



Higher growth induced upon *Potato Virus X* invasion in Messenger treated tomato plants

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Abstract: This investigation aimed to examine the possible role of foliar Messenger (a bio-product based on harpin protein) application in reducing the *Potato virus X* (PVX) development on tomato plants treated with 3 different doses of Messenger (M1: 0.5g/l, M2: 1g/l, M3: 0.25g/l). For that reason, seventy-two hours post treatment (hpt) a group of tomato plants was inoculated with PVX. Besides, another group of tomato plants were inoculated 24 hours afterwards to check the effect of plant age on PVX infection. Plants treated with M1 at 72h prior to the PVX inoculation showed the maximum recovery as an increase in chlorophyll content (25.78±0.67), plant height (31±4.58) cm and leaf area (361.07±76.15) cm². However, M1-enhancement in plant height (27±0.4) cm and leaf area (289.83±45.04) cm² of the non-inoculated tomato plants was lower than that in the inoculated ones. This indicated that M1 induction was most distinct after viral inoculation with higher growth of tomato plants than before introducing the virus. Furthermore, mature plant resistance against PVX was developed with as short plant age increase as to 24 hours.

Keywords: Messenger, harpin, plant height, chlorophyll content, leaf area

Messenger uygulanan domates bitkilerinde PVX bulaşıklığı sonucu bitki gelişiminin teşvik edilmesi

Öz: Bu araştırma, 3 farklı dozda (M1: 0.5g/L, M2: 1g/L, M3: 0.25g/L) Messenger (harpin proteine dayalı bir biyo-ürün) yaprak uygulamasının domates bitkilerinde Patates X virüsü (*Potato virus X*, PVX)'nün gelişimini azaltmada olası rolünü ortaya koymak amacıyla yapılmıştır. Bu nedenle, uygulama yapılan bir grup domates bitkisine uygulamadan yetmiş iki saat sonra PVX inokule edilmiştir. Bunun yanında, diğer bir grup domates bitkisi ise, bu uygulama zamanından 24 saat sonra bitki yaşının PVX enfeksiyonu üzerine etkisini kontrol etmek amacıyla inokule edilmiştir. PVX inokulasyondan 72 saat önce M1 uygulanan bitkilerin klorofil içeriğinde (25.78±0.67), bitki boyu (31±4.58) cm ve yaprak alanında (361.07±76.15) cm² artış meydana gelmiş ve bitkilerde maksimum toparlanma gözlenmiştir. Bununla birlikte, M1 uygulamasının, PVX aşılammamış domates bitkilerinin bitki boyu (27±0.4) cm ve yaprak alanı (289.83±45.04) cm² üzerindeki etkisi inokule edilen domates bitkilerinden daha düşük olmuştur. Bu da M1 aktivitesinin, domates bitkilerinin büyümesini virüs inokule edildikten sonra, virüs verilmeden önceye göre daha belirgin olarak etkilediğini ortaya koymuştur. Ayrıca, PVX'e karşı olgun bitki direnci, kısa zamanda bitki yaşı artışı ile birlikte 24 saat içerisinde gelişmiştir.

Anahtar Kelimeler: Messenger, harpin, bitki boyu, klorofil içeriği, yaprak alanı

1. Introduction

Improving the resistance of plant to the attack of a pathogen, such as a virus, is prominent and cheap. Various inducible systemic defenses may develop against further infections in plant parts distant from the site of primary infection, this kind of resistance is known as systemic acquired

resistance (SAR) and is considered, following the hypersensitive response (HR), to be the second phenotype of defense in most R-gene-mediated resistance responses. SAR can be triggered using some biotic and abiotic factors called elicitors; such as Messenger. The manufacture of that Messenger product (Eden Bioscience, Bothell,

WA, USA) is based on harpin protein and show efficacy in the field against range of pathogen infections based on induced resistance. Harpin was first isolated from the causative agent of fire blight of apple and pear; *Erwinia amylovora* (Wei et al., 1992), and its foliar application could effectively elicit resistance without developing HR response and enhance plant growth (Walters et al., 2007). It was also reported that harpin, as a heat stable protein involved in membrane transport, seems to mimic local lesion pathogens in the biological SAR systems and stimulate the accumulation of salicylic acid (Guest and Brown, 1997).

Potato virus X (PVX; the type member of the genus Potexvirus) is one of the most important models for studies of plant-virus interactions. Alone it is usually moderately pathogenic and to some extent causes symptomless infection (Lapierre and Signoret, 2004), however severe symptoms develop when PVX interacts synergistically with another unrelated virus (mainly *Potato virus Y* (PVY; the type member of the genus Potyvirus) in a mixed infection causing a significant economic loss (Vance et al., 1995). Many strains have been described by the means of host range, severity and type of symptoms produced, serological reactions and pH stability (Loebenstein et al., 2013). In its natural host, symptoms of PVX are always masked and most likely to be expressed during cooler temperatures or when the plant is also infected with other viruses (Kucharek et al., 2003). Occasionally yield losses of symptomless plants infected by

PVX may exceed 15 % when compared to virus-free plants (Strand, 2006). Although, in tomato it causes mild leaf mosaic and a slight growth reduction, PVX is considered to be of less economic importance to tomato production than other tomato viruses. Destruction of chloroplasts in the affected leaf areas by plant viruses reduces the photosynthetic rate and increase the susceptibility to photoinhibition (Ryšlavá et al., 2003). The decrease in photosynthetic rate of the infected leaves is often associated with development of the symptoms and loss of pigments that accompanied viral infections (Platt et al., 1979). Therefore, this study was worthwhile to demonstrate the change of chlorophyll content in symptomless infected plants and whether harpin eliciting SAR had a stimulation effect on chlorophyll formation and therewith plant growth.

2. Material and Methods

Plant Materials and Pathogen

The plant materials used in our greenhouse experiment included tomato plants (cultivar San Pedro) and the growing conditions consisted of a 16h light at 25°C and 8h dark at 18°C and Relative Humidity (RH) was kept 90-95 % with periodical irrigation by hand. The PVX (Spanish isolate SPCP1 strain) was maintained as lyophilized infected material. The overall plants were at 2-leaf stage (3-weeks old) when they were ready for the trial. Plants with similar size were chosen and sorted (as shown in table 1) into different groups (3 plants per group) :

Table 1. The experimental plant groups

Çizelge 1. Çalışmada kullanılan bitki grupları

Treatment	H ₂ O	M 1 (0.5g/l)	M2 (1g/l)	M3 (0.25g/l)
V0 (No virus)	(1) H ₂ O-Control	(2) Messenger only 2.a (M1V0) 2.b (M2V0) 2.c (M3V0)		
V (72hpt-PVX)	(3) Virus only	(4) Messenger + Virus 4.a (M1V) 4.b (M2V) 4.c (M3V)		

V0: no virus inoculation, V: virus inoculation at 72hpt (hour post treatment)

(1) Water control (0-control): Healthy plants treated with only water.

(2) Messenger only (M): The plants were treated only with Messenger and subdivided into three subgroups (M1V0, M2V0 and M3V0) on 3 different Messenger doses (M1: 0.5g/l, M2: 1g/l, M3: 0.25g/l).

(3) Virus only (V): Plants were only inoculated with PVX. It is subdivided for a further objective (Table 2) into two groups on plant age at 24h interval.

(4) Messenger + 72hpt-PVX (MV): That tested group of plants was divided into three subgroups (M1V, M2V and M3V) where they were treated with Messenger of 3 different doses and 72 hours post treatment (hpt) they were then inoculated with the PVX inoculum.

Exogenous application of Messenger

Except for water control, all other plants had their entire leaves sprayed once at 2-leaf stage with Messenger (%3 Harpin protein). The product was examined at three different doses M1: 0.5 g/l (the normal dose); M2: 1g/l and M3: 0.25g/l.

PVX inoculation and detection

Inoculum was prepared by macerating lyophilized PVX-infected leaves with a 1:4:w:v ratio of a neutral 0.1M phosphate buffer. The virus preparation was gently rubbed with carborundum onto the surface of the youngest leaves and the incidence of infected plants was detected after 2-3 weeks using DAS-ELISA test as described by Clark and Adams (1977). To test the effect of plant age on the PVX infection in tomato plants, two groups of plants with identical age were inoculated at 24h interval and monitored for the virus infection.

Assessment of aerial and physiological parameters

Symptoms were too weak to be scaled and the damage on all inoculated plants was assessed with measuring plant height (in cm) of the main plant stem and leaf area (in cm²) of each leaflet. Their leaf chlorophyll content was determined by using a SPAD (Soil Plant Analysis Development) meter

with an average of about fifteen records (unit-less SPAD values) for the whole plant.

Statistical analysis

Collected data of three plants per each treatment were analyzed as comparing their means by ANOVA at a significance level of $P \leq 0.05$. Means were calculated together with their associated standard deviation and when applicable, differences among groups were assessed applying the Tukey's post-hoc test. IBM SPSS was used for statistical analysis.

3. Results and Discussion

The PVX on Tomato Plants

The PVX infections in tomato plants significantly decreased the chlorophyll content to 66.25 %, plant height to 67.60 % and leaf areas to 45.41 % as compared to H₂O-control (Fig.1 and 2). These reductions were referred to the high demands on energy in the form of ATP in infected cells as result of the viral replication might induce alterations in the chlorophyll content and interfere with photochemical activity of infected leaves that disturbed the plant growth (Goncalves et al., 2005).

Low levels of chlorophyll content were reported in many virus infected plants (Hooks et al., 2008; Pazarlar et al., 2013; Goncalves et al., 2005; Rahman et al., 2008; Singh and Shukla, 2009; Song et al., 2009; Spoustová et al., 2013; Afreen et al., 2011 and Khalil et al., 2014).

A severe reduction in leaf areas due to virus infection was supported by number of different studies on different plants by Pazarlar et al. (2013), Hooks et al. (2008), Mofunanya and Edu (2015), El-Dougdoug et al. (2007) and Guo et al. (2005). They showed a significant decrease in leaf areas as compared to the control plants after the virus infection. In retrospect, Nandi and Raychaudhuri (1966) reported low growth rate caused by PVX infection on tomato plants. Furthermore, to about 65.62 % reduction in plant height was reported in PVX infected tomato plants (Balogun et al., 2002).

In our study, tomato plants showed distinct mature plant resistance against PVX and that was

clearly shown when the destructive/negative effects of the virus on the chlorophyll content, plant height and leaf areas were diminished on inoculating one group of plants 24h after the other ones. Plants inoculated at 26 days produced higher chlorophyll content (22.33 ± 1.53), plant height (15.4 ± 0.33 cm) and leaf areas

(255.55 ± 26.07 cm²) than those inoculated at 25 days after planting (Table 2, Fig.1).

This remarkable decrease of infection had left us a notice that mature plant resistance may have gradually launched/developed with as short plant age increase as to a one day.

Table 2. The effect of PVX different inoculating times on tomato plants.

Çizelge 2. Farklı zamanlarda yapılan PVX inokulasyonunun domates bitkileri üzerine etkisi

H2O Infection	Leaf area		Plant height		Chlorophyll content	
	Mean±Std. Dev.	%*	Mean±Std. Dev.	%*	Mean±Std. Dev.	%*
V0	373.43b±96.38	100	20.37c±0.70	100	26.67c±2.52	100
V1 (20 dai)	169.58a±28.26	45.41	13.77a±0.45	67.60	17.67a±0.58	66.25
V2 (19 dai)	255.55a,b±26.07	68.43	15.4b±0.33	75.60	22.33b±1.53	83.73

V0: no virus inoculation, V1: 1st virus inoculation (25 days old plants) , V2: 2nd virus inoculation (26 days old plants). * Ratios were expressed as a percentage of the water control mean value. The samples were collected at 20 days after the first inoculation (i.e. 19 days after the second inoculation). Mean values in each column followed by identical letters are not statistically different according to Tukey's Post Hoc Test ($p < 0.05$)

Bald (1937) was the first to suggest the mature plant resistance after he noticed the increased incubation period of TSWV in mature than in young tomato plants. Since then and further studies were reported on the occurrence of mature

plant resistance in virus infected plants (Schein, 1965; Crowley, 1967; Venekamp and Beemster, 1980; Wislocka, 1984; Sigvald, 1985; Beemster, 1987; Smit and Parlevliet, 1990; Gibson, 1991; Buiell and Parlevliet, 1996).



Figure 1. The effect of PVX different inoculating times on the average plant heights of tomato plants. V0: no virus inoculation, V1: 1st virus inoculation (25 days old plants) , V2: 2nd virus inoculation (26 days old plants).

Şekil 1. Farklı zamanlarda yapılan PVX inokulasyonunun domates bitkilerinde ortalama bitki boyuna etkisi. V0: virüs inokule edilmemiş, V1: 1. virus inokulasyonu (25 günlük bitkiler), V2: 2. virüs inokulasyonu (26 günlük bitkiler)

This type of resistance was often observed in both the resistant and the susceptible cultivars as well with much smaller infection in mature plant and plant tissues than the younger ones (Smit and Parlevliet, 1990). The rate of mature plant resistance increased with an increase of plant age to a few days and that resulted from the limitation

of the protein-synthesizing capacity in older leaves that could lead to sever declination in the virus multiplication or its translocation to younger leaves that would reduce plant fitness (Venekamp and Beemster, 1980; Buiell and Parlevliet, 1996).

The Messenger on Tomato Plants

The effects of the treatments of different doses of Messenger on the chlorophyll content, leaf areas and plant height of tomato plants after and

before the PVX virus inoculation were recorded. It showed that the dose M1 had slightly increased the chlorophyll content to 100.75 % of the corresponding water control plants (Table 3).

Table 3. The effect of Messenger doses on chlorophyll content of the 72hpt-PVX-inoculated tomato plants

Çizelge 3. Messenger dozlarının uygulamadan 72 saat sonra PVX inokule edilen domates bitkilerinde klorofil içeriğine etkisi

Product	Chlorophyll content (SPAD units)			
	Non-inoculated		Inoculated (72 hpt)	
	M.±S.D.	%*	M.±S.D.	%*
H ₂ O (n=3)	26.67a±2.52	100	17.67a±0.58	66.25
M1 (n=3)	26.87a±1.92	100.75	25.87b,c±0.67	97
M2 (n=3)	23.8a±0.79	89.24	24.63b±0.99	92.35
M3 (n=3)	25.47a±0.45	95.5	23.27b±0.29	87.25

n: the number of replicates, M.±S.D.: means ± standard deviation, hpt: hours post treatment, M1= 0.5g/l, M2= 1g/l, M3= 0.25g/l * Ratios were expressed as a percentage of the water control mean value. Mean values in each column followed by identical letters are not statistically different according to Tukey's Post Hoc Test (p < 0.05)

In addition, the doses of M1 and M2 were considered to have positive effects on the average plant heights with an increase in the ratios as

compared to the water control up to 132.55 % and 106.38 %, respectively (Table 4, Fig. 2).

Table 4. The effect of Messenger doses on the average plant height of the 72hpt-PVX-inoculated tomato plants

Çizelge 4. Messenger dozlarının uygulamadan 72 saat sonra PVX inokule edilen domates bitkilerinde ortalama bitki boyuna etkisi

	Plant height (cm)			
	Non-inoculated		Inoculated (72 hpt)	
	M.±S.D.	%*	M.±S.D.	%*
H ₂ O (n=3)	20.37b,c±0.70	100	13.77a±0.45	67.60
M1 (n=3)	27b,c,d±0.4	132.55	31b± 4.58	152.18
M2 (n=3)	21.67a,b±2.52	106.38	18.37a± 0.78	90.18
M3 (n=3)	19.2a±1.05	94.26	19.23a± 2.93	94.40

n: the number of replicates, M.±S.D.: means ± standard deviation, hpt: hours post treatment, M1= 0.5g/l, M2= 1g/l, M3= 0.25g/l * Ratios were expressed as a percentage of the water control mean value. Mean values in each column followed by identical letters are not statistically different according to Tukey's Post Hoc Test (p < 0.05)

Yet, there was no significant increase in the leaf areas due to any of the doses (Table 5). The application of M1 aided recovery from these pathological effects caused by PVX infection with an increase in the chlorophyll content, plant height and leaf area to 25.87±0.67, 31±4.58 cm and 361.07±76.15 cm², respectively (Table 3, 4 and 5, Fig.2).

However, M1-enhancement in plant height (27±0.4 cm) and leaf area (289.83±45.04 cm²) of the non-inoculated tomato plants was lower than that in the inoculated ones. This indicated that M1 induction was most distinct after viral inoculation with higher growth of tomato plants than before introducing the virus. Several studies are required to underline the experimental relationships

between the harpin inducing more growth of plants in the PVX-infected ones.

For our knowledge there was no previous work using the Messenger on the PVX-infection on tomato, however a number of studies have been made of SA application on PVX-infected plants. Naylor et al. (1998) reported a

salicylhydroxamic acid (SHAM)-mediated resistance in tomato plants with a decline in the PVX replication as a result of SA treatment. However, a recent study supported the involvement of alternative oxidase (AOX) in that SA-induced resistance (Rivas-San Vicente and Plasencia, 2011).



Figure 2. The effect of Messenger doses on the average plant height of the 72hpt-PVX-inoculated tomato plants. M1= 0.5g/l, M2= 1g/l, M3= 0.25g/l. V0: no virus inoculation, V: virus inoculation at 72hpt (hour post treatment).

Şekil 2. Messenger dozlarının uygulamadan 72 saat sonra PVX inokule edilen domates bitkilerinde ortalama bitki boyuna etkisi. M1= 0.5g/l, M2= 1g/l, M3= 0.25g/l. V0: Virüs inokule edilmeyen, V: Uygulamadan 72 saat sonra virüs inokule edilen.

Table 5. The effect of Messenger doses on leaf area of the 72hpt-PVX-inoculated tomato plants

Çizelge 5. Messenger dozlarının uygulamadan 72 saat sonra inokule edilen domates bitkilerinde yaprak alanına etkisi

Product	Leaf area (cm ²)			
	Non-inoculated		Inoculated (72 hpt)	
	M.±S.D.	%*	M.±S.D.	%*
H ₂ O (n=3)	373.43a±96.38	100	169.58a±28.26	45.41
M1 (n=3)	289.83a±45.04	77.61	361.07b±76.15	96.69
M2 (n=3)	258.44a±56.54	69.21	217.32a±24.85	58.2
M3 (n=3)	240.74a±20.16	64.47	199.59a±29.76	53.45

n: the number of replicates, M.±S.D.: means ± standard deviation, hpt: hours post treatment, M1= 0.5g/l, M2= 1g/l, M3= 0.25g/l * Ratios were expressed as a percentage of the water control mean value. Mean values in each column followed by identical letters are not statistically different according to Tukey's Post Hoc Test (p < 0.05)

4. Conclusion

PVX decreases plant height, leaf areas and chlorophyll content of the tomato plants causing lower infection with plant age increase up to 24h. Messenger as a harpin based product activates the growth of tomato plants and induces resistance mechanism to the virus infection. Applying the normal dose of Messenger (M1:0.5g/l) seventytwo hours after the PVX inoculation aided to recovery of the plants to grow faster than the untreated ones or before even introducing the

virus. That shows the distinct induction of the Messenger eliciting SAR in plants to the virus invasion. The molecular mechanism of the Messenger to launch SAR in plants shall be furtherly studied.

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