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IDENTIFICATION OF KEY MOLECULAR COMPONENTS OF THE RESISTANCE OF CHERRY TOMATO AGAINST *Phytophthora infestans*

Identificación de los principales componentes moleculares de la resistencia de tomate cherry contra *Phytophthora infestans*

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ABSTRACT

Cherry tomato *Solanum lycopersicum* var *cerasiforme* cv Matt's wild cherry is a very resistant cultivar to most *Phytophthora infestans* isolates. Two isolates were identified, US940480 and US970001 that cause an incompatible and a compatible interaction respectively. US970001 is one of the few isolates producing a compatible interaction with this cultivar. To identify genes with a differential gene expression between compatible and incompatible interactions, gene expression patterns were analyzed with tomato cDNA microarrays including 12,899 independent tomato cDNA clones at different time points after inoculation. A diverse set of statistical tools were used to identify key components of the plant response to the pathogen. Forty-three genes were up-regulated during the incompatible reaction at time point 36 hours, 15 globally at all time points and twelve were found both in globally and at 36 hours. Northern blots analysis was performed to confirm differential expression showed by microarray analysis and to study the differential expression of more plant resistance genes (PR) genes between compatible and incompatible interactions for this interaction.

Keywords: Compatible interaction, incompatible interaction, Matt's wild cherry, *Solanum lycopersicum* var. *cerasiforme*, *Phytophthora infestans*.

RESUMEN

El tomate cherry *Solanum lycopersicum* var *cerasiforme* cv Matt's es bastante resistente a la gran parte de aislamientos de *Phytophthora infestans*. Se han identificado dos aislamientos, US940480 y US970001 que causan interacción incompatible y compatible

respectivamente. US970001 es uno de los pocos aislamientos causantes de interacción compatible con este cultivo. Con el fin de identificar genes con expresión diferencial en interacciones compatible e incompatible, analizamos DNA copia de 12899 clones independientes en tres tiempos posteriores a la inoculación del patógeno. Se aplicaron diversas herramientas estadísticas para identificar componentes moleculares claves de la respuesta de la planta al patógeno. Cuarenta y tres genes fueron detectados como activados durante la interacción incompatible a las 36 horas posinoculación, 15 genes se detectaron como activados globalmente tomando en conjunto los 3 tiempos analizados y 12 genes tanto globalmente como a las 36 horas. Análisis de *Northern blot* permitieron confirmar la expresión diferencial detectada con los análisis de microarreglos y estudiar la expresión diferencial de otros genes de resistencia en plantas (PR) en interacciones compatible e incompatible en esta interacción.

Palabras clave: interacción compatible, interacción incompatible, tomate cherry Matt's, *Solanum lycopersicum* var. *cerasiforme*, *Phytophthora infestans*.

INTRODUCTION

Three well-defined types of host-pathogen interactions between tomato and *Phytophthora infestans* occur: highly compatible, partially compatible and incompatible interactions (Gallegly and Marvel, 1995). Several studies have focused in the partial compatible and compatible interaction between tomato and *Phytophthora infestans*. A previous study showed that partial compatibility in tomato against *P. infestans* acts independently of ethylene, Salicylic acid (SA), and Jasmonic Acid (JA) defense response pathways (Smart *et al.*, 2003). Other studies showed that compatibility is dependent upon salicylic acid (SA) and ethylene but apparently is not dependent upon the jasmonic acid (JA) signaling pathways (Niderman *et al.*, 1995; Fidantsef *et al.*, 1999; Stout *et al.*, 1999). Additionally, it is possible that patterns of ethylene-responsive plant resistance gene (PR) expression may be a general response to the biotrophic nature of highly compatible interactions between *P. infestans* and tomato rather than a specific defense response (Smart *et al.*, 2003). However, recent studies that could shed light to this matter and the involvement of other metabolic pathways in the compatibility between *P. infestans* and tomato are not found.

Highly compatible and partially compatible interactions were studied using the susceptible tomato cultivar Rio Grande inoculated with either a tomato-specialized isolate (highly compatible) or a non-specialized isolate (partially compatible) (Smart *et al.*, 2003). As expected, there was induction of the hypersensitive response (HR) earlier during the partially compatible interaction. However, contrary to our expectation, pathogenesis-related (PR) gene expression was not stimulated sooner in the partially compatible interaction.

In a general sense, the interaction between tomato and its pathogens is complex and besides, the aforementioned signaling pathways, it involves the co-regulation of gene expression, photosynthesis and sugar levels (Berger *et al.*, 2004). The repression of photosynthesis has been observed in cell suspension cultures of tomato treated with elicitors (Sinha *et al.*, 2002), in intact plants after the interaction with viral (Herbers *et al.*, 2000; Hanssen *et al.*, 2011), bacterial (Kocal *et al.*, 2008) and fungal pathogens

(Prokopová *et al.*, 2010) although in the latter case the impairment of photosynthesis was minimal. The more common explanation to this change in metabolism is the switch from normal to defense metabolism when a plant is challenged by a pathogen. In potato, down-regulation as a consequence of the *P. infestans* compatible interaction was observed for genes encoding proteins involved in photosynthesis (Restrepo *et al.*, 2005). The reason for the reduction in photosynthesis during the compatible interaction between potato and *P. infestans* is currently unknown.

Now, compatible interaction between an isolate of *P. infestans* (US970001) and a cherry tomato *S. lycopersicum* var *cerasiforme* cv Matt's wild cherry (Muller, 1940) were identified. This is a very resistant cultivar, which is only partially compatible with even the most aggressive tomato-specialized isolates (such as US980025) and incompatible with the isolate US940480. These interactions on Matt's wild cherry do allow us to compare host resistance responses between compatible and incompatible interactions.

MATERIALS AND METHODS

Plants, pathogen isolates, and inoculations

Four-week-old *Solanum lycopersicum* var *cerasiforme* cv Matt's wild cherry plants were placed into a humid chamber with a 16 h light period, 12 h per day of 100 % relative humidity, and a temperature of 15 °C. Plants were inoculated with *P. infestans* isolates US940480 (ATCC # 208834, a member of the US-8 clonal lineage) resulting in an incompatible interaction, or with isolate US970001 (ATCC # MYA-2350, a member of the US-17 clonal lineage) resulting in a compatible interaction. Another group of plants was sprayed with water (the mock-inoculated control). Inoculations were made with a sporangial suspension of 20,000/mL, and plants were sprayed until run-off as previously described (Smart *et al.*, 1998). Tissue (all leaflets) from three plants per group was collected at three time points (12, 36 and 72 hours after inoculation). The leaflets for each treatment at each time-point were pooled, flash frozen in liquid nitrogen, and stored at -80 °C. After the 72 h time-point, there were two plants left from each group (incompatible, compatible and mock-inoculated). These plants were kept in the humid chamber for an additional seven days to ensure that plants inoculated with the compatible isolate (US970001) became fully diseased, while those inoculated with the incompatible isolate (US940480) or mock-inoculated with water remained healthy. The entire experiment was repeated three times.

Tomato RNA isolation and preparation of array probes. Frozen plant tissues were ground using a cold mortar and a pestle. RNA was isolated from healthy tomato tissue, inoculated tomato tissue or *in vitro* grown *P. infestans*, using a previously described hot phenol method (Perry and Francki, 1992) with modifications described by Gu *et al.*, (2000). Ten µg of total RNA from each sample was separated electrophoretically on a 1.2 % formaldehyde-agarose gel, and transferred to Hybond-N membrane (Amersham Biosciences, Piscataway, NJ). Hybridizations were performed using Puregene hyb-9 hybridization solution (Gentra Systems, Plymouth, MN). The RNA was precipitated, pooled and stored at -80 °C.

For each array hybridization, cDNA was generated from total RNA (150 µg) isolated from Matt's wild cherry tomato leaflets inoculated with *P. infestans*, isolates US940480

(incompatible interaction) or US970001 (compatible interaction). cDNA from the incompatible interaction was labeled with one cyanine fluorophore (Cy3 or Cy5), while cDNA from the compatible interaction was labeled with the other. All protocols used to generate and label cDNA were as described by Hedge et al (Hedge *et al.*, 2000).

Microarray hybridization and scanning

Tomato cDNA arrays generated by the Center for Gene Expression Profiling (CGEP), Boyce Thompson Institute, Ithaca, New York, USA were used in these experiments. All clones, more than 12,800, were validated through resequencing and agarose gel electrophoresis prior to printing to confirm the sequence of the clone and the presence of an insert. All data on the EST sequences, the clones on the array, and annotation of the clones can be found at the Tomato Functional Genomics Database (TFGD) web site at http://ted.bti.cornell.edu/. Arrays were probed with infected tomato tissue (incompatible and compatible interaction) cDNA at each of the three time-points (12, 36 and 72 hpi). Two arrays per time point (3) were hybridized using the dye-swap design for each of the three biological replicates for a total of 18 arrays. Additionally, one dye swap experiment before infection was undertaken (2 arrays).

Arrays were pre-hybridized in order to block nonspecific background during hybridization. Slides were blocked in 5 X SSC, 0.1 % SDS, and 1 % bovine serum albumin at 42 °C for 45 min (Hedge *et al.*, 2000). Slides were washed in sterile distilled water followed by isopropanol and dried. Cy-3 and Cy-5 probes were combined, placed on the slide, and covered using a glass cover slip washed in 1 % SDS. Arrays were put into hybridization chambers (Corning Inc, NY) and hybridized overnight at 42 °C in a water bath. The slides were removed from the chambers and washed in 2 X SSC and 0.1 % SDS at 42 °C from 5 min., in 0.1 X SSC and 0.1 % SDS at room temperature for 5 min., and twice in 0.1 X SSC at room temperature for 5 min., then dried. Slides were scanned using an Axon GenePix 4100 microarray scanner (Axon Instruments Inc, Union City CA). The photomultiplier settings were adjusted in each channel to result in non-saturation of the most highly expressed genes.

Array data analysis

To determine fluorescence intensity and background intensity, 16-bit TIFF scanned images were analyzed using the software GenePix Pro version 4.1 (Axon Instruments Inc). The median pixel intensity within a given spot was used as the signal intensity. For the Cy5 dye, the background signal intensity determined by GenePix was occasionally higher than that of genes that were not highly expressed but that had previously been shown to be differentially expressed (Smart *et al.*, 2003). Therefore, to avoid negative values we utilized the strategy proposed by Frick and Schaller, (2002), and did not subtract the background signal. The log2-transformed expression values across all samples were standardized to a mean of zero and standard deviation of one (row standardization). Differentially expressed genes were determined using significance analysis of microarray (SAM) proposed by Tusher *et al.*, (2001). At each time-point, differentially expressed genes were detected comparing mean expression in each of the two conditions (incompatible and compatible interactions). An overall comparison of both conditions was done comparing mean expression of each condition along all time-

points. This global comparison could mask genes that are up-regulated at one time-point and down-regulated at another time point but allows the detection of genes that are consistently either up-or down-regulated.

Database submission of microarray data

The microarray data were prepared according to the Minimum Information about a Microarray Experiment (MIAME) recommendations (Brazma *et al.*, 2001) and submitted to the TED (Tomato Expression Database) database which is available at http://ted.bti.cornell.edu/.

Principal Component Analysis

A Principal Component Analysis (PCA) was conducted using genes as individuals and all time point hybridizations as variables by means of the ade4 R package (Dray *et al.*, 2007). The result of the PCA is the definition of new variables called Principal Components (PCs) that are linear combinations of the original variables and allow representing graphically in less dimensions (usually in two) the overall phenomena and data structure. Here the first two PC were used to represent the original 40 microarray conditions. The 40 microarray conditions correspond to 40 total RNAs hybridized: three time points, three biological replicates, two dye swaps per time point and two self-self hybridizations, a total of 20 hybridations, 40 RNAs, two RNAs (a Cy3 and a Cy5 – labeled RNA) are combined per hybridization.

PCA also produces the coordinates of all individuals (here genes) in the new two-dimensional space (plane). These coordinates are locations of the genes on a plane and can be used to plot genes on this space. Coordinates on the PC space of genes were used to characterize similarities between gene sets involved in resistance and disease processes. Based on previous studies we constructed a set of 187 genes involved in resistance and disease processes, see next section. The projection of genes on the PC space was plotted highlighting each of the gene sets with a different color and indicating their distance to the centroid (mean point) of each group. Moreover, the distances between genes of each group were obtained on the PC space of the first two components in order to compare the distance between and within groups.

Construction of gene sets implicated in resistance processes

Based on previous studies we constructed a set of 187 genes involved in resistance and disease processes. A total of 143 genes out of the 187 complete set, were filtered based solely in the gene description of the best BLAST hit and categorized as "*Phytophthora* inhibited protein", "Avr elicited" or "disease resistance". The remaining forty-four genes were classified as "inhibited by EPI1 and EPI2" (EPI: extracellular serine protease) (Tian *et al.*, 2004; Tian *et al.*, 2005; Tian *et al.*, 2007), WRKYs (Eulgem T. 2006) and "Pathogenicity associated" (Restrepo *et al.*, 2005).

Northern blot analysis

DNA probes were labeled using the Random Primers DNA Labeling System according to the manufacturers' protocol (Invitrogen, Carlsbad, CA). The PR genes used as probes were identical to those described by Gu *et al.*, (2000) and included; acidic glucanase

(GluA), basic glucanase (GluB), basic PR-1, and divinyl ether synthase (DES) and carbonic anhydrase (CA).

RESULTS AND DISCUSSION

A low number of expressed genes differentiates a compatible and an incompatible reaction

Expression analysis revealed a very low number of differentially expressed genes between compatible and incompatible interactions on Matt's wild cherry to *P. infestans*. Analysis of gene expression on the tomato (cv Matt's wild cherry) - *P. infestans* interaction compared two interactions, compatible and incompatible. In this study ratios correspond to expression in an incompatible interaction divided by expression in a compatible interaction. No differentially expressed genes were detected at 12 h or 60 h after inoculation. A total of 43/12899 up-regulated genes in the incompatible reaction were detected at time point 36. Combining all time points together, 15 of the 12899 clones represented on the TOM1 microarray were found to be up-regulated in the incompatible interaction vs. compatible interaction and 12 genes were up-regulated both at 36 h and globally (combining all time points). The pattern of expression was clearer when we considered genes up-regulated globally and at 36 h in the incompatible interaction since at least four genes out of 12 were involved in oxygen and free-radical metabolism, and a PR coding for proteins known for their involvement in signal transduction were also detected (supplementary table 1). Among the up-regulated genes at 36h in the incompatible cultivar (down-regulated for the compatible interaction), we could find PR proteins, photosystem I associated proteins and different types of kinases (supplementary table 1).

Novel statistical analyses reveal key molecular components of defense. Variability of gene expression fold change ratio after log normalization was analyzed and it was possible to detect that the overall variability is very low (standard deviations are 0.22 for the global experiment, 0.56 for the -12 timepoint and 0.33, 0.74, 0.27 for the 12, 36 and 60 timepoints respectively). This can be seen graphically in the boxplots depicted in figure 1. Interestingly, the most variable treatment was the experiment at timepoint -12. The most possible explanation for this can be that only one experiment (only one biological replicate) was available and the fold change is not applicable because it is not a real comparison of two conditions. This hybridization was only performed as a self-self control, the hybridization of two identical RNAs samples. This indicated that the variability of gene expression in this whole experiment is low, suggesting very few changes in gene expression in this particular pathosystem between a compatible and an incompatible interaction.

The PCA conducted on microarray data using genes as individuals was able to reduce the 40 original variables to two principal components (PCs) very successfully, as 75.3 % of the variance was retained in these first components. Moreover, the first PC retained 70.1 % of the variance indicating that most hybridizations are highly correlated and that differences in gene expression between time points is low or affects only few genes, which is on accordance with the low percentage of differentially expressed genes detected by SAM. As can be observed in figure 2 most variables are highly correlated with and

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
38	1-1-3.4.9.7	0,68	0,47	0,47	SGN-U143272	1-aminocyclopropane-1-carboxylate oxidase 1 (ACC oxidase 1) (Ethylene-forming enzyme) (EFE) (Protein pTOM 13)	e-103	SGN-U143272	1-aminocyclopropane-1-carboxylate oxidase 1 (ACC oxidase 1) (Ethylene-forming enzyme) (EFE) (Protein pTOM 13)	e-103	2	Ethylene related
39	1-1-8.4.7.3	0,64	0,41	0,34	SGN-U143274	1-aminocyclopropane-1-carboxylate oxidase 1 (ACC oxidase 1) (Ethylene-forming enzyme) (EFE) (Protein pTOM 13)	e-169	No sequence			2	Ethylene related
40	1-1-5.3.13.9	0,71	0,73	0,56	SGN-U143273	1-aminocyclopropane-1-carboxylate oxidase 2 (ACC oxidase 2) (Ethylene-forming enzyme) (EFE) (Protein GTOMA)	e-169	No sequence			2	Ethylene related
90	1-1-4.1.4.3	0,48	0,69	0,97	SGN-U143835	36.4 KD PROLINE-RICH PROTEIN	2e-028	SGN-U143835	36.4 KD PROLINE-RICH PROTEIN	2e-028	4	Protein biosynthesis
41	1-1-3.1.8.12	0,66	0,58	0,69	SGN-U143857	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase [*Solanum tuberosum*]	4e-039	SGN-U143858	Phospho-2-dehydro-3-deoxyheptonate aldolase 2, chloroplast precursor (Phospho -2-keto-3-deoxyheptonate aldolase 2) (DAHP synthetase 2) (3-deoxy-D-arabino-heptulosonate 7-phosphate synthase 2)	0.0	2	Glucose metabolism
1	1-1-7.1.9.16	0,84	0,50	0,60	SGN-U144956	AAA-type ATPase family [*Arabidopsis thaliana*]	e-114	SGN-U161319	No hits found		1	Transport
42	1-1-1.2.2.13	0,61	0,75	0,64	SGN-U144297	Acidic 26 kDa endochitinase precursor	e-149	SGN-U144297	Acidic 26 kDa endochitinase precursor	e-149	2	Defense
43	1-1-1.2.9.4	0,59	0,71	0,61	SGN-U144297	Acidic 26 kDa endochitinase precursor	e-149	SGN-U144297	Acidic 26 kDa endochitinase precursor	e-149	2	Defense
44	1-1-6.3.14.8	0,64	0,65	0,49	SGN-U144297	Acidic 26 kDa endochitinase precursor	e-149	SGN-U144297	Acidic 26 kDa endochitinase precursor	e-149	2	Defense
86	1-1-1.4.7.7	0,7	0,941	0,49	SGN-U143678	actin [imported] - Malva pusilla	0.0	No sequence			3	Uncategorized
2	1-1-4.3.15.6	0,83	0,57	0,73	SGN-U148131	aldehyde dehydrogenase (NAD) (EC 1.2.1.3) 2A precursor, mitochondrial-common tobacco	9e-027	No sequence			1	Stress responses
3	1-1-8.2.6.4	1,29	1,07	1,52	SGN-U149077	aux/IAA protein [*Populus tremula* x *Populus tremuloides*]	6e-073	No sequence			1	Auxin-related
116	1-1-2.2.10.11	0,85	1,68	1,63	SGN-U145913	auxin-regulated protein [*Arabidopsis thaliana*]	3e-054	SGN-U145913	auxin-regulated protein [*Arabidopsis thaliana*]	3e-054	5	Auxin-related
45	1-1-4.3.10.8	0,81	0,67	0,65	SGN-U146581	calcium-transporting ATPase [*Arabidopsis thaliana*]	0.0	SGN-U146582	potential calcium-transporting ATPase 9, plasma membrane-type (Ca2+-ATPase, isoform 9) [*Arabidopsis thaliana*]	3e-020	2	Transport
117	1-1-4.1.3.20	0,84	1,68	1,62	SGN-U147088	CBL-interacting protein kinase 23 [*Arabidopsis thaliana*]	0.0	SGN-U147088	CBL-interacting protein kinase 23 [*Arabidopsis thaliana*]	0.0	5	Signaling
118	1-1-1.2.7.16	0,83	1,33	2,01	SGN-U153535	CCR4-associated factor-related protein [*Arabidopsis thaliana*]	3e-076	SGN-U153535	CCR4-associated factor-related protein [*Arabidopsis thaliana*]	3e-076	5	Transcription
4	1-1-7.2.20.1	0,74	0,50	0,67	SGN-U145369	CER1 protein [*Arabidopsis thaliana*]	0.0	SGN-U145369	CER1 protein [*Arabidopsis thaliana*]	0.0	1	Uncategorized
46	1-1-6.4.1.2	0,93	0,64	0,61	SGN-U143697	CIG1 [*Nicotiana tabacum*]	0.0	SGN-U143697	CIG1 [*Nicotiana tabacum*]	0.0	2	Amino acid metabolism
119	1-1-2.1.4.6	0,72	1,68	1,62	SGN-U153930	CONSTANS B-box zinc finger family protein [*Arabidopsis thaliana*]	2e-040	SGN-U152950	putative zinc finger protein [*Oryza sativa*]	3e-027	5	Unknown

		Fold change			5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
5	1-1-8.3.20.1	0,82	0,40	0,63	SGN-U147362	CTV.15 [*Poncirus trifoliata*]	1e-040	No sequence			1	Defense
47	1-1-6.2.13.13	0,81	0,64	0,60	SGN-U148364	cyclic nucleotide-gated calmodulin-binding ion channel [*Nicotiana tabacum*]	1e-095	No sequence			2	Transport
120	1-1-4.3.9.3	0,74	1,34	1,46	SGN-U147907	cytochrome b6/f complex subunit VIII [*Arabidopsis thaliana*]	5e-008	SGN-U147907	cytochrome b6/f complex subunit VIII [*Arabidopsis thaliana*]	5e-008	5	Photosynthesis
121	1-1-4.2.1.19	0,88	1,97	1,82	SGN-U147993	DEAD/DEAH box helicase, putative [*Arabidopsis thaliana*]	8e-099	SGN-U153743	DEAD/DEAH box helicase, putative [*Arabidopsis thaliana*]	1e-015	5	Protein biosynthesis
122	1-1-1.3.9.9	0,87	1,62	1,89	SGN-U144008	dehydration-induced protein ERD15 [*Lycopersicon esculentum*]	3e-087	No sequence			5	Uncategorized
6	1-1-4.2.20.15	0,78	0,40	0,55	SGN-U148625	Delta12 fatty acid desaturase (EC 1.14.99.-) [imported] - Commerson's wild potato	e-157	SGN-U148625	Delta12 fatty acid desaturase (EC 1.14.99.-) [imported] - Commerson's wild potato	e-157	1	Uncategorized
48	1-1-2.4.2.20	1,53	1,40	1,59	SGN-U146266	dihydroxypolyprenylbenzoate methyltransferase [*Arabidopsis thaliana*]	e-113	No sequence			2	Respiration/electron transfer
49	1-1-3.4.10.20	0,76	0,59	0,60	SGN-U145711	disease resistance response protein-related/ dirigent protein-related [*Arabidopsis thaliana*]	1e-039	SGN-U145711	disease resistance response protein-related/ dirigent protein-related [*Arabidopsis thaliana*]	1e-039	2	Defense
50	1-1-4.3.10.20	0,75	0,55	0,51	SGN-U145711	disease resistance response protein-related/ dirigent protein-related [*Arabidopsis thaliana*]	1e-039	SGN-U145711	disease resistance response protein-related/ dirigent protein-related [*Arabidopsis thaliana*]	1e-039	2	Defense
51	1-1-6.4.4.14	0,70	0,54	0,55	SGN-U145711	disease resistance response protein-related/ dirigent protein-related [*Arabidopsis thaliana*]	1e-039	SGN-U145711	disease resistance response protein-related/ dirigent protein-related [*Arabidopsis thaliana*]	1e-039	2	Defense
91	1-1-4.3.5.8	0,89	1,55	1,58	SGN-U149635	Dof zinc finger protein [*Arabidopsis thaliana*]	2e-026	SGN-U149635	Dof zinc finger protein [*Arabidopsis thaliana*]	2e-026	4	Transcription
92	1-1-3.1.7.6	0,86	1,34	1,52		*E coli* genome sequence			*E coli* genome sequence		4	Uncategorized
52	1-1-2.4.20.10	0,74	0,62	0,49	SGN-U146408	Elicitor inducible gene product Nt-SubE80 [*Nicotiana tabacum*]	9e-053	SGN-U146408	Elicitor inducible gene product Nt-SubE80 [*Nicotiana tabacum*]	9e-053	2	Defense
7	1-1-4.3.11.6	0,81	0,54	0,76	SGN-U143822	embryo-abundant protein EMB [*Pisum sativum*]	3e-073	SGN-U143823	embryo-abundant protein -related [*Arabidopsis thaliana*]	6e-089	1	Uncategorized
8	1-1-1.3.17.21	0,80	0,56	0,66	SGN-U143823	embryo-abundant protein -related [*Arabidopsis thaliana*]	6e-089	SGN-U143823	embryo-abundant protein -related [*Arabidopsis thaliana*]	6e-089	1	Uncategorized
9	1-1-7.1.15.12	0,81	0,50	0,73	SGN-U143823	embryo-abundant protein -related [*Arabidopsis thaliana*]	6e-089	SGN-U143823	embryo-abundant protein -related [*Arabidopsis thaliana*]	6e-089	1	Uncategorized
93	1-1-1.3.10.11	0,8	1,09	1,64	SGN-U144127	Ethylene-responsive proteinase inhibitor I precursor	8e-010	SGN-U144127	Ethylene-responsive proteinase inhibitor I precursor	8e-010	4	Defense
10	1-1-7.3.11.12	0,89	0,51	0,63	SGN-U144164	expressed protein [*Arabidopsis thaliana*]	7e-047	SGN-U144164	expressed protein [*Arabidopsis thaliana*]	7e-047	1	Unknown
53	1-1-5.4.10.3	0,91	0,72	0,54	SGN-U143510	expressed protein [*Arabidopsis thaliana*]	4e-015	SGN-U143510	expressed protein [*Arabidopsis thaliana*]	4e-015	2	Unknown
123	1-1-1.4.11.2	0,77	1,52	1,56	SGN-U144273	expressed protein [*Arabidopsis thaliana*]	2e-095	SGN-U144274	expressed protein [*Arabidopsis thaliana*]	8e-055	5	Unknown
124	1-1-3.1.4.18	0,74	1,38	1,67	SGN-U149186	expressed protein [*Arabidopsis thaliana*]	5e-056	SGN-U149186	expressed protein [*Arabidopsis thaliana*]	5e-056	5	Unknown
125	1-1-4.3.6.6	0,82	2	2,31	SGN-U145103	expressed protein [*Arabidopsis thaliana*]	1e-061	SGN-U145103	expressed protein [*Arabidopsis thaliana*]	1e-061	5	Unknown

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
126	1-1-7.3.3.18	0,66	1,92	2,24	SGN-U156747	expressed protein [*Arabidopsis thaliana*]	1e-020	SGN-U156747	expressed protein [*Arabidopsis thaliana*]	1e-020	5	Unknown
127	1-1-8.1.20.9	0,77	1,8	1,92	SGN-U155502	expressed protein [*Arabidopsis thaliana*]	9e-067	No sequence			5	Unknown
54	1-1-2.4.18.19	0,87	0,85	0,69	SGN-U155826	F1K23.23 [*Arabidopsis thaliana*]	2e-050	No sequence			2	Uncategorized
55	1-1-6.4.6.15	0,76	0,71	0,79	SGN-U149705	F-box protein family [*Arabidopsis thaliana*]	5e-043	SGN-U149705	F-box protein family [*Arabidopsis thaliana*]	5e-043	2	Signaling
128	1-1-2.2.10.9	0,81	1,6	1,75	SGN-U144121	Fructose-1,6-Bisphosphatase, cytosolic (D-Fructose-1,6-Bisphosphate 1-Phosphohydrolase) (FBPASE) (CY-F1)	0.0	No sequence			5	Glucose metabolism
56	1-1-6.3.20.16	0,73	0,57	0,34	SGN-U146640	Glucan endo-1,3-beta-glucosidase A precursor ((1->3)-beta-glucan endohydrolase A) ((1->3) -beta-glucanase A) (Acidic beta-1,3-glucanase) (Beta-1,3-endoglucanase A)	0.0	SGN-U161922	Glucan endo-1,3-beta-glucosidase A precursor ((1->3)-beta-glucan endohydrolase A) ((1->3)-beta-glucanase A) (Acidic beta-1,3-glucanase) (Beta-1,3-endoglucanase A)	2e-061	2	Defense
129	1-1-8.3.15.18	0,84	1,63	1,75	SGN-U147319	glutamic acid-rich protein [*Arabidopsis thaliana*]	1e-047	SGN-U172975	No hits found		5	Uncategorized
57	1-1-8.3.10.19	0,75	0,59	0,47	SGN-U148248	hexose transporter [*Lycopersicon esculentum*]	e-118	No sequence			2	Transport
58	1-1-1.1.13.12	0,63	0,67	0,56	SGN-U144852	Histidine decarboxylase (HDC) (TOM92)	e-167	SGN-U144852	Histidine decarboxylase (HDC) (TOM92)	e-167	2	Amino acid metabolism
59	1-1-4.1.15.4	0,65	0,59	0,68	SGN-U144852	Histidine decarboxylase (HDC) (TOM92)	e-167	No sequence			2	Amino acid metabolism
60	1-1-6.4.20.13	0,66	0,60	0,72	SGN-U147710	Histidine decarboxylase (HDC) (TOM92)	e-164	SGN-U147710	Histidine decarboxylase (HDC) (TOM92)	e-164	2	Amino acid metabolism
61	1-1-7.4.14.15	0,67	0,59	0,63	SGN-U144851	Histidine decarboxylase (HDC) (TOM92)	2e-074	SGN-U144852	Histidine decarboxylase (HDC) (TOM92)	e-167	2	Amino acid metabolism
130	1-1-6.1.10.21	0,72	1,94	1,98	SGN-U145398	H-Protein precursor [*Flaveria pringlei*]	3e-073	SGN-U161488	No hits found		5	Amino acid metabolism
131	1-1-5.1.17.9	0,78	1,49	1,65	SGN-U145273	hypothetical protein [*Arabidopsis thaliana*]	e-100	SGN-U145273	hypothetical protein [*Arabidopsis thaliana*]	e-100	5	Unknown
62	1-1-8.1.12.17	1,38	1,23	1,37	SGN-U145387	hypothetical protein [*Cicer arietinum*]	e-133	SGN-U145387	hypothetical protein [*Cicer arietinum*]	e-133	2	Unknown
11	1-1-2.2.8.9	0,85	0,47	0,69	SGN-U146043	hypothetical protein F9K20.18 [imported] - *Arabidopsis thaliana*	4e-031	SGN-U146043	hypothetical protein F9K20.18 [imported] - *Arabidopsis thaliana*	4e-031	1	Unknown
132	1-1-4.4.1.13	0,72	1,6	1,49	SGN-U147270	isoflavone reductase-related [*Arabidopsis thaliana*]	e-166	No sequence			5	Uncategorized
79	1-1-1.1.2.19	0,84	1,04	0,6	SGN-U144587	late embryogenesis (Lea)-like protein ER5, ethylene-responsive - tomato	7e-083	SGN-U144587	late embryogenesis (Lea)-like protein ER5, ethylene-responsive - tomato	7e-083	3	Stress responses
133	1-1-2.2.2.19	0,8	2,37	2,71	SGN-U143432	late-embryogenesis protein homolog - tomato	7e-039	SGN-U143432	late-embryogenesis protein homolog - tomato	7e-039	5	Stress responses
134	1-1-2.3.9.11	0,86	2,06	2,37	SGN-U143432	late-embryogenesis protein homolog - tomato	7e-039	No sequence			5	Stress responses
135	1-1-5.3.4.1	0,81	2,23	2,59	SGN-U143432	late-embryogenesis protein homolog - tomato	7e-039	SGN-U143432	late-embryogenesis protein homolog - tomato	7e-039	5	Stress responses

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
12	1-1-3.4.11.5	0,86	0,52	0,66	SGN-U144292	late-embryogenesis protein lea5 - common tobacco	9e-021	SGN-U144292	late-embryogenesis protein lea5 - common tobacco	9e-021	1	Stress responses
136	1-1-8.1.15.18	0,84	1,85	1,69	SGN-U153282	leucine-rich repeat transmembrane protein kinase, putative [*Arabidopsis thaliana*]	7e-099	No sequence			5	Signaling
94	1-1-2.4.15.13	0,7	1,01	1,61	SGN-U155590	LEXYL2 [*Lycopersicon esculentum*]	2e-063	SGN-U155591	LEXYL2 [*Lycopersicon esculentum*]	0.0	4	Uncategorized
13	1-1-2.4.20.9	0,70	0,48	0,66	SGN-U143305	lipoxygenase (EC 1.13.11.12) LX-3 - potato	0.0	SGN-U143305	lipoxygenase (EC 1.13.11.12) LX-3 - potato	0.0	1	Defense
14	1-1-5.2.16.18	0,70	0,43	0,65	SGN-U143302	lipoxygenase (EC 1.13.11.12) LX-3 - potato	6e-058	SGN-U143305	lipoxygenase (EC 1.13.11.12) LX-3 - potato	0.0	1	Defense
137	1-1-2.4.14.7	0,85	1,4	2,08	SGN-U151601	MA3 domain-containing protein [*Arabidopsis thaliana*]	9e-029	SGN-U148525	topoisomerase-like protein [*Arabidopsis thaliana*]	e-109	5	Uncategorized
95	1-1-5.4.9.19	0,91	1,15	1,67	SGN-U144892	malonyl CoA:anthocyanin 5-O-glucoside-6''' -O-malonyltransferase [*Perilla frutescens*]	1e-091 -	SGN-U144892	malonyl CoA:anthocyanin 5-O-glucoside 6'''-O-malonyltransferase [*Perilla frutescens*]	1e-091	4	Transcription
15	1-1-1.2.20.19	0,82	0,51	0,74	SGN-U146362	MAP kinase phosphatase [*Zea mays*]	1e-032	SGN-U146535	Pectinesterase 3 precursor (Pectin methylesterase 3) (PE 3)	0.0	1	Signaling
63	1-1-2.2.11.1	0,80	0,82	0,58	SGN-U147347	metallo proteinase -related [*Arabidopsis thaliana*]	e-109	SGN-U147347	metallo proteinase -related [*Arabidopsis thaliana*]	e-109	2	Protein degradation
96	1-1-2.3.9.7	0,97	1,53	1,6	SGN-U146340	nine-cis-epoxycarotenoid dioxygenase4 [*Pisum sativum*]	0.0	SGN-U146340	nine-cis-epoxycarotenoid dioxygenase4 [*Pisum sativum*]	0.0	4	Respiration/electron transfer
16	1-1-1.2.20.3	0,80	0,43	0,58	SGN-U150460	No hits found		SGN-U152907	No hits found		1	Unknown
17	1-1-3.1.10.5	1,44	0,70	0,88	SGN-U143163	No hits found			No sequence		1	Unknown
18	1-1-8.2.1.1	1,46	0,71	0,83	SGN-U143163	No hits found		SGN-U143163	No hits found		1	Unknown
64	1-1-2.3.5.1	0,74	0,65	0,56	SGN-U160699	No hits found			No sequence		2	Unknown
97	1-1-7.4.7.6	0,83	1,21	1,41	SGN-U163944	No hits found		SGN-U163944	No hits found		4	Unknown
138	1-1-5.3.15.20	0,83	1,55	1,69	SGN-U153140	No hits found		SGN-U156588	No hits found		5	Unknown
139	1-1-6.1.5.1	0,76	1,87	1,91	SGN-U155051	No hits found		SGN-U156584	expressed protein [*Arabidopsis thaliana*]	3e-032	5	Unknown
140	1-1-7.4.14.4	0,75	1,77	1,82	SGN-U153491	No hits found		SGN-U156094	P0686C03.7 [*Oryza sativa* (japonica cultivar-group)]	0.003	5	Uncategorized
141	1-1-8.4.2.11	0,83	1,57	1,48	SGN-U156155	No hits found		SGN-U156155	No hits found		5	Unknown
19	1-1-2.1.3.12	0,76	0,36	0,62		No sequence			No sequence		1	Unknown
20	1-1-4.1.18.17	0,82	0,55	0,83		No sequence		SGN-U146139	myb family transcription factor (MYB108) [*Arabidopsis thaliana*]	9e-057	1	Transcription
21	1-1-5.2.20.16	0,80	0,56	0,80		No sequence			No sequence		1	Unknown
22	1-1-8.1.13.3	0,74	0,42	0,52		No sequence			No sequence		1	Unknown
23	1-1-8.3.3.4	0,80	0,43	0,54		No sequence			No sequence		1	Unknown
65	1-1-1.3.16.1	0,76	0,62	0,52		No sequence			No sequence		2	Unknown
66	1-1-2.2.12.4	0,68	0,63	0,55		No sequence			No sequence		2	Unknown

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
67	1-1-3.3.13.19	0,82	0,78	0,62		No sequence			No sequence		2	Unknown
68	1-1-3.3.16.11	0,72	0,56	0,45		No sequence			No sequence		2	Unknown
69	1-1-3.3.5.1	0,70	0,79	0,62		No sequence			No sequence		2	Unknown
70	1-1-6.1.1.8	0,68	0,66	0,50		No sequence			No sequence		2	Unknown
71	1-1-6.1.15.9	0,76	0,73	0,58		No sequence			No sequence		2	Unknown
72	1-1-7.4.12.21	0,67	0,64	0,57		No sequence			No sequence		2	Unknown
73	1-1-8.3.5.14	0,68	0,56	0,53		No sequence			No sequence		2	Unknown
80	1-1-1.3.19.19	0,67	0,91	0,39		No sequence			No sequence		3	Unknown
84	1-1-1.1.12.13	0,66	1,04	0,44		No sequence			No sequence		3	Unknown
85	1-1-1.1.14.7	0,64	1	0,42		No sequence			No sequence		3	Unknown
87	1-1-2.1.5.18	0,7	0,98	0,4		No sequence			No sequence		3	Unknown
98	1-1-3.2.7.1	0,92	1,13	1,39		No sequence			No sequence		4	Unknown
99	1-1-8.2.12.12	1,45	2,04	1,98		No sequence			No sequence		4	Unknown
100	1-1-8.3.19.6	0,85	1,19	1,38		No sequence			No sequence		4	Unknown
101	1-1-8.4.12.9	1,02	1,32	1,35		No sequence			No sequence		4	Unknown
102	1-1-1.2.9.9	0,77	1,25	1,417		No sequence			No sequence		4	Unknown
103	1-1-1.4.6.21	1,43	2,13	2,11		No sequence			No sequence		4	Unknown
104	1-1-2.4.20.7	0,64	0,73	1,57		No sequence			No sequence		4	Unknown
142	1-1-1.1.3.7	0,67	1,59	1,59		No sequence			No sequence		5	Unknown
143	1-1-1.4.16.11	0,74	1,46	1,62		No sequence		SGN-U146584	glucosyltransferase IS5a (EC 2.4.1.-), salicylate-induced - common tobacco	e-140	5	Glucose metabolism
144	1-1-2.1.15.17	0,82	1,51	1,65		No sequence			No sequence		5	Unknown
145	1-1-2.2.15.1	0,73	1,42	1,59		No sequence			No sequence		5	Unknown
146	1-1-3.2.10.16	0,76	1,66	2,01		No sequence			No sequence		5	Unknown
147	1-1-3.2.10.17	0,74	2,09	2,34		No sequence			No sequence			
148	1-1-4.2.14.17	0,86	2,01	1,74		No sequence			No sequence		5	Unknown
149	1-1-4.4.12.3	0,8	1,6	2,45		No sequence			No sequence		5	Unknown
150	1-1-5.1.12.15	0,79	1,5	1,65		No sequence			No sequence		5	Unknown
151	1-1-5.4.16.1	0,87	1,62	1,92		No sequence			No sequence		5	Unknown
152	1-1-6.3.15.21	0,81	1,39	1,71		No sequence			No sequence		5	Unknown
153	1-1-6.4.12.6	0,96	2,02	2,05		No sequence			No sequence		5	Unknown
154	1-1-6.4.20.12	0,83	1,57	1,88		No sequence			No sequence		5	Unknown
155	1-1-7.4.12.8	0,83	1,74	1,87		No sequence			No sequence		5	Unknown

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
156	1-1-8.1.3.6	0,72	1,66	1,99		No sequence			No sequence		5	Unknown
157	1-1-8.2.10.18	0,59	2,35	2,12		No sequence			No sequence		5	Unknown
175	1-1-4.3.8.12	0,81	1,98	2,14		No sequence			No sequence		5	Unknown
24	1-1-5.2.16.8	0,92	0,42	0,50	SGN-U148184	omega-6 fatty acid desaturase [*Sesamum indicum*]	e-173	SGN-U148184	omega-6 fatty acid desaturase [*Sesamum indicum*]	e-173	1	Defense
105	1-1-8.4.13.15	0,98	1,27	1,7	SGN-U143658	oxidase like protein - tomato	0.0	SGN-U143658	oxidase like protein - tomato	0.0	4	Respiration/electron transfer
81	1-1-4.2.9.3	0,69	1	0,32	SGN-U143238	Pathogenesis-related leaf protein 4 precursor (P4)	3e-094	SGN-U143238	Pathogenesis-related leaf protein 4 precursor (P4)	3e-094	3	Defense
89	1-1-5.2.16.3	0,65	0,99	0,35	SGN-U143238	Pathogenesis-related leaf protein 4 precursor (P4)	3e-094	SGN-U143238	Pathogenesis-related leaf protein 4 precursor (P4)	3e-094	3	Defense
82	1-1-5.1.9.3	0,63	0,97	0,36	SGN-U143242	Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein)	4e-094	SGN-U143242	Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein)	4e-094	3	Defense
83	1-1-5.3.4.17	0,72	0,91	0,39	SGN-U143242	Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein)	4e-094	SGN-U143242	Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein)	4e-094	3	Defense
88	1-1-2.3.14.10	0,65	0,93	0,45	SGN-U143242	Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein)	4e-094	SGN-U143242	Pathogenesis-related leaf protein 6 precursor (P6) (Ethylene-induced protein P1) (P14) (P14A) (PR protein)	4e-094	3	Defense
74	1-1-7.4.9.4	0,69	0,70	0,56	SGN-U161489	Pathogenesis-related protein P2 precursor	3e-036	SGN-U145399	Pathogenesis-related protein P2 precursor	8e-083	2	Defense
25	1-1-8.4.17.3	0,78	0,47	0,82	SGN-U146284	pathogen-inducible alpha-dioxygenase [*Nicotiana attenuata*]	0.0	SGN-U146284	pathogen-inducible alpha-dioxygenase [*Nicotiana attenuata*]	0.0	1	Defense
106	1-1-5.1.9.12	0,58	0,66	1,17	SGN-U143291	Peptidyl-prolyl cis-trans isomerase (PPIase) (Rotamase) (Cyclophilin) (Cyclosporin A-binding protein)	1e-088	SGN-U145787	unknown protein [*Phytophthora sojae*]	2e-066	4	Uncategorized
26	1-1-3.2.20.9	0,81	0,66	1,03	SGN-U144043	peroxidase	0.0	SGN-U144043	peroxidase	0.0	1	Stress responses
27	1-1-1.1.13.15	0,90	0,66	0,74	SGN-U143849	peroxidase [*Nicotiana tabacum*]	e-128	SGN-U143849	peroxidase [*Nicotiana tabacum*]	e-128	1	Stress responses
28	1-1-4.2.20.3	0,86	0,45	0,64	SGN-U143214	Photosystem II 10 kDa polypeptide, chloroplast precursor	2e-065	No sequence			1	Photosynthesis
158	1-1-8.1.9.9	0,89	1,89	2,07	SGN-U143993	photosystem II reaction center 6.1KD protein [*Arabidopsis thaliana*]	1e-032	SGN-U160895	photosystem II reaction center 6.1KD protein [*Arabidopsis thaliana*]	2e-019	5	Photosynthesis
29	1-1-6.4.16.7	0,79	0,48	0,60		potential chimera			potential chimera		1	Unknown
75	1-1-2.1.14.21	1,43	1,20	1,31	SGN-U143261	probable chaperonin 60 beta chain precursor, chloroplast (clone potbchap1) - potato	0.0	No sequence			2	Stress responses
107	1-1-7.2.18.7	1,25	1,5	1,6	SGN-U147590	probable inorganic diphosphatase (EC 3.6.1.1), soluble - potato	e-112	SGN-U147590	probable inorganic diphosphatase (EC 3.6.1.1), soluble - potato	e-112	4	Uncategorized

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
30	1-1-7.1.6.15	0,88	0,66	0,86	SGN-U152499	probable replication control protein - *Arabidopsis thaliana*	e-162	SGN-U152499	probable replication control protein - *Arabidopsis thaliana*	e-162	1	Nucleic acid-related
159	1-1-1.3.4.21	0,75	1,44	1,61	SGN-U156470	probable UDP-glucuronosyltransferase (EC 2.4.1.-) - garden pea	2e-043	SGN-U155585	Oxygen-evolving enhancer protein 1, chloroplast precursor (OEE1) (33 kDa subunit of oxygen evolving system of photosystem II) (OEC 33 kDa subunit) (33 kDa thylakoid membrane protein)	0.0	5	Glucose metabolism
31	1-1-2.2.6.15	0,92	0,54	0,69	SGN-U148414	proteasome inhibitor, putative [*Arabidopsis thaliana*]	1e-049	SGN-U148414	proteasome inhibitor, putative [*Arabidopsis thaliana*]	1e-049	1	Protein degradation
108	1-1-8.3.8.15	0,94	1,58	1,71	SGN-U149916	putative AP2 domain transcription factor [*Oryza sativa*]	1e-030	No sequence			4	Transcription
160	1-1-7.3.4.16	0,71	1,76	1,75	SGN-U145156	putative aquaporin PIP2-2 [*Vitis berlandieri x Vitis rupestris*]	3e-042	No sequence			5	Transport
109	1-1-6.3.9.6	1,4	1,93	2,55	SGN-U143454	putative chloroplast thiazole biosynthetic protein [*Nicotiana tabacum*]	e-173	SGN-U143454	putative chloroplast thiazole biosynthetic protein [*Nicotiana tabacum*]	e-173	4	Amino acid metabolism
32	1-1-1.3.6.16	0,86	0,57	0,69	SGN-U149647	putative F-box protein [*Arabidopsis thaliana*]	7e-040	No sequence			1	Signaling
110	1-1-8.3.9.6	1,51	2,21	2,17	SGN-U143803	putative gamma TIP [*Nicotiana glauca*]	e-129	SGN-U143803	putative gamma TIP [*Nicotiana glauca*]	e-129	4	Uncategorized
76	1-1-2.2.20.4	1,37	1,07	1,19	SGN-U155709	putative leucine rich repeat containing protein kinase [*Oryza sativa* (japonica cultivar-group)]	1e-039	SGN-U152818	putative leucine rich repeat containing protein kinase [*Oryza sativa* (japonica cultivar-group)]	8e-038	2	Signaling
111	1-1-3.4.13.21	0,72	0,83	1,09	SGN-U144671	putative peroxidase [*Solanum tuberosum*]	0.0	SGN-U144671	putative peroxidase [*Solanum tuberosum*]	0.0	4	Stress responses
161	1-1-4.1.12.4	0,8	1,43	2,05	SGN-U144902	putative polyprotein [*Nicotiana tabacum*]	2e-050	No sequence			5	Transcription
162	1-1-4.2.12.14	0,81	1,86	1,93	SGN-U145646	putative proteasome regulatory non-ATPase subunit [*Oryza sativa* (japonica cultivar-group)]	0.0	No sequence			5	Protein degradation
112	1-1-6.2.16.6	1,36	1,79	1,77	SGN-U159154	putative protein kinase LESK1 [*Lycopersicon esculentum*]	4e-083	SGN-U144533	omega-3 fatty acid desaturase [*Lycopersicon esculentum*]	0.0	4	Signaling
33	1-1-1.3.19.16	0,77	0,53	0,70	SGN-U146036	putative pseudo-response regulator [*Oryza sativa* (japonica cultivar-group)]	4e-011	No sequence			1	Transcription
34	1-1-1.4.20.1	0,69	0,49	0,61	SGN-U144138	pyruvate decarboxylase [*Solanum tuberosum*]	0.0	SGN-U144138	pyruvate decarboxylase [*Solanum tuberosum*]	0.0	1	Uncategorized
113	1-1-5.2.6.4	1,37	2,12	2,29	SGN-U150766	RelA-SpoT like protein RSH1 [*Nicotiana tabacum*]	4e-054	SGN-U161667	RelA-SpoT like protein RSH1 [*Nicotiana tabacum*]	6e-045	4	Uncategorized
163	1-1-3.4.4.9	0,66	1,74	1,83	SGN-U144915	ribosomal protein L17, chloroplast - common tobacco	8e-093	SGN-U144915	ribosomal protein L17, chloroplast - common tobacco	8e-093	5	Protein biosynthesis
164	1-1-6.4.10.16	0,69	1,72	1,86	SGN-U144915	ribosomal protein L17, chloroplast - common tobacco	8e-093	SGN-U165701	leucine-rich repeat extensin family [*Arabidopsis thaliana*]	0.008	5	Protein biosynthesis

		Fold change				5' sequence annotation			3' sequence annotation			Functional category
No	ArrayID	12h	36h	60h	SGN Unigene ID	SGN unigene nr best hit	e value	SGN Unigene ID	SGN unigene nr best hit	e value	Cluster	
165	1-1-6.3.18.2	0,82	1,48	1,69	SGN-U155534	Ribulose bisphosphate carboxylase small chain 3A/3C, chloroplast precursor (RuBisCO small subunit 3A/3C)	9e-097	SGN-U155534	Ribulose bisphosphate carboxylase small chain 3A/3C, chloroplast precursor (RuBisCO small subunit 3A/3C)	9e-097	5	Photosynthesis
166	1-1-4.4.14.8	0,73	1,47	1,95	SGN-U144153	serine carboxypeptidase [*Arabidopsis thaliana*]	e-100	No sequence			5	Signaling
35	1-1-5.1.18.19	0,66	0,36	0,56	SGN-U148626	serine/threonine protein kinase, putative [*Arabidopsis thaliana*]	4e-018	SGN-U148627	serine/threonine protein kinase, putative [*Arabidopsis thaliana*]	1e-053	1	Signaling
77	1-1-7.3.7.18	1,48	1,49	1,43	SGN-U148658	short-chain dehydrogenase/reductase family protein (tropinone reductase, putative) [*Arabidopsis thaliana*]	2e-075	SGN-U162374 -like [*Arabidopsis thaliana*]	short chain alcohol dehydrogenase 7e-010	2	Uncategorized	
36	1-1-7.1.16.21	0,84	0,46	0,67	SGN-U149579	SNARE BS14b [*Arabidopsis thaliana*]	7e-045	SGN-U149579	SNARE BS14b [*Arabidopsis thaliana*]	7e-045	1	Transport
167	1-1-3.3.4.19	0,86	1,7	1,73	SGN-U145894	sugar transporter, putative [*Arabidopsis thaliana*]	e-105	No sequence			5	Transport
168	1-1-6.4.5.8	0,88	1,7	2,03	SGN-U153352	sulfate transporter -related [*Arabidopsis thaliana*]	4e-072	No sequence			5	Transport
169	1-1-4.3.3.10	0,88	1,75	2,15	SGN-U148044	thylakoid lumenal 16.5 kDa protein, chloroplast precursor [*Arabidopsis thaliana*]	7e-049	SGN-U148044	thylakoid lumenal 16.5 kDa protein, chloroplast precursor [*Arabidopsis thaliana*]	7e-049	5	Uncategorized
170	1-1-4.4.10.9	0,74	1,56	2,34	SGN-U148044	thylakoid lumenal 16.5 kDa protein, chloroplast precursor [*Arabidopsis thaliana*]	7e-049	SGN-U148044	thylakoid lumenal 16.5 kDa protein, chloroplast precursor [*Arabidopsis thaliana*]	7e-049	5	Uncategorized
171	1-1-7.3.1.19	0,78	1,71	1,95	SGN-U148044	thylakoid lumenal 16.5 kDa protein, chloroplast precursor [*Arabidopsis thaliana*]	7e-049	SGN-U148044	thylakoid lumenal 16.5 kDa protein, chloroplast precursor [*Arabidopsis thaliana*]	7e-049	5	Uncategorized
172	1-1-8.1.14.6	0,95	1,81	1,77	SGN-U149443	TMV response-related gene product [*Nicotiana tabacum*]	4e-029	SGN-U148651	TMV response-related gene product [*Nicotiana tabacum*]	1e-026	5	Defense
173	1-1-4.1.14.8	0,72	2,22	2,4	SGN-U145351	ultraviolet-B-repressible protein [*Trifolium pratense*]	2e-017	SGN-U145351	ultraviolet-B-repressible protein [*Trifolium pratense*]	2e-017	5	Stress responses
114	1-1-1.4.12.11	0,82	1,3	1,5	SGN-U143389	unknown [*Prunus armeniaca*]	1e-092	No sequence			4	Unknown
174	1-1-1.4.4.10	0,56	1,57	1,67	SGN-U144728	unknown protein (sp|P72777) -related [*Arabidopsis thaliana*]	2e-056	No sequence			5	Uncategorized
78	1-1-2.3.3.5	0,71	0,94	0,70	SGN-U145441	vacuolar processing enzyme-1b [*Nicotiana tabacum*]	0.0	SGN-U145441	vacuolar processing enzyme-1b [*Nicotiana tabacum*]	0.0	2	Protein degradation
115	1-1-2.4.13.7	1,01	1,06	1,45	SGN-U143548	Wound-induced proteinase inhibitor I precursor	3e-057	No sequence			4	Stress responses
37	1-1-5.3.13.18	0,87	0,65	0,78	SGN-U143747	WRKY family transcription factor [*Arabidopsis thaliana*]	3e-029	SGN-U143747	WRKY family transcription factor [*Arabidopsis thaliana*]	3e-029	1	Transcription

Table S1. Differentially expressed tomato genes between a compatible and an incompatible interaction.
Each clone can have 2 GenBank accession numbers since both 5' and 3' sequences were generated.
No sequence means that no re-amplification was obtained from that particular clone.

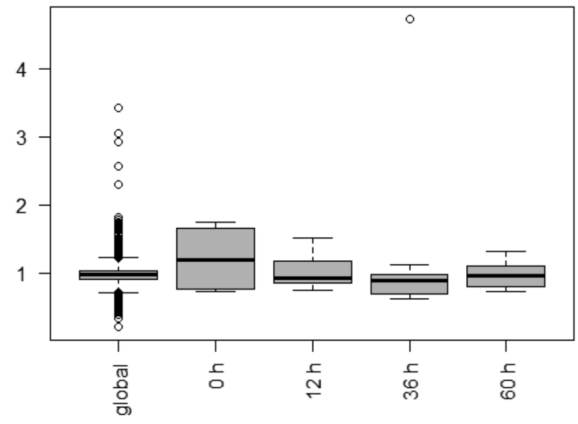

Figure 1. Boxplots of log2 transformed gene expression ratio between incompatible/compatible interaction.

therefore represented by PC1. It is worth mentioning that variables with most different behavior are V36 and V35, which correspond to replicates of time point 60. As differential expression was only detected at time point 36 between compatible and incompatible interactions, variability of gene expression at time point 60 is apparently not due to differential expression between these two conditions but to random variability due to differences between individuals at this time point.

Mean correlations of each one of the hybridizations with all other hybridizations are shown in table 1. As expected, correlations of V35 and V36 to the other hybridizations are lower (Table 1) as these variables representing expression at 60h showed a distinct

Variable	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Condition	C	I	I	C	C	I	I	C	C	I
Timepoint	T-12	T-12	T-12	T-12	T12	T12	T12	T12	T12	T12
Mean Correlation	0.65	0.67	0.68	0.69	0.75	0.74	0.77	0.76	0.74	0.74
Variable	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22
Condition	I	C	C	I	I	C	C	I	I	C
Timepoint	T12	T12	T12	T12	T12	T12	T36	T36	T36	T36
Mean Correlation	0.70	0.68	0.70	0.67	0.65	0.61	0.71	0.73	0.77	0.67
Variable	V23	V24	V25	V26	V27	V28	V29	V30	V31	V32
Condition	C	I	I	C	C	I	I	C	C	I
Timepoint	T36	T36	T36	T36	T36	T36	T36	T36	T60	T60
Mean Correlation	0.69	0.76	0.70	0.67	0.75	0.73	0.76	0.72	0.72	0.74
Variable	V33	V34	V35	V36	V37	V38	V39	V40	V41	V42
Condition	I	C	C	I	I	C	C	I	I	C
Timepoint	T72	T72	T72	T72	T72	T72	T72	T72	T72	T72
Mean Correlation	0.71	0.69	0.37	0.53	0.64	0.64	0.73	0.76	0.71	0.67

Table 1. Mean correlations of each array to the rest of the arrays. Arrays with low mean correlations to the rest are highlighted in grey. C: not inoculated, I: inoculated. Colons are numbered consecutively for identification on plots.

behavior (Fig. 2). Moreover, correlations between all other experiments are around 0.7 confirming that all experiments are related even though they belong to different conditions (incompatible and compatible interaction) and time points, confirming again that very few genes are differentially expressed between a compatible and an incompatible interaction in this pathosystem.

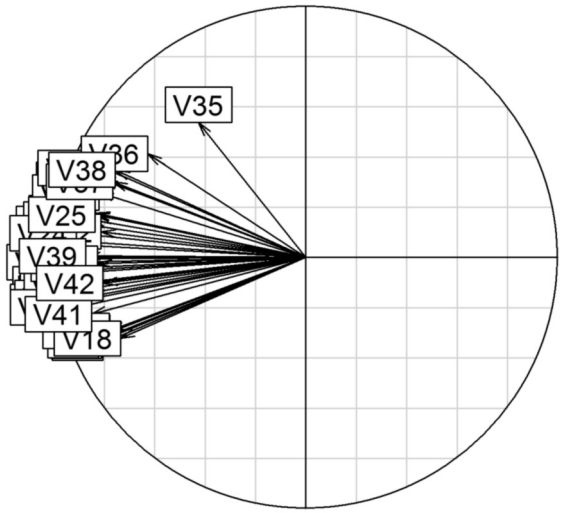

Figure 2. Corcircle of variable (condition replicates) projection on the first two PC space of PCA, showing that most arrays are correlated to the first Principal Component.

When genes are plotted on the plane of the first two PCs, (which represent 75.3 % of the information contained in the whole data set), no apparent structure in the data was detected. The behavior of most genes is neither differentiated nor characteristic, showing expression values around zero (the mean expression value in normalized and centered data). Therefore most of the genes are placed around the origin of the PC space. Only few genes behave in a different way, mainly in relationship to the first PC, which means that they behave differently than most of the other genes. Nevertheless, these genes do not group in a separate cluster but each one has a unique behavior (Fig. 3). Among these genes we could detect some defense-, wound-, harpin and Avr9- induced genes and enzymes belonging to the ethylene synthesis pathway. However, of high interest was the finding in this set of differentially expressed genes has a high number of genes related to photosynthesis and to the Halliwell-Asada enzyme pathway: ascorbate oxidase, monodehydroascorbate reductase, glutathione peroxidase, superoxide dismutase, glutathione-S-transferase, Rubisco, chlorophyll a/b binding proteins and carbonic anhydrase among others. All these genes were up-regulated in the incompatible interaction or down-regulated in the compatible interaction confirming previous results in other *P. infestans* hosts (Restrepo *et al.*, 2005).

For the purposes of this study, a set of genes involved in resistance and disease processes was built. Genes of interest and belonging to disease-associated gene-sets are highlighted in figure 4. Globally, all these genes behave similarly. However, two genes, one with WRKY

motif (SGN-U214610; AAAR98818) and one inhibited by EPI1 or EPI10 (SGN-U212610; AAA80496) showed a strikingly different behavior.

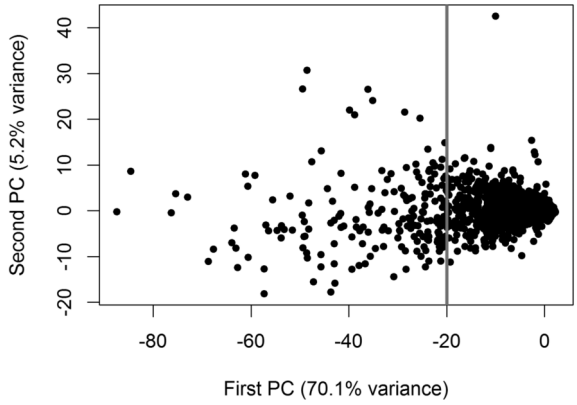

Figure 3. Gene projection of individuals (genes) on the first two PC space of PCA showing 75.3 % of the information of the original 40 arrays. Red vertical line indicates the -20 threshold.

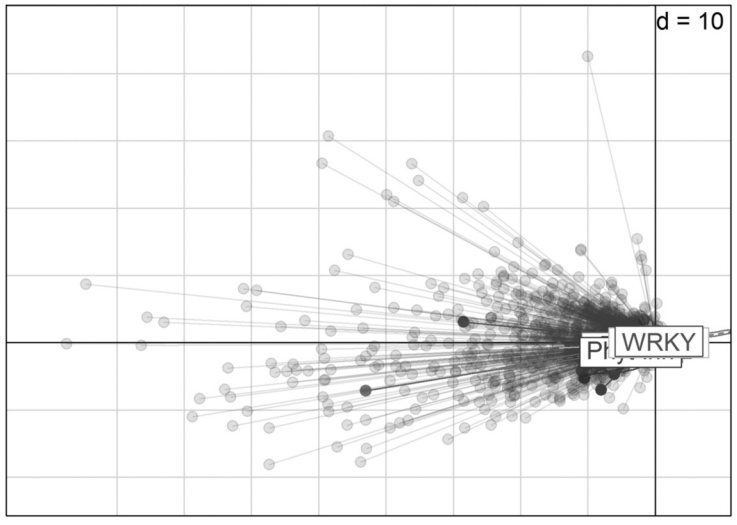

Figure 4. Projection of individuals (gene) on the first two PC space of PCA. Genes in light blue do not make part of a known resistance categories. Genes colored differently make part of the following categories: Phyt-inh (*Phytophtora* inhibited proteins), ResistG (Disease Resistance in general), WRKY, Patog (Associated to Pathogenicity (Restrepo *et al.*, 2005), Avr (Avr elicited), EPI1-10 (Inhibited by EPI1 y EPI10, Tian *et al.*, 2004) Mean point of these categories (centroids) are indicated with the name of the category.

Validation of differential gene expression by Northern Blots

Northern blots are the most reliable and robust analysis to confirm differential gene expression. Northern analysis confirmed the similarity of gene expression between a

compatible and incompatible interaction in the Matt's wild cherry tomato. Expression patterns of PR genes and carbonic anhydrase (CA) in the compatible and incompatible interactions in tomato cultivar Matt's wild cherry were investigated. In general no differential expression was observed for the selected genes, confirming the microarrays results. For GluA (Acidic glucanase), a higher level of expression was observed during the compatible interaction at 60 h after inoculation. For GluB (Basic glucanase) and PR1b we could detect induction at 12 h after inoculation and more expression at 36 h for the compatible interaction. For DES (divinyl ether synthase), a transient induction at 12 h in both interactions was detected but at higher levels for the compatible interaction. Finally, for CA we observed an expression at high levels in healthy plants but repression after 36 h in the compatible interaction (Fig. 5).

Combining statistical and Biological evidence towards the elucidation of defense mechanisms on Matt's Cherry tomatoes

From previous microarrays studies different groups have performed, it is now evident that more robust and creative ways of data analysis are needed to detect key genes in the evaluated treatments. It is known that the traditional statistical analyses of microarrays could discard interesting genes that do not show dramatically expression changes. Additionally, the appropriate combination of statistical analyses can help in the selection of genes to be functionally evaluated by different approaches including VIGS. This technology is not always successful in tomato, and even when it is successful it occurs as a mosaic (Rotenberg *et al.*, 2006), so the careful selection of genes to be silenced will reduce general costs of the whole experiment. The success in finding the differences among the reactions (compatibility and incompatibility) and identifying the key molecular components of resistance was the use of a novel combination of several statistical tools. The most intriguing result obtained in this study was the almost lack of molecular differences between compatible and resistant reactions of the Matt's wild cherry tomato against *Phytophthora infestans*. Using a common microarray analysis, the Significance Analysis of Microarrays (SAM), we observed that differential gene expression was very late when comparing resistant and susceptible reactions of the Matt's wild cherry tomato against two genotypes of the pathogen *P. infestans*. No differentially expressed genes were detected before 36 hours after inoculations. Actually, no differences in gene expression between the two types of interactions were observed at 60 hours suggesting that molecular processes involved in resistance in the Matt's cherry tomato cultivar could be due to i) the genes detected at 36 h in this study (Supplementary table 1) or ii) to the activity of one major gene up-regulated very early during the interaction, before 12 hpi and complemented with the genes detected in this study or iii) to the activity of a gene or the activities of a set of genes not detected at the thresholds defined in our SAM microarray analysis.

We therefore used a novel combination of old statistical techniques trying to detect key components of defense not detected by SAM. The PCA indicated that most of the time points (here the variables) have correlated behaviors. This shows that differences between replicates and also between compatible and incompatible interaction conditions are globally very weak and that the differences in gene expression that exist are not enough for a change in data structure. Moreover, the projection of the genes on the PC space

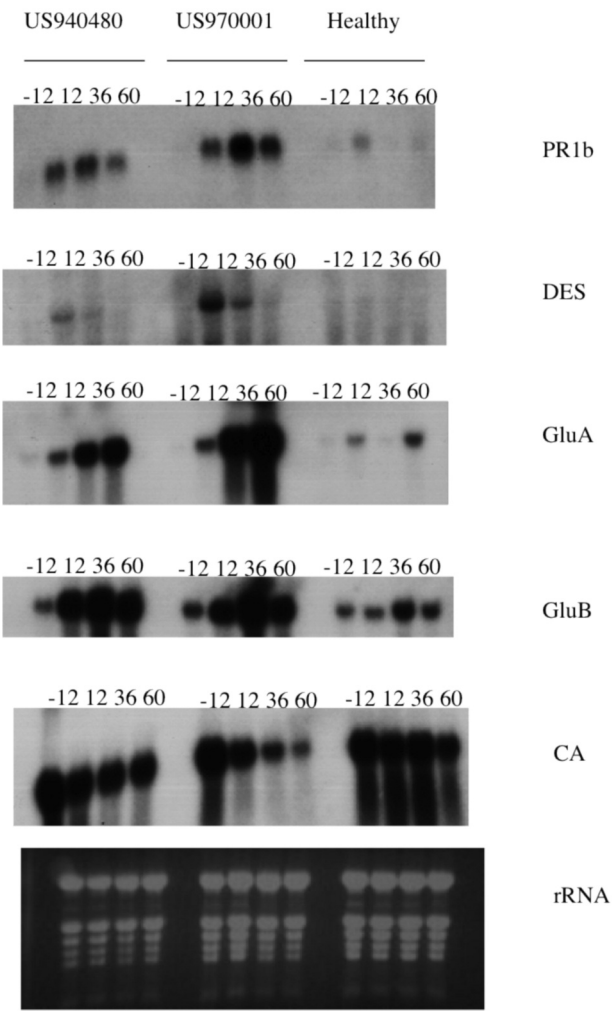

Figure 5. Northern analysis of pathogenesis-related (PR), DES and carbonic anhydrase gene expression in tomato plants during the interaction with two strains of *Phytophthora infestans* and in uninoculated plants under greenhouse conditions. Probes used for each blot are identified to the left and include: PR1b, divinyl ether synthase (DES), acidic glucanase (GluA), basic glucanase (GluB), and carbonic anhydrase (CA). Numbers at the top of the figure refer to the number of hours after inoculation the tissue was collected. Duplicate RNA gel blots were hybridized with each probe. Tomato – US8: incompatible interaction; tomato – US17: compatible interaction.

allows stating that most genes have very low expression and do not change this behavior along the conditions analyzed. This confirms the result of a low number of differentially expressed genes. However, PCA allowed the detection of a set of differentially expressed genes that had been identified in previous studies on potato late blight (Restrepo *et al.*, 2005). As previously shown, the largest group of genes with a different behavior corresponds to photosynthesis-related genes (Schenk *et al.*, 2000; Mysore *et al.*, 2002;

Gibly *et al.*, 2004; Restrepo *et al.*, 2005). These genes can be considered as down-regulated in the compatible interaction. As for the study of compatibility in potato challenged by *P. infestans* (Restrepo *et al.*, 2005), results shown herein suggest that under stressful conditions, the alteration of expression of carbonic anhydrase impacts photosynthesis through the Halliwell-Asada pathway and glutamate metabolism (Restrepo *et al.*, 2005; Pinzón *et al.*, 2010). Then, during compatibility, other genes such as those encoding JA-pathway proteins in potato could be down-regulated and result in disease (Restrepo *et al.*, 2005) but in resistant genotypes this effect might be rapidly counteracted.
Regarding the known disease associated genes that we grouped in five gene sets, the PCA revealed that they do not have a particular behavior as a group but most of them are expressed weakly and do not change in the analyzed conditions. Only two genes showed a particular behavior, one with WRKY motifs and one inhibited by EPI1 or EPI10. Again, the involvement of these genes in defense or compatibility has to be functionally validated even if the WRKY role in resistance is very well documented as a positive and negative regulator.

CONCLUSIONS

In conclusion, very few differences between the gene induction in the susceptible and resistant interactions between Matt's wild cherry tomato and its pathogen *P. infestans* could be observed. Lack of marked differences in gene expression was also observed for the signaling pathways. In previous studies, it was shown that differences between compatible and incompatible interactions can be mainly explained quantitatively (Tao *et al.*, 2003). However, a novel combination of statistical tools helped us to identify the key components of resistance in a tomato genotype showing interesting levels of resistance to its pathogens.

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