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Research Article

Application of plant growth-promoting rhizobacteria to protect bell pepper against *Tobacco mosaic virus*

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Abstract: *Tobacco mosaic virus* (TMV) is one of the economically important plant viruses which causes disease in various crops throughout the world. It has been reported that plant growth-promoting rhizobacteria (PGPR) can be used as potential biocontrol agents against plant viruses. Herein, greenhouse experiments were conducted to undertake the trilateral interactions among PGPR, bell pepper, and TMV. To this end, four-leaf-stage bell pepper seedlings were pre-treated by the PGPR, including *Pseudomonas fluorescens*, *P. putida*, and *Bacillus subtilis* in single and multiple application methods. The plants were then mechanically inoculated with TMV and visually inspected for symptom development till 28 days post-inoculation (dpi). The TMV accumulation in inoculated plants was quantitatively measured by Indirect-ELISA 28 dpi. Analysis of the extinction values showed that application of the PGPR was associated with the least significant ($p < 0.05$) value (0.08) compared to the positive control (0.77). Inoculation of PGPR triggered the biosynthesis of the defense-related enzymes such as catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase, mediating the biochemical protection against TMV in bell pepper plants. In addition to the disease control, a significant ($p < 0.05$) increase in growth parameters was observed in PGPR-treated plants compared to the control plants. In conclusion, these results indicated that multiple applications of PGPR strains enhanced the plant vigor and provided an increased level of TMV suppression in bell pepper plants.

Keywords: PGPR, Indirect-ELISA, virus accumulation, enzyme

Introduction

Bell pepper *Capsicum annum* L. is an important species commercially cultivated worldwide (Gniffke *et al*., 2013). *Tobacco mosaic virus* (TMV) is a mechanically-transmitted plant virus classified in the genus *Tobamovirus*, family *Virgaviridae*, affecting peppers worldwide (Moury and Verdin, 2012). Susceptibility to virus infection is one of the most significant problems in cultivated peppers, and no effective or efficient chemical solutions have been found yet (Sofy *et al*., 2014). Therefore, exploration for plant viral disease management by induction of plant's natural defenses seems necessary. In recent years, more attention has been focused on providing plant growth-promoting rhizobacteria (PGPR) as one of the promising approaches in controlling plant viruses under greenhouse and field

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conditions (Maksimov *et al*., 2019; Palukaitis *et al.*, 2017). These bacteria can reduce the virus concentration within plant cells and deteriorate viral disease symptoms in plants (Maksimov *et al.*, 2019). One of the most effective mechanisms of PGPR is the capacity to cause induced systemic resistance (ISR) against plant diseases (Raupach *et al*., 1996; Beris *et al*., 2018). Similarly, several studies have revealed that non-pathogenic rhizobacteria can stimulate plant defense enzymes when applied to the roots (Chen *et al*., 2000; Beneduzi *et al*., 2012; Konappa *et al.*, 2020). Suryadi *et al*. (2019) showed a successful reduction of the disease severity by PGPR, namely, *Bacillus subtilis* and *Pseudomonas fluorescens. Bacillus amyloliquefaciens* has been shown to interact with its plant host by inducing systemic resistance and acts as a biological control agent against *Tomato spotted wilt virus* (TSWV) and *Potato virus Y* (PVY) (Beris *et al*., 2018; Dimopoulou *et al.*, 2019). Several studies have found that PGPR strains as bio-elicitors protect against viruses; however, the efficacy of some PGPR strains varies. These variations have necessitated more investigations evaluating the effect of multiple applications of PGPR strains on host resistance against biotic challenges. It has been demonstrated that various applications of several PGPR strains can lead to more significant disease suppression than a single application of PGPR (Abdalla *et al*., 2017; Jetiyanon and Kloepper, 2002). In tomato plants, multiple applications of *P. fluorescens*, *P. putida*, and *B. subtilis* resulted in the reduced accumulation of TMV in leaves compared to the control (Aeini *et al*., 2018).

Management strategies to control plant viruses in Iran are mainly limited to controlling insect vectors and using resistant cultivars. However, using chemicals as the most effective control measure of insect vectors has led to environmental damage. Moreover, resistant cultivars are not always available and, if available, may not completely show resistance to virulent strains of the viruses. To date, far too little attention has been paid to managing viral diseases using PGPR. Therefore, these considerations have motivated us to 1) evaluate the ability of the individual PGPR strains and their combination to promote growth and reduce disease severity, 2) assess the induction of defense-related enzymes by PGPR against TMV in bell pepper.

Materials and Methods

PGPR strains, compatibility assay, and inoculum preparation

The three wild-type strains of PGPR were supplied by the Microbiology Lab of the Faculty of Agriculture at Shahid Chamran University of Ahvaz, Khuzestan, Iran. PGPR strains used in this study included *P. fluorescens* (Sc21), *P. putida* (Sc42), and *B. subtilis* (Sc13). These strains had been shown to induce systemic protection in tomatoes against TMV (Aeini *et al*., 2018). Compatibility test among bacterial isolates was carried out according to the method described by Fukui *et al.* (1994). To this end, each PGPR strain was first stored at 80 °C in tryptic soy broth (TSB) (Difco Laboratories, US) amended with 20% autoclaved glycerol. The strains were removed from ultra-cold storage, streaked onto tryptic soy agar (TSA) (Difco Laboratories, US), and incubated at 28 °C for 24 h to check their purity. After transferring single colonies, bacteria were cultured on nutrient agar (NA) (Difco Laboratories, US) medium and incubated at 27 °C for 48 h. The plates were then scraped off into sterilized distilled water. The concentration of bacterial suspensions was adjusted to 10^8 CFU/µl by measuring the optical density (OD) of each bacterial suspension at 600 nm wavelength (OD_{600}).

Experimental set up for the virus challenge and the PGPR treatment

The experiments were conducted in a temperature-controlled greenhouse to evaluate PGPR to protect bell pepper plants against TMV. Each biopreparation was mixed into a soilless growth medium (Speedling, US) at a ratio of 1:40 (vol/vol). Bell pepper seeds were sown into the amended soilless growth medium and maintained in the greenhouse. The greenhouse's ambient air temperature was 25 and 20 °C (day and night, respectively).

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Irrigation was carried out daily without any fertilization. In this experiment, nine treatments were included: three treatments consisted of single PGPR strains, three treatments consisted of two-strain mixtures, one treatment consisted of three-strain mixtures, absolute control (non-treated healthy control), and non-treated TMV-challenged control. The PGPR mixtures were prepared by combining equal proportions of each strain before application. Each seedling was transplanted to a 2-liter pot containing soilless potting medium Pro-Mix (Premier Peat, Canada) 21 days post-germination. The PGPR treatments were applied twice at one-week intervals. The experiment was repeated two times. The experiment was arranged in a completely randomized design with two plants per treatment and four replications.

TMV inoculum

An Iran-originated isolate (TMV-PU1), previously characterized by Alishiri *et al.* (2013), was used for inoculation. The virus was propagated on tobacco plants (*Nicotiana tabacum* var. Turkish). Then, a symptomatic leaf of TMV-infected tobacco plant was used as inoculum for the mechanical inoculation of bell pepper plants. To this end, the leaf tissue was homogenized into 0.01 M KH_2PO_4 solution, and the resulting extract was gently rubbed on carborundum-dusted leaves of bell pepper seedlings at the four-leaf stage. The inoculated plants were kept under greenhouse conditions for symptom development till 28 days post-inoculation (dpi).

Evaluation of plant growth characteristics and the disease assessment

Plant growth characteristics for bio preparation and control treatments were evaluated. The disease severity of TMV was determined 14 and 28 dpi using a 0-4 rating scale. Based on the symptoms developed on the plants, they were scored from 0 to 4 [0, no symptom; 1, mild mosaic; 2, mosaic and malformation; 3, severe mosaic and malformation; 4, death] (Zehnder *et al.*, 2000). The disease severity was calculated as follows: disease severity (%) = [Σ (the number of diseased plants in disease rating × disease rating)/(total number of plants investigated × highest disease rating)] × 100.

A chlorophyll meter SPAD-502DL (Konica Minolta Sensing Inc., Japan) was used to estimate the seeming chlorophyll content. Three SPAD readings per leaf (650/940 nm wavelength transmittance ratio) were taken at three points of each leaf, 15-35 mm from one side of the midrib (depending upon the leaf growth stage).

Twenty-eight days post-inoculation (dpi), the plants were harvested according to the procedure described by Radwan *et al*. (2007). Plants were carefully dislodged, taking special care not to sever the fine root hairs. The roots were washed entirely, and growth indices, including plant height and fresh weight, were measured. The plants were then placed in paper bags and kept in an oven for 2-3 days for dry weight determination.

Indirect- The TMV accumulation in foliar tissues was determined by Indirect- ELISA with the polyclonal antibody of TMV (BIOREBA, Switzerland) using the method of Clark and Adams (1977) at the end of experiments. The systemic leaf (fourth leaf from top) was harvested from each plant 28 dpi and subjected to Indirect-ELISA. TMV-infected tobacco *N. tabacum* var. Turkish sample was used as the positive control. Plant tissue of a healthy bell pepper plant was also used as the negative control. Three replications were considered for each type of control. ELISA plate was analyzed using Thermo Labsystems microplate reader (Thermo Scientific, Germany), and the OD was measured at the wavelength of 405 nm. The samples with an OD value more than $M + 3SD$ (M; mean OD value of negative controls, SD; mean standard deviation value of negative controls) were considered positive. As described below, four ELISA-positive samples were selected for further molecular assays as the representatives of their sampling sites.

Amplification of the viral genome

Total RNA was isolated from ELISA-positive samples using the RNA extraction kit (Denazist, Iran). The quality and quantity of extracted RNA were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA).

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Total RNA was then subjected to cDNA synthesis using QuantiTect Reverse Transcription Kit (QIAGEN, USA). The resulting cDNA was used as a template in polymerase chain reaction (PCR) assay using TMV-specific primer pair (TMV-spec: 5'-CGGTCAGTGCCGAACAAGAA-3' and Tob-Uni 1: 5'-ATTTAAGTGGASGGAAAAVC ACT-3'), which has been shown to amplify the CP gene (~694 bp) of the viral genome (Letschert *et al*., 2002). To this end, 25 µl reaction mixture containing 2 µl of cDNA, 0.5 µl of each primer (10 µM), 13 µl of RealQ Plus Master Mix Green (AMPLIQON, Denmark), and 9 µl of ddH_2O were prepared. The reaction mixture was pre-heated for 2 min at 96 °C and then subjected to a 35-cycle PCR program of 60 s at 96 °C, 30 s at 58 °C, 60 s at 72 °C, and a final extension phase at 72 °C for 10 min. Positive (TMV-infected tobacco samples) and negative (virus-free hibiscus samples) controls were used as two sets of the PCR control. Electrophoresis was then performed by running approximately 5 µl of the PCR products onto a 1.0% agarose gel with TAE buffer (40 mM Tris-acetic acid, 0.1 mM EDTA, pH 8.2-8.4 [at 25 °C]) previously stained with DNA Safe Stain (CINACLONE, Iran) and visualized under the UV light.

Plant defense-related enzyme assay

Catalase activity

Catalase (CAT) activity was assayed in plants 28 dpi according to the method described by (Chandlee & Scandalios, 1984). The assay mixture contained 2.6 µl of 50 mM/L potassium phosphate (KH_2PO_4) buffer (pH 7.0), 0.4 µl of 15 mM/L H_2O_2, and 0.04 µl of catalase enzyme extract. Changes in absorbance were read at 240 nm. The enzyme protein was estimated by the method of Bradford (1976) for all the enzymes. Three biological replicates with three technical replicates each were performed. Data were reported as the arithmetic mean ± standard deviation (SD).

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was determined according to the method of Beauchamp and Fridovich (1971). The reaction mixture containing 13 mM methionine, 2 mM riboflavin, 0.1 mM EDTA, and 75 µM nitro blue tetrazolium (NBT) salts was dissolved in 3 µl of 50 mM sodium phosphate (NaH_2PO_4) buffer (pH 7.8), and 100 µl of SOD enzyme extract was added. The mixtures were illuminated by Philips 40-W fluorescent tubes in triplicates, and absorbance was read at 560 nm in a spectrophotometer. The SOD activity is expressed in U/ mg protein (U = change in 0.1 absorbance h/mg protein under experimental conditions). Three biological replicates with three technical replicates each were performed. Data were reported as the arithmetic mean ± standard deviation (SD).

Ascorbate peroxidase activity

Ascorbate peroxidase (APX) activity was determined 28 dpi spectrophotometrically by recording the decrease in absorbance at 290 nm due to the ascorbate oxidation in 3 µl of the reaction mixture containing 50 mM phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM sodium ascorbate, 0.1 mM EDTA and a suitable amount of the enzyme extract. One unit of the APX activity was assumed as the amount of the enzyme which oxidized 1 µM ascorbate/min at 30 °C. Three biological replicates with three technical replicates each were performed. Data were reported as the arithmetic mean ± standard deviation (SD).

Peroxidase activity

Peroxidase activity was assessed in plants 28 dpi, according to Thomas *et al*. (1982). A solution comprising 0.2 µl of the enzyme extract, 5.8 µl of KH_2PO_4 buffer, and 2 µl of 20 mM H_2O_2 was used to evaluate peroxidase (POX). When 3 µl of 20 mM pyrogallol was added, the increase in the pyrogallol absorption was measured within 60 s at 470 nm and 25 °C using a UV spectrophotometer (Thomas *et al*., 1982). Three biological replicates with three technical replicates each were performed. Data were reported as the arithmetic mean ± standard deviation (SD).

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Data analysis

Data represent mean values ± SEM and statistical analysis was performed using SAS, version 9.2 (SAS Institute, USA) (Rohlf, 2002). A completely randomized design was applied for all experiments and any differences among the treatment means were detected using Duncan's Multiple Range Test (DMRT) at significant levels of $p = 0.05$.

Results

The TMV Detection

The absence of an inhibition zone indicated that the PGPR strains could be used together in multiple applications. The viral disease symptoms in control plants and PGPR-treated plants appeared seven dpi. Mechanically inoculated plants showed the typical symptoms of TMV infection, including leaf mosaic and malformation (data not shown). Indirect-ELISA results indicated the presence of TMV particles within the symptomatic plant samples (Fig. 1). The highest value of Indirect-ELISA extinction was observed in the TMV-challenged control sample. The remaining TMV-inoculated plants pre-treated with PGPRs, including *P. fluorescens* (Sc21), *P. putida* (Sc42), and *B. subtilis* (Sc13), showed a significant ($p \leq 0.05$) reduction in Indirect-ELISA extinction value, among which the plants pre-treated with the mixture of three PGPR strains (Sc21 + Sc42 + Sc13) exhibited the lowest value (Fig. 1). In the case of double applications, no significant difference was found among the extinction values in plants pre-treated with *P. putida* & *B. subtilis* (Sc42 + Sc13), *P. fluorescens* & *B. subtilis* (Sc21 + Sc13), and *P. fluorescens* & *P. putida* (Sc21 + Sc42). Similarly, a single application of Sc42 and Sc13 resulted in no significant difference between the extinction values. One treatment, Sc21, showed a significantly different value (Fig. 1).

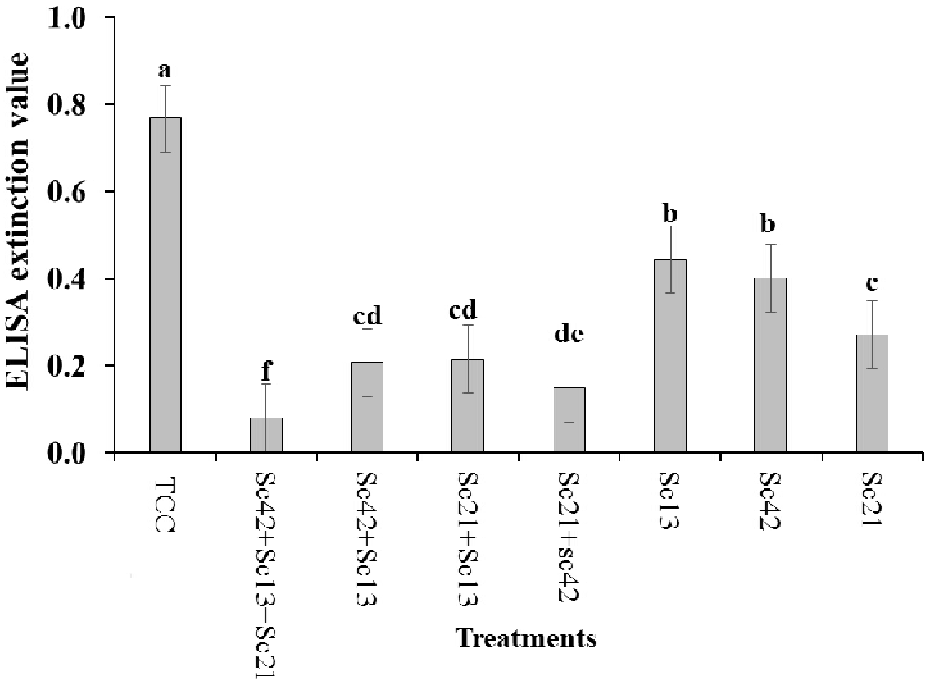

Figure 1 Indirect-ELISA extinction values from TMV-inoculated bell pepper plants treated with different rhizobacteria including *Pseudomonas fluorescens* (Sc21), *Pseudomonas putida* (Sc42) and *Bacillus subtilis* (Sc13) compared to TMV-challenged control (TCC) 28 dpi.

[DOR: 20.1001.1.22519041.2021.10.4.11.1]

PCR assays using TMV-specific primers (TMV-spec/Tob-Uni-1) resulted in amplifying viral DNA fragments of the expected size of ~694 bp (Fig. 2). These results confirmed the TMV infection of the inoculated plants.

Growth parameters and the disease severity

The results from chlorophyll measurement showed that the highest index belongs to the bell pepper plants pre-treated with Sc21 + Sc42 + Sc13 (Table 1). Growth parameters of the different treatments and control plants are shown in Table 1. According to the results, plants pre-treated with PGPR exhibited a significant ($p \leq 0.05$) increase in plant height, among which the mixture treatment (Sc21 + Sc42 + Sc13) resulted in the highest value compared to TMV-challenged control. No significant differences were observed for plant height, among other treatments.

Plant fresh weight of the mixture treatments was significantly more than control plants (Table 1). Bell pepper plants pre-treated with a mixture of PGPR (Sc21 + Sc42 + Sc13) showed a highly significant ($p \leq 0.05$) increase in fresh and dry weight of root and aerial parts in healthy and infected seedlings (Table 1). The data from disease severity measurement in TMV-inoculated plants 14 and 28 dpi are shown in Table 2. Accordingly, all PGPR-treated plants exhibit lower disease severity than TMV-challenged control (Table 2).

ROS scavenging enzymes

Applying a mixture of PGPR (Sc21 + Sc42 + Sc13) in the rhizosphere significantly induced an antioxidant defense system in bell pepper plant leaves challenged with TMV. Under TMV stress, PGPR-treated bell pepper plants exhibited a significant increase in CAT, SOD, APX, and POX compared to control plants (Fig. 3).

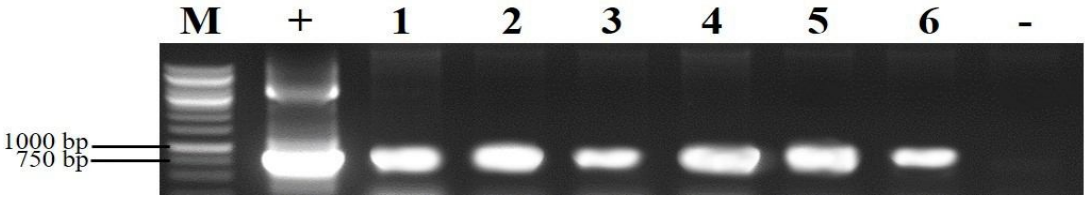

Figure 2 Electrophoresis pattern of RT-PCR products obtained using a TMV-specific primer pair (TMV-F/Tob-Uni-1) designed to amplify a ~694 bp fragment of the virus genome. (1), TMV-challenged tobacco (+, positive control); (2) 1-6, Indirect-ELISA-positive bell peppers; (3), absolute control (-, negative control); M, 1000 bp DNA ladder (Thermo Fisher Scientific, Germany).

Table 1 Mean comparison of optical density, chlorophyll index, and plant growth parameters in PGPR-treated bell pepper plants challenged with *Tobacco mosaic virus.*

Treatments	Absorbance value	Chlorophyll index	Plant Height (cm)	Fresh weight of root (g)	Fresh weight of aerial parts (g)	Dry weight of root (g)	Dry weight of aerial parts (g)
Sc21	0.27^{c}	34.03^{b}	27.30^{b}	1.39^{cd}	24.50^{cb}	0.49^{cb}	3.20^{cd}
Sc42	0.40^{b}	32.16^{ab}	29.13^{b}	2.42^{cbd}	24.30^{cb}	0.41^{c}	2.80^{cd}
Sc13	0.45^{b}	25.23^{b}	27.80^{b}	2.01^{cbd}	25.10^{cb}	0.50^{cb}	3.90^{bc}
Sc21 + Sc42	0.14^{de}	26.00^{b}	29.30^{b}	3.08^{cb}	23.00^{cb}	0.53^{cb}	3.50^{bc}
Sc13 + Sc21	0.21^{cd}	27.00^{b}	29.10^{b}	3.93^{cb}	21.30^{cb}	0.45^{c}	4.00^{bc}
Sc42 + Sc13	0.20^{cd}	28.16^{b}	28.10^{b}	3.80^{cb}	28.00^{ab}	0.48^{cb}	3.20^{dc}
Sc21 + Sc42 + Sc13	0.08^{f}	37.20^{a}	37.60^{a}	6.64^{a}	38.10^{a}	0.84^{a}	8.30^{a}
TMV-challenged control	0.76^{a}	17.50^{c}	21.45^{c}	0.94^{d}	14.90^{c}	0.37^{c}	1.60^{c}
Absolute control	0^{f}	37.00^{a}	29.90^{b}	3.23^{cb}	27.50^{ab}	0.72^{ab}	5.70^{b}
SEM	0.160	2.286	1.180	0.370	2.020	0.040	0.500
P-value	$p < 0.0001$	$p < 0.0002$	$p < 0.005$	$p < 0.003$	$p < 0.02$	$p < 0.0098$	$P < 0.0001$

Statistical comparisons are among treatments within a single column. The different letters indicate significant differences using the Duncan's test at $P = 0.05$.

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Table 2 Disease severity of *Tobacco mosaic virus* (TMV)-inoculated bell pepper plants treated with plant growth-promoting rhizobacteria strains in comparison with control plants 14 and 28 dpi.

Treatment	Disease severity	
	14 dpi	28 dpi
Sc21	66.30^{b}	66.66^{b}
Sc42	58.36^{bc}	55.60^{dc}
Sc13	57.33^{cb}	58.60^{cbd}
Sc21 + Sc42	52.30^{c}	50.60^{d}
Sc13 + Sc21	55.60^{c}	54.00^{dc}
Sc42 + Sc13	59.00^{cb}	60.33^{bc}
Sc21 + Sc42 + Sc13	32.00^{d}	31.00^{e}
TMV-challenged control (Positive control)	78.66^{a}	82.66^{a}
Absolute control (healthy plant)	0^{e}	0^{f}
SEM	1.70	1.67
P-treat	$p < 0.0001$	$p < 0.0001$

Means were compared based on Duncan test at $p = 0.05$. Values within a column followed by the same letter(s) do not differ at $p = 0.05$ level.

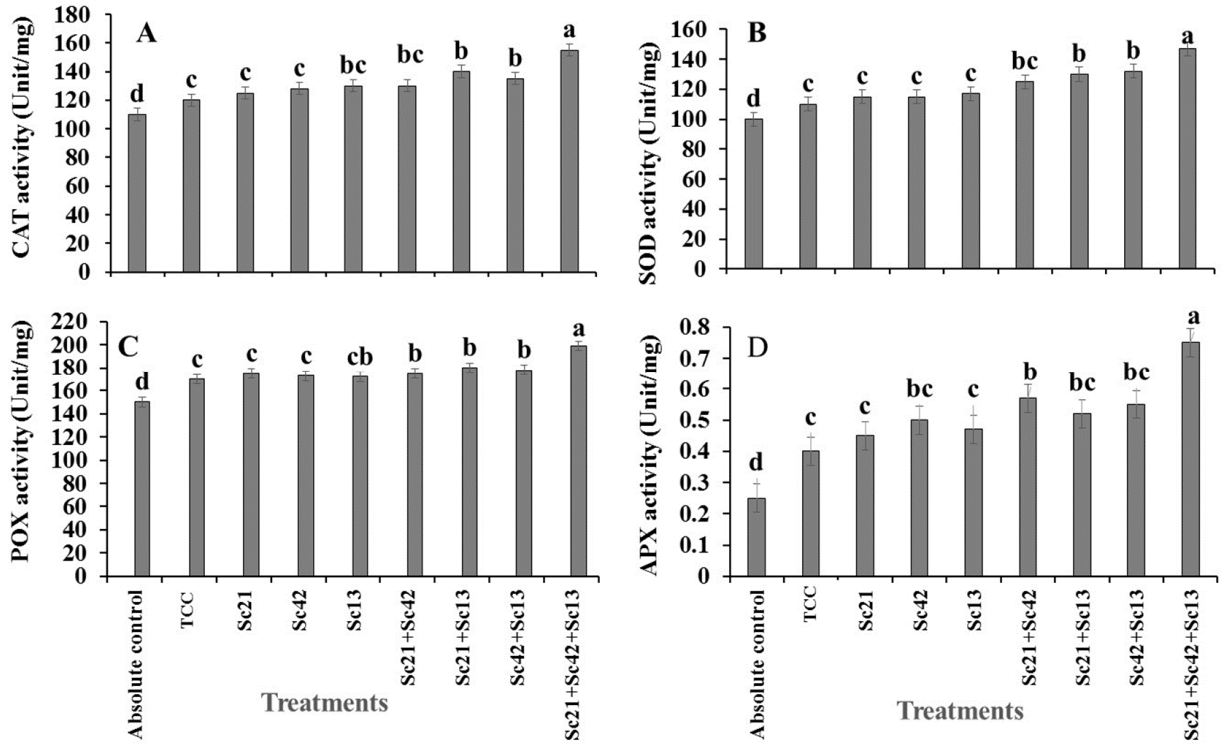

Figure 3 Effect of the rhizobacterial treatment on (A) catalase (CAT), (B) peroxidase (Pox) (C) superoxide dismutase (SOD) and (D) ascorbate peroxidase (APX), activity in the leaf fraction of the bell pepper under absolute control and TMV-challenged control (TCC). Bell pepper seedlings were treated with individual rhizobacterial isolates, and the antioxidative defense enzymes were measured in the leaf lysate preparation 28 dpi. According to the Duncan test, the different letters (a, b, c, d) are significantly different at 0.05%.

Discussion

This study was set out to evaluate PGPR to induce defense-related enzymes and suppression of TMV within bell pepper plants. In plant-virus interactions, the chloroplast is a common target for viral pathogenesis/ propagation, and photosynthesis-related factors

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play an essential role in developing disease symptoms (Li *et al*., 2016). The virus-induced disruption of chloroplasts' structure and function has been suggested to be associated with typical photosynthesis-related symptoms (Zhao *et al*., 2016). It has been shown that a decline in photosynthetic pigments of the tomato plant (Vitti *et al*., 2016) and cucumber plant (Sofy *et al*., 2020) occurs upon the viral infection. Bell pepper plants treated with the mixture of PGPR showed a significant increase in photosynthetic pigments, which might enhance the host's tolerance to the disease caused by TMV. Taken together, vegetative growth in PGPR-treated plants was significantly more than the virus-infected control. Plants treated with the mixture of PGPR (Sc21 + Sc42 + Sc13) had significantly lower disease severity scores compared to other PGPR-treated plants. These results suggest that the mixture of PGPR isolates can reduce the virus infection by reducing TMV disease symptoms and severity. This finding agrees with Murphy's *et al*. (2003) and Dashti's *et al*. (2012) results which showed a significant increase in the vegetative growth and decrease in the disease severity in CMV-inoculated tomato plants treated with the PGPR mixture. The enhanced growth parameters due to the presence of PGPR are considered as an advantage for the enhanced protective effect (Dashti *et al.,* 2012). As mentioned before, the simultaneous application of PGPR strains can reduce the damages due to the viral infection more than a single application. The results obtained in this study corroborate the findings of a great deal of the previous work in which combination of *Streptomyces fradiae* MML1042, *Bacillus licheniformis* MML2501 + *Pseudomonas aeruginosa* MML2212 + *Bacillus* sp. MML2551 contributed significantly to protecting the sunflower plants from sunflower necrosis virus disease (SNVD) and increased seed germination, plant height, and yield parameters (Srinivasan and Mathivanan 2009; Yu *et al*., 2019). Also, Srinivasan and Mathivanan (2009) demonstrated adding more microorganisms to the microbial mixture, including *Trichothecium roseum* MML005 and *Streptomyces* sp. PM5, could enhance the plant defense against SNVD. The maximum inhibition of *Cotton leaf curl virus* (CLCuV) was observed in the plants treated with a mixture of bacterial isolates, including *Pseudomonas aeruginosa* (S1HL3), *Burkholderia* sp. (S1HL4), and *Bacillus* sp. (JS2HR4 and JS3HR2) (Ramzan *et al.*, 2016). These findings highlight the effectiveness of PGPR's multiple applications to increase resistance against viral diseases.

One of the significant indirect mechanisms of PGPR is the stimulation of ISR. PGPR activates the biosynthesis of defense-related molecules in the host plant. Antioxidative defense enzymes protect the cells from oxidative damage scavenging by transforming ROS into non-toxic end products (Hussain *et al*., 2016; Sofy *et al*., 2021). It is, therefore, likely that such correlations exist between an increase in antioxidant enzyme activity and induced resistance against the virus (Backer *et al*., 2018). Based on Li *et al*. (2016), *Enterobacter asburiae* BQ9 induces resistance against *Tomato yellow leaf curl virus* (TYLCV) in pre-treated plants by a significant increase in expression of antioxidant enzymes, phenylalanine ammonia-lyase, peroxidase, catalase, and superoxide dismutase. Conclusively, the deteriorated symptoms on the TMV-infected bell pepper plants might result from an increase in defense-related enzymes stimulated by the PGPR application.

Conclusion

The present study was designed to determine the effect of combined PGPR strains to reduce the disease severity of TMV in bell pepper plants. The results showed that the compatible mixture of *P. fluorescens*, *P. putida*, and *B. subtilis* could stimulate antioxidant defense enzymes and protect the bell pepper plants against TMV. Interestingly, the combination of the PGPR strains resulted in an improvement of plant growth characteristics. These findings provide novel information for multiple

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applications of PGPR strains to control TMV in bell pepper plants. Treated plants with the mixture of PGPR showed a significant increase in photosynthetic pigments, which might enhance the host's tolerance to the disease caused by TMV.

Conflict of interests

The authors declare that they have no conflicting interests.

Authors' contributions

Milad Aeini prepared statics and wrote the article, Parnian Pooladi conducted experiments, Mohamad Hamed Ghodum Parizipour and Seyed Abdollah Eftekhari interpreted the data.

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References

Abdalla, O. A., Bibi, S. and Zhang, S. 2017. Application of plant growth-promoting rhizobacteria to control *Papaya ringspot virus* and *Tomato chlorotic spot virus*. Archives of Phytopathology and Plant Protection, 50: 11-12.

Aeini, M., Ghodoum Parizipour, M. H. and Pooladi, P. 2018. Management of *Tobacco mosaic virus* by some probiotic bacteria in tomato plant. Biological Control, 7(2): 117-127.

Alishiri, A., Rakhshandehroo, F., Zamanizadeh, H. R. and Palukaitis, P. 2013. Prevalence of *Tobacco mosaic virus* in Iran and evolutionary analyses of the coat protein gene. The Plant Pathology Journal, 29(3): 260.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S. and Smith D. L. 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science, 9: 1473.

Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44(1): 276-87.

Beneduzi, A., Ambrosini, A. and Passaglia, L. M. 2012. Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology, 35(4): 1044-51.

Beris, D., Theologidis, I., Skandalis, N. and Vassilakos, N. 2018. *Bacillus amyloliquefaciens* strain MBI600 induces salicylic acid dependent resistance in tomato plants against *Tomato spotted wilt virus* and *Potato virus Y*. Scientific Reports, 8(1): 10320.

Bradford, M. M. 1976. Determinación de proteínas: método de bradford. Analytical Biochemistry, 72: 248-54.

Chandlee, J. M. and Scandalios, J. G. 1984. Analysis of variants affecting the catalase developmental program in maize scutellum. Theoretical and Applied Genetics, 69(1): 71-77.

Chen, C., Belanger, R. R., Benhamou, N. and Paulitz, T. C. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiological and Molecular Plant Pathology, 56(1): 13-23.

Clark, M. F. and Adams, A. N. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, 34: 475-83.

Dashti, N. H., Ali, N. Y., Cherian, V. M. and Montasser, M. S. 2012. Application of plant growth-promoting rhizobacteria (PGPR) in combination with a mild strain of *Cucumber mosaic virus* (CMV) associated with viral satellite RNAs to enhance growth and protection against a virulent strain of CMV in tomato. Canadian Journal of Plant Pathology, 34(2): 177-86.

Dimopoulou, A., Theologidis, I., Liebmann, B., Kalantidis, K., Vassilakos, N. and Skandalis,

[DOR: 20.1001.1.22519041.2021.10.4.11.1]

N. 2019. *Bacillus amyloliquefaciens* MBI600 differentially induces tomato defense signaling pathways depending on plant part and dose of application. Scientific Reports, 9(1): 1-2.

Fukui, R., Schroth, M. N., Hendson, M. and Hancock, J. G. 1994. Interaction between strains of Pseudomonads in sugar beet sphermospheres and the relationship to pericarp colonization by *Pythium ultimum* in soil. Phytopathology, 84: 1322-1330.

Hussain, S., Khan, F., Cao, W., Wu, L. and Geng, M. 2016. Seed Priming Alters the Production and Detoxification of Reactive Oxygen Intermediates in rice seedlings grown under sub-optimal temperature and nutrient supply. Frontiers in Plant Science, 7: 439.

Jetiyanon, K. and Kloepper, J. W. 2002. Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biological control, 24(3): 285-91.

Konappa, N., Krishnamurthy, S., Arakere, U. C., Chowdappa, S. and Ramachandrappa, N. S. 2020. Efficacy of indigenous plant growth-promoting rhizobacteria and *Trichoderma* strains in eliciting resistance against bacterial wilt in a tomato. Egyptian Journal of Biological Pest control, 30(1): 106.

Letschert, B., Adam, G., Lesemann, D. E., Willingmann, P. and Heinze, C. 2002. Detection and differentiation of serologically cross-reacting tobamoviruses of economical importance by RT-PCR and RT-PCR-RFLP. Journal of Virological Methods, 106(1): 1-10.

Li, H., Ding, X., Wang, C., Ke, H., Wu, Z., Wang, Y., Liu, H. and Guo, J. 2016. Control of *Tomato yellow leaf curl virus* disease by *Enterobacter asburiae* BQ9 as a result of priming plant resistance in tomatoes. Turkish Journal of Biology, 40: 150-159.

Maksimov, I. V., Sorokan, A. V., Burkhanova, G. F., Veselova, S. V., Alekseev, V., Yu, Shein, M. Yu., Avalbaev., A. M., Dhaware, P. D., Mehetre, G. T., Singh, B. P. and Khairullin, R. M. 2019. Mechanisms of plant tolerance to RNA viruses induced by plant-growth-promoting microorganisms. Plants, 8: 575.

Moury, B. and Verdin, E. 2012. Viruses of pepper crops in the Mediterranean basin: a remarkable stasis. Advances in Virus Research, 84: 127-162.

Murphy, J. F., Reddy, M. S., Ryu, C. M., Kloepper, J. W. and Li, R. 2003. Rhizobacteria-mediated growth promotion of tomato leads to protection against *Cucumber mosaic virus*. Phytopathology, 93(10): 1301-7.

Gniffke, P. A., Shieh, S. C., Lin, S. W., Sheu, Z. M., Chen, J. R., Ho, F. I., Tsai, W. S., Chou, Y. Y, Wang J. F., Cho. M. C. and Schafleitner, R. 2013. Pepper research and breeding at AVRDC - The World Vegetable Center. XV EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant pp. 305-311.

Palukaitis, P., Yoon, J. Y., Choi, S. K and Carr, J. P. 2017. Manipulation of induced resistance to viruses. Current Opinion in Virology, 26: 141-8.

Radwan, S. S., Dashti, N., El-Nemr, I. and Khanafer, M. 2007. Hydrocarbon utilization by nodule bacteria and plant growth-promoting rhizobacteria. International Journal of Phytoremediation, 9(6): 475-86.

Ramzan, M., Tabassum, B., Nasir, I. A., Khan, A., Tariq, M., Awan, M. F., Shahid, N., Rao, A. Q., Bhatti, M. U., Toufiq, N. and Husnain, T. 2016. Identification and application of biocontrol agents against *Cotton leaf curl virus* disease in *Gossypium hirsutum* under greenhouse conditions. Biotechnology & Biotechnological Equipment, 30(3): 469-78.

Raupach, G. S., Liu, L., Murphy, J. F., Tuzun, S. and Kloepper, J. W. 1996. No Induced systemic resistance in cucumber and tomato against *Cucumber mosaic cucumovirus* using plant growth-promoting rhizobacteria (PGPR). Plant Disease, 80: 891-894.

Rohlf, F. 2002. NTSYSpc: Numerical Taxonomy System, ver. 2.1. Setauket, NY: Exeter Publishing Ltd.

Sofy, A. R., Dawoud, R. A., Sofy, M. R.,

[DOR: 20.1001.1.22519041.2021.10.4.11.1]

Mohamed, H. I., Hmed, A. A. and El-Dougdoug NK.2020. Molecules improving regulation of enzymatic and non-enzymatic antioxidants and stress-related gene stimulation in *cucumber mosaic cucumovirus*-infected cucumber plants treated with glycine betaine, chitosan and combination. Molecules, 25(10): 2341.

Sofy, A. R., Sofy, M. R., Hmed, A. A., Dawoud, R. A., Alnaggar, A. E., Soliman, A. M. and El-Dougdoug, N. K. 2021. Ameliorating the adverse effects of *Tomato mosaic tobamovirus* infecting tomato plants in egypt by boosting immunity in tomato plants using zinc oxide nanoparticles. Molecules, 26(5): 1337.

Sofy, M. R., Sharaf, A. M., Noufl, M. and Sofy, A. R. 2014. Physiological and biochemical responses in cucurbita pepo leaves associated with some elicitors-induced systemic resistance against *Zucchhini yellow mosaic virus*. International Journal of Modern Botany, 4(2): 61-74.

Srinivasan, K. and Mathivanan, N. 2009. Biological control of *Sunflower necrosis virus* disease with powder and liquid formulations of plant growth promoting microbial consortia under field conditions. Biological control, 51(3): 395-402.

Suryadi, Y., Susilowati, D. N. and Fauziah. F. 2019. Management of plant diseases by pgpr-mediated induced resistance with special reference to tea and rice crops. In: Sayyed R. (Eds.), Plant Growth Promoting Rhizobacteria for Sustainable Stress Management. Microorganisms for Sustainability, vol 13. Springer, Singapore. pp. 65-110.

Thomas, R. L., Jen, J. J. and Morr, C. V. 1982. Changes in soluble and bound peroxidase-iaa oxidase during tomato fruit development. Journal of Food Science, 47(1): 158-61.

Yu, A. V. and Yu, S. M. 2019. Mechanisms of plant tolerance to rna viruses induced by plant-growth-promoting microorganisms. Plants, 8(12): 575.

Vitti, A., Pellegrini, E., Nali, C., Lovelli, S., Sofo, A., Valerio, M., Scopa, A. and Nuzzaci, M. 2016. *Trichoderma harzianum* T-22 induces systemic resistance in tomato infected by *Cucumber mosaic virus*. Frontiers in Plant Science, 107: 1520.

Zehnder, G. W., Yao, C., Murphy, J. F., Sikora, E. R. and Kloepper, J. W. 2000. Induction of resistance in tomato against *Cucumber mosaic cucumovirus* by plant growth-promoting rhizobacteria. BioControl, 45(1): 127-37.

Zhao, J., Zhang, X., Hong, Y. and Liu Y. 2016. Chloroplast in plant-virus interaction. Frontiers in Microbiology, 7: 1565.

[DOR: 20.1001.1.22519041.2021.10.4.11.1]

کاربرد باکتری‌های افزایش‌دهندهٔ رشد جهت محافظت از فلفل دلمه‌ای در برابر ویروس موزاییک تنباکو

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چکیده: ویروس موزاییک توتون یکی از ویروس‌های مخرب گیاهی بوده که به انواع مختلف محصولات آسیب می‌زند. گزارش‌هایی از توان مهار زیستی باکتری‌های افزایش‌دهندهٔ رشد علیه ویروس‌ها موجود است. در این تحقیق آزمایش گلخانه‌ای برای تعیین اثر متقابل TMV، فلفل دلمه‌ای و باکتری‌های افزایش‌دهندهٔ رشد طراحی شد. جهت انجام این آزمایش جدایه‌هایی از *Pseudomonas fluorescens*، *Pseudomonas putidae* و *Bacillus subtilis* به‌صورت تکی و یا مخلوطی از جدایه‌ها به رایزوسفر گیاهچه چهاربرگی فلفل دلمه‌ای اضافه گشت. سپس گیاهان فلفل دلمه‌ای با ویروس مایه‌زنی شده و برای ظهور علائم به‌مدت ۲۸ روز نگه‌داری شدند. هم‌چنین ۲۸ روز پس از مایه‌زنی میزان غلظت ویروس با استفاده از روش الایزای غیرمستقیم تخمین زده شد. نتایج نشان داد که کاربرد باکتری‌های PGPR سبب کاهش میزان غلطت نوری در مقایسه با شاهد مثبت می‌شوند. هم‌چنین میزان آنزیم‌های مؤثر در مقاومت (پروکسیداز، کاتالاز، آسکوربیت پروکسیداز و سوپراکسید دیسموتاز) افزایش معنی‌داری نسبت به شاهد داشتند. علاوه بر مهار بیماری، مایه‌کوبی باکتری‌های PGPR سبب افزایش معنی‌دار مؤلفه‌های رشدی در مقایسه با شاهد در فلفل دلمه‌ای شد. به‌طورکلی نتایج این تحقیق نشان داد که مایه‌کوبی باکتری‌های افزایش‌دهندهٔ رشد سبب افزایش مؤلفه‌های رشدی و بازدارندگی از بیماری ناشی TMV در فلفل دلمه‌ای می‌شوند.

واژگان کلیدی: الایزای غیرمستقیم، تجمع ویروس، آنزیم، ویژگی رشدی

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