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Differential expression of CsWRKY genes reduced damage in Soy protein hydrolysate-treated cucumber plants infected with *Fusarium oxysporum*

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ABSTRACT

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The biostimulant activity of soy protein hydrolysate on *Fusarium oxysporum*-inoculated cucumber plants was investigated in comparison with salicylic acid (SA). Cucumber seedlings were treated with trypsin-digested soybean (PrH) and SA followed by *F. oxysporum* inoculation, and were assessed for gene expression pattern, disease incidence (DI%), growth rate and biochemical responses. Results showed that *F. oxysporum* infection in PrH-treated plants decreased shoot and root dry weights by 4 and 18.2%, respectively, while these parameters were decreased 45 and 66.5% in SA-treated, and 42 and 65.9% in control plants. Glutathione peroxidase (GPx) activity was decreased in PrH-treated plants upon infection with higher rate compared to control plants while it was increased in SA-treated plants. Gene expression analysis revealed that, compared to other treatments, CsWRKY2 was expressed earlier and in higher rate in PrH-treated plants, and was negatively correlated with disease incidence leading to lowest disease infection (11.3 %) among treatments. These results suggest that PrH activates defense responses in cucumber plants against infection at the expense of reduced plant growth. Although the increase in CsWRKY2 expression enhances plant defense, but its over-expression higher than a threshold will negatively affect plant growth. By contrast, CsWRKY35 expression was negatively correlated with plant growth and its resistance against pathogen. The findings of the present study may pave the road for exploration of *WRKY* genes in cucumber breeding programs.

Key words: Plant immune system, *WRKY* genes, defense response, cucumber, fusarium wilt

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is the most important vegetable crop in Cucurbitaceae family which is widely cultivated in temperate regions of the world (Jayaraman *et al*., 2011). Several fungal diseases infect cucumber cultures in humid regions leading to huge losses and not only increase production costs but also reduce the fruit quality (Lebeda *et al*., 2007). Cucumber fusarium wilt disease caused by *Fusarium oxysporum* f. sp. radicis cucumerinum (FOR) is one of the most important and devastating diseases which specially affects greenhouse cultured cucumber plants (Liu *et al*., 2004; Yang *et al.,* 2007). As a soil-borne disease that occurs at all growing stages of cucumber, FOR reduces the quality and quantity of the final product (Li *et al.,* 2009).

A wide variety of control measures are being used to manage this disease, e.g. resistant varieties, grafting onto resistant rootstocks, fungicide application, soil fumigation, biological control and exploitation of transgenic plants (Neycee *et al*., 2012a, Zhang *et al.*, 2008; Dehestani *et al*., 2009; Dolatabadi *et al*., 2014). Plants have evolved complicated defense strategies to survive in environments with harsh biotic/abiotic stresses (Keramati *et al*., 2016; Barzegargolchini *et al*., 2017; Ghorbanpour *et al*., 2018). Application of plant innate immune system is one the most sophisticated strategies which has gained much attention during the past decade. To activate immune system of plants prior to disease invasion, it is necessary to identify or develop special inducer(s) and then investigate their effects (Caillot *et al.*, 2012).

Plants can detect invading organisms through sensing a wide array of pathogen-derived molecular patterns and activate their immune system via specialized signaling pathways. The activated defense system in turn leads to enhanced production of defense-related biochemicals which can alleviate pathogen damages (Romeis, 2001; van Loon *et al.*, 2006). Artificial induction of plant immune systems has been at the center of several investigations as an alternative measure to control pathogens (Zhang *et al*., 2008, Mofidnakhaei *et al*., 2016). There are several reports of successful application of biological and/or chemical agents for enhancing plant defense responses before pathogen invasion and various plant strengthener formulations have been commercialized (Lachhab *et al*., 2015; Ertani *et al*., 2013; Ertani *et al*., 2014; Kondo *et al*., 2006).

Resistance elicitors or inducers are substances from different sources (Aziz *et al.*, 2004; Ramezani *et al*., 2018) that elicit defense responses in the host at the early phase of interaction between plant and pathogen. These early defense responses include several signaling pathways as well as reactive oxygen species (ROS) accumulation which eventually leads to enhanced production of defense enzymes and metabolic modifications (Ramezani *et al.,* 2017a; Moradi *et al.*, 2016; Sanzani *et al*., 2010).

Recent studies have revealed transcript level of many genes including signal transducers and transcription factors, pathogenesis-related proteins and some regulatory genes are significantly altered during plant defense induction (Ramezani *et al.* 2017b; Dehestani *et al*., 2009; Eulgem, 2005). These changes greatly depend on defense-related hormones such as SA or jasmonic acid (JA) (Sato *et al*., 2010; Tsuda *et al*., 2009).

Salicylic acid (SA) is a phenolic compound and the most investigated natural plant defense hormone that plays critical role in plant physiological processes including biotic stresses resistance (Klessig and Malamy, 1994; Morris *et al*., 2000; Stacey *et al*., 2006). It has been well documented that increased levels of the SA in plant tissues, e.g. via exogenous SA application, can improve plant immunity against biotrophic and hemibiotrophic pathogens (Glazebrook, 2005). These efforts have revealed SA and its derivative have important roles in signal transduction for activating local resistance and systemic acquired resistance (SAR) disease resistance. As a general rule of thumb, the earlier the plant detects pathogen and activates defense responses through transcription factors, the lower is the damage of pathogen.

Proteins isolated from natural sources exhibit some special properties, e.g. nutritional value, free radical-scavenging, antioxidant, antibacterial and antifungal activity, and are used for various agricultural purposes (Chen and Qiu, 2003; Fu, 2003; Zhao *et al*., 1997; Neycee *et al.*, 2012b). Chemical or enzymatic hydrolysis of the protein sources endows them with some new improved characteristics making

them more appropriate for various applications (Chen and Qiu, 2003). The mixture derived from enzymatic hydrolysis of proteins comprise short peptides and free amino acids (monomer and simple oligomers) with enhanced beneficial properties and/or reduced disadvantages of intact proteins (Matsumiya and Kubo, 2011; Lamsal *et al.*, 2007).

Although protein hydrolysates from different sources have been used as plant defense inducers, the exact mode of action and the underlying biological processes have not as yet been understood. A better understanding of the mechanisms by which protein hydrolysate alleviates the pathogen damage would pave the road for its sophisticated application as plant strengthener. The main objective of the present study was to evaluate the effects of soy meal protein hydrolysate (PrH) on the genetic and physiologic responses of cucumber plants against *F. oxysporum*. Salicylic acid (SA) was included in the study as a comparable resistance inducer to achieve a better understanding of the controlling efficiency of PrH in a comparative study.

MATERIALS AND METHODS

Plant material and growth conditions

The cucumber cultivar "Sultan" which is susceptible to Fusarium wilt disease (Molavi *et al.*, 2009) was used in this study. Eighteen seeds used for each treatment were sterilized with 2.5% NaClO and sown individually in eight centimeters diameter plastic pots (containing equal volumes of sterilized perlite and coco peat), which totally occupied an area about 50 by 70 cm^2, and were grown under controlled environment**.** Pots were placed in a greenhouse at 25 ± 2°C/20 ± 2°C (day/night), with a photoperiod of 16 h light/8 h dark, and a relative of humidity (RH) of 60% ±10%. Pots were arranged as randomized complete block design with three replications, where each replication consisting three pots inoculated in a single infection event. Three plants used for sampling at each time course, and were used as independent replications for molecular and biochemical analyses. The rest of plants were used for measuring growth parameters and disease severity assays.

Pathogen growth and inoculation

F. oxysporum f. sp. radicis-cucumerinum (FOR) was used for plant inoculation (Vakalounakis *et al*., 2004; Molavi *et al.*, 2009). This isolate was obtained from Agriculture and Natural Resources Research and Education Center of Tehran Province, Varamin, Iran (Sharyari, personal communication). It was previously isolated and purified and its pathogenesis and aggressiveness were evaluated as moderate (Molavi *et al*., 2009). Conidial suspension of this pathogen was prepared as described by Mandeel and Baker (1991) with minor modifications. FOR isolate was first cultured on potato dextrose agar medium (PDA) in eight centimeter Petri dishes at 25°C in the dark for 10 days.

To prepare conidial suspension, plates were flooded with sterile distilled water and gently scraped with spatula. Then, the conidia were filtered from mycelia fragments through three layers of sterile wound dressing and the final concentration was adjusted to 10^6 conidia ml^{-1} using a hemocytometer to create the same condition for all treatments. Furthermore, at this concentration, the disease severity is very similar to natural conditions (Molavi *et al*., 2009; Mandeel and Baker, 1991). Plants were inoculated with FOR using dipping method (Vakalounakis *et al.*, 2004). Both inoculated and control seedlings were replanted back into their original pots and incubated at above-mentioned conditions. To confirm absence of any contamination in the used Fusarium isolate, the pathogen was again isolated from infected plants after sample collection and was evaluated in plant protection

laboratory.

Plant treatment with resistance inducers and sample collection

Soy protein was isolated and hydrolyzed and Salicylic acid (SA) obtained from Sigma Co. Ltd. Three days before pathogen inoculation, each seedling was sprayed with 15 ml of 3, 5, and 7 mM of SA (dissolved in 10% ethanol) or PrH (2% w/v dissolved in sterile distilled water with pH=7). We had previously evaluated several PrH concentrations following similar studies conducted on other plant species (Colla *et al*., 2014; Colla *et al*., 2017) and selected 2% as the optimum dose. Control plants were sprayed with 10% ethanol. For biochemical and molecular analysis, three cucumber seedlings were harvested as independent replications and their leaves and roots were collected separately at 0, 6, 12, 24, 36, 72, and 168 hours post-inoculation (hpi). Samples were immediately frozen in liquid nitrogen and stored at -80°C.

Glutathione Peroxidase (GPx) Activity

The decrease of NADPH was measured at 340 nm at 0 and 4 minutes (Hopkins and Tudho, 1973). Briefly, 0.1 ml of enzyme extract in 100 mM phosphate buffer was diluted with 2.58 ml of 0.15M phosphate buffer (pH 7.0). Then, the following mixture was added: 0.1 ml of 0.0084 M-NADPH, 0.005 ml of GSSG-R (yeast preparation containing 200 e.u. per ml (Sigma Co. Ltd), 0.01 ml of 1.125 M NaN3, and 0.1 ml of 0.15 M GSH (Glutathione). The reagents were incubated at ambient condition for 5 min. Then, 0.1 ml of 0.0022 M H_2O_2 was added to initiate the reaction. The absorbance was measured at 340 nm every minute up to 5 minutes.

Gene expression analysis

The expression profiles of two defense related transcription factors including CsWRKY2 and CsWRKY35 were assessed through Real-time PCR analysis. Gene-specific primers for CsWRKY2 and CsWRKY35 were designed using Oligo software (ver. 5) and the actin gene was used as control (Table 1). Total RNA from control and treated plants was isolated using RNX- Plus solution (CinnaGen Inc, Iran) and were then treated with DNase I. The first-strand cDNA was synthesized using RevrtAid reverse transcriptase kit (Thermo Scientific, USA) according to manufacturer`s instructions.

Table 1. Primer sequences of the WRKY genes used for cucumber plants. Actin gene was used as control for qPCR analysis.

Primer Name	Oligo Sequence 5'--> 3'	Primer length (bp)
CsWRKY2 F:	GGGGAATTGCGGTCTGACTA	20
CsWRKY2 R:	TCTCCGTGATCTGTCCATCTA	21
CsWRKY11 F:	GAAAACCAGAAAACGAAAAG	20
CsWRKY11 R:	GGAATCATTATCATCGCTAC	20
CsWRKY35 F:	CAGAGGCTATTTCAGATGCA	20
CsWRKY35 R:	GGTAGGTGACTTCAAACGTG	20
Actin (AB010922) F:	GGCAGTGGTGGTGAACAT	18
Actin (AB010922) R:	CTGGTATCGTGCTGGATT	18

The real-time PCR reactions were performed using Maxima SYBR Green/ROX qPCR Master Mix kit (Thermo Scientific, USA) in a Bio-Rad CFX-96 instrument (Bio-Rad, USA). Real time RT-PCR reactions were carried out under the following program: 95°C for 15 min, 15 S of denaturation, 30 S of annealing and extension at 61 °C for 40 cycles. Melt curve analysis was performed to check the specificity of the amplified product. Relative fold expression of the genes was analyzed using $2^{-\Delta\Delta CT}$ method using Bio-

Rad CFX Manager software (BioRad, USA).

Growth charcteristics and disease evaluation

Growth factors including biomass (shoot and root weights) 15 days after inoculation. Plant samples were oven dried at 65ºC for 24 hours and then weighed to measure dry weight. Disease incidence was estimated as percentage of symptomatic plants to total plants 15 days after pathogen inoculation. The incidence of Fusarium wilt disease was recorded by observation of visual symptoms of the disease such as wilting, rot and death of the plant (Kareem *et al.*, 2016).

Statistical analysis

Analysis of variance (ANOVA) was performed and Dunnett's multiple comparison test employed for mean comparisons using Statistix 8 software.

RESULTS AND DISCUSSION

Shoot Dry Weight (SDW)

PrH treatment did not significantly change SDW (only +1.8%) whereas SA treatment significantly increased SDW by 69.6% compared to control plants (Fig. 1a). Fusarium inoculation in PrH-treated plants did not change SDW significantly, but significantly reduced SDW in SA-treated and Fusarium-inoculated plants (by -45% and -42%, respectively) compared to control plants (Fig. 1c).

Root Dry Weight (RDW)

Salicylic acid (SA) treatment significantly increased RDW by 79.5% while PrH treatment reduced it by 37.5% (Fig. 1b). Pairwise comparisons of each treatment for this trait compared to its corresponding control showed that Fusarium inoculation in PrH^+Fu^+ plants reduced RDW by 18.2%, which was lower than those for SA^+Fu^+ and CFu^+ (66.5% and 65.9%, respectively) (Fig. 1d). Thus in contrast to SA^+ treatment, PrH^+ treatment reduced or did not change plant growth characteristics.

Many researchers have studied the effects of PrH on plant metabolism and physiology as well as the stress tolerance and reported that PrH from different sources not only did not exhibit any negative effects but also their application at low concentrations improved plant performance including increase in shoot and root biomass (Ertani *et al*., 2014; Colla *et al*., 2014). However, there are several reports indicating phytotoxicity and reduced growth following foliar application of animal-derived PrHs (Cerdan *et al*., 2009). These detrimental effects can be attributed to high salinity (Colla *et al*., 2014), special micronutrients, composition and concentration of amino acids (Oaks *et al*., 1977). Also, researches have showed that exogenous application of SA stimulated growth and physiological responses in several plant species under normal and stressed conditions (Najafian *et al.*, 2009; Gutierrez-Coronado *et al*., 1998).

Glutathione Peroxidase (GPx) Activity Assay

Glutathione Peroxidase (GPx) activity generally decreased in all treatments, with different rates, during the 7-days period. GPx activity was increased by PrH treatment for a short time, but was decreased with SA treatment. Therefore, maximum GPx activity was observed in PrH^+ treatment 36 hpi and then decreased whereas SA^+ had maximum GPx activity at 12 hpi. GPx activity in all treatments was at the same level at the end of 7-days period (Fig. 2a).

Fusarium inoculation in control plants (CFu^+) significantly reduced GPx activity up to 36 hpi and then significantly increased it at 96 hpi and thereafter (Table 2). This situation with higher level was also observed in PrH^+Fu^+ and SA^+Fu^+ treatment and following Fusarium inoculation. The highest GPx activity was observed in SA^+Fu^+ and PrH^+Fu^+ treatments, respectively (Fig. 2b; Table 2).

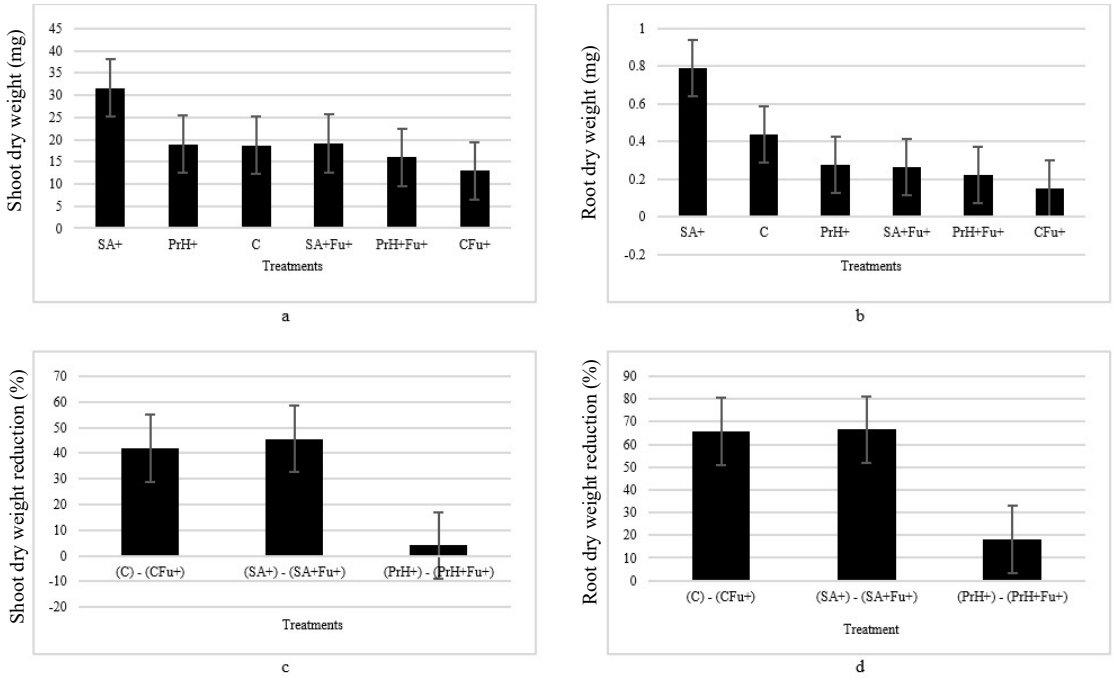

Fig. 1. Variation in: a) shoot dry weight (SDW), b) root dry weight (RDW), c) shoot dry weight (SDW) reduction (%) after *Fusarium oxysporum* inoculation in each treatment and its corresponding control, d) root dry weight (RDW) reduction (%) after *F. oxysporum* inoculation in each treatment compared to control. SA^+: three mM salicylic acid, PrH^+: soy protein hydrolysate, C: treatment against control; Fu: *F. oxysporum*

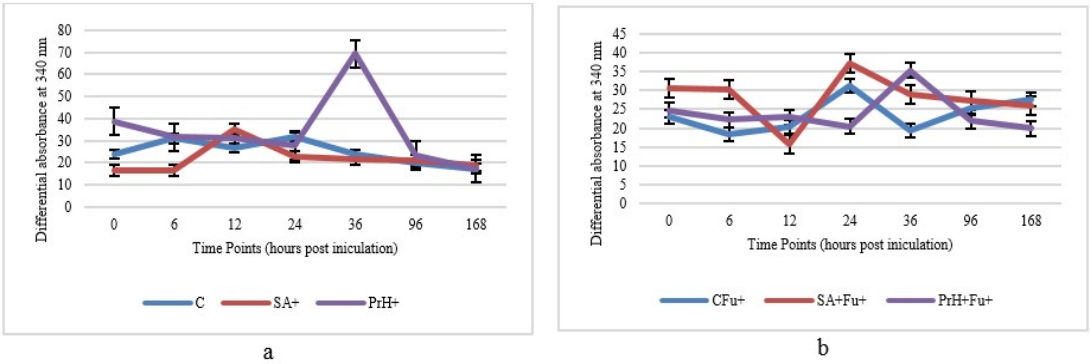

Fig. 2. Variation in glutathione peroxidase (GPx) activity in cucumber plants treated with three mM salicylic acid (SA^+) and soy protein hydrolysate (PrH^+) in comparison with control plants (C) a) non-inoculated with *Fusarium oxysporum*, b) inoculated with *F. oxysporum* (Fu^+).

Glutathione peroxidase (GPx) is a member of primary antioxidant enzymes required for life in all oxygen metabolizing cells (Cesaratto *et al.*, 2004) and plays an important role in repairing damages induced by ROS and protection of cells against oxidative damage (Liu *et al.*, 2018; Singh and Shichi, 1998). Expression levels of GPx determine intracellular concentrations of ROS and also their biological properties. It has been reported that a significant increase in GPx levels was observed in cucumber plants inoculated with *F. oxysporum*, hence, there was a direct correlation between GPx levels and disease tolerance (Heidarzade *et al*., 2018).

Paiva *et al.* (2018), using *OsGPX3*-RNAi silenced rice plants (GPX3s), showed GPX3s plants were more sensitive to salinity. A resistant finger millet (*Eleusine*

Table 2. Changes in glutathione peroxidase (GPx) activity at different time points in cucumber plants treated with three mM salicylic acid (SA+) and soy protein hydrolysate (PrH+) in comparison with control plants (C) either inoculated (Fu+) or non-inoculated with *Fusarium oxysporum* (Fu).

	Time Points (hours post inoculation)						
Treatment	**0**	**6**	**12**	**24**	**36**	**96**	**168**
PrH+	**38.71**	**31.50**	**31.29**	**27.57**	**69.29**	**23.14**	**17.29**
PrH+Fu+	**24.71**	**22.29**	**22.86**	**20.43**	**35.29**	**21.86**	**20.00**
SA3	**16.29**	**16.57**	**35.14**	**22.71**	**21.40**	**21.20**	**18.60**
SA3Fu+	**30.57**	**30.29**	**15.57**	**37.14**	**29.00**	**27.43**	**25.86**
C	**23.86**	**30.86**	**26.86**	**31.57**	**23.71**	**19.71**	**17.29**
CFu+	**22.86**	**18.29**	**20.29**	**31.14**	**19.29**	**25.29**	**27.71**

coracana (L.) Gaertn.) genotype, the trend of expression of GPx was descending but it was ascending in a susceptible genotype from 48 hpi to 96 hpi (Jacob *et al.*, 2019). Khosravi *et al*. (2017) also reported a positive correlation between *gpx* gene expression and resistance to *Phytophthora capsici* in cucumber plants.

Gene expression analysis

Expression level of CsWRKY2 was gradually increased up to 10.4 folds in PrH^- treated plants at 12 hpi and then was decreased during the experiment (Fig. 3a). This increase was associated with significant decrease in root growth. After Fusarium inoculation, the expression level of CsWRKY2 gene increased in control plants (CFu^+) and with higher level in SA^+Fu^+ treatment (Fig. 3b). In contrast, Fusarium inoculation decreased the expression level of CsWRKY2 gene in PrH^+Fu^+ treatment, but still it was the highest expression level among all treatments (Table 3). Xu *et al*. (2015) found that CsWRKY2 gene expression was increased against cold, drought and salinity stresses. It was also revealed that expression of CsWRKY2 was significantly increased 24 hours after SA treatment which indicated its important role in SA induced defense responses.

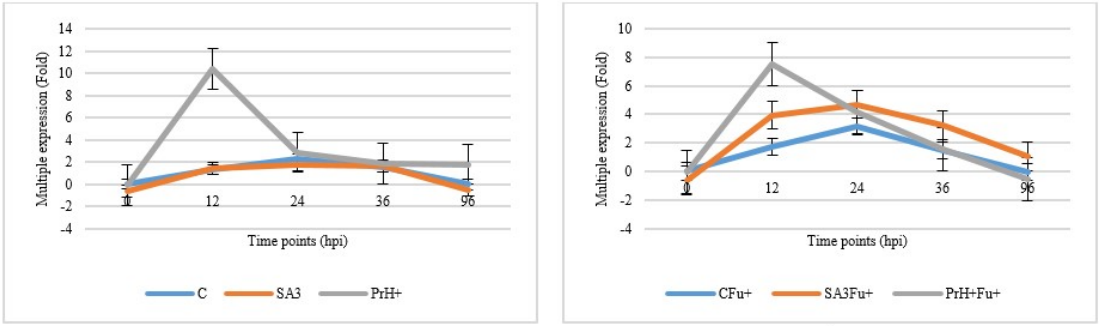

Fig. 3. Variation in expression (as multiple expression, fold) of CsWRKY2 gene in cucumber plants treated with three mM salicylic acid (SA^+) or soy protein hydrolysate (PrH^+) in comparison with control plants (C)

Expression analysis demonstrated that CsWRKY35 gene in control (C) plants increased up to seven fold at 24 hpi and then decreased (Fig. 4a). Also, this change pattern was observed in PrH-treated plants but maximum expression occurred earlier (12 hpi). Interestingly, SA treatment decreased the expression level and postponed its expression enhancement (96 hpi) (Fig. 4b). Infection with

Table 3. Changes in expression (as fold increase) of CsWRKY2 gene in *Fusarium oxysporum* (Fu)-inoculated cucumber plants treated with three mM salicylic acid (SA^+) and soy protein hydrolysate (PrH^+) in comparison with control plants (C).

	Time Points (hpi)				
Treatment	**0**	**12**	**24**	**36**	**96**
C	**1.00**	**1.32**	**2.29**	**1.55**	**-0.05**
CFu+	**1.00**	**1.74**	**3.15**	**1.48**	**-0.02**
SA+	**-0.63**	**1.45**	**1.77**	**1.63**	**-0.53**
SA+Fu+	**-0.63**	**3.93**	**4.69**	**3.22**	**1.03**
PrH+	**-0.07**	**10.37**	**2.86**	**1.82**	**1.77**
PrH+Fu+	**-0.07**	**7.54**	**4.18**	**1.57**	**-0.55**

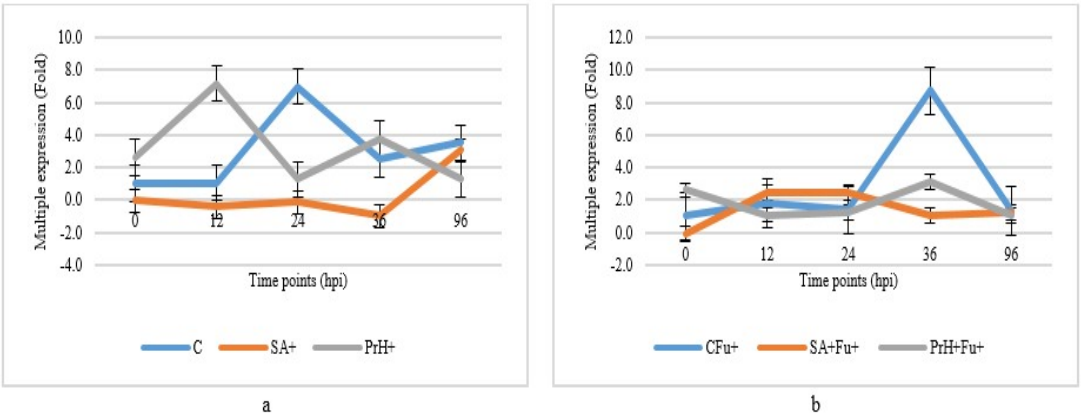

Fig. 4. Variation in expression (as multiple expression, fold) of CsWRKY35 gene in cucumber plant treated with three mM salicylic acid (SA^+) or soy protein hydrolysate (PrH^+) in comparison with control plants (C)

F. oxysporum induced an 8.7-fold increase in expression of CsWRKY35 gene in control (CFu^+) plants while this increase was observed with a lower rate in SA^+Fu^+ plants. By contrast, expression rate of this gene was decreased in PrH^+Fu^+ plants upon infection with pathogen. As a general result, expression of WRKY35 was higher in control (CFu^+) plants compared with all other treatments (Table 4). Xu *et al*. (2015) reported that *Phytophthora melonis* infection and SA treatment increased expression rates of WRKY35 in cucumber plants and determined its involvement in plant defense pathways.

WRKY transcription factors are a group of regulatory proteins which are involved in several biological processes in plants. Although they are important for their role in plant interaction with biotic/abiotic stresses, they are involved in almost all plant metabolic processes (Ding *et al.*, 2015; Liu *et al*., 2015; Li *et al*., 2015; Eulgem and Somssich, 2007). Our results of the expression analysis of CsWRKY2 and CsWRKY35 also indicated that they are actively involved in SA-related immune responses against *Fusarium oxysporum* infection in cucumber plants.

Disease Incidence

Diseases infection (DI) of 11.3% was recorded for PrH+Fu+ plants (Fig. 5). This value was 33.7% in SA+Fu+ plants which was not significantly different from control plants (CFu+, 36.4%). Based on these results, PrH reduced the disease damage significantly and the shoot and root wet weights as well as their dry weights were not significantly reduced by the pathogen

Table 4. Changes in expression (as fold increase) of CsWRKY35 gene in *Fusarium oxysporum* (Fu)-inoculated cucumber plants treated with three mM salicylic acid (SA^+) and soy protein hydrolysate (PrH^+) in comparison with control plants (C).

	Time points (hpi)				
Treatment	**0**	**12**	**24**	**36**	**96**
C	**1.0**	**1.0**	**7.0**	**2.5**	**3.5**
CFu+	**1.0**	**1.8**	**1.4**	**8.7**	**1.3**
SA+	**0.0**	**-0.4**	**-0.1**	**-1.0**	**3.1**
SA+Fu+	**-0.2**	**2.5**	**2.4**	**1.8**	**3.4**
PrH+	**2.6**	**7.2**	**1.3**	**3.7**	**1.3**
PrH+Fu+	**2.6**	**1.1**	**1.2**	**3.1**	**1.0**

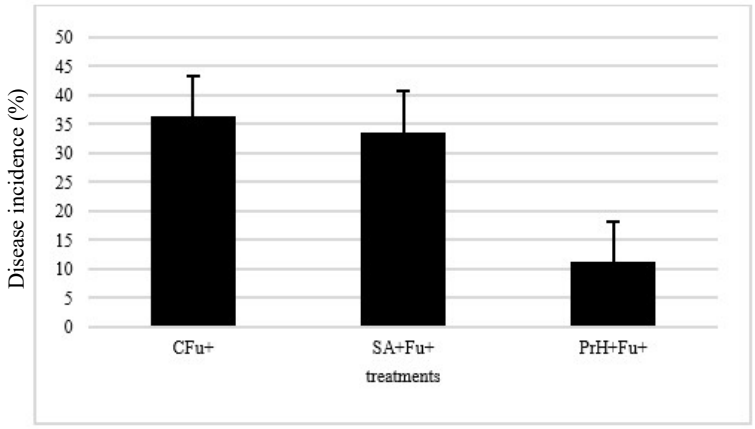

Fig. 5. Mean of disease incidence on cucumber plants inoculated with *Fusarium oxysporum* (Fu^+) in three mM salicylic acid (SA^+) and soy protein hydrolysate (PrH^+) treatments compared to control plants (C)

attack. Lachhab *et al.* (2015) reported that application of PrH significantly reduced the incidence of decay resulted from green mold diseases of citrus fruits caused by *Penicillium digitatum*.

Huffakera *et al.* (2013) demonstrated that a peptide from maize plant, called ZmPep3, acts as an elicitor, stimulated genes that contribute to metabolite production, and regulated pathogen defense responses. On the other hand, SA treatment was not efficient in reducing the disease incidence and plant root and shoot mass production was considerably reduced (Fig. 1). Our results are in contrast with most of the previous reports indicating a mitigating role for SA in plants infected with fungal pathogens. Metraux *et al.* (1990) reported that increased levels of SA significantly reduced the disease damage in cucumber plants. Qi *et al.* (2012) reported that SA application inhibited *Fusarium graminearum* growth in wheat tissue and significantly reduced the disease damage.

CONCLUSION

The resistance inducing activity of soybean protein hydrolysate (PrH) was evaluated in comparison with SA-treated and control cucumber plants. Pre-treatment of plants with PrH affected various plant characteristics including growth parameters and biochemical properties with a special rate and trend leading to higher Fusarium resistance (expressed as DI%) in cucumber plants. Our results revealed that reduction of shoot and root weights due to Fusarium inoculation in PrH-treated plants were significantly lower than those observed in the control and SA-treated plants. In addition, disease incidence probability

(11.3 %) in this treatment (PrH^+Fu^+) was lower than that in other treatments.

Furthermore, increase in Fusarium resistance was associated with alterations in cucumber's biochemical properties. In general, highest GPx activity was observed in PrH^+ treatment that was associated with highest reduction in plant growth Also, after Fusarium inoculation highest reduction in GPx activity was observed in PrH^+Fu^+ and resulted in equal GPx activity in PrH^+Fu^+ and SA^+Fu^+ treatments that were higher than control (CFu^+). This observation was associated with the lowest reduction in growth rate after Fusarium inoculation in PrH^+Fu^+ treatment than others. Thus, increase in GPx activity up to a given threshold probably improved plant resistance to pathogen, but its higher levels reduce plant growth.

Furthermore, Gene expression analysis revealed that PrH^+ treatment intensely induced CsWRKY2 after and before Fusarium inoculation that was associated with lower growth losses and DI%. Thus, this gene is probably involved in immune responses against *Fusarium oxysporum* infection in cucumber plants leading to reduced pathogen damage. Also, expression of CsWRKY35 in PrH^+Fu^+ and SA^+Fu^+ was highly significant but lower than control (CFu^+). Therefore, increase in CsWRKY35 expression up to a given threshold may contribute to Fusarium resistance but over expression may negatively affect plant growth and pathogen resistance. This gene was over expressed in PrH^+ treatment earlier than other treatments. These findings would increase our knowledge of the behavior of transcription factors upon pathogen attack which eventually can be used for exploration in cucumber breeding program for fusarium wilt disease resistance.

AUTHOR CONTRIBUTION

MZ performed research, analyzed data, and wrote the manuscript. AD, GN, and KK designed the research and interpreted the data. All authors read and approved the final manuscript

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