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MicroRNAs as biomarkers in Tomato Leaf Curl Virus (ToLCV) disease

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ABSTRACT

MicroRNAs are ~21- 25 nt long RNA species that are critical regulators of transcriptome across the eukaryotes. Growing number of evidences clearly supports their involvement in plant leaf development. ToLCV infection severely affects the morphology of mature tomato leaves. To investigate the mechanism underlying the virus- host interaction, we focussed our studies on expression of microRNAs and their respective targets under normal and ToLCV infection. We have cloned Myb33, ARF4 homolog, Argonaute1, Apetala2, SBP transcription factor and RBOH from tomato and checked their expression by RT-PCR. Our work suggests that miR159 is upregulated while miR164 and miR171 are downregulated under viral infection. Our studies shed light on the impact of ToLCV infection on host transcriptome.

INTRODUCTION

MicroRNAs are a class of single stranded, endogenously expressed small RNA that are 21-24 nt long. They bind to and cleave targets bearing complementarity or suppress their translation (1, 2). These regulatory molecules are attributed to govern plethora of biological processes in both plants and animals (3, 4, 5, 6). Recent evidences support their role in conferring bacterial (7) and viral resistance (8). Interestingly, in plants, artificial miRNAs have been demonstrated to dictate leaf development and provide viral resistance (9, 10).

Tomato Yellow Leaf Curl Virus (TYCLV) is a common pathogen of tomato that contributes to the crops destruction worldwide.

Many mutants exhibits leaf deformation and could be a candidate to study the mechanism behind viral mediated leaf curling. We searched for such genes in tomato and found DR12 (a ARF4 homolog) (11) and Respiratory Burst Oxidase Homolog (RBOH) (12) mutants displaying severe leaf curl phenotypes. Although number of studies on leaf mutants exists but these are under permanent genetic backgrounds. How ToLCV temporarily effect leaf morphology remained largely unknown. The present research is aimed to study the interference by pathogen with miRNA-mediated regulation of target mRNAs, which have potential roles in defensive and developmental processes in plants.

RESULTS AND DISCUSSION

1. We have cloned Myb33, ARF4 homolog, Argonaute1, Apetala2, SBP transcription factor and RBOH genes from tomato (Pusa Ruby) that are known to affect the leaf or flower development.
2. The Reverse-transcription PCR clearly reveals that upon viral infection, the expression level of the above mentioned genes get markedly altered. The northern analysis of few genes further confirms the variation of expression under infection.
3. MicroRNA northern analysis with probe against miR159, miR164 and miR171 indicates that ToLCV infection indeed lead to deregulation of miRNA levels. However, the LA1777 which itself has abnormal leaf size, shows different pattern in the northern analysis against miR164. This suggests that miR159 or miR171 can be used effectively as biomarkers under ToLCV infection.

Figures:

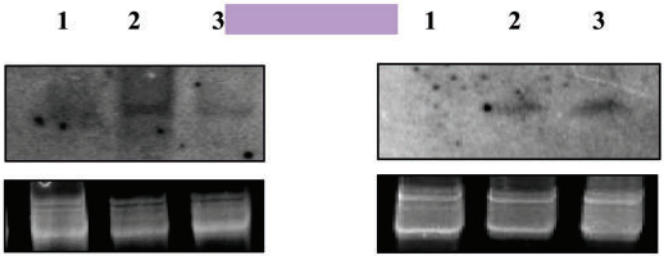

Fig. A. Northern analysis of miR159 in (1)Healthy, (2) ToLCV infected and (3) LA1777 leaves. Similar experiment was performed with probe against miR159 in (1) Healthy, (2) Agro-inoculated viral

genome and (3) Natural ToLCV infected tomato leaves. Lower panel shows the EtBr stained loading controls.

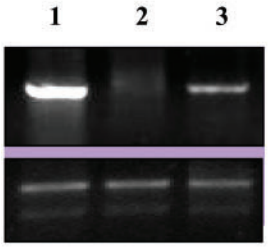

Fig. B. Reverse-Transcription PCR for Myb33 homolg in tomato using gene specific primers in (1)Healthy, (2) ToLCV infected and (3) LA1777 tomato leaves. Lower panel shows the actin control.

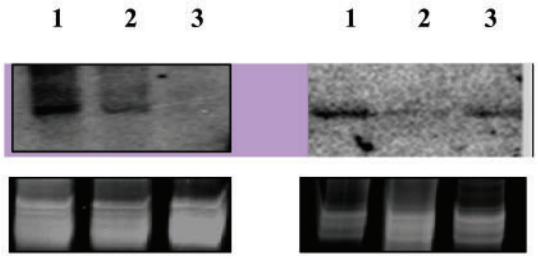

Fig. C. Northern analysis of miR164 and miR171 in (1)Healthy, (2) ToLCV infected and (3) LA1777 tomato leaves. Lower panel shows the EtBr stained loading controls.

CONCLUSION

The studies conducted indicate that miR159, miR164 and miR171 are altered during viral infection, however miR159 and miR171 could be potential biomarker against ToLCV infection. The analysis of RT-PCR and northern hybridization data together suggests that ToLCV infection in tomato provides us a better system to study the roles of miRNA in leaf development. The work done provides us a glimpse of the intricacy of host- virus arms race during infection establishment.

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