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An insight into differentially regulated genes in resistant and susceptible genotypes of potato in response to tomato leaf curl New Delhi virus-[potato] infection

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ABSTRACT

Apical leaf curl disease, caused by tomato leaf curl New Delhi virus-[potato] (ToLCNDV-[potato]) is one of the most important viral diseases of potato in India. Genetic resistance source for ToLCNDV in potato is not identified so far. However, the cultivar Kufri Bahar is known to show lowest seed degeneration even under high vector levels. Hence, microarray analysis was performed to identify differentially regulated genes during ToLCNDV-[potato] infection in a resistant (Kufri Bahar) and a susceptible cultivar (Kufri Pukhraj). Under artificial inoculation conditions, in Kufri Pukhraj, symptom expressions started at 15 days after inoculation (DAI) and then progressed to severe symptoms, whereas no or only very mild symptoms were observed in Kufri Bahar up to 35 DAI. Correspondingly, qPCR assay indicated a high viral load in Kufri Pukhraj and a very low viral load in Kufri Bahar. Microarray analysis showed that a total of 1111 genes and 2588 genes were differentially regulated (|log2 (Fold Change)| > 2) in Kufri Bahar and Kufri Pukhraj, respectively, following ToLCNDV-[potato] infection. Gene ontology and mapman analyses revealed that these altered transcripts were involved in various biological & metabolic processes. Several genes with unknown functions were 5 to 100 fold expressed after virus infection and further experiments are necessary to ascertain their role in disease resistance or susceptibility. This study gives an insight into differentially regulated genes in response to ToLCNDV-[potato] infection in resistant and susceptible cultivars and could serve as the basis for the development of new strategies for disease management.
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1. Introduction

Geminiviruses are the most damaging plant viruses worldwide and pose a severe threat to global food security. They are abundant in tropical and subtropical environments, where vectors that transmit these viruses are abundant (Leke et al., 2015). The genus, *Begomovirus* in the family *Geminiviridae* consists of whitefly-transmitted geminiviruses having ssDNA genomes encapsidated in unique twin particles (18 × 30 nm) and infect only dicotyledonous plants (Fauquet and Stanley, 2003; Fauquet et al., 2008; Stanley, 1985). *Tomato leaf curl New Delhi virus* belongs to the genus *Begomovirus* (Brown et al., 2015), and its variants infect a wide range of crops including tomato, cucurbits, chili pepper, okra, brinjal, papaya and potato (Jyothsna et al., 2013; Kanakala et al., 2013; Kumar et al., 2012; Pratap et al., 2011).

In potato, a variant of ToLCNDV (ToLCNDV-[potato]) causes apical leaf curl disease (Usharani et al., 2004) and now it is one of the most important diseases of potato in India. The incidence of this disease was initially observed in western part of Uttar Pradesh state and later in Hisar district of Haryana (Garg et al., 2001; Lakra, 2002). The virus was transmitted to potato from sponge gourd (Sohrab et al., 2013) and tomato (Saha et al., 2014) due to overlapping planting period and early planting of potato where the potato is exposed to high temperature and whitefly population. Now, this disease is widely spread almost in all potato growing states (Jeevalatha et al., 2012, 2013; Saha et al., 2014). The disease incidence is higher particularly in Indo-Gangetic plains (40–100% infection) and cause heavy yield losses in susceptible varieties (Lakra, 2002; Venkatasalam et al., 2005, 2011). Up to 40% incidence is reported from West Bengal (Saha et al., 2014). The ToLCNDV-[potato] is a bipartite begomovirus with two genomic components referred as DNA-A and DNA-B. The DNA A components of the ToLCNDV-[potato] isolates shared more than 90.0% similarity to ToLCNDV isolates (Usharani et al., 2004) from cucurbitaceous crops

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such as tomato and okra, 89.0–90.0% to papaya isolates and 70.4-74.0% to other ToLCVs (Jeevalatha et al., 2012). The DNA-A encodes two proteins in the virion sense *viz*., the coat protein (AV1; CP) and AV2. In the complementary sense, it encodes the replication-associated protein (AC1; Rep); the transcriptional activator protein (AC2; TrAP); the replication enhancer protein (AC3; REn); AC4 and AC5 with unknown functions. The DNA-B encodes the nuclear shuttle protein (BV1; NSP) and the movement protein (BC1; MP), both of which are vital for systemic spread and symptom expression.

Microarray has been used to identify the gene expression changes during virus infection in plants (Musser et al., 2014; Pierce and Rey, 2013; Shahid et al., 2015). Infection of plants by both RNA and DNA viruses leads to a similar pattern of alteration in the plant transcriptome. However, in addition to the induction of pathogen response, dependency of the geminiviruses on the host machinery for their replications makes them unique to induce changes in the expression of genes associated with cell cycle regulation and DNA replication (Ascencio-Ibanez et al., 2008). Geminiviruses activate genes required for transition into S phase to establish DNA replication competent environment in mature cells (Desvoyes et al., 2006; Egelkrout et al., 2001, 2002). Geminiviruses-encoded proteins are thought to interact with a host protein, the retinoblastoma-related protein (pRBR), to induce transcription of genes encoding host replicative enzymes (Egelkrout et al., 2001, 2002; Kong et al., 2000). Cabbage leaf curl virus (CaLCuV) altered expression of cell cycle-associated genes, preferentially activating genes expressed during S and G2 and inhibiting genes active in G1 and M in *Arabidopsis thaliana* (Ascencio-Ibanez et al., 2008). Geminiviruses could also influence host gene expression by altering signal transduction pathways through interactions with host protein kinases (Ascencio-Ibanez et al., 2008). CaLCuV triggered pathogen response *via* the salicylic acid pathway and induced expression of genes involved in programmed cell death, genotoxic stress, and DNA repair (Ascencio-Ibanez et al., 2008). Naqvi et al. (2011) found induction of 20 tomato ESTs belonged to the class of genes that play crucial roles in innate immunity, plant metabolism and ethylene signalling after infection with ToLCNDV. Differential expression analyses of the pepper leaf transcriptome from symptomatic and recovered stages of pepper golden mosaic virus (PepGMV) infection revealed a total of 309 differentially expressed genes between healthy (mock) and symptomatic or recovered tissues. A set of genes with novel roles in defense responses was identified in pepper, including genes involved in histone modification indicating that post-transcriptional and transcriptional gene silencing may be one of the major mechanisms involved in the recovery process (Góngora-Castillo et al., 2012). A total of 2206 genes were up-regulated and 1398 genes were down-regulated in tomato yellow leaf curl Sardinia virus (TYLCSV) infected tomato plants, with an over representation of genes involved in hormone metabolism and responses, nucleic acid metabolism, regulation of transcription, ubiquitin-proteasome pathway, autophagy, primary and secondary metabolism, phosphorylation, transcription and methylation-dependent chromatin silencing (Miozzi et al., 2014). Genes encoding for WRKY transcriptional factors, R genes, protein kinases and receptor (-like) kinases were down-regulated in the susceptible line while these genes were up-regulated or not differentially expressed in the resistant line upon infection with tomato yellow leaf curl virus (TYLCV) (Chen et al., 2013). Many R genes have been known to be responsible for the indirect recognition of viruses. Recent development in sequencing technology has facilitated the identification of resistant genes, particularly in the *Solanaceae* family (Zouine et al., 2012).

Genetic resistance source for ToLCNDV in potato is not identified so far. However, lowest seed degeneration was observed in Kufri Bahar (4.5% yield reduction) even under a high whitefly vector population pressure and with repeated use of the same seed stock, while other varieties showed faster degeneration under field conditions (Lakra, 2003). The mechanism behind the resistance is not clearly understood. Hence, a study was carried out to identify the genes that are differentially regulated during ToLCNDV-[potato] infection in Kufri Bahar and in a susceptible cultivar Kufri Pukhraj using microarray. To our knowledge, our research represents the first microarray study of the transcriptional responses induced by a begomovirus in potato.

2. Materials and methods

2.1. Plant materials and inoculations

A resistant potato cultivar Kufri Bahar, a cultivar Kufri Pukhraj susceptible to ToLCNDV-[potato] and pure culture of the virus maintained in infected potato plants in insect proof glass house were used in the study. Two weeks old plants were graft inoculated with a twig from infected plants and un-grafted plants served as controls. Three replicates were maintained for each treatment and observed periodically for symptom expression. Leaf samples were collected randomly from top and middle part of the plants at 15, 20, 25, and 30 days after inoculation (DAI) and were immediately frozen in liquid nitrogen and stored at −80 °C. Microarray analysis was carried out with four composite leaf samples collected at 15 DAI; Kufri Bahar control (KBC), Kufri Bahar inoculated (KBI), Kufri Pukhraj control (KPC) and Kufri Pukhraj inoculated (KPI). Three independent biological replicates were pooled to get one composite sample. Three technical replicates of each sample were used in microarray analysis.

2.2. Total DNA extraction and viral DNA quantification

Total DNA was extracted from leaf samples of inoculated and un-inoculated Kufri Bahar and Kufri Pukhraj collected at 15, 20, 25, and 30 DAI using GenElute™ Plant Genomic DNA Miniprep kit (Sigma-Aldrich, Missouri, USA) following the manufacturer's instructions and the concentration and quality of DNA was checked using a Nanodrop 2000 spectrophotometer (Thermoscientific, Leon-Rot, Germany). Real time PCR assay was carried out to quantify the viral load using coat protein gene specific primers (LCV-CPFP- 5′-ACCGTCGTCCTACAGGATCTC-3′ and LCV-CPRP- 5′-GCTCGGTTCATTGTCAAA CATGT- 3′) and increase in viral load was compared between susceptible and resistant potato cultivars by plotting C_T values at different days of post inoculation. Absolute quantification of the viral load was performed using serially diluted (10^1 to 10^9 copies) plasmid carrying coat protein gene of ToLCNDV-[potato]. Leaf samples collected from top and middle parts of the plants of three biological replicates were pooled and used in real-time PCR assay. Three technical replicates were maintained for each sample.

2.3. Oligonucleotide microarray construction

Potato genome available (PGSC DM assembly version 3) at Potato Genome Sequencing Consortium (PGSC) database (http://potatogenomics.plantbiology.msu.edu/) was used to construct 60-mer oligonucleotide microarray chips by Roche NimbleGen Inc., Madison, USA using a multi-step approach to select probes with optimal predicted hybridization characteristics. Three oligonucleotide probes were selected per UniGene, comprising a probe set, and each probe set is represented on the final array by two replicates. The arrays were of 12 × 135 K format, which contained 12 arrays on a single slide (25 mm × 76 mm) and each array with 135,000 probe features targeting 39,031 genes.

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