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RESEARCH

Efficacy of the sterile fungus GU23-3 on growth characters of cucumber plants and resistance against *Papaya ring spot virus*

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ABSTRACT

Background: Papaya ring spot virus (PRSV) is a major threat to the production of cucurbits all over the world. The sterile fungus GU23-3 is a rhizosphere fungus conferring positive effects on plant growth and health..
Objective: The aim of this study is to evaluate the effects of GU23-3 on cucumber growth and resistance against PRSV..
Methods: Disease severity and virus titer were evaluated in cucumber plants treated with barley grain (BGI) or culture filtrate (CF) of GU23-3 under greenhouse conditions. Additionally, the expression levels of defense genes were assessed by reverse transcription PCR (RT-PCR) analysis.
Results: Both of virus severity and titer were significantly reduced in treated plants in comparison with the control. All growth characters and yield of cucumber plants were increased in GU23-3 treated plants than the control. RT-PCR analysis revealed increased expressions of different pathogenesis related genes (Chitinase, β 1-3 Glucanase, PR1, POX, PAL and LOX) explaining the role of these genes in the mechanism of induced resistance against PRSV by GU23-3.
Conclusion: GU23-3 could be considered a new and alternative strategy to control PRSV infection.

Keywords: Papaya ring spot virus; induced resistance; plant growth promoting fungi; cucumber; RT-PCR.

BACKGROUND

Papaya ring spot virus (PRSV) belongs to the genus potyvirus of the family potyviridae, has a worldwide distribution and is responsible for significant yield loses in cucurbit crops (Quiot-Douine et al., 1990). Potyviruses are positive, single-stranded, RNA viruses, and represent one of the most economically important and largest groups of plant viruses (Shukla et al., 1994). PRSV is an important pathogen of papaya and cucurbits (Riechmann et al., 1992).

PRSV induces a large array of symptoms in papaya and cucurbit cultivars, such as vein clearing, mottling, malformed leaves, filiformy, ringspots and streaks on fruits, stem and petioles, and stunting. Variation in symptoms is dependent on virus isolate, stage of infection, plant size and vigour, and temperature (Conover, 1964 and Purcifull et al., 1984). Distortion of young leaves that sometimes results in shoestring appearance caused by the extreme reduction of leaf laminae similar to that caused by broad mites (Heu et al., 2002).

All plants have active defense mechanisms against pathogen attacks. If defense mechanisms are triggered by a stimulus prior to infection by a virulent plant pathogen, disease symptoms can be reduced (Pieterse et al., 2002; Ryu et al., 2004; Lee et al., 2005 and Kang et al., 2007).Treatment of plants with biotic and a biotic elicitors could enhance resistance in plants to pathogens (Bellamy et al., 1995; Beck et al., 1996; Lawton et al., 1996; Van Loon et al., 1998 and Beauchamp et al., 2002). Induced systemic resistance (ISR) in plants to fungal, bacterial and viral pathogens has been demonstrated after pre inoculation with weakly aggressive, a virulent or

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incompatible forms of the disease-causing organisms. This response of plants after induction is known as systemic acquired resistance (SAR) and has been demonstrated in several plant–pathogen systems (Ross et al., 1961; Kuć, 1983; Ishiba et al., 1981). Resistance against *Cucumber mosaic virus* (CMV) increased in tobacco plants treated with *Bacillus sp*. (Wang et al., 2009). Additionally, plant growth promoting rhizobacteria mediated protection against CMV in *A. thaliana* (Ryu et al., 2004).

Plant growth-promoting fungi (PGPF) are a class of non-pathogenic soil-borne filamentous fungi that have beneficial effects on plants (Dewan and Sivasithamparam, 1989 and Hyakumachi, 1994). ISR by PGPF have also been intensively studied and is thought to be one of those mechanisms involved in plant disease management ((Meera et al., 1995 and Murali et al., 2012). *Phoma* sp. and the sterile fungus used to induce systemic resistance against anthracnose caused by *C. orbiculare* in cucumber plants (Meera, 1994). Several PGPF have been reported such as species belonging to the genera *Trichoderma, Fusarium, Penicillium*, *Phoma* and sterile fungi (Ahmad and Baker, 1988; Hyakumachi, 1994; Kleifeld and Chet, 1992; Windham et al., 1986 and Meera, 1994).

Studies of PGPF have concentrated on the mechanisms stimulating plant growth. PGPF have been reported to produce substances such as plant hormones (Blanchard and Björkman, 1996), to allow plants to utilize decomposing organic matter through mineral solubilization (Altomare et al., 1999 and Harman et al., 2004), and to suppress plant pathogens in the rhizosphere by antagonistic mechanisms, such as the production of hydrolytic enzymes, aggressive mycoparasitism, competition for saprophytic colonization, and the induction of plant systemic resistance (Benítez et al., 1998; Benítez et al., 2004;Haran et al., 1996; Lorito1996; Meera et al., 1994 and Meera et al., 1995).

Salicylic acid (SA), jasmonates (JA) and ethylene (ET) are well known to play crucial roles in plant disease and pest resistance (Robert-Seilaniantz et al., 2007). Culture filtrates (CFs) of PGPF isolates also induced resistance against anthracnose. CF-treated plants expressed resistance to pathogen infection by an alteration of various metabolisms, such as high increases in activities of chitinase, β-1, 3-glucanase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase, indicating that an elicitor substance(s) existed in the CFs (Meera, 1994). Defense mechanism against CMV infection had increased when plant treaded with CF of SKT-1 mediated the expression of a majority of the various pathogen related genes. While BGI treatment induced systemic resistance against CMV through SA signaling cascade in *Arabidopsis* plants (Elsharkawy et al., 2013). Tobacco plants treated with CF of *Penicillium* sp.GP16-2 elicited ISR against CMV via multiple pathways in tobacco plants. While, BGI of GP16-2 induced systemic resistance against CMV through an SA signaling cascade (Elsharkawy et al., 2017). Defense mechanism against CMV infection had increased when plants treaded with CF of SKT-1 mediated the expression of a majority of the various pathogen related genes. While BGI treatment induced systemic resistance against CMV through SA signaling cascade in *Arabidopsis* plants (Elsharkawy et al., 2013).

Therefore, the aim of the present study was to test the ability of the PGPF (sterile fungus GU 23-3) to induce systemic resistance in cucumber plants and to improve plant growth and protection against PRSV.

MATERIALS AND METHODS

Fungal inoculation

Barley grain inoculum (BGI)

The PGPF used in this study was the sterile fungus GU23-3 which was isolated from turf grass rhizosphere (Hyakumachi, 1994). GU23-3 was grown in 2 % potato dextrose agar (PDA) in petri plates for 7 days at 25°C. Twenty mycelial disks (5 mm) were obtained from GU23-3 cultures from the growing margin of a colony on PDA and were transferred to a 500-ml Erlenmeyer flask containing autoclaved barley grains (100 g of barley grains and 100 ml of distilled water). After 10 days of incubation at 25°C in the dark with shaking every 2 days, the completely colonized barley grains were air-dried at room temperature (23–25°C). The dried BGI was ground to a 1–2-mm particle size and stored at 4°C until used.

Cell-free culture filtrate (CF)

GF 23-3 was cultured on PDA medium for 7 days. Twenty mycelial disks (5 mm) of GU 23-3 cultures were taken from the growing margin of a colony and transferred to a 500-mL Erlenmeyer flask containing 200 mL potato dextrose broth (PDB). The fungal culture was then maintained at room temperature (25°C) for 10 days without shaking. The crude culture filtrate was separated from the mycelia and filtered through two layers of Whatman No. 2 filter paper, and then filter-sterilized through (0.22 μm Millipore filters, Millipore products division, Bedford, USA).

Cultivation of plants in soil

Cucumber seeds were sterilized by immersion in 70% ethanol for 2 min followed by 2% (v/v) NaOCl for 2 min, thoroughly rinsed three times in sterile distilled water, and socked in 150 ml of distilled water flask for 1 day at 28°C in the absence of light. The seeds were incubated in sterile petri dishes with Whatman No. 2 filter paper wet of distilled water and stored in incubator for 2 days at 28°C to synchronize germination. Sterilized pots were filled with approximately 100 g of autoclaved compost materials and transplanted with cucumber plants.

Papaya ring spot virus (PRSV) inoculation.

The virus was maintained in squash plants (*Cucurbita pepo* cv. Eskandarani).The PRSV inoculum used throughout the experiments consisted of infected sqaush leaf tissues which were singled out cloned by successive local lesion transfers to *Chenopodium amaranticolor* as local lesion host plants (**Yeh et al., 1984**). A single local lesion was used to inoculate squash as propagative host plant. Young symptomatic squash leaves were ground in 0.2 M potassium phosphate buffer (pH 7) containing 0.02 M sodium sulfite and Carborundum as an abrasive. The extract was rubbed onto cucumber cotyledons and true leaves.

Disease severity assessment

Disease severity rating for cucumber plants is: 0, no symptoms; 2, mild deformation and mosaic of the youngest two leaves; 4, pronounced leaf deformation and mosaic of the youngest two leaves with progression of symptoms into sequentially older leaves; 6, pronounced leaf deformation and mosaic progressed beyond the two youngest leaves with all leaves expressing mosaic symptoms and sever blister ; 8, similar symptoms as described for a rating of 6 with plants also being stunted in growth and shoes string (note that this stunting included both reduced internodes extension and smaller leaves); and 10, plants were severely stunted with a majority of leaves being small, severely deformed and tightly bunched together. The results of

the disease severity represent the mean values of 10 samples in each treatment starting from 14 days after PRSV inoculation to 45day.

Enzyme-linked immunosorbent assay (ELISA)

PRSV titer was determined by indirect ELISA using a commercial kit (Loewe Biochemica GmbH, Germany) according to the manufacturer's instructions. Optical densities (OD) were recorded at 405 nm with a microplate reader (Stat Fax 4200, USA) and samples showing OD values higher than twice the average of the negative control were considered positive **(Mnari-Hattab et al., 2009).**

One-step reverse-transcriptase polymerase chain reaction (RT-PCR) analysis

For the RNA analysis, leaves were harvested 0, 1, 2, 4, and 6 days post virus inoculation (DPI) and stored at −80°C until being used. Each treatment included three replications with two plants per replicate. Total RNA was extracted following protocol of easy-RED™ Total RNA Extraction Kit (for liquid sample, Cat. No. 17063 100 ml, iNtRON Biotechnology, Inc.). Maxime RT-PCR PreMix Kit for 20µl rxn (Cat. No. 25131) was used for reverse transcription and PCR amplification. The RT-PCR reaction was set up in 20µl as follow manufacturer's instructions.

Effect of plant growth promotion fungi on cucumber plants

Two experiments were carried out under unheated plastic house. The field experiment was conducted in the experimental farm of Faculty of Agriculture, Kafrelsheikh University during two successive autumn seasons. Leaf area for the 6^{th} leaf (dm^2) was calculated using the fresh weight method at 45 days after transplanting, by five discs from the leaves with 1.13 cm^2 for each disc were used for this estimation, according to the following formula:

$$\text{Leaf area} = \frac{\text{leaves fresh weight} \times \text{discs area}}{\text{Discs fresh weight}}$$

Early fruit yield was determined as weight (kg) and number of fruits per plant and square meter as sum of the first 9 picking (48 days of transplanting). Total fruit yield was determined as weight (kg) and number of fruits per m^2 for all picking which were 28 in both seasons. Marketable and unmarketable fruit yield included fruits yield as weight (kg) and number per plant and square meter from 28 pickings in both seasons. Moreover, leaves fresh and dry weights and dry matter.

Data analysis

The experiment included two treatments (culture filtrate and barely grain inoculum of sterile fungi GU 23-3). A split-plot system in a randomized blocks design was used. The experiments were repeated at least thrice, and treatment means were tested by analysis of variance, according to Little and Hills (1972). Duncan's multiple rang test was used for the comparisons among treatments means (Duncan, 1955).

RESULTS

Effect of BGI of the sterile fungus GU 23-3 on systemic protection against PRSV in cucumber plants

Symptoms of PRSV appeared 7 days post inoculation (DPI) and ranged from vein clearing in young non-inoculated leaves to severe mosaic and deformation. Cucumber plants

grown in soil amended with the BGI of GU 23-3 exhibited a dramatic reduction in PRSV symptoms as compared with the non-treated control plants, which showed sever mosaic with sever blisters and small deformed leaves (Figs 1, 2 and 3). All cucumber plants treated with the BGI of GU 23-3 showed significant reduction in disease severity rating after PRSV inoculation (Table1). Based on ELISA measure, PRSV accumulation at 7, 14 and 21 days after the virus challenge inoculation, PRSV titer was significantly reduced in cucumber plants treated with BGI GU 23-3 (Table 2).

Fig. 1: Disease symptoms after 14 days of inoculation of PRSV on cucumber plants.
A, healthy plant and B, infected plants non -treated, C cucumber plants treated with BIG of GF 23-3 and D treated with CF of GF 23-3

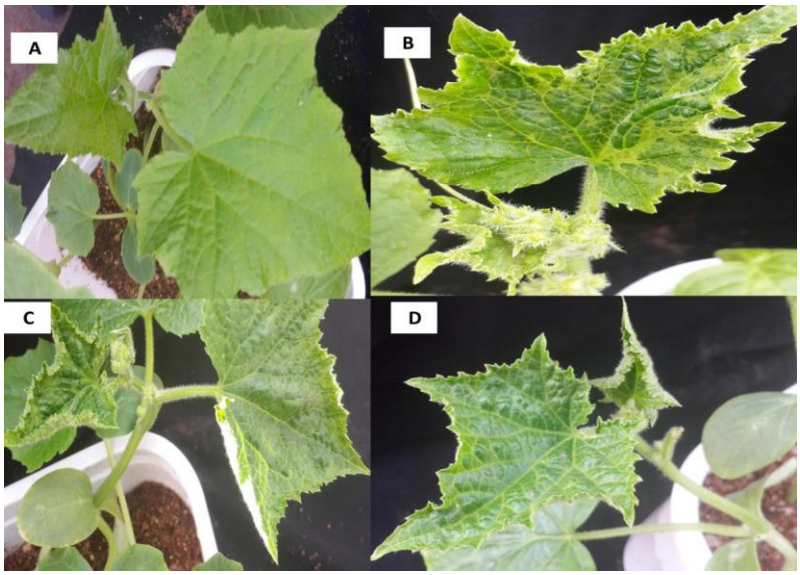

Fig. 2: Disease symptoms after 21 days of inoculation of PRSV on cucumber plants. A, healthy plant and B, infected plants non -treated, C cucumber plants treated with BIG of GF 23-3 and D treated with CF of GF 23-3.

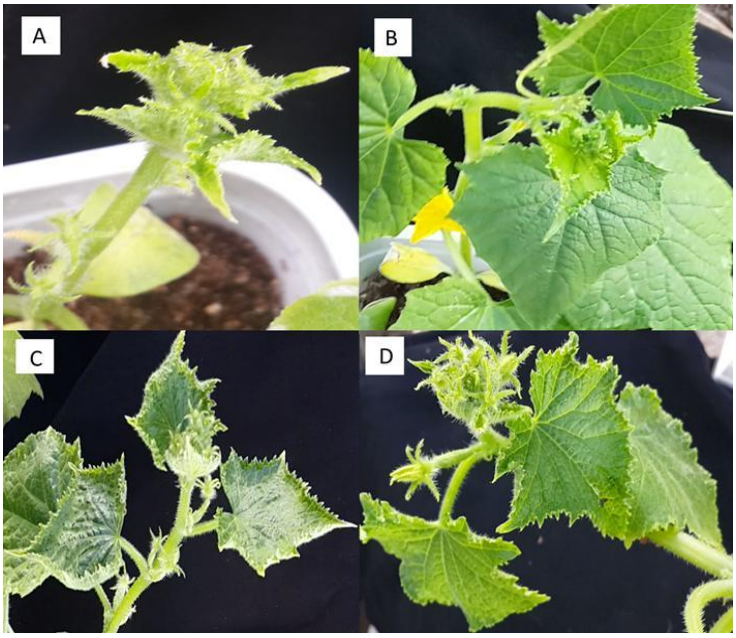

Fig. 3: Disease symptoms after 45 days of inoculation of PRSV on cucumber plants. A, infected plants and B, healthy plant treated, C cucumber plants treated with BGI of GU 23-3 and D treated with CF of GF 23-3.

Table (1): Disease severity in PRSV-inoculated cucumber plants treated with sterile fungus GU23-3 or with BTH, relative to non-treated control plants

Treatments	Disease severity					
	1W	2W	3W	4W	5W	6W
Plant with PRSV	3.333a	6.667a	8.667a	10.00a	10.00a	10.00a
CF of GF 23-3	0.6673b	3.333b	4.667b	6.667b	7.333b	7.333b
BGI of GF 23-3	1.334 b	2.667 b	5.333b	5.333b	5.333b	6.667b
BTH	0.6673b	2.000 b	4.667b	6.000b	6.667bc	7.333bc
F. test	*	**	**	*	**	**

**and* indicate significant differences at $P \leq 0.01$, $P \leq 0.05$, respectively, according to F test. Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test

Table (2): *Papya ringspot virus* accumulation in leaves of cucumber plants treated with sterile fungus GU23-3 or with BTH, relative to non-treated control plants

Treatments	**Virus concentration by ELISA**		
	1W	2W	3W
Plant with PRSV	1.676a	2.543a	3.433a
CF of GU 23-3	0.760b	1.446b	2.246b
BGI of GU 23-3	0.862b	1.673b	2.497b
BTH	0.635b	1.641 b	2.637b
F. test	*	**	**

**and* indicate significant differences at P≤ 0.01, P≤0.05, respectively, according to F test. Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test

Effect of CF of GU23-3 on systemic protection against PRSV

The severity of PRSV was significantly reduced in cucumber plants pre-treated with the CF of GU 23-3 compared with the untreated control plants (Table 1). ELISA demonstrated that PRSV titer was significantly reduced in cucumber plants treated with CF of GU 23-3 relative to the controls (Table 2). These results confirm that the CF of GU 23-3 was as effective as the BGI of GF 23-3. In addition, no significant differences were observed between the protection values induced by the BGI of GU 23-3 or its CF in cucumber plants (Table 2).

Effect of BGI of sterile fungi GU 23-3 on the growth of cucumber plants under greenhouse conditions

Our results showed that the growth parameters such as plant shoot fresh weight and dry weight, dry matter and leaf area were significantly decreased in infected plants compared with control plants. Treatment of infected plants with BGI of GU23-3 significantly increased shoot fresh and dry weight and dry material, leaf area as compared with non-treated plants (Table 3). The results showed that total green color (TGC) concentrations were significantly reduced in infected leaves by 25.39 %, 26.78% during first and second seasons, respectively compared with the control (Figure 4). However, infected cucumber plants treated with GU 23-3 increased TGC by 22.67%, 19% during first and second seasons, respectively compared with the non -treated control plants (Table 4). Cucumber plants treated with BGI of GU 23-3 exhibited a significant increase in early yield per m^2 by 103 %, 180 % during first and second seasons, respectively compared with the non -treated control plants (Table 4). Total yield were significantly increased by 109%, 115.31% during first and second seasons per m^2, respectively compared with the non -treated control plants (Table 5). Treatments with GU 23-3 significantly decreased unmarketable fruits of cucumber plants by 36.13%, 29.24% and significant increased marketable (Figure 5) fruits per plant as well as m^2 by 22.58%, 26.03% during first and second seasons, respectively compared with the non-treated plants (Table 6). However, virus infection increased unmarketable fruits (Figure 5) by 127.58%, 108.73% during first and second seasons, respectively compared with the control plants (Table 6).

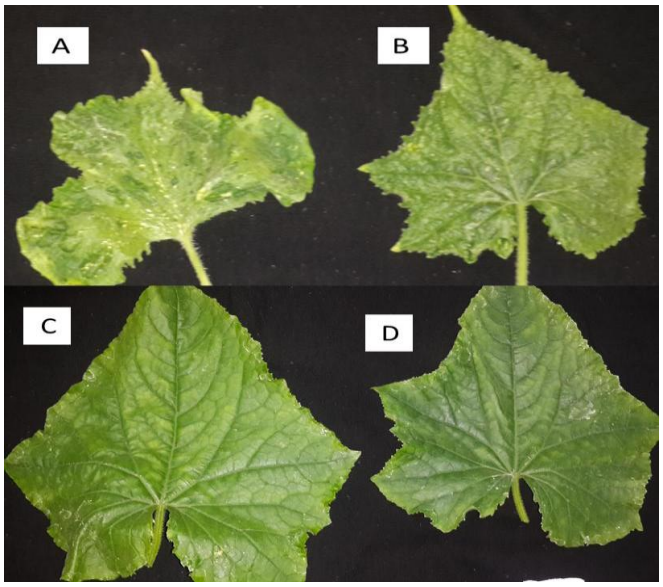

Fig. 4: Disease symptoms after 45 days of inoculation of PRSV on cucumber plants. A, infected plant with PRSV non-treated, B treated plant with GU 23-3, C healthy plant treated, and D healthy plant non-treated in green house.

Table (3): Effect of plant growth promotion fungi (GU23-3) treatments on leaf area/plant, leaf area index and leaves dry matter content of cucumber plant during first and second seasons.

Treatments	Leaf area/ plant (dm^2)	Fresh weight	Dry weight	Leaves dry matter	Leaf area/plant (dm^2)	Fresh weight	Dry weight	Leaves dry matter
	45 days after transplanting							
	1st season				2nd season			
Cont. h.	98.430b	23.27b	4.47b	3.69b	86.960b	14.667b	1.506b	3.423b
Plant + PRSV	27.267d	7.540d	0.899d	2.53d	18.463d	6.600d	0.363d	1.746c
GU 23-3+PRSV	40.867c	11.25c	1.53c	3.00c	37.020c	9.167c	0.830c	3.015b
GU 23-3	148.600a	29.02a	6.19a	4.12a	112.450a	17.100a	2.126a	4.154 a
F. test	**	**	**	**	**	**	**	**

** indicate significant differences at P≤ 0.01 according to F test. Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Table (4): Effect of plant growth promotion fungi (GU 23-3) treatment on early fruit yield and total green color of cucumber plant during first and second seasons.

Treatments	Early fruit yield								Total green color (SPAD	
	weight(kg)				Number					
	1st season		2nd season		1st season		2nd season		1st season	2nd season
	Per plant	Per m^2	Per plant	Per m^2	Per plant	Per m^2	Per plant	Per m^2		
Cont. h.	1.371b	3.222b	1.161b	1.990b	16.00a	37.600a	13.667a	21.667a	32.50a	39.033b
Plant with PRSV	0.487d	1.143d	0.366c	0.843c	8.00b	18.917b	5.333d	10.333b	24.26c	28.600d
GU 23-3+PRSV	0.989c	2.325c	1.028b	1.580b	10.67b	25.067a	12.000a	17.333a	29.76b	34.067c
GF 23-3	1.672a	3.930a	1.560a	2.507a	17.33a	40.733a	16.667a	25.667a	45.00a	44.967a
F. test	**	**	**	**	**	**	**	**	**	**

** indicate significant differences at *P≤ 0.01* according to F test. Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Fig. 5: Disease symptoms evolution on fruits of cucumber plants under greenhouse conditions. A, left infected and right healthy control, B on the left infected fruits and on the right healthy fruits treated with GU 23-3 stopped virus symptoms.

Table (5): Effect of plant growth promotion fungi (GU 23-3) treatment on total fruit yield and average fruit weight of cucumber plant during 2016/2017 and 2017/2018 seasons.

Treatments	Total fruit yield							
	1st season				2nd season			
	weight(kg)		Number		weight(kg)		Number	
	Per plant	Per m^2	Per plant	Per m^2	Per plant	Per m^2	Per plant	Per m^2
Cont. h.	3.114b	7.317b	36.667b	86.167b	4.674b	7.402b	50.917ab	83.033b
Plant with PRSV	1.343c	3.155c	18.000d	42.300d	1.985c	2.846d	24.283c	36.817d
GF 23-3+PRSV	2.809b	6.601b	28.667c	67.367c	3.712b	6.128c	40.717b	68.933c
GF 23-3	4.148a	9.747a	43.000a	101.05a	5.882a	9.548a	60.317a	99.483a
F. test	**	**	**	**	**	**	**	**

** indicate significant differences at *P≤ 0.01* according to F test. Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Table (6): Effect of plant growth promotion fungi (GU 23-3) treatment on Marketable fruit yield and Nonmarketable fruit Yield of cucumber plant during 2016/2017 and 2017/2018 seasons.

Treatments	Marketable fruit yield								Nonmarketable fruit Yield			
	1st season				2nd season				1st season		2nd season	
	Weight (kg)		Number		Weight (kg)		Number		Weight (kg)	Number	Weight (kg)	Number
	Per plant	Per m^2	Per plant	Per m^2	Per plant	Per m^2	Per plant	Per m^2	Per m^2	Per m^2		Per m^2
Cont. h.	2.524b	5.928b	29.00b	68.283b	2.570b	6.039	28.00	65.80	1.385bc	18.17b	1.363	17.233c
Plant with PRSV	0.000c	0.000c	0.00d	0.000d	0.000d	0.000	0.00	0.00	3.152a	42.28a	2.845	36.717a
GF 23-3+PRSV	4.042b	4.589b	18.67c	43.867c	1.902c	4.467	17.00	39.95	2.013b	23.50b	2.129	28.983b
GF 23-3	7.653a	8.651a	39.33a	92.433a	3.730a	8.765	38.667	83.817	1.096c	8.617c	0.782	8.617d
F. test	**	**	**	**	**	**	**	**	**	**	**	**

** indicate significant differences at P≤ 0.01 according to F test. Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Effect GU23-3 on expression of SA- and JA/ET-inducible defense-related genes

RT-PCR results demonstrated that the expression levels of *Chitinase, β-1, 3 Glucanase*, *PR1* and *Pox* genes in BGI treatment were quickly elevated at 1 day after virus inoculation and continue elevated up to 6 days after virus inoculation (Fig. 6). In contrast, their expressions were slowly elevated in CF treatment and decreased again at 4 and 6 DPI. Although, the expression levels of JA- expressing genes (*PAL* and *LOX*) in BGI treatment were started to increase at 2 days after virus inoculation and decreased again at 4 and 6 DPI, the expression level of *PAL* gene was quickly increased in CF treatment and continue elevated up to 6 DPI (Fig. 6).

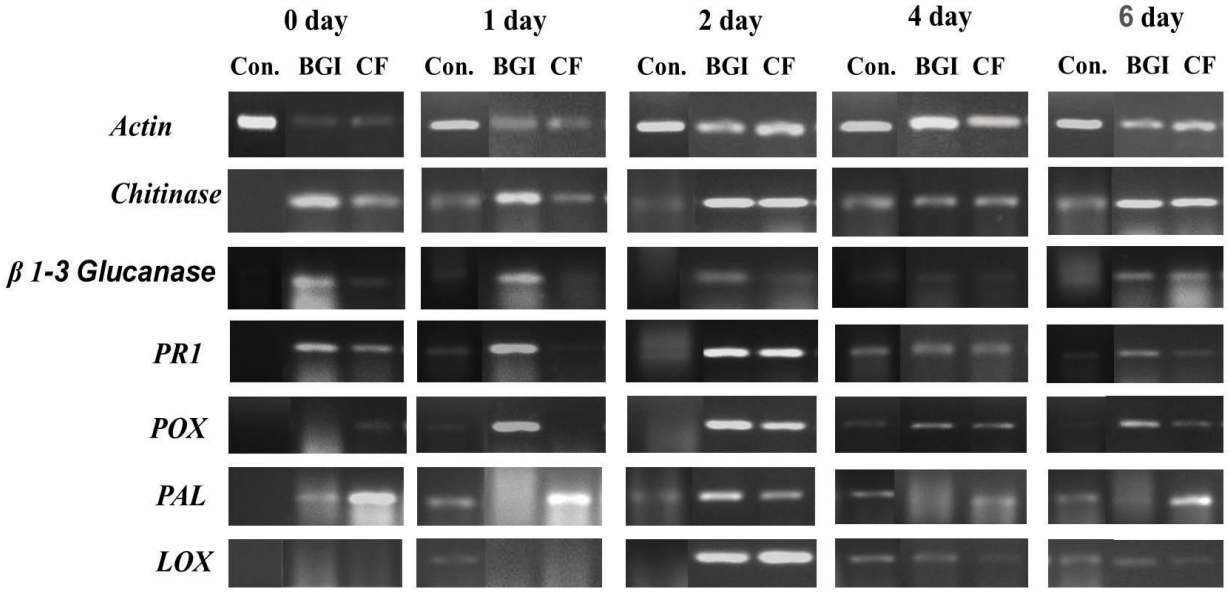

Fig. 6: Expression of defence-related genes in leaves of cucmber plants treated with BGI or CF of sterile fungus 1 day before challenge inoculation with *Papaya ringspot virus*. A constitutively expressed Actin was used as a control in RT-PCR.

DISCUSSION

Papya ringspot virus (PRSV) particles are flexuous filaments (760-800 nm x 12 nm) made of monomers of a single polypeptide species of about 36 KDa which encapsidate a single stranded translational-sense RNA molecule of approximately 10.300 bases (Yeh et al., 1992; Wang and Yeh, 1997). Ringspot and distortion of leaves and stunting produce deformed fruits with some ringspot were important symptoms for PRSV and also a range of other symptoms such as leaf mosaic and chlorosis, water-soaked oily streaks on the petiole and upper part of the trunk (Persley, 1999). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance; in both SAR and ISR, plant defenses are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by a pathogen or parasite (Gary and Goodman, 2004). Our results showed that prior treatment with the combined inoculation of GU 23-3 induced SAR protected cucumber plants against PRSV for more than 21 DPI. The protection conferred on the treated plants was manifested in the reduction of disease severity and PRSV accumulation in the leaves. In our study system, the GU 23-3 treatment were applied to the roots, while PRSV was inoculated in the leaves, and the treatments remained spatially separated on the plant. Therefore, the observed disease suppression may operate through activation of induced systemic resistance. As a result of induced systemic resistance, a reduction of disease severity and increased plant growth have been observed following CMV infection in many crops (Ryu et al., 2004; Ryu et al., 2007; Ipper et al., 2008; Wang et al., 2009 and Elsharkawy et al., 2012). Raghavendra et al. (2013) reported that controlling bacterial blight of cotton caused by *Xanthomonas campestris pv. malvacearum* (Xcm), *P. fluorescens, T. harzianum* and *B. subtilis* found that disease incidence was significantly reduced when compared to the control treated with water. Decreasing of virus symptoms by PGPR and PGPF application has been previously reported (Kloepper et al., 1980; Elsharkawy et al., 2012a, b and Elsharkawy et al., 2013). According to Murphy et al., 2003, PGPR can potentially induce ISR against CMV to reduce symptoms or reduce viral

accumulation in infected periwinkle plants. Based on the obtained data, the PGPF isolates were able to reduce virus infection by reducing PRSV disease symptoms and severity, which indicates the importance and involvement of ISR mechanism.

In the present study, we examined nine growth parameters to analyze growth promotion effect, shoot fresh and dry weights, dry material, leaf area, total green color, and early and total yield marketable and unmarketable fruit yield. Inoculation with GU 23-3 significantly increased the shoot biomass, leaf area, and total green color at 45 day after transplanting when compared to the control plants. Our results have shown that the growth parameters such as plant shoot fresh and dry weights, dry material and leaf area, were significantly decreased due to virus infection compared with control plants. Many studies have proposed the usage PGPF as an eco-friendly and sustainable tool to enhance the yield of different crop plants. Under greenhouse conditions, *P. fluorescens, T. harzianum* and *B. subtilis* treated seeds enhanced the seed germination and plant growth of cotton plants and improved growth attributes pertaining to stem girth, plant height, weight of the boll and number of bolls/plant when compared with control (Raghavendra et al., 2013). Additionally, the major noticeable effects of seed/ root inoculation with ACC deaminase- producing rhizobacteria are the plant root elongation, promotion of shoot growth, and enhancement in rhizobial nodulation and uptake of N, P and K as well as mycorrhizal colonization in various crops (Nadeem et al., 2007; Shaharoona et al., 2008; Nadeem et al., 2009). Arpana and Bagyaraj (2007) and Elsharkawy et al. (2012) showed that *Glomus mosseae* (Gm) and *Fusarium equiseti* GF18-3 inoculation, both individually and in combination, significantly increased the leaf area, number of leaves, plant height and dry weight of Kalmegh plants (*Andrographi paniculata*).

Result showed that cucumber plants treated with BGI of GU 23-3 exhibited a significant increase in early and total yield. In lettuce, strawberry, pea, and chickpea, crop yields were also increased significantly following the application of *Trichoderma* spp. (Bal and Altintas 2006; Elad et al., 2006; Hossain et al., 2013; Akhtar et al., 2015). In present study, PRSV was significantly decreased leaf area. Treatment of infected plant with BGI of GU23-3 significantly increased leaf area compared with untreated control. As a result to increasing the leaf area, the production will be increased and the use of chemical fertilizers will be reduced. A severe reduction in leaf areas due to virus infection was supported by number of different studies on different plants (Guo et al., 2005; El-Dougdoug et al., 2007; Mofunanya and Edu, 2015; Hooks et al., 2008 and Pazarlar et al., 2013). PGPF have been reported to produce substances such as plant hormones (Blanchard and Björkman, 1996), to allow plants to utilize decomposing organic matter through mineral solubilization (Altomare et al., 1999 and Harman et al., 2004), and to suppress plant pathogens in the rhizosphere by antagonistic mechanisms, such as the production of hydrolytic enzymes, aggressive mycoparasitism, competition for saprophytic colonization, and the induction of plant systemic resistance (Benítez et al., 1998; Benítez et al., 2004; Haran et al., 1996; Lorito, 1996; Meera et al., 1994 and Meera et al., 1995). Colonization of the roots is considered one of the most important characteristics of PGPF, and helps them to interact with plants to enhance growth and protection. The results showed that total green color (TGC) concentrations were significantly reduced in infected leaves compared with non-treated plant. Several studies have focused on interactions between different plants and virus combinations. *Yellow vein mosaic virus* (YVMV) effect on total chlorophyll (Chl) and carotenoids (Car) concentrations were significantly reduced in infected leaves by 64% and 62%, respectively compared with control plant (Palanisamy et al., 2009). Additionally, *Zucchini yellow mosaic virus* (ZYMV) infected leaves of pumpkin showed severe symptoms as mosaic, green blisters,

size reduction and deformation and the virus infection diminished the Chl a (48%), Chl b (53%) and carotenoid contents (52%). Treatments with GU 23-3 significantly decreased unmarketable fruits of cucumber plants and significantly increased marketable and reduced the non-marketable fruits, thus reducing the economic losses resulting from viral infection. Shivanna et al. (2005) showed that cucumber plants accumulated more inorganic minerals, such as Ca, Mg, and K, in their aerial shoots and cucumber plants increased productivity of marketable fruits due to increased levels of soil nutrients made available by PGPF and displayed better growth.

CONCLUSION

In conclusion, the data showed that treatment with the BGI of GU 23-3 enhanced the growth of cucumber plant and early and total yield of marketable fruits compared with control plants. Additionally, the disease severity and ELISA data demonstrated that treatment with the BGI or CF of GU 23-3 suppressed PRSV in cucumber plants. The RT-PCR analysis showed that the treatment with the GU 23-3 BGI or its CF stimulated the expression of defense genes resulting in resistance induction against PRSV.

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