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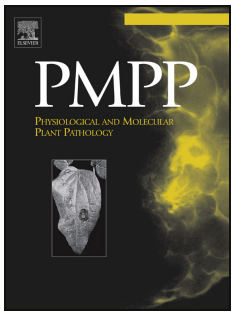

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Title

Transcriptome profiling of *Fusarium solani* f. sp. *eumartii* -infected potato tubers provides evidence of an inducible defense response

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Abstract

Fusarium solani f. sp. *eumartii* is a phytopahtogenic fungus that causes dry rot in potato tubers. To analyze and characterize the transcriptomic profile of *Solanum tuberosum* cv. Spunta against *F. solani* f. sp. *eumartii* infection, a cDNA collection of genes differentially over expressed 24 h after *F. solani* f. sp. *eumartii* infection was generated. In this work, the identification and functional classification of over seven hundred potato cDNA clones up-regulated by *F. solani* f. sp. *eumartii* infection was reported. Statistically overrepresented functional categories allowed the identification of both well known defense response gene groups as well as other not typically related to biotic stress. A macroarray generated to validate the cDNA collection and to quantify gene expression confirmed that over 78% of the cDNA clones were significantly up-regulated in tubers at 24 h after *F. solani* f. sp. *eumartii* infection. Identified genes and pathways evidenced the existence of a comprehensive and complex inducible defense response of potato tubers to *F. solani* f. sp. *eumartii* infection.

1. Introduction

Plants do not have specialized cells to carry out immune functions, however they have both structural and biochemical pre-formed barriers that present a first obstacle against pathogen attacks. When these constitutive defenses are overcome by a pathogen, recognition leads to a complex signaling cascade of inducible defense responses. These responses include cell wall strengthening, oxidative burst, metabolic changes and the expression of a large amount of defense-related genes [1-4]. Transcriptional regulation of gene expression has been recognized as an important part of the plant's induced response resulting in changes at biochemical, cellular and physiological level [5,6]. Transcriptome studies during plant-pathogen interactions offer powerful tools to unravel the possible mechanisms of stress tolerance. Microarray and macroarray analysis of crops challenged with fungal pathogens allowed the identification of organ specific and new up-regulated gene products as well as the identification of plant pathways likely to be involved in plant resistance [7-10].

Fusarium spp. are ubiquitous fungal pathogens in a wide variety of crops. Particularly, potato (*Solanum tuberosum*) dry rot caused by *Fusarium solani* f. sp. *eumartii* is a serious threat in various locations of the United States, as well as Argentina, Brazil and Canada [11]. Dry rot is an important post-harvest disease that affects potato tubers in storage and seed pieces after planting. *F. solani* f. sp. *eumartii* infects tubers at wounded sites causing lesions on the surface and extends deeply in the tuber tissue producing a visible dry rot [12]. Fusarium seed-piece decay is commonly controlled by preplant applications of chemical fungicides. Nevertheless, their massive use has lead to environmental pollution problems. In addition, resistance to chemicals seems to be widespread among strains of *Fusarium* spp [13]. Despite the

importance of dry rot as one of the most economically important post-harvest diseases of potato tubers, limited research has been performed at genetic and molecular level. Currently there is no commercial potato cultivar exhibiting resistance to *Fusarium* spp. However, a reduced susceptibility to *F. solani* f. sp. *eumartii* infection was observed in cv. Spunta [14]. Some genes previously related to stress responses have been reported to be also up-regulated in cv. Spunta after *F. solani* f. sp. *eumartii* attack. That is the case of *StCyp,* coding for a cyclophilin; *StMBF1*, a transcriptional coactivator; *St-ACO3* coding for an ACC oxidase and *StMPK1* coding for a MAP kinase [15-18].

A collection of potato cDNA clones was isolated through a differential screening strategy from a 24 h *F. solani* f. sp. *eumartii* infected-tuber cDNA library [15]. In this work, differential potato cDNA clones were identified and classified into functional categories and further screened by means of a macroarray study. Analysis of up-regulated transcripts during fungal establishment provided a first global insight into the complexity of potato-*F. solani* f. sp. *eumartii* interaction which might in turn lead to the discovery of novel strategies for dry rot management.

2. Materials and Methods

2.1. Plant and fungal material

Potato (*Solanum tuberosum* subsp *tuberosum*) tubers from the commercial cultivar, cv. Spunta were harvested in the late summer and stored at 4 °C in the dark. *Fusarium solani* f. sp. *eumartii*, isolate 3122 was obtained from INTA Collection, Balcarce, Argentina. The fungus was grown on potato dextrose agar (PDA) with fluorescent light (150 $mmol.m^{-2}.s^{-1}$) under a 14 h photoperiod at 25 °C for 3 weeks.

2.2. Fungal infection of potato tubers

Tubers were acclimatized before treatments at 25 °C for 24 h in the dark, surface sterilized by immersion in 0.5% sodium hypochlorite for 5 min and rinsed with sterile distilled water. Potato tubers were inoculated with a PDA disc colonized by *F. solani* f. sp. *eumartii* (10^5 spores) by using the hollow punch method [19]. As control, mock-inoculation treatments were made by using a sterile PDA disk. Tubers were kept at 25 °C for 24 h in the dark. Tissue samples from approximately 0.5 cm around the inoculated and mock-inoculated sites were collected, frozen in liquid nitrogen and stored at -80 °C.

2.3. RNA extraction

Total RNA was extracted using the guanidinium-HCl and LiCl precipitation as previously described [20]. Total RNA was quantified using an Ultrospec 1100 Pro spectrophotometer and stored at -80 °C.

2.4. Clone isolation, sequencing and analysis

Several hundred cDNA clones were isolated from a 24 h *F. solani* f. sp. *eumartii* infected-tuber cDNA library differentially screened with a *F. solani* f. sp. *eumartii* inoculated -subtracted probe, and a mock inoculated -control probe as described previously [15]. A total of 703 clones were amplified by PCR using T3 and T7 universal primers. PCR steps were performed for 40 cycles at the following times and temperatures: 50 s at 94 °C, 50 s at 53 °C, and 1 min at 72 °C. PCR products were purified by using PureLink PCR Purification or PureLink Quick Gel Extraction (Invitrogen) and sequenced with T3 universal primer using a ABI 377 automated DNA Sequencer (Applied Biosystem USA) at High-Throughput Genomics Unit, Department of Genome Sciences, Washington University, USA. Clones were

identified through simultaneous database searches using BLASTn algorithm at the NCBI network service to the GenBank nr database (http://www.ncbi.nlm.nih.gov/BLAST/) and at the Solanaceae Genomics Network (SGN, http://sgn.cornell.edu/). Additionally, the best hits obtained were compared to the *Arabidopsis thaliana* genome at GenBank database using BLASTx algorithm in order to identify its ortholog. Similarities with expected value (E) smaller to 10^{-20} were considered significant. Functional categorization of identified ESTs was carried out through Munich Information Center for Protein Sequences (MIPS, http://mips.gsf.de/proj/thal/db/index.html) using the Functional Categories described for *Arabidopsis* proteins [21]. Sequences were submitted to GenBank EST database (accession numbers from GT888386 to GT889003, from GT982694 to GT982778 and from GW672437 to GW672441).

2.5. Macroarrays

Preparation, cDNA labeling, hybridization and quantification of the array were carried out at the DNA Chips Service-SCSIE, Department of Biochemistry and Molecular Biology, University of Valencia, Spain. Briefly, BioGrid (BioRobotics, UK) was used as the spotting robot and macroarrays were made by printing 358 independent PCR products in triplicate spots onto positively charged nylon membranes (Amershan Hybond N+) [22]. Total RNA extracted from 24h *F. solani* f. sp. *eumartii* -inoculated and control tubers were labeled with [α-^{33}P] dCTP by reverse transcription using SuperScript II (Invitrogen) following manufacture's indications. Unincorporated nucleotides were removed by using MicroSpin S-300 HR columns (Amersham Bioscience, USA). Three biological replicates for each treatment were used as independent RNA source and probe generation. The hybridization protocol used was as follows: macroarrays were pre-treated with 0.5% SDS for 30 min at 80 ºC

to remove particles deposited during array printing and filters were pre-hybridized with 5 ml pre-hybridization solution (the same as used for hybridization but without the radioactive sample). The pre-hybridization solution was then replaced with 5 ml of the same solution containing the radioactive sample and hybridized for 17 h. After washings, membranes were kept humid, sealed in Saran wrap and exposed to an imaging plate (BAS-MP, FujiFilm). For new hybridizations, filters were stripped by pouring 3 × 150 ml boiling stripping buffer over the membrane. Exogenous control genes were used to normalize macroarray data [23]. A mix of three mRNAs that are derived from *Bacillus subtilis* tRNAs lys (ATCC87482), thr (ATCC87484), and phe (ATCC87483) cloned into a vector that contains a stretch of As was used. These RNAs were generated by in vitro transcription using a T3 in vitro transcription kit (Roche) of the linearized DNA template with the appropriate restriction enzyme. PCR products, corresponding to these clones, were spotted at multiple sites (12 spots for each clone) on the DNA macroarray. Additionally, negative controls (trp from *B. subtilis*; bioB, bioC, and bioD from *E. coli*) were included in the macroarray. For methodology details see [22]. Images were acquired using a FujiFilm FLA3000 Phosphorimager. Spot intensities were measured as ARM density (artifact-removed density), background and sARM density (background-corrected ARM density) by using the Array Vision software (Imaging Research, Canada).

2.6. Northern blot assay

Total RNA (10 μg/lane) isolated from *F. solani* f. sp. *eumartii* -inoculated or control tubers were separated on denaturing 1.0% agarose gels. After migration, RNAs were transferred onto a nylon membrane N+ (Amersham Biosciences, USA) according to the manufacturer's indications and hybridized with the indicated radioactive probe. cDNAs probes were labeled with [α-^{32}P] dCTP by random priming

using Megaprime DNA labeling system (Amersham Biosciences, USA). Nylon membranes were pre-hybridized with 20 ml of pre-hybridization solution (0.5 M buffer phosphate pH 7.2, 7% SDS and 10 mM EDTA pH 8.0) for 20 min at 65 °C. Hybridization was performed in the same pre-hybridization solution at 65 °C for 24 h then, washed twice with 2 × SSC, 0.1% SDS for 15 min at 65 °C, 1 × SSC, 0.1% SDS for 30 min at 65 °C and 0.5 × SSC, 0.1% SDS for 15 min at 65 °C. Membranes were exposed in an Imaging Plate (Fujifilm) at room temperature for 4 days. Images were obtained from a Storm Scanner (Amersham Biosciences, USA). Densitometry analyses were performed using Image J Program (version 1.42q, National Institute of Health, USA, http://rsbweb.nih.gov/ij/); values were normalized to the level of the ribosomal RNA in each sample.

2.7. Data Analysis

MIPS functional categories statistically overrepresented in the potato cDNA library were determined using hypergeometric distribution for *P*-value calculation described in (http://mips.gsf.de/proj/funcatDB/help_p-value.html). A *P*-value <0.001 was considered significant.

Clones significantly up- or down-regulated in the macroarray study were determined using a z-Test (two sample assuming unequal variance) from Microsoft Excel data analysis tool. A *P*-value <0.05 was considered significant.

3. Results

3.1. Up-regulation of potato genes by F. solani *f. sp.* eumartii *infection*

Previous light microscopy and ultrastructure studies comparing *F. solani* f. sp. *eumartii* infection on cv. Spunta and cv. Huinkul -a highly susceptible cultivar- placed

the hyphal penetration between 12 and 24 h post-inoculation [24]. Selected time point for this study (24 h) allowed the identification of a wide spectrum of stress responsive genes. A total of 703 cDNA clones were isolated from a potato cDNA library of 24 h *F. solani* f. sp. *eumartii*-infected tubers by means of a differential screening using control and a subtracted probe [15]. Following PCR amplification, electrophoresis and purification, clones were sequenced and analyzed through BLASTn searches using SGN and nr Genbank databases. After manual clustering of redundant sequences, a total of 578 unique ESTs were identified and sorted into functional categories using the Munich Information Center for Protein Sequences (MIPS) Functional Catalogue Database based in their *Arabidopsis* orthologs (Fig. 1). The functional category Metabolism (FunCat 01) showed the highest number of entries (112). Protein metabolism related categories; *Protein fate* (FunCat 14) and *Protein synthesis* (FunCat 12) presented a high number of entries (63 and 55 respectively). Functional categories closely related with defense responses, such as C*ell rescue, defense and virulence* (FunCat 32, 59 entries) and *Interaction with the environment*, both *cellular* (FunCat 34, 52 entries) and *systemic* (FunCat 36, 25 entries) were also highly represented.. *Cellular transport, transport facilities and transport routes* (FunCat 20, 47 entries), *Transcription* (FunCat 11, 32 entries) and *Energy* (FunCat 02, 28 entries) completed the list of the most populated categories. Additionally, a total of 66 ESTs orthologs were present in the catalog as unclassified proteins and another 72 ESTs orthologs were either absent in the catalog or were classified only under FunCats 70 (*Subcellular localization*) or 16 (*Protein with binding function or cofactor requirement*), which were not included in this analysis for functional classification. Moreover, 58 ESTs had no *Arabidopsis* orthologs and they could not be included in

this classification. On the other hand, 7 ESTs were identified as *F. solani* f. sp. *eumartii* genes given their high similarities to fungal sequences (not shown).

Statistically significant overrepresentation of functional categories (*P*-value < 0.001) was obtained from the MIPS FunCat system using hypergeometric distribution for *P*-value calculation. Categories overrepresented were *Metabolism* (FunCat 01), *Energy* (FunCat 02), *Protein synthes*is (FunCat 12), *Cell rescue, defense and virulence* (FunCat 32), *Interaction with the environment* (FunCat 34) and *Systemic interaction with the environment* (FunCat 36) (Table 1). Other categories, even when not overrepresented as a whole, contained specific overrepresented subcategories, such as *RNA processing* (FunCat 11.04), *Protein folding and stabilization* (FunCat 14.01), *Enzymatic activity regulation* (FunCat 18.02.01) and *Electron transport* (FunCat 20.01.15).

A complete list of cDNA clones including their most similar GenBank entries, matching potato SGN Unigene and *Arabidopsis* orthologs is provided (Supplementary Table S1).

3.2. Macroarray study: validation of subtracted cDNA clones

A macroarray analysis was performed to validate the differential expression of potato cDNA clones isolated from the *F. solani* f. sp. *eumartii* -infected tubers. Three hundred fifty eight randomly selected cDNA clones were arrayed in triplicates and hybridized with newly obtained cDNA from 24 h *F. solani* f. sp. *eumartii* -inoculated or control tubers. Table 2 shows a partial list of cDNA clones analyzed in the macroarray. A complete list is presented in the Supplementary Table S2. Data analysis confirmed that 282 clones (more than 78%) were significantly up-regulated by *F. solani* f. sp. *eumartii* infection. Most up-regulated genes (52.5%) showed an expression increase between 2 to 3 fold in infected tubers as compared to the control.

Furthermore, over 20% of the clones exceeded a 3-fold increase in expression level. On the other hand, 5 cDNA clones were significantly downregulated (*P*-value <0.05) by the fungal infection.

3.3. Confirmation of a set of up-regulated genes by Northern blot

To validate the macroarray analysis, differential expression of transcripts showing very different fold-induction in the macroarray were tested by Northern blot assay. For this purpose, newly prepared RNA samples from independent assays were used. Clones GT888588 (FQR1), GT888556 (PR-P2) and GT888498 (Splicing factor) up-regulated 13.3, 4.12 and 1.9 folds respectively in the macroarray, showed fold induction comparable to those detected by the array, asserting the robustness of the analysis (Fig. 2A). Additionally, differential expression of a set of up-regulated genes belonging to the overrepresented *RNA processing* functional category was further confirmed by Northern blot assays. Steady-state levels of transcripts GT982695 (KH domain RNA BP), GT888692 (DEAD box RNA helicase -STRS1), GT982720 (DEAD box RNA helicase -RH15), GT888504 (Poly A^+ BP), GT888843 (Glycine-rich RNA BP -SGRP-1) and GT888642 (Glycine-rich RNA BP) were analyzed in 24 h *F. solani* f. sp. *eumartii* -inoculated and control tubers (Fig. 2B). Densitometry quantification of these assays confirmed that all of them were up-regulated in tubers by fungal infection and showed levels of induction similar to the macroarray data (Fig 2C). The expression patterns of *DEAD box RNA helicase STRS1*, *DEAD box RNA helicase RH15*, *Poly A^+ BP*, *Glycine-rich RNA BP -SGRP-1* and *Glycine-rich RNA BP* were also investigated at earlier inoculation time points (4 h and 8 h). As presented in Fig. 2D, expression of these transcripts did not show significant changes at earlier time points, suggesting that genes functionally related with RNA processing may be part of a later onset response.

3.4. MapMan visualization of defense genes up-regulated in F. solani *f. sp.* eumartii *-infected potato tubers*

In order to display macroarray data onto pathway diagrams, MapMan software tools developed to map transcriptome data from Solanaceous species [25] were used. Genes classified as involved in plant response to pathogen or pest attacks were visualized by MapMan (Fig. 3). It can be observed that a major proportion of the genes represent final steps in the defense paths depicted: pathogenesis-related proteins, heat shock proteins (HSP), proteolysis related proteins, cell wall related proteins and peroxidases. However, signal transduction components and transcription factors were scarcely represented at 24 h after fungal inoculation. Additionally, MapMan tools allowed the proper classification of some genes previously classified as unknown or unclassified under MIPS based classification, as defense-related genes or proteolysis related genes (e.g. clone GW672441 -pathogenesis related protein PR10-like, SGN-U268443, clone GT888767 -similar to F-box protein SKP1 interacting partner 2, SGN-U280452).

4. Discussion

4.1. Overrepresented functional categories in the potato-F. solani *f. sp.* eumartii *interaction showed the activation of an inducible defense response*

Analysis of potato transcripts up-regulated by *F. solani* f. sp. *eumartii* infection allowed the identification of functional categories of genes involved in potato defense response to fungal stress. Generally, a high representation of a category reflects the activation of particular physiological processes that might contribute to the defense

response against fungal infection. Thus, analysis of such functional categories helps to elucidate potato tuber transcriptional responses during the host-fungal interaction.

Overrepresentation of defense-related categories as *Cell rescue, defense and virulence* (FunCat 32), *Interaction with the environment* (FunCat 34), and *Systemic interaction with the environment* (FunCat 36) supports the existence of an inducible defense response during potato-*F. solani* f. sp. *eumartii* interaction. Genes found in these categories, such as chitinases, peroxidases and PR-proteins have been largely demonstrated to participate in the defense response of multiple plant-pathogen systems [26-28].

High representation of main functional categories as *Metabolism* (FunCat 01), in particular metabolism of amino acids, carbohydrates, vitamins, cofactors and prosthetic groups and *Protein synthesis* (FunCat 12) indicates that a metabolically active response was triggered by *F. solani* f. sp. *eumartii* infection. Similarly, these categories have been the most represented in expression studies of *Arabidopsis-Alternaria brassicicola* interaction [29] and rice infected by *Magnaporthe grisea* [30]. The parallel activation of *Energy* (FunCat 02) and *Electron transport* (FunCat 20.01.15) pathways suggests an increase in energy requirement, probably as a result of mechanisms set off by the potato defense response. As it is also well known, the induction of the defense mechanisms involves a massive redistribution of energy toward the defense response, making plant defense an intensive energy demanding process [31].

Overrepresented *Protein folding and stabilization* subcategory (FunCat 14.01) included five different molecular chaperones Hsp70 and five different Dna-J co-chaperone proteins. Since chaperone proteins play a key role maintaining protein homeostasis under environmental stress conditions [32], up-regulation of this set of

genes suggests that a requirement of this proteins may exist, either to prevent aggregation, promote folding to the native state and/or refold aggregated proteins affected by fungal action in potato tubers.

In addition, functional category *Regulation of enzymatic activity* (FunCat 18.02.01) was also highly represented. This category included genes involved in posttranscriptional modifications, suggesting that activation of regulatory mechanisms is also required to control the fungal infection. The differential clone collection comprised twelve different families of protein kinases, including members of MAPK (mitogen-activated protein kinase) family, MPK4 and MPK20, whose cascade-mediated signaling constitute essential steps in the establishment of resistance to pathogens [33]. A SOS-like kinase was also identified, which has been related to the regulation of the expression and activity of ion transporters such as SOS1 [34]. A CIPK (calcineurin B-like protein-interacting protein kinase) whose rice ortholog and paralogs are differentially induced by stresses as drought, salinity, cold, polyethylene glycol, and ABA treatment [35] was also detected as well as a SAPK8 (osmotic stress/ABA–activated protein kinase 8) whose *Arabidopsis* ortholog is activated by ABA signal as well as by hyperosmotic stress [36]. Other kinases included a CTR1-like kinase, which was reported in tomato to play a role in ethylene signaling, development and defense [37] and a shaggy related kinase, whose tomato ortholog LeCTR2 regulates the signaling of brassinosteroids [38]. Differential activation of these protein kinases is also an indicator of the wide physiological remodeling triggered by fungal attack in potato tubers.

Taking together, overrepresentation of the identified and monitored functional categories exposes the existence of a transcriptome reprogramming as part of an inducible defense response in potato tubers challenged by *F. solani* f. sp. *eumartii*

infection. This response correlates with the low susceptibility of cv. Spunta to *F. solani* f. sp. *eumartii* infection [14]. However, defense response described for cv. Spunta tubers does not confer a full resistance to the pathogen.

4.2. Participation of RNA processing genes in potato-F. solani *f. sp.* eumartii *interaction*

A significant number of potato genes related with transcription were also identified. It resulted in the overrepresentation of the specific *RNA processing* subcategory (FunCat 11.04), suggesting that proteins involved in RNA metabolism, as RNA binding proteins, splicing factors and helicases may play a role in potato tubers during fungal infection. Our potato cDNA collection contained 9 different RNA binding proteins -including glycine rich, RNA recognition motif (RRM), KH domain, Poly A^+ binding protein (PABP) and Pumilio family types, 6 different helicases and 8 different splicing factors.

RNA processing comprises pre-mRNA splicing, capping, polyadenylation, transport, localization, translation and stability. In particular, mRNA stabilization and alternative splicing are processes that play a key role in the regulatory responses activated by different stress situations [39,40]. In plants, however, participation of RNA processing proteins in stress responses has been reported only very recently and its bibliography is far less abundant as compared to animal systems [41]. Glycine rich RNA binding proteins were reported to contribute to abiotic stress tolerance in *Arabidopsis* [42-44]. Accumulation of zinc finger motif RNA binding proteins has been also described upon water stress and cold in wheat [45]. Another RNA binding protein type, the Pumilio family is also up-regulated upon exposure to *Verticillium dahliae* toxins in *Arabidopsis* [46]. On the other hand, helicases have been recently related to abiotic stress in plants. Soybean DEAD-box RNA helicase *GmRH* is

induced in response to low temperature and high salinity stresses suggesting an important role in RNA processing during such stress conditions [47]. Ectopic expression of *Medicago sativa* helicase MH1 improves seed germination and plant growth under drought, salt and oxidative stress in *Arabidopsis* [48]. In addition, helicase transcripts *AtRH9* and *AtRH25* are markedly up-regulated in response to cold stress and *AtRH25* overexpression enhances freezing tolerance in *Arabidopsis* plants [49]. Alternative splicing is also involved in stress response. An *Arabidopsis* mRNA splicing factor, *STA1* is required for the turnover of unstable transcripts and it has an important role in response to abiotic stresses [50]. Although alternative splicing might confer adaptive advantages to plants, the signaling pathways that link stress conditions to splicing machinery are yet poorly understood [51].

Additionally, macroarray results on genes belonging to the RNA processing category were validated by Northern blot assays. A close agreement of values was observed for all the clones analyzed, supporting macroarray data. Also, a temporal analysis of the expression of these RNA processing genes was carried out by investigating expression profiles at earlier infection times. However, no changes in expression at 4 and 8 h post-infection were measured in any of the analyzed transcripts, suggesting that defense response involving these particular RNA processing proteins might not be established in the initial hours of the infection.

4.3. Participation of genes with unknown function and Solanaceae specific genes in potato-F. solani *f. sp.* eumartii *interaction*

About 20% of the clones were either unclassified or classified as unknown using MIPS classification. This relatively large fraction of genes suggests that still undiscovered pathways might participate in the potato defense response to *F. solani* f. sp. *eumartii* infection. Thus, thirteen percent of the cDNA clones were not included in

the functional classification as they lacked an *Arabidopsis* ortholog. These genes showed either no significant homology to any sequence in the databases or presented homology to genes absent in *Arabidopsis*. Remarkably, the group of genes lacking an *Arabidopsis* ortholog includes a number of genes coding for proteins previously related with pathogen response in Solanaceae, including protease inhibitors (metallocarboxypeptidase inhibitor and Kunitz-type trypsin inhibitor), class I chitinases and pathogenesis-related proteins (types STH-2, STH-21 and PR10-like) and a myb-related transcription factor, whose tobacco ortholog has been involved in the regulation of defense related genes [52]. In addition, a *WRKY* type transcription factor and a *C3HC4-type RING finger* were also part of this group, both genes belonging to gene families largely involved in defense responses [53,54]. Another clone included in the group of unclassified genes was the transcript coding for FQR1, which showed the highest up-regulation level in the macroarray analysis. FQR1 was identified as primary auxin responsive gene in Arabidopsis [55]. In addition, Mammalian FQR1 orthologs has been recently reported to play a role as a regulator of proteasomal degradation [reviewed in [56]. The presence of this kind of cDNA clones in this collection strongly suggests that novel proteins and pathways involved in the potato-*F. solani* f. sp. *eumartii* interaction might be identified through this analysis.

A comparative analysis of this work with previous transcriptomic studies in other systems shows that many differentially expressed genes are common to different plant-fungal interactions [57]. Likewise, several genes presented in this work were also identified in potato tuber tissues infected by *Phytophthora infestans* [58], suggesting that a shared background of transcriptomic defense response exists, despite the singularities of each specific interaction.

In conclusion, the methodology employed in this work, combining the generation of a differential cDNA collection followed by macroarray and Northern blot validation constituted a rigorous and reliable practical approach. In addition, several hundred novel potato sequences were generated and added to the GenBank ESTs database, providing new useful information from species whose genomic sequence is still to be finished. Further studies focusing on the biological functions of the differentially expressed potato genes will help to provide a better understanding of potato-*Fusarium* interaction.

Supplementary material

Table S1 List of 703 cDNA clone collection obtained through differential screening using a subtracted probe (24 h of *F. solani* f. sp. *eumartii* infection).

Table S2 Macroarray analysis. List of 282 cDNA clones differentially over expressed after 24 h of *F. solani* f. sp. *eumartii* infection. Expression ratios were calculated from three biologically independent replicates.

Table S3 List of defense-related cDNA clones included in MapMan

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Figure Legends

Fig. 1 Functional classification of a cDNA collection of genes differentially over expressed after 24 h of *F. solani* f. sp. *eumartii* infection. Classification was carried out according to the MIPS functional categories descript for *Arabidopsis* proteins. Number of cDNA clones included in each category is indicated. Dark gray bars highlight defense related FunCats.

Fig. 2 Northern blot validation of potato transcripts up-regulated by *F. solani* f. sp. *eumartii* infection. Blots containing *F. solani* f. sp. *eumartii* inoculated (I) and control (C) total RNA (20 μg/lane) were hybridized with the indicated [^{32}P]-labeled cDNA probes. Validation of selected clones showing different up-regulation ratios (A). Relative expression ratios obtained in the macroarray analysis are shown under the panels. Validation of *RNA processing* FunCat clones: representative images from 24 h-treated tuber samples (B), densitometry of 24 h treated tubers (C). Representative images from 4 h and 8 h treated tubers (D). Etidium bromide stained agarose gels before blotting are showed at the bottom of each set of hybridization images as loading controls.

Fig. 3 MapMan regulation overview map showing differences in transcript levels between infected and control potato tubers. Displayed are genes associated with pathogen response. Squares in darker tones (using a gray tone scale) represent higher gene expression in infected as compared with control tubers. Circles indicate no gene associated. The list of depicted genes with their normalized expression values are given in Supplementary Table S3.

Table 1 Functional Categories overrepresented in the 24 h *F. solani* f. sp. *eumartii* -infected potato tuber clone collection. Number of entries in the clone collection and percentages respect to the total number of classified clones are indicated. Genome % corresponds to percentages of genes found for each Category in *A. thaliana* genome. Genes were classified according to MIPS Functional Categories.

FunCat	Library Entries (*%*)	Genome %	*P*-value
01 ***Metabolism***	112 (*25.2*)	17.3	$1.49e^{-05}$
01.01 ***Amino acid metabolism***	17 (*3.83*)	1.20	$3.09e^{-03}$
01.05 C-compound and carbohydrate metabolism	48 (*10.8*)	5.79	$2.43e^{-05}$
01.07 Metabolism of vitamins, cofactors & prosthetic groups	14 (*3.16*)	0.88	$4.49e^{-05}$
02 ***Energy***	28 (*6.32*)	1.59	$8.18e^{-10}$
02.01 ***Glycolysis and gluconeogenesis***	8 (*1.80*)	0.25	$1.82e^{-05}$
02.07 ***Pentose-phosphate pathway***	5 (*1.12*)	0.16	$7.30e^{-04}$
11 *Transcription*			*ns*
11.04 ***RNA processing***	15 (*3.38*)	1.46	$2.52e^{-03}$
12 ***Protein synthesis***	59 (*13.3*)	4.68	$5.37e^{-13}$
12.01 ***Ribosome biogenesis***	47 (*10.6*)	1.50	$3.57e^{-26}$
12.04 ***Translation***	55 (*12.4*)	4.28	$1.69e^{-12}$
14 Protein fate			*ns*
14.01Protein folding and stabilization	16 (*3.61*)	0.87	$2.05e^{-06}$
18 *Regulation of metabolism and protein function*			*ns*
18.02.01 ***Enzymatic activity***	10 (*2.25*)	0.77	$2.60e^{-03}$
20 *Cellular transport, transport facilities & routes*			*ns*
20.01.15 ***Electron transport***	22 (*4.96*)	2.52	$8.19e^{-03}$
32 ***Cell rescue, defense and virulence***	59 (*13.3*)	5.01	$8.58e^{-12}$
32.01 ***Stress response***	45 (*10.1*)	0.92	$5.22e^{-13}$
32.07 ***Detoxification***	14 (*3.16*)	0.93	$8.37e^{-05}$
34 ***Interaction with the environment***	52 (*11.7*)	5.81	$1.22e^{-06}$
34.11 ***Cellular sensing and response to external stimulus***	52 (*11.7*)	5.24	$5.18e^{-08}$
36 ***Systemic interaction with the environment***	25 (*5.64*)	2.66	$3.97e^{-04}$
36.20 ***Plant / fungal specific systemic sensing and response***	21 (*4.74*)	2.43	$3.11e^{-03}$
36.25 ***Animal specific systemic sensing and response***	7 (*1.58*)	0.37	$1.46e^{-03}$
42 *Biogenesis of cellular components*			*ns*
42.02 ***Eukaryotic plasma membrane***	2 (*0.45*)	0.01	$1.43e^{-03}$

ns: not significant

Table 2. Selection of 24 h *F. solani* f. sp. *eumartii* -infected potato tuber clones analyzed in a macroarray study. Genes were classified according to MIPS Functional Categories. Fold induction represents the ratio of expression in infected tubers as compared to the control (mean ± sd of three independent biological replicates). *S.l., S. lycopersicum; N.b., N. benthamiana.*

Accession no.	Putative function	Closest ncbi entry (Blastn e value)	SGN-Potato Unigene	Fold induction
Metabolism				
01-*Metabolism*				
GT888893	Ketol-acid reductoisomerase	EU616546 ($1E^{-161}$)	U268055	**3.46** ±0.34
GT982712	Dynamin-related protein ADL2	AJ538434 (0)	U276140	**3.04** ±0.90
GT888557	Guanylate kinase	AC212431 (0)	U290992	**2.42** ±0.34
GT982704	Riboflavin kinase /FMN hydrolase	XM_002276579 ($7E^{-47}$)	U435008 (*S.l.*)	**2.04** ±0.34
GT888658	Transketolase	Y15781 (0)	U268937	**3.98** ±0.25
GT888686	Sucrose synthase 2	AY205084 (0)	U268212	**5.62** ±0.41
GT888478	Decoy	AK321108 (0)	U270297	**4.49** ±0.25
GT888500	Endo-1,3-beta-D-glucanase (glub2)	U01901.1 (0)	U269323	**4.65** ±0.01
GT888920	Fatty acid multifunctional protein	AK321545 (0)	U271314	**2.16** ±0.54
GT982775	Lipoxygenase	X95512.1 (0)	U268109	**2.10** ±0.35
GT982738	3-hydroxy-3-methylglutaryl CoA reductase	AB041031 ($5E^{-116}$)	U268122	**4.31** ±0.47
02-*Energy*				
GT888887	ATP synthase delta chain, mitochondrial	AK319936 (0)	U579799 (*S.l.*)	**3.81** ±0.48
GT888619	Succinyl- CoA ligase beta subunit-like	DQ200398 (0)	U268134	**5.28** ±1.25
GT888488	Vacuolar H+-atpase A1 subunit	AK323678 (0)	U269858	**2.89** ±0.35
Information pathways				
11-*Transcription*				
GT888598	CBF-B/NF-YA family transcription factor	AK324716 (0)	U274337	**2.22** ±0.44
GT888468	Myb family transcription factor	XM_002276490 ($1E^{-99}$)	U285867	**2.74** ±0.23
GT982695	KH domain/zinc finger RNA BP	AK329271 (0)	U277772	**1.93** ±0.26
GT888692	DEAD box RNA helicase (STRS1)	AK322041 (0)	U275212	**2.93** ±0.42
GT888898	RNA helicase	BT012737 (0)	U270694	**3.81** ±1.07
GT982720	DEAD box RNA helicase RH15-like	AK324442 (0)	U281221	**2.00** ±0.58
GT888504	Poly(A)-binding protein (PABP)	AF190657 ($7E^{-72}$)	U269827	**3.35** ±0.79
GT888862	RRM-containing protein	XM_002515366 (0)	U277568	**2.68** ±0.72
GT888843	Glycine rich RNA binding protein (SGRP-1)	DQ252483 ($3E^{-122}$)	U281341	**7.78** ±0.94
14-*Protein fate*				
GT888501	ATP binding, heat shock protein	XM_002269978 ($3E^{-99}$)	U271545	**2.55** ±0.23
GT888708	Dnaj protein family, similar to AHM1	AK326219 (0)	U274249	**2.65** ±0.22
GT982728	F-box protein FBL2	AC216650 (0)	U282464	**2.18** ±0.13
GT888747	Polyubiquitin (ubq8)	AK321059 (0)	U270488	**2.93** ±0.31
GT888689	Ubiquitin conjugating enzyme E2-like	DQ222513 (0)	U268938	**2.50** ±0.58
GT888908	Subtilisin-like serine protease	AK321831 (0)	U283743	**2.47** ±0.15
GT888628	Calcium-dependent protein kinase CDPK5	FJ026805 (0)	U509608 (*N.b*)	**2.63** ±0.68
18-*Regulation of metabolism and protein function*				
GT888774	Shaggy-related protein kinase alpha	DQ252508 (0)	U269309	**1.98** ±0.01
GT888921	Sphingosine kinase	XM_002270907 ($4E^{-126}$)	U284268	**1.98** ±0.27
GT888629	Beta4 proteasome subunit	AK246286 (0)	U277014	**2.43** ±0.33
GT888814	Protein kinase, leucine-rich repeat protein	AK321463 (0)	U271620	**2.02** ±0.17
GT888523	Mitogen-activated protein kinase	AB062141 (0)	U273705	**3.08** ±0.57
GT888996	Elongation factor 1B alpha-subunit	DQ207867 (0)	U268627	**3.28** ±0.08
Transport				
20-*Cellular transport, transport facilities & routes*				
GT888668	Importin alpha subunit	AK321447 (0)	U269549	**2.12** ±0.18

GT982735	Monooxygenase	AB061259 ($9E^{-171}$)	U270436	**12.58** ±2.74
GT888963	Calcium-transporting ATPase 7	AF195029 (0)	U295863	**2.34** ±0.33
GT888776	ADP/ATP translocator-like	DQ235188 (0)	U268233	**3.70** ±0.76
GT888415	Major facilitator superfamily protein	AK324052 (0)	U284961	**2.73** ±0.42
GT982694	Syntaxin	AK324207 ($4E^{-68}$)	U270051	**3.29** ±0.67
Perception and response to stimuli				
32-*Cell rescue, defense and virulence*				
GT888477	Alcohol dehydrogenase 3 (ADH-3)	gb|M25152 (0)	U269406	**4.03** ±0.75
GT888890	Cysteine protease (cyp gene)	AJ245924 (0)	U268427	**2.00** ±0.02
GT888480	Peroxidase, putative	AK329501 ($4E^{-145}$)	U270433	**5.89** ±0.63
GT888779	Chitinase (chtb4)	U02608.1 (0)	U268802	**4.74** ±0.36
GT888666	Class II chitinase (chta3)	AF024537 (0)	U270641	**6.14** ±1.53
GT982746	Class I chitinase (chtc2)	AF043248 (0)	U268802	**2.87** ±0.38
GT888798	NBS-LRR disease resistance protein	AK326650 ($2E^{-100}$)	U272417	**3.23** ±0.53
GT888900	Glutaredoxin	AK326095 ($8E^{-108}$)	U275112	**2.41** ±0.40
GT888489	Glutathione peroxidase	Y14762.1 (0)	U268729	**5.23** ±0.92
GT982724	Catalase (CAT2)	AY500290 (0)	U268645	**2.27** ±0.10
34 -*Interaction with the environment*				
GT888884	Multiprotein bridging factor 1	AF232062 ($8E^{-127}$)	U269924	**2.78** ±0.46
GT888943	MATE efflux protein	AC235792 ($2E^{-49}$)	U279442	**2.53** ±0.09
GT888927	Wound-induced protein (WIN2) precursor	X13497.1 (0)	U271959	**4.24** ±0.60
GT888556	Pathogenesis-related protein P2 (PR-P2)	X58548.1 (0)	U268690	**4.14** ±0.19
GT888986	Dihydrolipoamide dehydrogenase	AF542182 (0)	U275629	**3.89** ±0.44
36- *Systemic interaction with the environment*				
GT888981	CBL-interacting protein kinase 1 (CIPK1)	AK247548 (0)	U290226	**2.39** ±0.19
GT982736	NADPH-cytochrome P450 oxidoreductase	DQ099545 ($2E^{-82}$)	U291746	**2.69** ±0.26
GT982754	Formate dehydrogenase	Z21493.2 (0)	U268611	**3.44** ±0.17
GT888679	HHP4 (heptahelical protein 4)	AJ608716 ($3E^{-93}$)	U598786 (*S.l*)	**3.18** ±0.01
Developmental processes				
42- *Biogenesis of cellular components*				
GT888844	Nonspecific lipid transfer protein 2	AK321239 (0)	U272742	**2.71** ±0.79
GT888540	Expansin-related protein 1	BT013002 (0)	U283573	**5.85** ±0.95
GT888979	SAG18 (senescence associated gene 18)	AK327995 (0)	U278795	**1.99** ±0.36
GT888852	Peptidoglycan-binding LysM protein	AK323499 (0)	U292118	**3.22** ±0.41
Unclassified				
GT888879	TIP41-like family protein	AK322268 (0)	U295696	**6.03** ±2.84
GT888850	F-box protein, zinc finger protein	BT014418 (0)	U291421	**2.38** ±0.16
GT888472	Short-chain dehydrogenase/reductase	XM_002276237 ($8E^{-11}$)	U282108	**5.13** ±0.68
GT888758	F-box protein, SKP1 interacting partner 2	AK324851 ($1E^{-99}$)	U280452	**3.37** ±0.74
GW672441	Pathogenesis related protein PR10-like	AK319457 (0)	U268443	**2.73** ±0.42
GT982734	Stress-associated protein 5 (SAP5)	FJ442191 ($4E^{-119}$)	U276536	**3.98** ±0.46
GT888782	Pathogenesis-related protein (STH-21)	M25156.1 (0)	U268440	**2.43** ±0.60
GT982744	Pumilio-family RNA binding protein	XM_002271074 ($5E^{-131}$)	U271518	**2.30** ±0.47
GT888863	Zinc finger protein (PMZ) -related	AK320321 (0)	U278173	**3.02** ±0.64
GT888588	Flavodoxin-like quinone reductase (FQR1)	AK329294 (0)	U273087	**13.29** ±0.69
GT888507	NADH-ubiquinone oxidoreductase	AK246611 ($2E^{-100}$)	U273604	**2.14** ±0.45
GT888420	F-box protein, zinc finger protein	AK323597 ($3E^{-150}$)	U285123	**2.12** ±0.06
GT888808	RNA binding protein	DQ200389 (0)	U277572	**2.78** ±0.16
GT888642	Glycine-rich RNA-binding protein	AK321634 ($1E^{-142}$)	U272857	**5.34** ±1.00
GT888498	Arginine/serine-rich splicing factor	AK323395 (0)	U269590	**1.82** ±0.09