expansin-like protein precursor	4.03	2.95	3.58	3.86
A_96_P011891	β-mannosidase enzyme	4.96	3.38	4.65	2.01
A_96_P158731	Pectin methylesterase	3.23	2.74	3.27	1.93
A_96_P041966	UDP-glucose:protein transglucosylase-like	2.72	2.09	2.71	1.78
A_96_P248462	Glycine-rich protein	3.65	4.57	3.85	3.97
A_96_P000731	Expansin	2.28	3.06	1.87	3.23
A_96_P045776	Expansin 8	2.11	3.04	2.31	3.51
A_96_P012411	Extensin (class I)	2.53	5.11	2.63	2.85
A_96_P011936	α-L-arabinofuranosidase	1.72	1.95	2.16	2.65
A_96_P100389	Xyloglucan specific endoglucanase inhibitor3	2.28	3.44	2.31	1.99
A_96_P074214	Cellulose synthase (CesA2)	1.81	3.78	2.15	3.48
A_96_P038656	Expansin 4	-6.50	-4.50	-6.62	-7.21
A_96_P012926	Expansin2	-6.58	-6.00	-7.13	-7.75
A_96_P010741	xyloglucan endotransglycosylase	-4.80	-2.98	-3.19	-3.85
A_96_P204879	Xyloglucan endotransglycosylaseXTH4	-4.60	-3.99	-3.28	-3.67
A_96_P005651	UDP-GlcNac-dolichyl-phosphateN-acetylglucosaminephosphotransferase	-4.33	-5.17	-4.36	-6.18
A_96_P061456	Xyloglucan-specific endoglucanase inhibitor 4	-1.19	-1.73	-1.78	-3.32
A_96_P204789	Polygalacturonase inhibitor protein	-2.27	-3.34	-3.08	-4.58
A_96_P228099	Pectinesterase	-4.41	-5.09	-6.09	-5.22
A_96_P009231	Expansin11	-3.12	-3.01	-3.79	-4.93
A_96_P029251	Cellulose synthase (CesA1)	-3.13	-7.39	-4.69	-9.02
A_96_P004486	Arabinogalactan	-3.04	-4.27	-2.95	-5.62
A_96_P256572	Polygalacturonase-like protein-like	-2.49	-6.14	-2.32	-4.44
A_96_P076124	Methionine rich arabinogalactan	-1.24	-3.40	-1.82	-4.33
A_96_P014166	Expansin12	-1.88	-4.51	-3.38	-6.20
A_96_P012911	Expansin10	-1.22	-2.06	-1.79	-2.93
A_96_P000556	Expansin A4	-1.47	-1.26	-1.64	-1.95
A_96_P014751	Xyloglucanendotransglucosylase-hydrolase XTH7	-1.69	-3.93	-2.58	-5.09
A_96_P262677	Xyloglucan galactosyltransferase	-1.43	-2.32	-1.45	-2.12
A_96_P251522	Cellulose synthase (CesA3)	-1.11	-4.15	-1.76	-3.98
A_96_P253037	UDP-apiose/xylose synthase	-1.35	-2.07	-1.85	-2.24

Fold changes in up- (with no prefix) and down-regulated (with negative mark prefix) between mock- and pathogen (*M. grisea* and *A. alternata* f. sp. *lycopersici)* inoculated tomato leaves are given.

non-host interactions of hot-pepper against *Xanthomonas axonopodis* pv. *glycines*.

Differential activation of secondary metabolism-related genes

The activation of the phenylpropanoid pathway produces many secondary metabolites, such as lignins, flavonoids and isoflavonoids (Whitbred and Schuler, 2000). Phenylalanine ammonia-lyase (PAL) is a key biosynthetic catalyst in phenyl propanoid pathway. In our study, we have identified that transcript encoding PAL1 was down-regulated in tomato during non-host and compatible interactions (Table 4). Further, the transcripts encoding enzymes involved in the biosynthetic pathway of alkaloids and terpenoids were also down-regulated after inoculation with non-host and compatible pathogens. But, transcripts encoding enzymes involved in flavonoid synthesis like flavonoid 3', 5'-hydroxylase, flavanone 3 β-hydroxylase and flavonol synthase increased in both the interactions. But, transcript encoding isoflavone reductase (IFR) was down-regulated (Table 4).

Regulation of primary metabolism related genes

Photosynthesis and carbohydrate metabolism-related transcripts were down-regulated in tomato leaves inoculated with non-host and compatible interactions compared to mock-inoculated leaves (Table S1). Most of the photosynthesis-related genes were significantly down-regulated in both the interactions except respiratory burst oxidase protein (rbohF). Repression of photosynthesis-related

Table 3. MAPK pathway and hormone-related transcripts that are differentially regulated during compatible and non-host interactions compared to mock-inoculated tomato leaves. hpi-hours post inoculation.

Agilent probe ID	Annotations	*M. grisea*		*A. alternata*	
		6hpi	24hpi	6hpi	24hpi
Mitogen-activated kinase pathway					
A_96_P015986	MPK3	10.34	9.04	10.43	9.69
A_96_P117882	Mitogen-activated protein kinase	4.37	5.21	3.75	5.21
A_96_P113192	MAP3K-like protein kinase	3.06	4.26	3.46	3.98
A_96_P184109	MAPKK	2.24	2.35	1.97	2.58
A_96_P014206	WIPK	1.79	1.22	2.36	1.82
A_96_P017116	MAPK7	1.94	1.59	2.12	2.61
A_96_P020686	MEK2	1.93	2.06	2.22	2.18
A_96_P021096	MKK4	2.08	2.61	2.03	2.17
A_96_P117882	MPK4	4.37	5.21	3.75	5.21
A_96_P033771	MKP1	1.42	1.89	2.27	2.06
A_96_P081289	SERK3B	1.81	1.74	1.79	1.78
A_96_P247652	SERK1	3.54	3.87	2.87	3.90
Hormone related					
A_96_P011951	Auxin-regulated protein	4.33	4.08	5.02	4.64
A_96_P232704	Allene oxide synthase 1	8.42	8.47	8.73	8.33
A_96_P000016	Allene oxide synthase 2	1.73	1.64	1.72	1.15
A_96_P020931	ACC oxidase	8.08	7.90	6.45	6.11
A_96_P012551	ACC synthase	6.18	5.16	6.30	5.81
A_96_P000131	Putative ethylene receptor protein	2.87	3.00	2.95	2.22
A_96_P083929	Methyl jasmonate esterase	-5.15	-4.51	-4.37	-5.62
A_96_P135992	Zeaxanthin epoxidase	-5.84	-4.08	-4.57	-5.90
A_96_P076504	Gibberellin 20-oxidase-1	-6.22	-6.19	-7.09	-5.52
A_96_P125177	Gibberellin 7-oxidase	-7.26	-5.77	-7.16	-7.59
A_96_P230164	Gibberellin 2-oxidase 1	-9.46	-6.96	-9.18	-8.14
A_96_P231099	Neoxanthin synthase	-1.93	-2.51	-2.00	-2.04
A_96_P017766	Putative ethylene receptor	-2.60	-3.13	-2.82	-3.15
Lipid signaling pathways					
A_96_P232679	Calmodulin 5/6/7/8-like protein	5.27	6.98	6.32	8.43
A_96_P140802	Calcium-dependent protein kinase 4	2.49	3.20	2.17	2.83
A_96_P095489	Calcium dependent protein kinase	2.17	3.23	2.22	3.18
A_96_P011991	Phospholipase PLDa2	2.96	2.40	2.86	2.91
A_96_P100049	phospholipase C PLC3	1.75	2.36	1.80	2.28
A_96_P250357	phospholipase C PLC2	1.40	2.11	1.90	2.59
Sugar signaling					
A_96_P014026	SNF1 kinase complex anchoring protein	2.31	2.74	2.58	3.93

Fold changes in up- (with no prefix) and down-regulated (with negative mark prefix) between mock- and pathogen (*M. grisea* and *A. alternata* f. sp. *lycopersici)* inoculated tomato leaves are given.

genes was observed in incompatible host-pathogen interactions (Matsumura et al., 2003). Transcripts related to carbohydrate metabolism like glycolysis, Calvin cycle and Krebs cycle were down-regulated in both host and non-host interactions (Fig 3), indicating that activation of NHR imposes a metabolic cost to the plant. Such a relationship between growth and defense responses was not uncommon (Berger et al., 2004).

The transcripts encoding hexose transporters (HXT1, HXT3) and sucrose transporter (SUT1) were up-regulated at high levels in tomato leaves inoculated with the pathogens. (Table S2). Hexoses, generated by the hydrolysis of sucrose, act as signaling molecules during pathogen attack which in turn induce defense-related genes (Ehness et al. 1997; Gomez-Ariza et al., 2007). The expression of transcripts required for starch degradation (α-amylase, and α-glucosidase) was elevated in tomato leaves inoculated with the pathogens. Increase in starch degradation was also reported in potato-*Erwinia* interaction (Stewart et al., 1994). Our observations indicate a possible co-ordination of defense responses (including NHR responses) and growth in plants, with metabolic resources shunted to defense responses.

Changes in fatty acid and amino acid metabolism genes

The transcripts encoding 9-LOX and 13-LOX were up-regulated significantly in both the interactions (Table S3). Rance et al. (1998) reported that 9-LOX activity was up-regulated during tobacco-*Phytophtora parasitica* var. *nicotianae* interactions. Two 9-LOX-derived compounds with antimicrobial activity, colneleic and colnelenic acids are synthesized upon pathogen infection in the potato-*P. infestans* interaction (Weber et al., 1999). Further, the divinyl ether synthase gene involved in synthesis of colnelenic acid was up-regulated highly (8-fold) in tomato leaves inoculated with non-host and compatible pathogens.

In tomato, a small family of decarboxylases (LeAADC1A, LeAADC1B, and LeAADC2) was involved in conversion of phenylalanine to phenethylamine and tyrosine to tyramine (Tieman et al., 2006) (Table S3). Tyramine was shown to be involved in synthesis of antimicrobial compounds like (feruloyl-CoA-tyramine FT) and *P*-coumaroyl tyramine (CT). In our study, three AADC isozymes were up-regulated in tomato during non-host and compatible interactions (Table

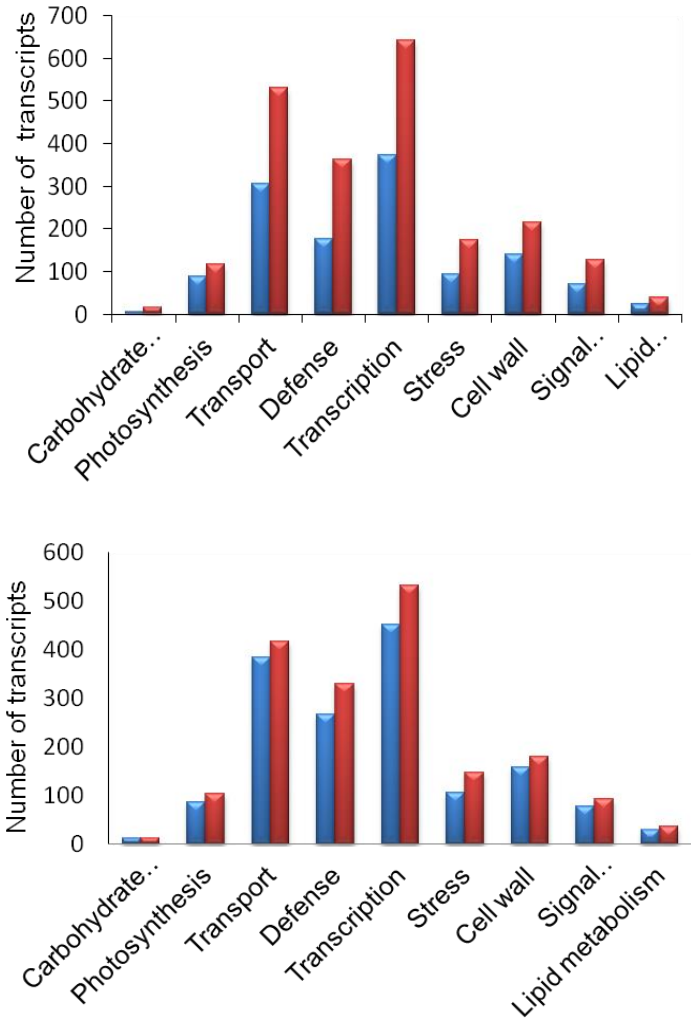

Fig 2. Functional categories of differentially expressed transcripts during non-host and compatible interactions. Assigned functional categories of differentially expressed transcripts using cut-off statistical parameter $P<0.01$ with $\log_2$ expression value of ≥ 1 and ≤ -1. Differentially expressed transcripts in *A. alternata* f. sp. *lycopersici*-challenged (A) and *M. grisea*-challenged (B) tomato leaves compared to mock-inoculated tomato leaves. The blue and red bars represent the differentially regulated transcripts at 6 and 24 hpi in each functional category, respectively.

S3). There were no reports on the role of AADC and 2-phenylethanol in plant defense responses.

Differential expression of genes encoding transcriptional factors

The transcriptional factors belonging to different families, like WRKY, MYB and NAM/NAC factors were differentially regulated (Table S4). The WRKY transcription factors 2 and 71 were up-regulated in non-host and compatible interactions of tomato. Mohr et al. (2010) also reported that WRKY transcription factors regulate expression of surveillance genes at the top of the defense-signaling cascade, including the positive regulation of an *R* gene by one or more WRKY proteins.

Different transcripts of NAC/NAM were induced in tomato during non-host and compatible interactions (Table S4). The NAC are a family of genes specific to plants and play a role in defense and abiotic stress responses as well as in a diverse set of developmental processes. CUP-SHAPED COTYLEDON (CUC), a part of a larger NAC (for NAM, ATAF, and CUC) protein family of transcription factors was also up-regulated in tomato during non-host and compatible interactions (van Esse et al., 2009). The barley NAC gene HvNAC6 was implicated in basal defense against the barley powdery mildew pathogen *Bgh* (Jensen et al., 2007).

Transcripts that are differentially regulated either in non-host or in compatible interactions

Some of the transcripts were differentially regulated either in non-host or in compatible interactions. The transcript encoding WRKY-type DNA binding protein was up-regulated in non-host interactions, and down-regulated in compatible interactions. Branched chain α-keto acid dehydrogenase E1-α subunit, involved in amino acid degradation to generate precursor molecules and energy to cells, was up-regulated only in non-host interaction. Peroxisomal acyl-CoA oxidase 1A, which is involved in fatty acid oxidation in peroxisomes, was up-regulated only in compatible interactions. TSW12 (non-specific lipid-transfer protein 1) was down-regulated only in compatible interactions. Torres-Schumann et al. (1992) reported that TSW12 was induced in tomato during seed germination and its level increases after NaCl treatment or heat shock.

Materials and Methods

Plant material and pathogen inoculation

Tomato (*Lycopersicon esculentum* cv. Money maker) plants were grown in soil in a growth chamber with a 16 h photoperiod at 350 lE/m2 light intensity at 24 ºC and at constant (70%) humidity. One month-old tomato plants were inoculated with conidial suspension of *M. grisea* and *A. alternata* f. sp. *lycopersici* containing 1 x 10^6 spores per mL. A conidial suspension was obtained by washing 7 days-old PDA slant cultures with distilled water containing 0.02% Tween-20. Mock inoculation was done with 0.02% Tween-20 in distilled water.

Experimental design and GeneChip analysis

Sample collection

All samples were collected in two independently repeated experiments at 6, and 24 h post inoculation (hpi). For each sample, leaf material was harvested from three plants inoculated with non-host and host pathogens, pooled separately, and flash frozen in liquid nitrogen. Leaves collected from mock-inoculated plants were used as the reference sample to which all other samples were compared.

RNA isolation

Total RNA was isolated from 100 mg of the frozen leaves using NucleoSpin RNA plant kit (Machery Nagel, Duren, Germany). RNA samples were analyzed on Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA, U.S.A.) prior to GeneChip hybridization. RNA was considered to be of good quality when the rRNA 28S/18S ratios were greater than or equal to 1.5, with the rRNA contribution being 30% or more and an RNA integrity number (RIN) was ≥7.0.

Table 4. Secondary metabolism-related transcripts that are differentially regulated during compatible and non-host interactions compared to mock-inoculated tomato leaves. hpi-hours post inoculation.

Agilent probe ID	Annotation	*M. grisea*		*A. alternata*	
		6 hpi	24 hpi	6 hpi	24 hpi
A_96_P208614	Flavonol synthase	7.54	7.88	7.63	8.46
A_96_P212304	Flavanone 3-β-hydroxylase	4.39	4.16	4.38	4.95
A_96_P086204	Flavonoid 3',5'-hydroxylase	4.17	4.26	4.33	4.92
A_96_P085909	Caffeoyl-CoAO-methyl transferase	2.77	2.94	3.23	3.03
A_96_P099654	Chalcone isomerase	1.41	1.54	1.60	1.78
A_96_P091574	4-coumarate-CoA ligase 1	5.16	4.19	6.01	4.34
A_96_P015671	3-hydroxy-3-methylglutaryl coenzyme A synthase	3.26	3.46	3.81	3.11
A_96_P145376	N-hydroxycinnamoyl-CoA:tyramineN-hydroxycinnamoyl transferase THT1-3	3.93	3.76	4.80	4.28
A_96_P229424	N-hydroxycinnamoyl-CoA:tyramine N-hydroxycinnamoyl transferase THT7-1	2.20	1.79	2.81	2.53
A_96_P012713	N-hydroxycinnamoyl-CoA:tyramine N-hydroxycinnamoyl transferase THT7-8	1.67	1.72	2.48	3.32
A_96_P103969	Flavonoid3-glucosyl transferase	-8.73	-5.51	-6.21	-4.00
A_96_P214559	Chalcone synthase 2	-6.27	-5.86	-6.36	-6.33
A_96_P063816	Tropinone reductase II	-4.36	-2.66	-5.30	-2.68
A_96_P088214	Phytoene synthase	-4.59	-4.90	-4.55	-5.13
A_96_P060786	PAL 1	-4.04	-6.01	-4.37	-6.46
A_96_P054696	Lycopene beta-cyclase	-3.67	-3.89	-3.99	-4.47
A_96_P020766	Tropinone reductase I	-3.60	-3.96	-3.48	-5.40
A_96_P001996	Monoterpene synthase 1	-3.30	-2.35	-2.88	-3.81
A_96_P249197	Carotenoidcleavage oxygenase	-3.60	-3.72	-3.92	-2.98
A_96_P039156	Cinnamoyl CoA reductase 2	-3.02	-3.20	-2.80	-3.46
A_96_P219504	1-deoxy-D-xylulose-5-phosphate reductoisomerase activity	-2.59	-2.77	-2.18	-2.48
A_96_P013701	Putative tropinone reductase	-2.23	-2.24	-1.65	-4.48
A_96_P085554	HMG-CoA reductase	-2.46	-2.73	-3.35	-3.33
A_96_P122147	Lycopene epsilon-cyclase	-1.26	-4.37	-1.62	-5.30
A_96_P214374	Chalcone synthase	-2.22	-3.75	-2.48	-4.55
A_96_P010611	9-cis-epoxy-carotenoid dioxygenase 1	-3.83	-3.40	-3.97	-3.19
A_96_P027786	Cycloartenol synthase	-1.00	-3.27	-1.69	-4.08
A_96_P191299	Isoflavone reductase homolog	-1.79	-3.64	-1.71	-4.85

Fold changes in up- (with no prefix) and down-regulated (with negative mark prefix) between mock- and pathogen (*M. grisea* and *A. alternata* f. sp. *lycopersici)* inoculated tomato leaves are given.

RNA labeling

Double-stranded cDNA was synthesized from poly(A)+ mRNA present in the total RNA using MMLV-reverse transcriptase (Agilent Quick Amp Kit, USA) and a primer encoding a T7 RNA polymerase promoter sequence fused to (dT) 24. The double-stranded cDNA was purified and used as a template in the subsequent *in vitro* transcription reaction. Fluorescent complimentary RNA (cRNA) was generated from cDNA for one-color processing using Agilent's Quick Amp Labeling Kit (USA). The amplification of cRNA was carried out in the presence of T7 RNA polymerase, cyanine 3-labeled CTP and NTPs mix.

Hybridization and data collection

The labeled target cRNA was purified, fragmented, and hybridized to a whole genome tomato 4X44K AMADID: 22270 gene chip arrays according to protocols provided by the manufacturer (Agilent, USA). Fragmentation of labeled cRNA and hybridization was d one using the Gene Expression Hybridization kit of Agil ent (Part Number 5188-5242). Hybridization was carried out in Agilent's surehyb chambers at 65 °C for 16 h. The hybridized slides were washed using Agilent's gene expression wash buffers (Part No. 5188-5327) and scanned using the Agilent microarray scanner G Model G2565BA at 5 µ resolution.

Data analysis

The scanned images were manually verified and found to be devoid of uneven hybridization, streaks, blobs and other artifacts. Hybridization across the slide was good based on number of feature that were "g is PosAndSignif" which indicates feature is positive and significantly above background. Feature Extraction (FE) 9.5.3 supported extraction of one-color .tif images of Agilent microarrays scanned on Agilent Scanner.

Normalization was done using Gene Spring GX v11.5 Software. Intra-array normalization, which deals with variability within a single array, was done among the controls using Percentile Shift Normalization method. In intra-array normalization, gProcessed signal (dye normalized background subtracted signal intensity) was log transformed, and for each of the array, the 75th percentile value was calculated separately. In each sample, the log transformed intensity value for each probe was subtracted by the calculated 75th percentile value of the respective array and expression values were obtained.

Feature extracted data was analyzed using Gene Spring GX v11.5 software from Agilent (USA). Signal quantification

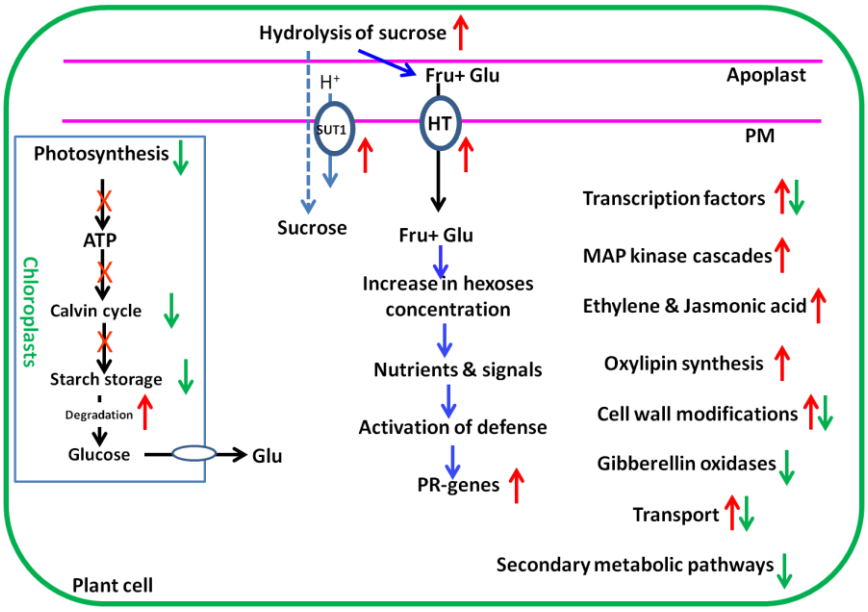

Fig 3. A model summarizing the different metabolic pathways affected during non-host and compatible interactions of tomato. A green arrow indicates that the number of genes involved in the corresponding metabolic pathway is down- regulated. A red arrow indicates the opposite. Green and red arrows drawn together indicate that the number of up-regulated genes and down-regulated genes are not much differed.

and data analysis were achieved using Gene Spring GX v11.5 software. Following local background subtraction, the signal for each spot was normalized based on the median value of the median intensity of all the spots for each array. Only genes for which the hybridization signal was greater than the average value plus two standard deviations of the controls were analyzed. Each ratio was converted to its $\log_2$ value, and the average $\log_2$ value for each gene of the two independent arrays corresponding to each experiment was calculated. Statistical significance of the gene expression differential over the course of the replicate experiments was calculated by using a Student's t test analysis. Only genes with high levels of significance ($P < 0.01$) and a minimum absolute value of $\log_2 > 1$ were systematically considered in this study, to minimize the false positive as up- or down-regulated. Expression profiles from each time point were clustered based on their similarity in expression pattern using a hierarchical average linkage clustering algorithm and Pearson correlation distance.

Annotation of probe set

Differentially expressed transcripts were annotated using the BLAST hit from the non-redundant database of NCBI http://www.ncbi.nlm.nih.gov) against *Solanum tuberosum* total genome. For GO, we used potato gene model for each probe set.

Conclusions

The transcripts that are differentially regulated during both the non-host and host interactions majorly belonged to basal disease response, known to be induced by all pathogens in plants. A few defense-related genes were expressed early during non-host interactions of tomato with *M. grisea*. The basal defense was overcome by *A. alternata* f. sp. *lycopersici*, but not by *M. grisea*. Genes involved in the synthesis of volatile compounds like 2-phenylethanol were highly up-regulated in both the non-host and compatible interactions.

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