J. Agr. Sci. Tech. (2018) Vol. 20: 571-582

Effects of *Xanthomonas citri* subsp. *citri* Infection on Chlorophyll Pigment Content, Chlorophyll Fluorescence and Proteins Change in *Citrus aurantifolia*

A. Rasoulnia[1], S. M. Alavi[1]*, H. Askari[2], N. Farrokhi[2], and M. Soltani Najafabadi[3]

ABSTRACT

Citrus canker has worldwide distribution and is still a devastating disease caused by the bacteria *Xanthomonas citri* subsp. *citri* (Xcc). With the aim of evaluating *C. aurantifolia* response mechanism, plant leaves non-inoculated and inoculated with the bacteria were collected in 1, 4, and 7 days post-inoculation. Consequently, the chlorophyll pigment content and fluorescence were determined, and proteomics study was conducted. Results indicated that pathogen infection, despite the negative effect on chlorophyll pigment content, improved the physiological condition. The maximum efficiency of PSII photochemistry and PSII quantum Yield (YPSII) as well as photochemical quenching increase were observed in infected plants compared to the control, whilst non-photochemical quantum decreased during infection. Judging by the results, the proteomic analysis revealed that these responses were mirrored by rapid changes in the host proteome that included the up-regulation of carbohydrate metabolism proteins and down-regulation of the ATP generating proteins during pathogen infection. The results indicated that the pathogen manipulates the host homeostasis by its effector proteins to exploit in its favor.

Keywords: Citrus canker, Homeostasis, Pathogen, Proteomics.

INTRODUCTION

Citrus bacterial canker, caused by *Xanthomonas citri* subsp. *citri* (Xcc), affects almost all citrus species and cultivars(Ryan *et al.*, 2011). The subspecies appear as several pathotypes, among which, Type A* (Bock *et al.*, 2010), also known as Asiatic citrus canker, has shown to be the most widespread and severe pathotype (Garavaglia *et al.*, 2010).

Investigation of plant-pathogen interaction shows pathogens aim to overcome host defense responses, while plants employ a battery of responses to limit pathogen growth and disease development. Consequently, plant induces a defense mechanism which demands a reallocation of energy towards the defense response and this apportioning of energy for defense processes lead to the modification of primary metabolism (Bolton, 2009). As a result, the down-regulation of sugar-regulated photosynthetic genes is preceded by an accumulation of hexoses, probably due to an increase in invertase activity triggering a signaling pathway (Kocal *et al.*, 2008). Accumulation of carbohydrate represses photosynthesis through feedback inhibition of sucrose synthesis, decreased stromal availability of orthophosphate,

[1] Department of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Islamic Republic of Iran.
*Corresponding author; e-mail: mealavi@nigeb.ac.ir
[2] Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University G. C., Evin, Tehran, Islamic Republic of Iran.
[3] Oil Seeds Research Department, Seed and Plant Improvement Institute, Karaj, Islamic Republic of Iran.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

physical hindrance of CO_2 diffusion, and also physically disrupts thylakoid structure and decompartmentalizes photosynthetic membranes (Araya *et al.*, 2010). Furthermore, pathogen infection often leads to the development of chlorotic and necrotic areas and to a decrease in photosynthetic assimilate production (Berger *et al.*, 2007). The effect on photosynthesis can be analyzed by monitoring in vivo chlorophyll fluorescence, which is a very sensitive marker for the efficiency of photosynthesis and also responds to the changes in energy conversion at photosystem II reaction centers and detects any limitations in the dark enzymatic steps of the complex process of photosynthesis (Berger *et al.*, 2007). Literature review shows that this method is used not only to measure efficiency of photosystem II quantum yield in compatible interaction with biotrophic bacteria such as *Pseudomonas syringae* (Bonfig *et al.*, 2006), biotrophic fungi such as *Albugo candida* (Chou *et al.*, 2000), *Phoma lingam* (Hura *et al.*, 2014), *Puccinia coronata and Blumeria graminis* (Swarbrick *et al*., 2006), and viruses such as pepper mild mottle tobamovirus (Pérez-Bueno *et al.*, 2006), but also used in abiotic stress (Li *et al.*, 2013). It should be noted that a recent study showed the *Xcc* biotroph pathogen possess the plant natriuretic peptide-like molecules which can modulate the host homeostasis (Gottig *et al.*, 2008) and counteract the shut-down of photosynthesis. In this context, the proteomics research results showed that the carbon metabolism and photosynthetic-related protein such as Rubisco activase and ATP synthase upregulated in citrus plant inoculated with *Xcc* in comparison with healthy plant (Garavaglia *et al*., 2010).

Several pathogens have acquired the ability to modify plant systems. In this regard, *Xcc* has been demonstrated to affect both photosynthetic parameters and the host proteome after infection. Therefore, to assess the interaction between Iranian strain of *Xcc* and *C. aurantifolia*, Here, we aimed to investigate the effect of pathogen attack on chlorophyll fluorescence and plant proteome changes during pathogen infection. Therefore, to lightening molecular mechanism of plant-pathogen interaction, primary metabolism related proteins changes were analyzed by 2-DE proteomics.

MATERIALS AND METHODS

Plant Material and Inoculation

Citrus aurantifolia was used as the host plant for *X.citri*, NIGEB-088(A*). All plants were grown in a greenhouse in incandescent light at 28°C with a photoperiod of 16 hours. Briefly, bacterial inoculations and in planta growth assays were performed as described previously (Zhang *et al.*, 2010). Three leaves of three individual plants were selected for each treatment. In 1, 4, and 7 days post-inoculation, plant leaves, non-inoculated and inoculated with the bacteria, were evaluated for chlorophyll fluorescence, and were collected for further analysis.

Determination of Physiological Parameters

Chlorophyll and total carotenoid contents were estimated according to the method described by Arnon (1949). Chlorophyll fluorescence measurements were performed using a FMS2 fluorometer (Hansatech instrument, UK). *Fv/Fm* (maximum efficiency of PSII photochemistry), *Fm* (maximal Fluorescence intensity) and *F0* (minimal Fluorescence intensity) were determined based on literature (Maxwell and Johnson, 2000). Light-adapted measurements provided maximum (Fm' and steady-state Fluorescence, Fs). Chlorophyll fluorescence parameters are unitless and were calculated as detailed previously (Kramer *et al.*, 2004).

Protein Extraction and 2-DE:

Protein was extracted from the leaves according to TCA/acetone method (Damerval *et al.*, 1986). Briefly, the IEF was

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

performed at 20°C using Multiphore II and dry strip kit according to manufacturer procedure (Amersham pharmacia Biotech). The second dimension was developed by SDS-PAGE in a vertical slab of acrylamide (12.5% monomer, with 2.6% cross linker) using a PROTEAN II MULTI Cell (Bio-Rad, Hercules, CA, USA). GS-800 densitometer (Bio-Rad) at a resolution of 600 dots per square inch (dpi) for scanning the silver-stained gels. The scanned gels were saved as TIF images for subsequent analysis. Spot quantification was carried out using the Melanie Viewer 7.0 software (Gene Bio, Geneva, Switzerland). The molecular masses of proteins on gels were determined by electrophoresis of standard protein markers (Amersham Biosciences) and pI of the proteins determined by the migration of the protein spots on 18 cm IPG (pH 4–7, linear) strips. The percent volume of each spot was estimated and analyzed to determine protein abundance. Statistical analysis of the relative abundance of each matched protein spot among the control and treated leaves of tolerant and sensitive genotype was accomplished using a Two-tailed T-test and followed by considering only quantitative differences with a P-value of at least 0.05 levels. Protein spots were excised from preparative CBB- or silver-stained gels and then the trypsin digestion was performed based on literature (Askari, *et al.*, 2006) MALDI-MS was used to acquire PMFs and MS/MS spectra from tryptic fragments. Preparation of sample or MS was done according to the procedure of kit (Bruker Daltonik, Bremen, Germany). The PMF and MS/MS were performed using Ultraflex II TOF/TOF (Bruker Daltonik). The MS/MS was carried out by means of the LIFT-TOF/ TOF mode of the UltraflexII. The samples were measured, post processing, and identified in a fully automated fashion using the software AutoXecute TM, Flex-Analysis TM, and Proteinscape and, subsequently, mass spectral data were submitted to NCBInr database searching program MASCOT version 2.1.04 (http://www.matrixscience.com) for protein identification.

Statistical Analysis

Data were analyzed with ANOVA in factorial experiment based on RCBD, and, for differences between treatments, the means were separated using a post hoc Tukey multiple comparison test (Minitab 12.0 software, Minitab Inc., PA, USA).

RESULTS

Plant Inoculation and Bacterial Colonization in Leaf

The inoculation of citrus leaves with *Xcc* suspension (10^5 CFU mL^{-1}) induced visible disease symptom during infection, accordingly, the water-soaking and tissue hydration were observed 1 day after inoculation. However, obvious chlorosis was observed in 4 days after inoculation, (Figure 1-a). According to the results, increasing concentration of *Xcc* in the leaf apoplast was observed during this study (Figure 1.b).

Determination of Chlorophyll and Total Carotenoid Content

To determine pathogen damage on pigment contents, we estimated chlorophyll and carotenoid content. Results showed pigments were affected by pathogen infection. There were significant differences in total chlorophyll between the control and the infected plant (Figure 2). The infection time is considered important to affect pigment content. Chlorophyll a and b simultaneously with total chlorophyll showed a significant decrement in response to infection (Figure 2). The results shown in Figure 2 indicate that carotenoid content was affected by pathogen infection and showed significant decline.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

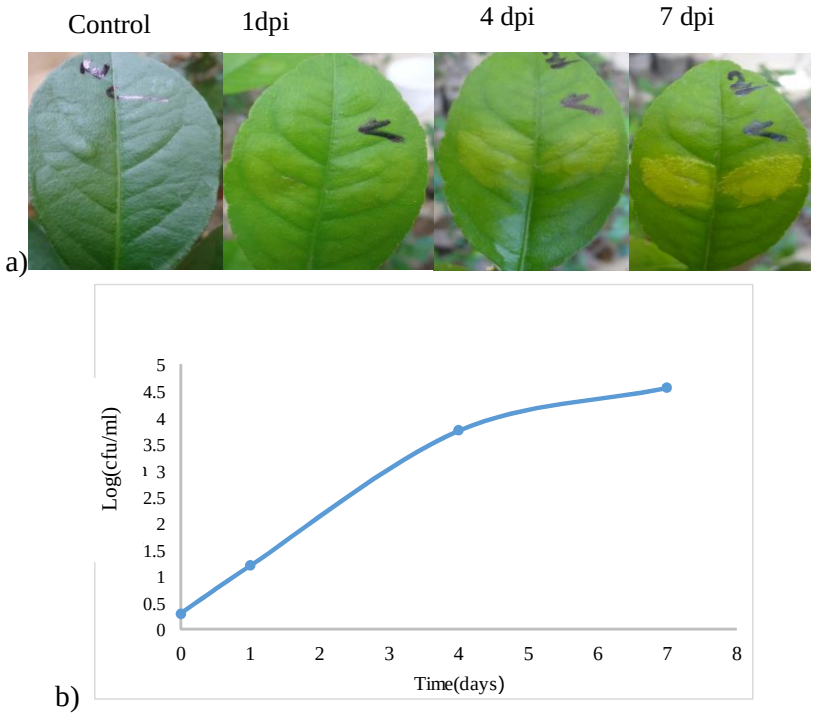

Figure 1. Differential pathogenicity of *Xcc* on citrus plants: (a) Leaves of Mexican lime infiltrated with *Xcc* suspension showing symptom of canker disease. Control infiltrated with distilled water (dpi shows days post inoculation), (b) Bacterial growth curve in planta system.

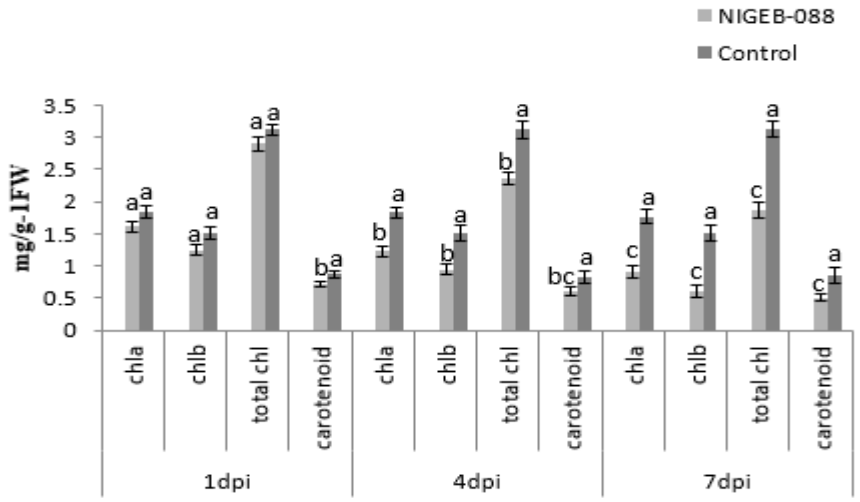

Figure 2. Effect of pathogen infection on photosynthetic pigments. Comparative estimation of chlorophyll a (cha), chlorophyll b (chb), total chlorophyll (total chl) and carotenoid of *Citrus aurantifolia* leaf in a time-dependent manner under pathogen infection. Dpi shows days post inoculation.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

Chlorophyll Fluorescence Parameters

In order to investigate the effect of *Xcc* infection on photosynthesis, changes in photosynthetic parameters were analyzed with a chlorophyll fluorometer. The parameters, maximum efficiency of PSII photochemistry (Fv/Fm), quantum Yield of PSII (YPSII), photochemical quenching (pq) and NonoPhotochemical Quenching (NPQ) were assessed after inoculation. In all parameters, significant effects of pathogen infection were evident between the control and infected plants (Table1). *Fv/Fm* and YPSII showed significant increment at 7 days after inoculation, whereas significant change in qp was observed 1 day after inoculation in infected plants compared with the control (Figure 3). It should be noted that negligible increment was detected in both *Fv/Fm* and YPSII at 1 and 4 days after inoculation. Interestingly, NPQ showed differential behavior from other parameters. In this connection, NPQ showed decrement in infected plants in comparison with the control plants.

Two-Dimensional Gel Electrophoresis Analysis of Protein

In order to evaluate *C.aurantifolia* leaf proteome in response to pathogen attack, proteomics approach was performed. Proteins were extracted from leaves of

Table 1. Mean analysis of main effect of inoculation and days after inoculation. Data presented shows mean of three biological replicates. Different letters show significant differences.

Effects		Chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	*Fv/Fm*	pq	YPSII	NPQ
Days after inoculation	1	2.955265^{a}	0.77018^{a}	0.761^{b}	0.521^{c}	0.562^{b}	0.745^{a}
	4	2.599707^{b}	0.68148^{b}	0.781^{b}	0.651^{b}	0.581^{b}	0.731^{a}
	7	2.3237^{c}	0.63636^{c}	0.823^{a}	0.862^{a}	0.681^{a}	0.712^{b}
Inoculation	NIGEB-088	2.62622^{b}	0.676007^{b}	0.788^{a}	0.678^{a}	0.608^{a}	0.7293^{b}
	Mock	3.12345^{a}	0.69600^{a}	0.758^{b}	0.421^{b}	0.544^{b}	0.781^{a}

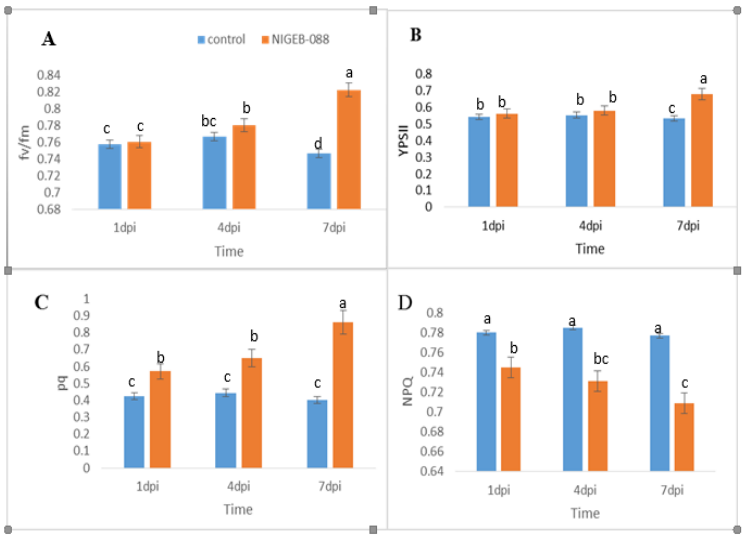

Figure 3. Chlorophyll fluorescence parameters in citrus leaves treated with *Xcc*: (A) Potential quantum efficiency of PSII (Fv/Fm); (B) PSII operating efficiency (YPSII); (C) Photochemical fluorescence quenching (pq); (D) Nonphotochemical Fluorescence Quenching (NPQ) of control and *Xcc*-infiltrated citrus leaves at the times stated. The results are the mean of three replicates and error bars represent the standard deviations.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

infected and non-infected plants at each time of infection and then characterized by 2-DE. The gels of each time point of infection were compared with gels of mock-inoculated leaves. Comparative analysis of proteome pattern indicated that numbers of spots differentially changed in response to pathogen infection; consequently, the spots with significant differences were excised from the gel and identified by mass spectrometry. Among the identified proteins, metabolism related proteins are presented in Table 2. The proteins including Oxygen-evolving enhancer protein 1 and Ribulose bisphosphate carboxylase/oxygenase activase are involved in photosynthesis. These proteins showed up-regulation at 1 and 4 days after inoculation in infected plants compared with the control, and then down-regulated. Interestingly, proteins involved in glycolysis including enolase, triose phosphate isomerase and 2,3-bisphosphoglycerate-independent phosphoglycerate mutase showed an increase at 1dpi, and then decreased. Glycine cleavage system H protein up-regulated in infected plants compared with the control. (Table 2, Figure 4).

DISCUSSION

Besides the investigation of defense responses upon pathogen attack, the analyses of changes in carbohydrate metabolism have received attention in recent years. Production of ROS including superoxide radical ($O_2^{\bullet-}$), hydrogen

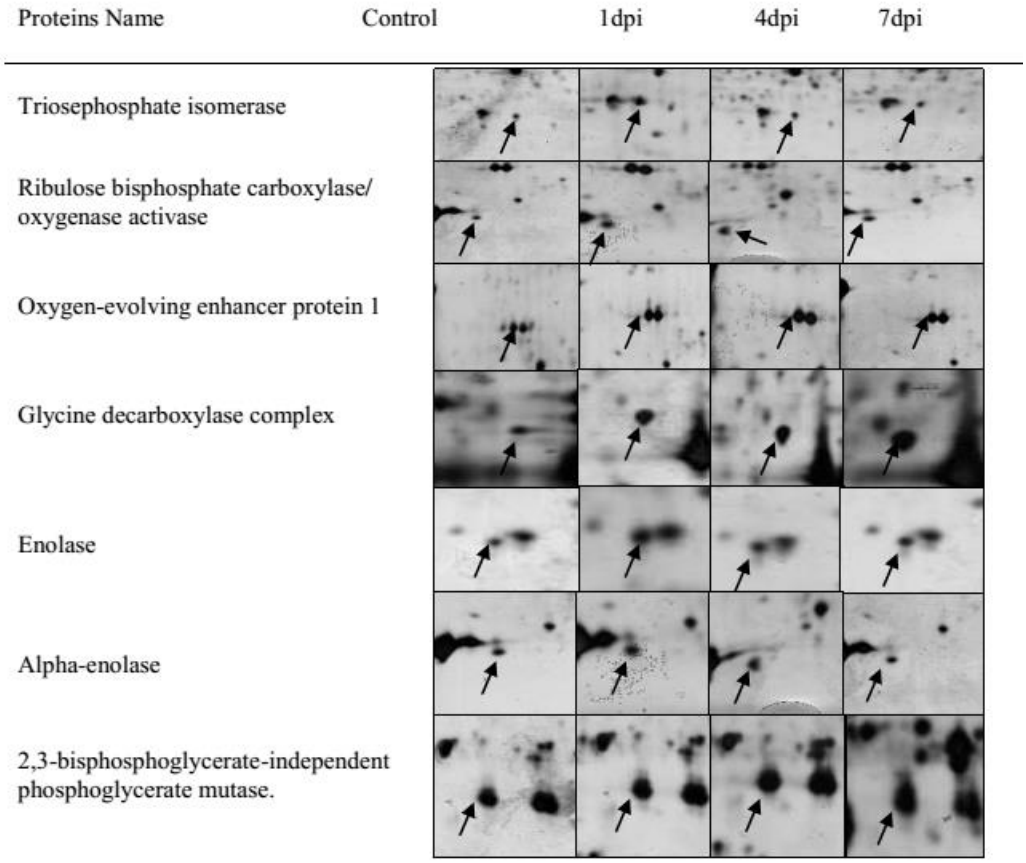

Figure 4. Time-dependent changes of the differentially expressed proteins. Boxed areas are magnified gel sections and related spots are given in Table 2 which were excised from 2-DE gel and identified by MS. Arrows show the related spot in each time course points.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

Table 2. Identification of *Xcc* induced proteins by MALDI-TOF mass spectrometry.

Spot no	Accession number	Protein name	Theoretical Mr/pI	Protein score	Peptide match	Time course fold change[a]		
						1dpi	4 dpi	7 dpi
1	AEE79384	Triosephosphate isomerase	47.8/6.3	406	9	2.23	5.06	4.6
2	AEE78714	Oxygen-evolving enhancer protein 1	35.4/5.7	83	17	2.5	1.2	-
3	AEC09100	Glycine decarboxylase complex	47.3/4.8	324	5	1.1	1.23	2.46
4	CAA41116	Enolase	47.8/5.5	153	25	0.6	0.8	0.54
5	AAQ7724	Alpha-enolase	47.8/5.5	143	27	2.67	1.34	0.34
6	XP015612991	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	61/5.6	145	27	1.1	1.3	-
7	AEC09716	Ribulose bisphosphate carboxylase/Oxygenase activase	53.8/7.2	73	9	1.03	2.35	1.7

peroxide (H2O2), and the hydroxyl radical (OH•) at the site of pathogen infection is the earliest response of plants to pathogen attack (Wojtaszek, 1997), which, consequently, leads to oxidative damage of nucleic acid, protein, pigment and lipid (Mandal *et al*., 2008). In this experiment, chlorophyll and carotenoid content showed decrement in 4 and 7 days after inoculation. Similar results concerning biotic stress have been observed in other experiments (Berova *et al.*, 2007; Garavaglia *et al.*, 2010). The decline in chlorophyll content may be associated with the process of detoxification of compounds derived from a pathogen.

Pathogen infection leads to induction of changes in carbohydrate metabolism of plants. Due to cost-intensive of defense mechanism, plants increase demand for assimilates (Berger *et al.*, 2007). On the other hand, pathogen withdrawal of nutrients causes an increase in assimilating requirement. One goal of our work was to investigate the effect of *Xcc* on photosynthesis, the process in which carbohydrates are synthesized. The reduction of photosynthesis in compatible interaction by biotroph and necrotroph pathogens is well-known. In contrast to the common down-regulation of the photosynthetic parameters *Fv/Fm* and YPSII, NPQ can be increased or decreased by biotic stresses. Similar to the our result, NPQ was decreased following infection with *P. syringae* (Bonfig *et al*., 2006). In contrast, an increase of NPQ was observed in response to the biotrophic fungus *Albugo candida* (Chou *et al*., 2000) and *Botrytis cinerea* (Berger *et al*., 2007). This may be occurring due to the accumulation of ROS and consequently damage the thylakoid membranes, leading to impairment of the structure and function of the photosystems (Kangasjärvi *et al.*, 2012). The most interesting finding was the increment in *Fv/Fm*, YPSII, and decrement in NPQ. However, the finding of the current study do not support the previous plat-pathogen interaction results, but there are evidence for supporting that the citrus canker causing

bacteria Xcc, but no other phytopathogen, possess a PNP-like gene that enable pathogen to modulate host homeostasis (Nembaware *et al*., 2004). PNPs are a class of extracellular, systemically mobile peptides that elicit a number of plant responses important in plant homeostasis and growth (Wang *et al*., 2011). Similar to this result, *Fv/Fm* and YPSII have been improved following the infection of *C.sinensis* with *Xcc* (Garavaglia *et al*., 2010). In summary, it seems that pathogen is counteracting the shutdown of photosynthesis to supply the nutrient for its survival.

The other goal of this study was investigation of metabolism related protein response to pathogen infection in association with the above finding. The physiological results presented here emphasize that pathogens can induce changes in host homeostasis modulation, which resulted in an improvement in the efficiency of photosynthesis. In this context, proteomics study revealed that the *Xcc* infection lead to decrease of the severity of reduction of key photosynthetic proteins in host plant (Garavaglia *et al*., 2010). Similarly, results showed that Ribulose bisphosphate carboxylase/oxygenase activase and Oxygen-evolving enhancer protein1 are two proteins involved in photosynthesis, which up-regulated in infected plant compared with the control. Rubisco activase involved in regulating Rubisco activity by hydrolyzing ATP to promote the dissociation of inhibitory sugar phosphates, and this even at limiting CO_2 concentration (Portis *et al.*, 1986). Up-regulation of Rubisco activase was observed in maize infected with *Setosphaeria turcica* (Kumar and Kirti, 2015).The results indicate that such an increased plant metabolism will lead to the net solute gain in affected tissues which could help biotroph pathogen to exploit across its favor.

Another photosystem II associated protein is Oxygen evolving enhancer protein, which was differentially up-regulated upon pathogen. The PSII Oxygen-Evolving Complex (OEC) oxidizes water to provide protons for use by PSI, and consists of OEE1 (PsbO), OEE2 (PsbP) and OEE3 (PsbQ). In PSII, the Oxygen-Evolving Complex (OEC) is responsible for catalyzing the splitting of water to O^2 and 4H+. Electrons stripped from water during this reaction are funneled back into photochemical reaction center II, then, transported through the electron transport chain to photosystem I, eventually to be used for the reduction of NADP (Mayfield *et al.*, 1987). Upregulation of Oxygen evolving enhancer protein has been observed in resistant cultivars of different plants under stress condition (Geddes *et al.*, 2008; Rasoulnia *et al.*, 2011).In the current study, increase of oxygen evolving enhancer protein indicates an important role of this protein in maintaining of photosystem II activity during pathogen attack. Mizobuchi and Yamamoto (1989) demonstrated that oxygen evolving enhancer protein1 was essential for oxygen-evolving activity and photosystem IISII stability. In citrus, despite the existence of obvious symptoms of chlorosis on the infected leaf, not only the reduction of photosynthesis apparatus function was not observed, but also induction of the oxygen evolving protein was observed, indicating the effect of the pathogen to enhance the function of photosynthesis apparatus in infected tissue compared with uninfected plants. Also, this increase can be correlated with the improvement of *Fv/Fm* and quantum yield of photosynthesis. Therefore, oxygen evolving protein may be attributed to maintaining the capacity of PSII for enhanced photosynthesis after *Xcc* attacks.

The supplying of the cellular energy required for plant processes such as plant defense response during plant-pathogen interaction by primary metabolism is well documented, therefore, energy is a critical issue during the plant-pathogen interaction because of more requirement of the energy for induction of defense response (Berger *et al*., 2007). Change in expression of the enolase, triose phosphate isomerase, and 2,

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

3-bisphosphoglycerate-independent phosphoglycerate mutase, which is involved in glycolysis process, suggests manipulating of the energy pathway. Enolase is an enzyme of the glycolytic pathway converting the 2-phosphoglycerate to phosphoenolpyruvate. The multifunctional properties of enolase suggest that the enzyme may have different functions in addition to participation in the glycolytic pathway alone, consequently, it is probably a relevant component of the plant response to bacterial infections (Dahal *et al.*, 2010). The increased expression of proteins involved in ATP-generating pathway during bacterial infection can be assumed as consequences of increased plant metabolism to compensate for the cost of defense. Induction of glycolytic proteins has been observed in previous research (Mahmood *et al*., 2006). With respect to the results, reduction of this protein after an increase in one-day post inoculation indicates that the pathogen can limit expression of the mentioned proteins in order to weaken the host defense.

The important role of glycine decarboxylase in the photorespiratory metabolism of plants was previously indicated by another researcher (Berger *et al*., 2004). Net photosynthetic CO_2 assimilation rates in C3 plants can be reduced by photorespiration, a process that results from the oxygenase activity of Rubisco. Increasing levels of glycine dehydrogenase occur in response to pathogen attack, which suggests metabolic changes during plant-pathogen interaction (Afroz *et al.*, 2009). With regard to the results, induction of this protein 4 days post-inoculation represents difficult condition of photosynthesis and suggests the initiation of photorespiration.

CONCLUSIONS

Plant pathogenic biotrophic bacteria, such as *Xcc*, enter the host plant through stomata and wounds, and proliferate into the intracellular space leading to chlorotic symptom and lesion. The apparent disease chlorotic symptom was detectable at 4 DPI, but the formation of a pustule was observed at 7 DPI. Pathogen infection leads to induction of changes in carbohydrate metabolism of plants. Results suggested that inoculation with *Xcc* resulted not only in an improvement in *Fv/Fm*, but also in an increase in Quantum yield of PSII (YPSII). In contrast, no differences were observed in the photochemical quenching (pq), whereas Non-Photochemical Quenching (NPQ) showed a significant decrease in energy loss as heat as a consequence of *Xcc* treatment. Proteomics analysis also showed a change in the metabolic pathway related proteins expression during infection. Oxygen-evolving enhancer protein and Ribulose bisphosphate carboxylase/oxygenase activase are two proteins involved in photosynthesis which showed an improvement in the expression until 4 days post inoculation, but proteins involved in ATP-generating pathway decreased during infection. We concluded that the pathogen can modulate the host metabolism in its favor. These results improved our insights about defense mechanism of citrus plant and will also facilitate future development in breeding program to select resistant or tolerant cultivars.

ACKNOWLEDGEMENTS

This work was financially supported by NIGEB under M-406 project.

REFERENCES

1. Afroz, A., Khan, M.R., Ahsan, N. and Komatsu, S. 2009. Comparative Proteomic Analysis of Bacterial Wilt Susceptible and Resistant Tomato Cultivars. *Peptide.*, **30:** 1600-1607.
2. Araya, T., Noguchi, K. and Terashima, I. 2010. Effect of Nitrogen Nutrition on the Carbohydrate Repression of Photosynthesis

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

in Leaves of Phaseolus vulgaris L. *J. Plant Res*., **123:** 371-379.
3. Arnon, D. I. 1949. Copper Enzymes in Isolated Chloroplasts Polyphenoloxidase in *Beta vulgaris. Plant Physiol*., **24:** 1- 15.
4. Askari, H., Edqvist, J., Hajheidari, M., Kafi, M. and Salekdeh, G. H. 2006. Effects of Salinity Levels on Proteome of Suaeda Aegyptiaca leaves. *Proteomics*., 6: 2542-2554.
5. Berger, S., Papadopoulos, M., Schreiber, U., Kaiser, W. and Roitsch, T. 2004. Complex Regulation of Gene Expression, Photosynthesis and Sugar Levels by Pathogen Infection in Tomato. *Physiol Plantarum*, **122:** 419-428.
6. Berger, S., Sinha, A. K. and Roitsch, T. 2007. Plant Physiology Meets Phytopathology: Plant Primary Metabolism and Plant–Pathogen Interactions. *J. Exp. Bot*., **58:** 4019-4026.
7. Berova, M., Stoeva, N., Zlatev, Z., Stoilova, T. and Chavdarov, P. 2007. Physiological Changes in Bean (*Phaseolus vulgaris* L.) Leaves, Infected by the Most Important Bean Disease (*Phaseolus vulgaris* L.). *JCEA*, **8**: 45-54.
8. Bock, C., Graham, J., Gottwald, T., Cook, A. and Parker, P. 2010. Wind Speed Effects on the Quantity of Xanthomonas citri Subsp. Citri Dispersed Downwind from Canopies of Grapefruit Trees Infected with Citrus Canker. *Plant Dis*., **94:** 725-736.
9. Bolton, M.D. 2009. Primary Metabolism and Plant Defense-Fuel for the Fire. *Mol. Plant Microbe In.*?? **22:** 487-497.
10. Bonfig, K. B., Schreiber, U., Gabler, A., Roitsch, T. and Berger, S. 2006. Infection with Virulent and Avirulent *P. syringae* Strains Differentially Affects Photosynthesis and Sink Metabolism in Arabidopsis Leaves. *Planta*., **225:** 1-12.
11. Chou, H. -M., Bundock, N., Rolfe, S.A. and Scholes, J. D. 2000. Infection of Arabidopsis Thaliana Leaves with *Albugo candida* (White Blister Rust) Causes a Reprogramming of Host Metabolism. *Mol. Plant Pathol*., **1:** 1- 15.
12. Dahal, D., Pich, A., Braun, H. P. and Wydra, K. 2010. Analysis of Cell Wall Proteins Regulated in Stem of Susceptible and Resistant Tomato Species afterInoculation with *Ralstonia solanacearum*: A Proteomic Approach. *Plant Mol. Biol*., **73:** 643-658.
13. Damerval, C., De Vienne, D., Zivy, M. and Thiellement, H. 1986. Technical Improvements in Two-Dimensional eElectrophoresis Increase the Level of Genetic Variation Detected in Wheat-Seedling Proteins. *Electrophoresis*, **7:** 52-54.
14. Garavaglia, B. S., Thomas, L., Gottig, N., Dunger, G., Garofalo, C. G., Daurelio, L. D., Ndimba, B., Orellano, E. G., Gehring, C. and Ottado, J. 2010. A Eukaryotic-Acquired Gene by a Biotrophic Phytopathogen Allows Prolonged Survival on the Host by Counteracting the Shut-Down of Plant Photosynthesis. *PLOS ONE*., **5:** 89-98.
15. Geddes, J., Eudes, F., Laroche, A. and Selinger, L. B. 2008. Differential Expression of Proteins in Response to the Interaction between the Pathogen *Fusarium graminearum* and Its Host, *Hordeum vulgare. Proteomics*, **8:** 545-554.
16. Gottig, N., Garavaglia, B.S., Daurelio, L. D., Valentine, A., Gehring, C., Orellano, E. G. and Ottado, J. 2008. *Xanthomonas axonopodis* pv. *citri* Uses a Plant Natriuretic Peptide-Like Protein to Modify Host Homeostasis. *P?? Natl. Acad. Sci. USA*, **105:** 18631-18636.
17. Hura, K., Hura, T. and Grzesiak, M. 2014. Function of the Photosynthetic Apparatus of Oilseed Winter Rape under Elicitation by *Phoma lingam* Phytotoxins in Relation to Carotenoid and Phenolic Levels. *Acta Physiol. Plant*, **36:** 295-305.
18. Kangasjärvi, S., Neukermans, J., Li, S., Aro, E. -M. and Noctor, G. 2012. Photosynthesis, Photorespiration, and Light Signalling in Defence Responses. *J. Exp. Bot*., **51:** 406-417.
19. Kocal, N., Sonnewald, U. and Sonnewald, S. 2008. Cell Wall-Bound Invertase Limits Sucrose Export and Is Involved in Symptom Development and Inhibition of Photosynthesis during Compatible Interaction between Tomato and *Xanthomonas campestris* pv *vesicatoria*. *Plant Physiol*., **148:** 1523-1536.
20. Kramer, D. M., Johnson, G., Kiirats, O. and Edwards, G. E. 2004. New Fluorescence Parameters for the Determination of QA Redox State and Excitation Energy Fluxes. *Photosynth. Res*., **79 :** 209-218.
21. Kumar, D. and Kirti, P. B. 2015. Transcriptomic and Proteomic Analyses of Resistant Host Responses in A*rachis diogoi* Challenged with Late Leaf Spot Pathogen,

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

Phaeoisariopsis personata. *PLOS ONE*., **10:** 117-1299.
22. Li, G. L., Wu, H. X., Sun, Y. Q. and Zhang, S. Y. 2013. Response of Chlorophyll Fluorescence Parameters to Drought Stress in Sugar Beet Seedlings. *Russ J. Plant Physiol.*, **60**: 337-342.
23. Mahmood, T., Jan, A., Kakishima, M. and Komatsu, S. 2006. Proteomic Analysis of Bacterial-Blight Defense-Responsive Proteins in Rice Leaf Blades. *Proteomics*, **6:** 6053-6065.
24. Mandal, S., Mitra, A. and Mallick, N. 2008. Biochemical Characterization of Oxidative Burst during Interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Physiol. Mol. Plant P*.??, 72: 56-61.
25. Maxwell, K., Johnson, G.N. 2000. Chlorophyll Fluorescence: A Practical Guide. *J. Exp. Bot*., **51**: 10-18.
26. Mayfield, S. P., Bennoun, P. and Rochaix, J. 1987. Expression of the Nuclear Encoded OEE1 Protein Is Required for Oxygen Evolution and Stability of Photosystem II Particles in *Chlamydomonas reinhardtii*. *Embo J*., **6:** 313-319.
27. Mizobuchi, A. and Yamamoto, Y. 1989. Assembly of Photosystem II Polypeptides and Expression of Oxygen Evolution Activity in the Chloroplasts of *Euglena gracilis* Z during the Dark-Light Transition. *BBA-Bioener.*, **977:** 26-32.
28. Nembaware, V., Seoighe, C., Sayed, M. and Gehring, C. 2004. A Plant Natriuretic Peptide-Like Gene in the Bacterial Pathogen *Xanthomonas axonopodis* May Induce Hyper-Hydration in the Plant Host: A Hypothesis of Molecular Mimicry. *BMC Evol. Biol*., **4:** 1- 10.
29. Pérez-Bueno, M. L., Ciscato, M., García-Luque, I., Valcke, R. and Barón, M. 2006. Imaging Viral Infection: Studies on *Nicotiana benthamiana* Plants Infected with the Pepper Mild Mottle Tobamovirus. *Photosynth. Res*., **90:** 111-123.
30. Portis, A. R., Salvucci, M. E. and Ogren, W. L. 1986. Activation of Ribulosebisphosphate Carboxylase/Oxygenase at Physiological CO2 and Ribulosebisphosphate Concentrations by Rubisco Activase. *Plant Physiol*., **82:** 967-971.
31. Rasoulnia, A., Bihamta, M. R., Peyghambari, S. A., Alizadeh, H. and Rahnama, A. 2011. Proteomic Response of Barley Leaves to Salinity. *Mol. Biol. Rep.*, **38:** 5055-5063.
32. Ryan, R. P., Vorhölter, F.-J., Potnis, N., Jones, J. B., Van Sluys, M. -A., Bogdanove, A. J. and Dow, J. M. 2011. Pathogenomics of *Xanthomonas*: Understanding Bacterium–Plant Interactions. *Nat. Rev. Microbiol*., **9:** 344-355.
33. Swarbrick, P. J., Schulze-Lefert, P. and Scholes, J. D. 2006. Metabolic Consequences of Susceptibility and Resistance (Race-Specific and Broad-Spectrum) in Barley Leaves Challenged with Powdery Mildew. *Plant Cell Environ.*, **29:** 1061-1076.
34. Wang, Y. H., Gehring, C. and Irving, H. R. 2011. Plant Natriuretic Peptides Are Apoplastic and Paracrine Stress Response Molecules. *Plant Cell Physiol*., **52:** 837-850.
35. Wojtaszek, P. 1997. Oxidative Burst: An Early Plant Response to Pathogen Infection. *Biochem. J*., **322:** 681-692.
36. Zhang, X., Francis, M. I., Dawson, W. O., Graham, J. H., Orbović, V., Triplett, E. W. and Mou, Z., 2010. Over-Expression of the Arabidopsis *NPR1* Gene in Citrus Increases Resistance to Citrus Canker. *Eur. J. Plant Pathol*., **128:** 91-100.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]

اثر آلودگی باکتری زانتوموناس بر روی محتوی کلروفیل ، کلروفیل فلورسانس و تغییرات پروتئینها در گیاه لیمو ترش

ا. رسول‌نیا، س. م. علوی، ح. عسکری، ن. فرخی، و م. سلطانی نجف‌آبادی

چکیده

با وجود گستردگی شانکر مرکبات، این بیماری هنوز هم از بیماریهای ویرانگر می باشد که به وسیله باکتری زانتوموناس ایجاد می شود. به منظور بررسی مکانیسم پاسخ گیاه، برگهای گیاه با باکتری آلوده شده و برگهای آلوده و سالم پس از 4،1و7 روز پس از آلودگی جمع آوری شدند. متعاقبا محتوی کلروفیل و کلروفیل فلورسانس اندازه گیری شدند و همچنین مطالعه پروتئومیکس انجام شد. نتایج نشان داد که آلودگی پاتوژن علیرغم اثر منفی بر روی محتوی کلروفیل باعث بهبود شرایط فیزیولوژیکی شد. با توجه به نتایج، ماکزیمم عملکرد بیوشیمیایی فتوسیستم2، عملکرد کوانتوم و دفع فتوشیمیایی در گیاهان آلوده نسبت به گیاهان سالم افزایش پیدا کرد ولی دفع غیر فتوشیمیایی دچار کاهش شد. نتایج نشان داد که این تغییرات همزمان با افزایش بیان پروتئینهای درگیر در فتو سنتز و کاهش بیان پروتئینهای تولید انرژی منعکس شد. این نتایج نشان می دهد که پاتوژن با دستکاری هموستازی گیاه بوسیله افکتور پروتئینها ، آن را در جهت بهره برداری خودش قرار می دهد.

[DOR: 20.1001.1.16807073.2018.20.3.6.2]