



Data in Brief

Microarray analyses for identifying genes conferring resistance to pepper leaf curl virus in chilli pepper (*Capsicum* spp.)



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ABSTRACT

Pepper leaf curl virus (PePLCV) is a serious threat to pepper (*Capsicum* spp.) production worldwide. Molecular mechanism underlying pepper plants response to PePLCV infection is key to develop PePLCV resistant varieties. In this study, we generated transcriptome profiles of PePLCV resistant genotype (BS-35) and susceptible genotype (IVPBC-535) after artificial viral inoculation using microarray technology and detail experimental procedures and analyses are described. A total of 319 genes differentially expressed between resistant and susceptible genotypes were identified, out of that 234 unique genes were found to be up-regulated >2-fold in resistant line BS-35 when compared to susceptible, IVPBC-535. The data set we generated has been analyzed to identify genes that are involved in the regulation of resistance against PePLCV. The raw data have been deposited in the Gene Expression Omnibus (GEO) database under accession number [GSE41131](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41131).

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Specifications

Organism/cell line/tissue	<i>Capsicum</i> species/leaf tissues
Sex	N/A
Sequencer or array type	Tomato gene expression microarray (Agilent Technologies, USA)
Data format	Raw and analyzed
Experimental factors	Susceptible and resistance chilli pepper plants inoculated with pepper leaf curl virus-Varanasi isolate
Experimental features	Susceptible and resistance genotypes were inoculated with pepper leaf curl virus and samples were collected after 24 h of inoculation for RNA isolation
Consent	N/A
Sample source location	IIVR, Varanasi

1. Direct link to deposited data

All samples' data are separately available at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41131>

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2. Introduction

Plants are constantly challenged by a large and diverse group of microorganisms; however, their defence system renders them resistant to the majority of potential invaders. Several mechanisms are known to be involved in plant defence against bacterial, fungal and viral infection which generally involves the orchestrated transcriptional activation of multiple genes and often activate hypersensitive response (HR) and systemic acquired resistance (SAR) [1,2]. Geminiviruses are major threat to production of diverse crops in tropical and subtropical countries [3]. Within family Geminiviridae, *Pepper leaf curl virus* (PePLCV) is a member of genus begomovirus transmitted by whiteflies with wide host range specificity [4]. It infects pepper (*Capsicum* spp.) crop worldwide and causes severe yield losses [5]. It was found in endemic form in major pepper growing regions of India during last few years and thus becoming a major threat to chilli and sweet pepper production [5].

Molecular mechanisms involved in the establishment of resistance or susceptibility of peppers to PePLCV has not been studied so far. There have been a few reports of studies on transcriptional profiling and analysis of gene function related to the host response to plant viruses. DNA microarray is one of the powerful techniques for analysing whole genome expression against a specific stimulus in a wide range of biological systems. This technology enables to perform a high sensitivity parallel screening of thousands of genes to determine their

expression profiles and quantitatively analyze a large amount of data [6, 7]. As a member of the Solanaceae, tomato provides a well-characterized system to anchor transcriptional profiles of various biotic and abiotic stresses. *Capsicum* shares genetic similarity with tomato and both are prone to whitefly transmitted leaf curl viruses. The characterization of the defence pathway in *Capsicum* using tomato microarray is desirable to enhance understanding of plant defence mechanisms against viruses. Hence this study was conducted to investigate the expression profiles in pepper in response to PepLCV inoculation, using a tomato DNA microarray. As far as we know, this is the first report on the characterization of gene expression for resistance to PepLCV in chilli pepper using tomato microarray.

3. Materials and methods

3.1. Plant materials and viral inoculation

Two chilli pepper genotypes, a highly resistant BS-35 (putative interspecific derivative landrace of *C. chinense* × *C. frutescens*) and susceptible IVPBC-535 (*C. annuum*, nonpungent paprika cultivar) were identified for this study through many years of field and artificial screenings against *Pepper leaf curl virus* [8]. Five plants of both genotypes were grown in glasshouse in plastic trays and plants were inoculated with viruliferous whiteflies following Rai et al. [5]. In brief, virus (PepLCV-Varanasi) culture was maintained on PepLCV susceptible sweet pepper (California Wonder) plants kept in an insect-proof cage made of 50-mesh nylon net. Adult whiteflies collected from the egg-plant plants were given an acquisition access period of 24 h on the infected sweet pepper plants. Each plantlet was inoculated at the three-leaf stage, using 10–12 viruliferous whiteflies per plantlet for an inoculation access period of 24 h. Inoculated seedlings were sprayed with imidacloprid (@ 0.3 ml/l) to kill the whiteflies so that uniform inoculation access period can be provided to each plants. After 24 h of inoculation, leaf samples were collected from both the genotypes and frozen in liquid nitrogen until processing.

3.2. RNA isolation and cRNA synthesis

High quality total RNA was isolated from 100 mg of frozen tissue using Trizol® following the manufacturer's instructions (Invitrogen, USA). Genomic DNA was eliminated by treatment with DNase I for 20 min at RT using DNase IH (Invitrogen, USA). RNA concentration was measured using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware USA). Purity and integrity of total RNA was determined by 260/280 nm ratio and checked by electrophoresis in 1% agarose gel. About 250 ng of total RNA was used to produce Cyanine 3-CTP labeled cRNA using the Low Input Quick Amp Labeling Kit, One-Color (Agilent Technologies) according to the manufacturer's instructions. Following 'One-Color Microarray-Based Gene Expression Analysis' protocol version 6.0 (Agilent Technologies), 2 µg of labeled cRNA was hybridized with a tomato gene expression microarray 44 K (Agilent Technologies). The microarray workflow quality control was implemented using the Agilent Spike-In Kit which consisted of a set of 10 positive control transcripts optimized to anneal to complementary probes on the microarray with minimal self-hybridization or cross-hybridization. The concentrated Agilent one Color RNA Spike-Mix stock was diluted in the buffer provided by the kit and mixed with the RNA samples prior to the amplification and labeling process to achieve the relative amounts recommended by the manufacturer.

3.3. Microarray hybridization, scanning and data analysis

For hybridization, Agilent tomato gene expression microarray 44K slide (Agilent Technologies, Santa Clara CA, USA) containing probes designed from the EST sequences deposited in three different databases, GeneBank (GB accessions), TIGR (TA accessions) and the Tomato Gene

Index (TC and NP accessions) were used. Slides were scanned in an Agilent Microarray Scanner (G3000) according to the manufacturer's protocol. Signal data were collected with dedicated Agilent Feature Extraction Software (v 9.5.1). Agilent Processed Signals were processed using GeneSpring software version 12.0 (Agilent Technologies). Box and whisker plot displaying the log ratio distribution of microarray data after normalization is shown in Fig. 1. The raw data are available from the GEO repository, accession number GSE41131.

3.4. MapMan analysis of viral responsive genes

The MapMan software version 2.0 was used to annotate functional BINs of all present tomato genes. The mapping file for the tomato gene expression microarray was downloaded from the MapMan website (<http://gabi.rzpd.de/project/MapMan/>). Log₂- values of the virus induced gene expression relative to control samples were used as input. Default settings were used to perform an uncorrected Wilcoxon rank test.

3.5. Quantitative RT-PCR analysis

A total of 10 highly expressed genes under PepLCV treatment were selected for further qPCR validation. Specific primers for qPCR were designed from each target sequence using Primer 3 with default parameters [9]. Total RNA was extracted from the leaves of resistant (BS-35) and susceptible (IVPBC-535) genotypes after 24 h of inoculation, in two biological replicates. For each sample, 500 ng RNA was reverse transcribed using SuperscriptIII first strand synthesis system (Invitrogen, USA) and random hexamers according to manufacturer's instructions (http://tools.invitrogen.com/content/sfs/manuals/superscriptIIIfirststrand_pps.pdf). qPCR was carried out in a 25 µl reaction mix containing 200 nM of each primer, 1 µl of cDNA sample and FastStart Universal SYBR Green Master (Roche Applied Science). Negative RT RNA control and non-template controls were incorporated in the assays. qPCRs were performed using a BioRad RT thermocycler (BioRad, USA). The thermal profile was set to 95 °C for 10 min, and 40 cycles of 95 °C for 15 s, and hybridization temperature for 1 min. Amplicon specificity was verified by melting curve analysis (60 to 95 °C) after 40 PCR cycles. To normalize the target gene expression, the difference between the CT of the target gene and the CT of Actin (constitutive control) for the respective template was calculated (Δ CT value). To calculate fold changes (FC) in gene expression, the Δ CT value was calculated as follows: Δ CT = CT (target gene) – CT (constitutive control gene). Relative transcript levels were calculated as: $1000 \times 2^{-\Delta$ CT.

4. Conclusion

This study describes the gene expression profiling of PepLCV resistant (BS-35) and susceptible (IVPBC535) genotypes after artificial inoculation of PepLCV. This is the first report of microarray analysis of pepper leaf transcriptome for PepLCV resistance using tomato microarray. In total, 319 genes differentially expressed between resistant and susceptible genotypes were identified, out of that 234 unique genes were found to be up-regulated >2-fold in resistant genotype BS-35 (ANOVA, $p < 0.05$) when compared to susceptible, IVPBC-535. However, only 85 genes were up-regulated in susceptible line IVPBC-535, indicating that gene expression in the resistant genotype responded strongly to PepLCV. These microarray data are important resources to explore and identify genes involved in expression of resistance reaction against PepLCV.

Conflicts of interest

The authors declare no conflicts of interest.

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