Mycopath (2019) 17(2): 55-58 **Research Article**

Effect of *Serratia marcescens* on induction of resistance in tomato against *Tomato leaf curl Palampur Virus*

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Abstract

Tomato leaf curl Palampur virus (ToLCPMV) is a bipartite begomovirus, which causes losses to several crops. In this research, we evaluated the effect of biofertilizer Phosphozist containing *Serratia marcescens*, on tomato resistance to ToLCPMV. Expression of *PR5* and *PR13* genes were evaluated using real-time PCR technique. Phosphozist-treated plants had significantly lower disease severity than the control. The control rate of ToLCPMV was 32.67%. The level of expression of *PR5* and *PR13* genes was significantly increased by biofertilizer Phosphozist. The results of this research indicated that *S. marcescens* reduced the severity of ToLCPMV disease and increased the expression of *PR5* and *PR13* genes in tomato plants and this reduction of the disease severity by *S. marcescens* was probably due to induction of resistance genes.

Keywords: Induced resistance, *PR5*, *Serratia marcescens*, *Tomato leaf curl Palampur virus*.

Introduction

Begomovirus consists of more than 320 species (Zerbini *et al*., 2017). *Tomato leaf curl Palampur virus* (ToLCPMV) is a bipartite begomovirus (Malik *et al*., 2011). ToLCPMV has been reported in India for the first time and the virus can emerge as one of the most serious begomovirus problems in future (Kumar, 2011). The virus has infected several crops in Iran (Heydarnejad *et al*., 2013; Abkhoo and Mehraban, 2020). Cucumber cultivar Kian showed a minimum infection and in comparison to other greenhouse cucumber cultivars, it is relatively resistant to ToLCPMV (Sabouri and Heydarnejad, 2013).

Previous studies have shown that *Serratia marcescens* elicited resistance against *Cucumber mosaic virus* (CMV) (Raupach *et al*., 1996; Ryu *et al*., 2004; Ryu *et al*., 2013). Pathogenesis-related (PR) proteins have been described in many plant species upon infection with fungi, bacteria, or viruses (Loon *et al*., 2006). *PR5* and *PR13* genes elicit systemic resistance in plants against pathogens (Padmanabhan *et al*., 2019; Rayapuram *et al*., 2008). Therefore, the objectives of this research were to investigate the effect of *S. marcescens* on tomato resistance to ToLCPMV through assessing level of resistance-related genes *PR5* and *PR13*.

Materials and Methods

Plants and biofertilizer

Solanum lycopersicum "Pardis F1" plants were used. The biofertilizer Phosphozist® containing *S. marcescens* was provided by Keshtkar gostar Nojan company.

Inoculation

The biofertilizer Phosphozist was diluted in sterile water according to prescribed and applied as drenching (50 mL plant^{-1}). Weekly drench application was provided (Beris *et al*., 2018). The first application was done in tomato plants at the three leaf stage. Virus inoculation was done one day after the second biofertilizer Phosphozist application (Beris *et al*., 2018) with infectious clones (Kumar, 2009) according to the described Gresimli *et al*. (1986) method. Plants treated with water served as control (16 plants per treatment).

Assessment of viral disease severity

Infected tomato plants were scored on a 1–5 scale as described by Sohrab *et al*. (2013) with slight modifications: 1 = no symptom, 2 = yellow dots, 3 = yellow dots, mosaic and slight curling, 4 = yellowing and leaf curling, and 5 = leaf curling and dwarf plants.

Disease index (DI) was calculated by using the following formula (Sun *et al*., 2016):

DI (%) = (∑(scale no. × no. of plants at corresponding disease scale) ×100/(highest scale × no. of total tested plants).

Control rate (%) was determined by the following formula (Guo *et al*., 2019):

[(Disease index of control - Disease index of biofertilizer Phosphozist treatment) / (Disease index of control)] × 100%.

Analysis of resistance-related gene expression

Gene expression was examined at 24 and 48 hours post viral inoculation. Total RNA was isolated from leaves by RNA extraction Ribospin™ plant (GeneAll Biotechnology, Seoul, Korea), and cDNA was synthesized using ExcelRT™ Reverse Transcription Kit (SMOBIO, Taiwan). The quantitative RT-PCR was performed in a Rotor-Gene

3000 (PCR program: 15 min at 95 °C, 40 cycles; 20 s at 95 °C, 20 s at 59 °C and 30 s at 72 °C). Each treatment had three biological replicates and two technical replicates. Primer sequences used in the present research are shown in Table 1. *Ubiquitin* (*UBI3*) was used as housekeeping gene (Rotenberg *et al.*, 2006). The expression levels of *PR5* and *PR13* were calculated using the REST software.

Statistical analysis

Statistical analyses were done using MSTAT-C package (v.1.42). Group means were compared using *t-test* ($P \leq 0.05$).

Results and Discussion

Phosphozist-treated plants had significantly lower disease severity than the controls (Fig. 1). The control rate of ToLCPMV was 32.67%.

Melting curve analysis and expression of genes shown in Fig. 2 and 3. The results showed significant differences between the expression of *PR5* gene in the leaves of infected Phosphozist-treated plants and controls at 48 hours after pathogen inoculation. Treatment with biofertilizer Phosphozist increased the level of *PR5* gene expression at 48 hours after inoculation. The results showed significant differences between the expression of *PR13* gene in the leaves of infected Phosphozist-treated plants and controls at 24 and 48 hours after treatment. Treatment with biofertilizer Phosphozist increased the level of *PR13* gene expression at 24 and 48 hours after pathogen inoculation.

In this study, the biofertilizer Phosphozist increased expression of *PR5* and *PR13* genes. Interestingly, *PR5* overexpressed plants conferred enhanced resistance, resulting in delay in accumulation of *Tomato spotted wilt tospovirus* and symptom expression (Padmanabhan *et al*., 2019). Rayapuram *et al*. (2008) reported that *PR-13* mediates bacterial resistance in *Nicotiana attenuata* in nature. Similarly, it was reported that treatment of cucumber seeds with *Pseudomonas fluorescens* strain 89B-27 and *S. marcescens* strain 90-166 significantly managed diseases (Raupach *et al*., 1996). *Arabidopsis thaliana* treated with *Bacillus pumilus* strain SE34 and *S. marcescens* strain 90-166 had significantly reduced symptom severity by CMV (Ryu *et al*., 2004). Ryu *et al*. (2013) studied the role of quorum sensing (QS) in the induced systemic resistance elicited by *S. marcescens* strain 90-166, in tobacco.

In conclusion, the results of this research indicated that *S. marcescens* reduces the severity of ToLCPMV disease and increases the expression of *PR5* and *PR13* genes in tomato plants and this reduction of the disease severity by *S. marcescens* was probably due to induction of resistance genes.

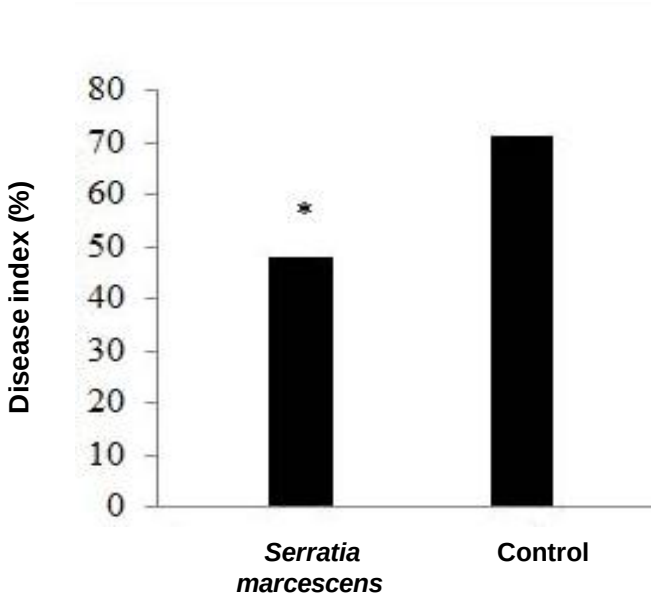

Fig. 1: Effect of biofertilizer phosphozist on disease severity of infected tomato with ToLCPMV (21 days after inoculation). *Differ significantly from control at $P \leq 0.05$.

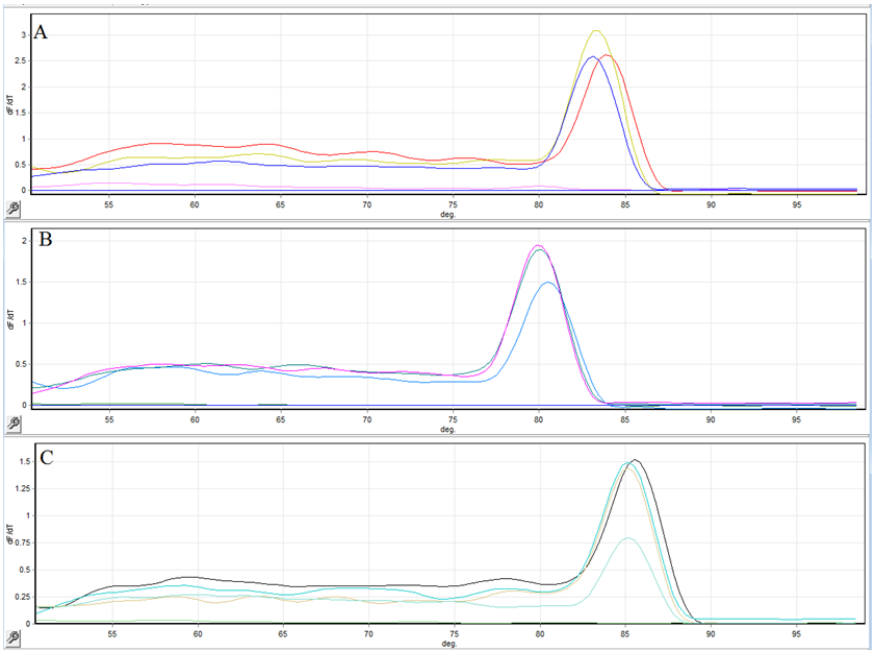

Fig. 2: Analysis of the melting curve for *PR5* (A), *PR13* (B) and *ubiquitin* (*UBI3*) (C) genes. Each peak represents the melting temperature of a PCR product.

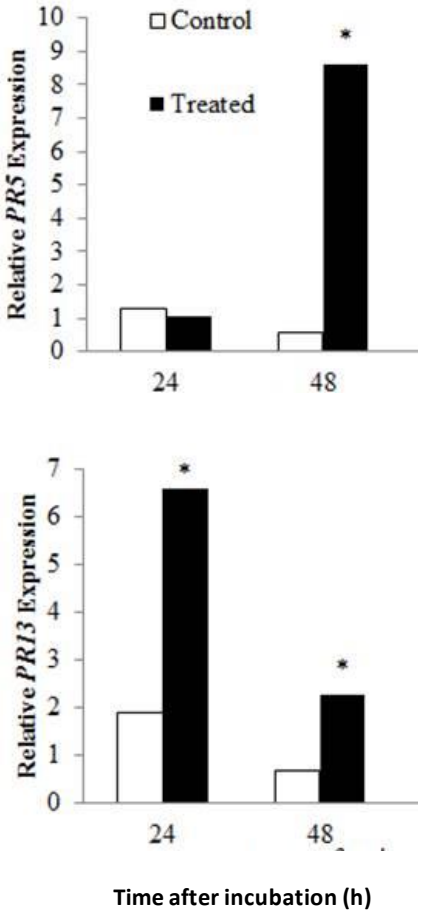

Fig. 3: Level of mRNA expression of *PR5* and *PR13* in Phosphozist-treated and non-treated (control) tomato plants after inoculation of *Tomato leaf curl Palampur virus* (ToLCPMV). Each sample is normalized for the amount of the template to the levels of *ubiquitin*. *$P \leq 0.05$ compared with control group (ToLCPMV alone).

References

Abkhoo J, Mehraban A, 2020. Genetic diversity of of *Tomato leaf curl Palampur virus* and its whitefly vector, *Bemisia tabaci*, in the Sistan region. *J. Microbe. World*, (In press).

Beris D, Theologidis I, Skandalis N, Vassilakos N, 2018. *Bacillus amyloliquefaciens* strain MBI600 induces salicylic acid dependent resistance in tomato plants against *Tomato spotted wilt virus* and *Potato virus Y. Sci. Rep*., **8**: 10320.

Heydarnejad J, Hesari M, Massumi H, Varsani A, 2013. Incidence and natural hosts of *Tomato leaf curl Palampur virus* in Iran. *Austral. Plant Pathol.*, **42**: 195-203.

Grimsley N, Hohn B, Hohn T, Walden R, 1986. Agroinfection, an alternative route for viral-infection of plants by using the Ti plasmid. *Proc. Natl. Acad. Sci*. *USA*, **83**: 3282-3286.

Guo Q, Li Y, Lou Y, Shi M, Jiang Y, Zhou J, Sun Y, Xue Q, Lai H, 2019. *Bacillus amyloliquefaciens* Ba13 induces plant systemic resistance and improves rhizosphere microecology against tomato yellow leaf curl virus disease. *Appl. Soil Ecol*., **137**: 154-166.

Kumar Y. 2011. Genome organization and mechanism of RNA silencing suppression of begomoviruses infecting some solanaceous crops. Ph.D. Thesis Institute of Himalayan Bioresource Technology.

Loon LC, Rep M, Pieterse CMJ, 2006. Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol*., **44**: 135-162.

Malik AH, Briddon RW, Mansoor S. 2011. Infectious clones of *Tomato leaf curl Palampur virus* with a defective DNA B and their pseudo-recombination with *Tomato leaf curl New Delhi virus*. *Virol. J*., **8**: 173.

Rotenberg D, Thompson TS, German TL, Willis DK, 2006. Methods for effective real-time RT-PCR analysis of virus-induced gene silencing. *J. Virol. Methods*, **138**: 49-59.

Ryu ChM, Murphy JF, Mysore KS, Kloepper JW, 2004. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against *Cucumber mosaic virus* by a salicylic acid and *NPR1*-independent and jasmonic acid-dependent signaling pathway. *Plant J*., **39**: 381-392.

Ryu CM, Choi HK, Lee CH, Murphy JF, Lee JK, Kloepper JW. 2013. Modulation of quorum sensing in acylhomoserine lactone-producing or degrading tobacco throughout plants leads to alteration of induced systemic resistance elicited by the rhizobacterium *Serratia marcescens* 90-166. *Plant Pathol. J*., **29**: 182-192.

Padmanabhan C, Ma Q, Shekasteband R, Stewart KS, Hutton SF, Scott JW, Fei Z, Kai-Shu Ling KS, 2019. Comprehensive transcriptome analysis and functional characterization of PR-5 for its involvement in tomato Sw-7 resistance to tomato spotted wilt tospovirus. *Sci. Rep*., **9**: 7673.

Rayapuram C, Wu J, Haas C, Baldwin IT, 2008. *PR-13*/Thionin but not *PR-1* mediates bacterial resistance in *Nicotiana attenuata* in nature, and neither influences herbivore resistance. *Mol. Plant Microbe Interact*., **21**: 988-1000.

Raupach GS, Liu L, Murphy JF, Tuzun S, Kloepper JW. 1996. Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth promoting rhizobacteria (PGPR). *Plant Dis*., **80**: 891-894.

Sabouri M, Heydarnejad J, 2013. Evaluation of the reaction of greenhouse cucumber cultivars inoculated by the infectious clone of *Tomato leaf curl Palampur virus*. *J. Agric. Biotechnol*., **5**: 83-96.

Sohrab SS, Karim S, Varma A, Abuzenadah AM, Chaudhary AG, Damanhouri GA, Mandal B, 2013 Characterization of *Tomato Leaf Curl New Delhi Virus* infecting cucurbits: Evidence for sap transmission in a host specific manner. *Afr. J. Biotechnol*., **12**: 5000-5009.

Someya N, Nakajima M,Watanabe K,Hibi T, Akutsu K, 2005. Potential of *Serratia marcescens* strain B2 for biological control of rice sheath blight. *Biocont. Sci. Technol*., **15**: 105-109.

Sun WJ, Lv WJ, Li LN, Yin G, Hang X, Xue Y, Chen J, Shi Z, 2016. Eugenol confers resistance to *Tomato yellow leaf curl virus* (TYLCV) by regulating the expression of *SlPer1* in tomato plants. *New Biotechnol*., **33**: 345-354.

Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E, Navas-Castillo J, Rivera-Bustamante R, Roumagnac P, Varsani A, 2017. ICTV virus taxonomy profile: Geminiviridae. *J. Gen. Virol*., **98**: 131-133.