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Microarray analyses during early stage of the tomato/*Alternaria solani* interaction

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ABSTRACT

Tomato early blight is an important threat and it has capacity to reduce the production in all major tomato producing areas. Molecular mechanism underlying the resistance against this is not well known. Therefore we studied this system to search the possible mechanism of resistance, which includes pathogenesis related protein, and pathways and transcription factors, which are responsible for resistance against this pathogen using affymetrix gene chip for tomato. Their differential expressions have enhanced the biochemical and other related products, which have, direct or indirect role in stopping the penetration of mycelia in the host plant.

1. Introduction

Early blight of tomato is economically the most important disease of tomatoes in the USA, Australia, Israel, the UK, and India, where significant reductions in yield (35 up to 78%) have been observed [1,2,3]. There is no complete resistant genotype for early blight has been found yet, but in different screening experiments few genotypes belonging to *Solanum habrochaites*, *Solanum arcanum* have shown moderate to high resistance [4,5]. The accession which have resistance for this disease mostly belong to wild types for example *S. arcanum*, *Solanum peruvianum*, *Solanum neorickii*, and *Solanum chilense*. *S. habrochaites* accessions were found to have both susceptibility and resistance against early blight. The resulting lines from crosses of tomato with these wild species have still no satisfying crop qualities. Therefore, disease control of early blight is mainly conducted with chemical protective agents. However, these agents do not always prevent the infestation of the fruits and severe losses can still occur. The long-term effects of these chemicals e.g. fungicides on humans are still unknown but may contribute to resistance against medications in humans with life-threatening infections. Also, this may cause mutations by permanent silencing or reprogramming normal genes, which can last for several generations.

Early blight (EB) resistance is a quantitative trait [6], which makes selection more difficult compared to qualitative traits [7,8]. In order to understand the genetic control of early blight resistance and to facilitate its introgression in tomato, molecular markers and QTL analysis has been carried out. With discoveries of new technologies, the research in this field has progressed with use of functional genomics tools to observe the mechanism of resistance again early blight. Determining the function of a set of resistance genes helps us understand the pathway leading to the resistance reaction of a host plant.

2. Specification

Organism/cell line/tissue	*Solanum lycopersicum*/cultivated and wild type, leaf tissues
Sex	N/A
Sequencer or array type	Tomato Genome Array (Affymetrix, USA)
Data format	Raw and analyzed
Experimental factors	Susceptible and resistant tomato plants, inoculated with *A. solani*. Leaves for RNA isolation were collected after 24 h of inoculation
Experimental features	Tomato plants inoculated with *A. solani* cultures and control plant were maintained and samples were collected at 24 hai
Consent	N/A
Sample source location	IIVR, Varanasi

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3. Materials and methods

3.1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE71428, Submission no. GSE71428. Also, one research article has been published by Upadhyay et al. in Journal of Plant Pathology and Microbiology [9].

3.2. Plant material

Plant materials utilized for this study were from the smut-tomato variety CO-3 and EC-520,061 as susceptible and resistant respectively. The basis of this selection was their performance against EB pathogen during screening experiment [5]. The seeds of these varieties were provided by Indian institute of Vegetable Research, Varanasi, Uttar Pradesh, India. The plants were grown in growth chamber under temperature-controlled condition at 25 °C.

3.3. Pathogen inoculum

Challenge inoculum consisted of Varanasi isolate of *Alternaria solani* (causal organism of early blight in tomato), that was isolated from the infected tomato leaves in Indian Institute of Vegetable Research, Varanasi. The culture was propagated on Potato Dextrose Agar (PDA) in 90-mm Petri dishes. The dishes were incubated at 25 °C under a cool-white fluorescent diurnal light with 12 h photoperiod for 10–15 d.

After 15-days culture was scraped and macerated together with sterile pestle and mortar. This culture was free from conidia but thickening of conidiogenous hyphae and chlamydospore like structures were observed in this. Before the formation of these structures, cultures did not have their usual aggressiveness and potential for infection. One and half month old plants were inoculated by a spraying suspension solution of *A. solani* under control condition. Tomato plants were sprayed with an inoculum (157 cfu ml^{-1}) to induce infection. The inoculated pots were kept at 28 °C and more than 95% humidity to create proper conditions for infection. Plants sprayed with sterile distilled water were treated as control. Leaf samples were collected in three biological replications after 24 h of inoculation which is a stage of penetration of leaf tissue by the fungal mycelia [10] and immediately stored in liquid nitrogen and then kept in a freezer at −80 °C.

3.4. Microarray experiment

RNA isolation was done using TRIzol reagent (Invitrogen, Carlsbad, CA) according to manufacturer's instructions and then its quantity and quality was accessed by spectrophotometry (optical density = 260/280) and on a 1% agarose gel containing formaldehyde.

Double stranded cDNA synthesis, in vitro transcription to synthesize biotin labeled aRNA, purification and fragmentation of aRNA, and hybridization of arrays were performed according to Affymetrix technical manual. The Affymetrix GeneChip Tomato Genome Array contains 10,038 probe sets, representing about 4600 unigenes. The hybridized chips were washed, stained and scanned using the GeneChip scanner to generate the CEL files. Fig. 1 shows the schematic presentation of the experiment.

The CEL files were imported into GeneSpring GX v12 (Agilent Technologies). Signal intensities were recorded for all the 10,038 probe sets. The data has been deposited at NCBI (http://www.ncbi.nlm.nih.gov), with accession number GSE71428. Using Robust Multi-array Average (RMA) algorithm (Irizarry et al. 2003) normalized the signal intensities. The Principal Component Analysis (PCA) in GeneSpring GX 10.1 established that the three biological replicates were located close to one another. The high correlation coefficient was observed among the three replicated samples, indicating less genetic background noise. To correct the variability in the normalized expression values, the probe sets with coefficient of variation <50% were retained, and the rest were discarded.

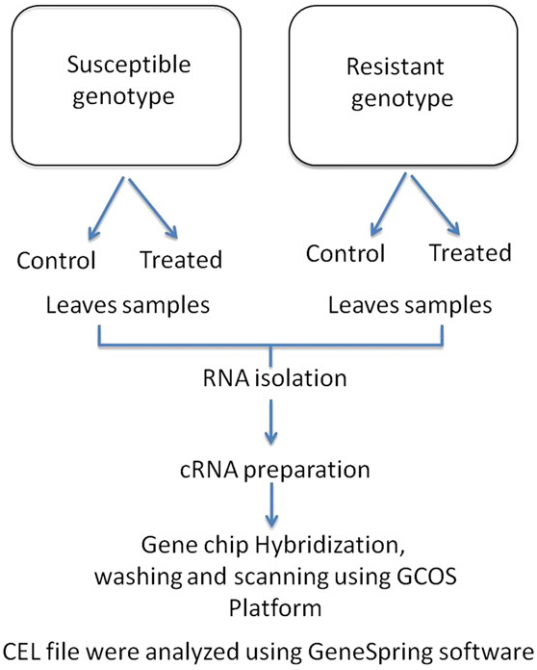

Fig. 1. Schematic presentation of expression profiling experiment.

3.5. Functional annotation of the differentially expressed probe sets

The tables of significant transcripts were generated at p values <0.05 and fold change value >2.0. For annotation of transcripts an annotated probe file was referred which was generated at Cornell University, USA (ted.bti.cornell.edu/*TFGD/array/Affy_probe_annotation.xls*) and NCBI website. Among those significantly differentially expressed transcripts, we selected the transcripts which had their function as regulation of transcription.

3.6. Screening of transcription factor from microarray data

The Tomato transcription factors analyzed in this experiment were described in the transcription factor database. According to the annotation of Affymetrix genome microarray, we screened for TF genes that were differentially induced or repressed after *A. solani* infection in CO-3 and EC-520061 with a fold change (FC) of >2.0 and a p-value of <0.05. The results were shown as a Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/*webcite*). Further probe filtering for TF genes that were significantly induced by *A. solani* or constitutively expressed in the resistant cultivar EC-520061 was performed with the fold-change tool in Genespring GX 11.5.

3.7. q-RT PCR validation

Total RNA was extracted from the leaves of the two genotypes (CO-3 and EC-520061) after 24 h of inoculation, in three biological replicates. First strand cDNA for each sample was synthesized by using SuperscriptTMIII first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Primers for quantitative real-time RT-PCR were designed using web based primer designing tool from IDT (http://eu.idtdna.com/Scitools/Applications/Primerquest/default.aspx).

Quantitative real time PCR was performed in three biological replicates using SYBR Green (Qiagen, USA) fluorescence dye and analyzed by using iQ-SYBR Green Supermix (Bio-Rad, CA, USA) according to the

manufacturer's protocols on iQ5 thermo cycler (Bio-Rad, CA, USA) with iQ5Optical System Software version 2.0 (Bio-Rad, CA, USA). To normalize the target gene expression, the difference between the C_T of the target gene and the C_T of Actin (constitutive control) for the respective template was calculated (ΔC_T value). To calculate fold changes (FC) in gene expression, the ΔC_T value was calculated as follows: $\Delta C_T = C_T$ (target gene) $- C_T$ (constitutive control gene). Relative transcript levels were calculated as: $1000 \times 2 - \Delta C_T$.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

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