

***Bacillus thuringiensis* - Mediated Priming Induces Jasmonate/Ethylene and Salicylic Acid-Dependent Defense Pathways Genes in Tomato Plants**

Masumeh Dezhabad¹, Hengameh Taheri^{1*} and Babak Pakdaman Sardrood²

¹Department of Plant Production and Genetics, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Khuzestan, Iran

²Department of Plant Protection, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Khuzestan, Iran

ABSTRACT: *Bacillus thuringiensis* Berliner as a biological control agent can play a crucial role in the integrated management of a wide range of plant pests and diseases. *B. thuringiensis* is expected to elicit plant defensive response through plant recognition of microbe-associated molecular patterns (MAMPs), however, there is little information on the molecular base of induced systemic resistance priming of tomatoes. Using q-RT-PCR technique, the transcription rate of the genes responsive to salicylic acid, SA (*Chi9*, *Chi3*, *PR1*), jasmonic acid, JA (*Pin2*), and of the signaling regulatory genes of jasmonate/ ethylene, JA/ ET hormones (*WRKY33*, *ERF1*, *MYC2*) were studied at the time of 6, 12, 24, 48, 72, and 96 hours after inoculation of tomato plants with *B. thuringiensis* strain IBRC-M 11096 as the promoting plant growth factor. The bacterial strain could prime tomato cultivar of Early Urbana through induction of all three hormonal signaling pathways (SA, JA, and ET) involved in the resistance to a broad range of necrotrophic as well as biotrophic pathogens. However, further transcription of *WRKY33*, *ERF1*, *MYC2*, and *Pin2* genes in the inoculated plants, indicated that the observed priming effect was mainly based on JA/ ET signaling pathway. These promising results indicate high potential of superior isolates of *B. thuringiensis* in the field management of the crops.

KEYWORDS: Beneficial soil microbes, Hormone signaling pathway, Induced systemic resistance, Systemic acquired resistance

INTRODUCTION

The control of soil-born plant pests and diseases has ever been as one of the global challenges in the world of agriculture which is because of multiple of problems such as the fixation of pesticides onto soil organics and mineral colloids, soil pollution, superficial and underground waters. Biological control is an eco-friendly and reliable alternative choice in hand of most farmers in the developing countries in their combat against soil-borne diseases such as fusarium vascular wilt of tomato, which

is one of the most important diseases of tomato plants [1]. The beneficial rhizosphere microbes include plant growth promoting rhizobacteria (PGPR) can stimulate plant tolerance against pathogens by inducing systemic defense response (ISR). These induced responses are modulated by signal transduction networks in which phytohormones jasmonate/ ethylene (JA/ET) and salicylic acid (SA) have crucial roles [2]. In contrast, SAR is systemic acquired resistance in response to biotrophic pathogens that results

*Corresponding author (✉): academichngthr@gmail.com

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in increased level of SA and activation of pathogenesis-related (PR) proteins [3].

ISR is associated with a potency of expression of defense-related genes, which is termed priming. Root colonization by beneficial soil microbes can induce a primed state that improves plants systemic immunity, therefore can be successfully used in integrated management of plant diseases and pests. In fact, priming-mediated resistance is more efficient in expression of basal defense responses upon invasion compared to non-primed plants [4]. It was shown that transcript levels of several important transcriptional factors like members of AP2/ERF family and MYC2 were notably enhanced in primed plants [5-6]. However, ISR transcriptome analysis of *Arabidopsis* before and after pathogen attack confirmed that transcriptional changes that occur in primed plants after pathogen attack are greater than that of unchallenged primed plants [7]. Although the priming phenomenon has been abundantly described, the molecular aspects underlying defense priming are less well understood [8]. Although some non-pathogenic rhizosphere microbes stimulate ISR through SA-dependent signaling pathway [9], most beneficial microbes activated ISR through JA/ET signaling pathway [10, 11]. However, in some cases, ISR functions through both the SA and JA/ET signaling pathways to confer increased resistance against hemibiotrophic and necrotrophic pathogens. For example, *Bacillus cereus* strain AR156-mediated ISR simultaneously activates the SA- and the JA/ET dependent signaling pathways [12].

Bacillus thuringiensis is regarded as globally the most abundantly applied biological control bacterial agent mostly against plant pests [13]. It has also been used successfully as potential biological control agent (BCA) to control plant pathogenic oomycetes and fungi such as *F. oxysporum* [14]. Some strains of the bacterium have been known that promote plant growth and development, and these PGPRs can also act as biofertilizers and biostimulants for improved crop yield under normal and stress conditions [15]. In addition, it has been reported the synergism between insecticidal activity of *B. thuringiensis* and ISR induction against Colorado potato beetle (*Leptinotarsa decemlineata* Say) [16]. There are documents indicating the ability of some strains of the bacterium to stimulate ISR in the crops like tomato [17-18]. To further understanding of hormone-dependent pathways in *B. thuringiensis*-mediated priming, we

focused on transcriptional changes of JA/ET and SA dependent genes in tomato.

MATERIALS AND METHODS

Plant materials

Seed of tomato cultivar, Early Urbana Y were superficially sterilized with 2% hypochlorite solution for 5 min and rinsed twice with sterile deionized water following the method applied by Akköprü and Demir (2005)[19]. Then, seeds were sown in seed trays. Two weeks after germination, seedlings were transplanted into a 1 L pot containing a vermiculite potting soil mixture that had been autoclaved for 20 min at 121 °C. Plants were incubated in greenhouse at 25°C under natural light: dark conditions of 16: 8 h.

Preparation of *B. thuringiensis* inoculum

An active culture of *Bacillus thuringiensis* strain IBRC-M 11096 was purchased from National Center of Iran Genetic and Biological Resources, Tehran. The suspension of bacterial cells was prepared following the method described by Lacy and Lukezic (2008), briefly, a loop of the overnight grown culture of the bacterium was inoculated into nutrient broth medium under sterile conditions. The flask was sealed and incubated at 25°C on a shaker (150 RPM) for 48 h. The culture of optical density (OD) of 0.9 at the wavelength of 600 nm was diluted to the ratio of 1: 100 in order to its density was adjusted to 10⁷ colony forming units (CFUs)/mL. The suspension was instantly applied for inoculation [20].

Plant treatments

30 mL of the bacterial suspension was added to soil of each pot after transplantation. For controls, 30 mL of autoclaved (121°C for 20 min) diluted nutrient broth (Biolife, Italy) was applied to soil of each pot after transplantation. Leaves from each plant in its four-leaf stage were collected 6, 12, 24, 48, 72 and 96 hours after inoculation (hai). For each sampling, three leaves from the apex were harvested, frozen in liquid nitrogen and then stored at -70°C until use.

RNA extraction and qRT-PCR

Total RNA was extracted from each frozen leaf sample using Super RNA Extraction Kit (Cat No. YT9080), Yekta Tajhiz Azma ltd., Tehran, Iran. The extraction was

Table 1. The primers applied in qRT-PCR reactions

Gene	Primer	Primer sequence (5'→ 3')	Accession number	Amplicon length (bp)
<i>PR1</i>	F	TCTTGCGGTTTCATAACGATG	DQ159948.1	104
	R	CAGCTCTTGAGTTGGCATAG		
<i>Chi9</i>	F	ATCTGGTTCTGGATGACTCC	Z15140.1	125
	R	TTTGTGATGACACCGAATCC		
<i>Chi3</i>	F	ACTATGGCAGAGGACCTATC	Z15141.1	108
	R	TCGCAACTAAATCAGGGTTG		
<i>Act</i>	F	CCATGTTCCCAGGTATTGC	U60480.1	120
	R	AGCCTCCAATCCAGACAC		
<i>PinII</i>	F	GCACTGGTTACAAGGGTTG	X94946.1	145
	R	TTTGCCAATCCAGAAGATGG		
<i>ERF1</i>	F	GGTCCTTGGTCTCTACTCAAT	AY044236.1	139
	R	TCTTGTGCTTGACTCTTCTAGT		
<i>MYC2</i>	F	GACGTGATTCAATGGCTCCT	KF428776.1	140
	R	CAGGGGAAGCAATGAAGAAG		
<i>WRKY33</i>	F	GTGGAAAGGGCATCACAAAG	NM_001319981.1	138
	R	TCGGTTAATTGAGTGGTTGC		

done according to the kit instructions. The extracted RNA samples were quantified by NanoDrop 2000C spectrophotometer (Thermo Fischer scientific Inc., USA). The first strand of complementary DNA (cDNA) was synthesized using oligo dT primer and 500 ng of total RNA according to the manufacturer's instructions of the applied Revert Aid™ First Strand cDNA Synthesis Kit (Fermentas, USA). The gene-specific primers (Table 1) were designed based on the available coding sequences of the genes using Primer Quest™ software available online at the website of Integrated DNA Technologies (www.idtdna.com). The designed primers were synthesized in SinaClone Company, Tehran, Iran. To confirm the efficacy of the designed primers in the specific amplification of the genes of interest, gradient PCR was carried out using Bio-Rad thermal cyclers (iCycler, USA) with tested temperatures ranged between 52-62°C.

Quantitative Real Time-PCR (qRT-PCR) was performed using HIFI SYBER Green kit (Mabnateb, Iran) Master Mix and Step One Plus® (ABI, USA) machine under the following conditions: an initial denaturation of 10 min at 95°C followed by 40 cycles, with one cycle consisting denaturation at 95°C for 15 s, annealing 60°C for 15 s and extension at 72°C for 20 s. The threshold cycle (Ct) for

each gene was normalized with *S. lycopersicum* Actin gene applied as the reference gene. Fold changes in transcript expression were calculated by the $2^{-\Delta\Delta CT}$ comparative method [21] and the obtained data were analyzed using the relative expression software, REST@ software as described by Pfaffl et al. (2002) [22]. Three individual plants were used as biological replicates. For each biological replicate, two technical replicates were run.

RESULTS

The transcription rate of the transcription factor gene, *ERF1* significantly increased in 6-48 hai, and reached its maximum rate, 5.5 fold of that in corresponding control plants (5.5 CCPs) 24 hai. With the *MYC2*, the transcription rate of the gene indicated significant increases in all studied hours after inoculation (6-96 hai) compared with corresponding control plants. The highest rate of *MYC2* transcription (8.6 CCPs) was recorded 24 hai. The transcription of *Pin2* gene significantly increased 12-48 hai, and attained its highest rate (12 CCPs) 48 hai. The transcription factor gene, *WRKY33* indicated significant increase of transcription 24-48 hai, where its highest rate of transcription (5.9 CCPs) was observed 24 hai.

The *PR1* gene exhibited significant transcriptional increase 12-48 hai, and its maximum rate of transcription (5.3 CCPs) was observed 24 hai. However, no significant increase in the transcription of the gene was found 6, 72, and 96 hai compared to that recorded with corresponding control plants. Transcription rate of the gene *Chi9*

increased 12-96 hai, where the maximum rate of *Chi9* transcription (8 CCPs) was attained 24 hai. The *Chi3* rate of transcription significantly increased 24-96 hai, where its highest rate of transcription, 9.4 times that of the corresponding control plants (9.4 CCPs), was determined 48 hai (Figure 1).

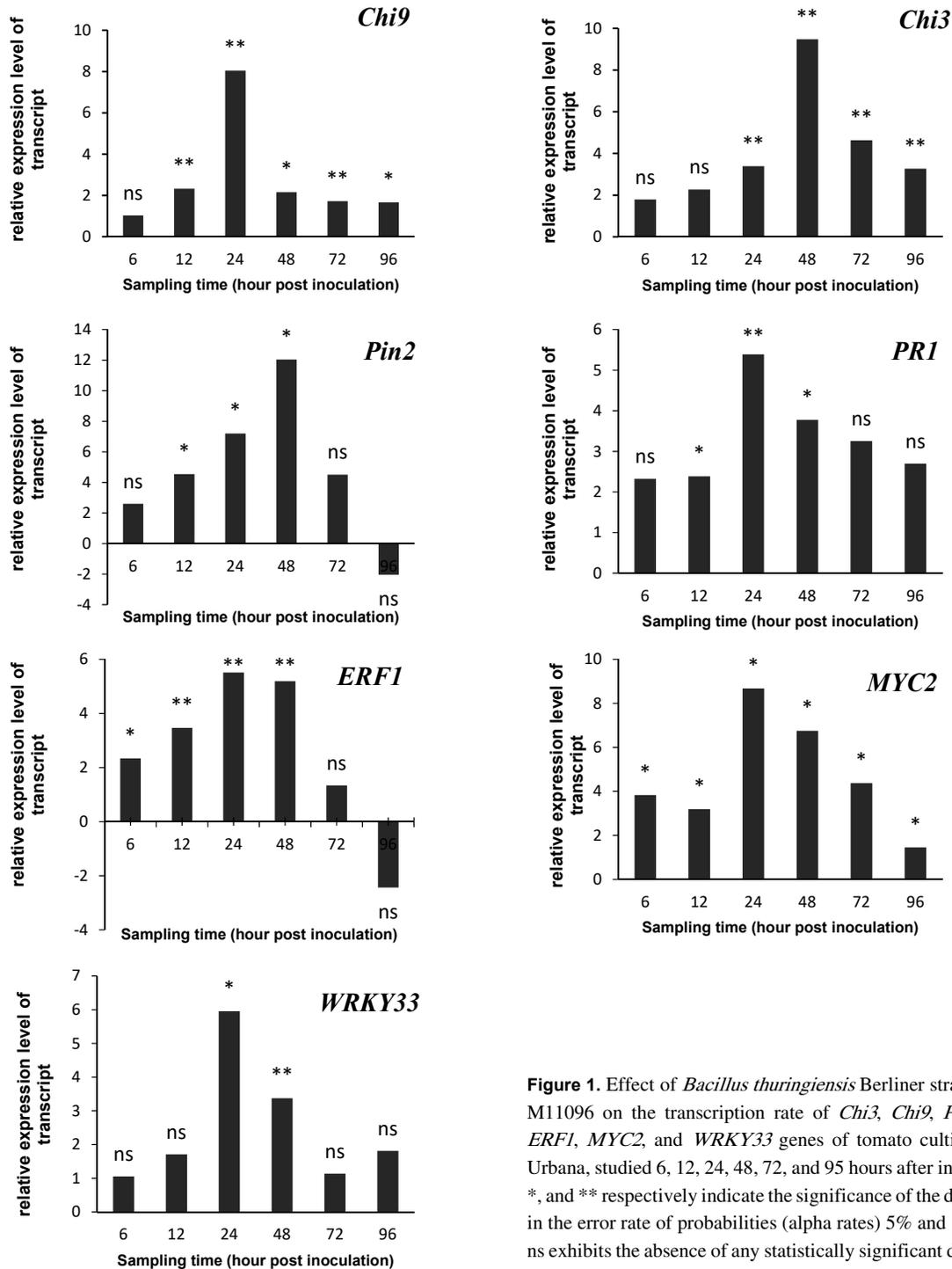


Figure 1. Effect of *Bacillus thuringiensis* Berliner strain IBRC-M11096 on the transcription rate of *Chi3*, *Chi9*, *Pin2*, *PR1*, *ERF1*, *MYC2*, and *WRKY33* genes of tomato cultivar Early Urbana, studied 6, 12, 24, 48, 72, and 95 hours after inoculation. *, and ** respectively indicate the significance of the differences in the error rate of probabilities (alpha rates) 5% and 1%, while ns exhibits the absence of any statistically significant difference.

DISCUSSION

The strain almost synchronously induced the transcription of the genes involved in either ISR (including *ERF1*, *MYC2*, *Pin2*, and *WRKY33*) or SAR (including *PR1*, *Chi9*, and *Chi3*) pathways in tomato cultivar. The induction of ISR pathway genes preceded that of the genes involved in SAR pathway. The bacterial induction of both defense pathways has previously reported with the model plant *Arabidopsis thaliana* [12]. Kwon et al. (2010) indicated that the plant growth promoting *Bacillus subtilis* strain GB03 induced the increased expression of *ERF1* as well as *PR1* genes in *A. thaliana* [23]. Additionally, the plant growth promoting *Paraburkholderia phytofirmans* PsJN induced the increased expression of *ERF1*, *MYC2*, and *PR1* genes and therefore, led to increased resistance of *A. thaliana* to the pathogenic *Pseudomonas syringae* pv. *tomato* DC3000. Interestingly, *P. phytofirmans* PsJN activated the signaling pathways related to three plant hormones SA, JA, and ET. The bacterium could induce plant resistance via the induction of the third pathway in the plants representative of mutational destructions in both hormonal pathways. Therefore, mutations in all three hormonal pathways were required to result in the susceptibility to the pathogen [24]. Here, the strain IBRC-M 11096 of *B. thuringiensis* was capable to induce the transcription of *ERF1* gene since the 6th hai. As the gene is connected to both JA, and ET pathways, therefore, it is expected that the strain IBRC-M11096 is also able to induce all three hormonal pathways involved in tomato systemic resistance. The transcription factor *MYC2* is considered as a key regulator in JA signaling pathway in *A. thaliana*. The activity of *MYC2* is necessary for the induction of ISR by soil beneficial microorganisms [25]. The increased expression of *MYC2* gene in *A. thaliana* and induction of ISR have been reported as the result of the treatment by plant growth promoting bacterial strain, *Pseudomonas fluorescens* WCS417r [25]. In this research, the rate of *MYC2* transcription significantly increased till 96 hai compared with corresponding non-inoculated control plants. Considering *MYC2* as one of the major factors regulating the response to JA signal, its increased transcription after inoculation with *B. thuringiensis* IBRC-M11096 strain confirms the strain capability in JA-dependent induction of tomato defense pathway. The increased transcription of *Pin2* observed 12-48 hai revealed the activation of JA signaling pathway

and ISR by the bacterial strain. The gene *Pin2* encodes proteinase inhibitor II, one of plant defensive proteins against insect invasion. As the gene activity is mainly regulated by JA, it is considered as a marker gene for JA pathway, and its rate of expression is investigated in the studies on hormonal pathways [26]. The transcription factor, *WRKY33* is known as a positive regulator of genes responsible for JA pathway, and as a suppressor of the genes responsible for SA pathway [27]. The induction of *WRKY33* has been reported as the result of treatment of *A. thaliana* with both virulent and avirulent strains of *P. syringae* pv. *tomato* DC3000 [28, 29]. With our research, the rate of *WRKY33* gene transcription significantly rose following inoculation with IBRC-M11096 strain of *B. thuringiensis*, confirming its potential in the induction of tomato JA pathway.

In this study, the treatment with *B. thuringiensis* strain IBRC-M11096 significantly induced transcriptional increase of *PR1* gene in tomato. The transcription of the gene is mainly regulated through SA pathway [30]. Therefore, the activation of *PR1* gene expression can be an indicator of SA pathway activation as the impact of the application of various elicitors. Reportedly, *B. cereus* first increased the expression of SA pathway-specific gene, *PR1* and then raised the transcription rate of JA pathway marker gene, *Pin2*. This means that *B. cereus* needs to activate both SA-, and JA-dependent pathways in order to induce plant systemic resistance [31]. Similar results have been obtained using other strains of *Bacillus* [32, 33]. Similarly, the expression of both *PR1*, and *Pin2* genes increased in tomato transplants as the result of the treatment with *B. thuringiensis* strain IBRC-M11096. However, there is no complete consensus that the ISR induced by plant growth promoting rhizobacteria (PGPR) is due to the expression of pathogenesis-related proteins (PR proteins). For instance, Pieterse et al. (1998) indicated that *P. fluorescens* WCS417r was capable to induce ISR without the activation of PR proteins. In contrast, there are reports of the role of *Bacillus* spp. in the induction of ISR via the induction of PR genes [34]. For example, SA signaling pathway was activated during induced systemic resistance to *P. syringae* pv. *tabaci* of tobacco plants treated by the T4 strain of *B. pumilus* [35]. Fatouros et al. (2018) indicated the effect of biological control agent, *Paenibacillus alvei* K165 on the level of defense gene expression in lettuce (*Lactuca sativa* L.), where the increased expression of *PR1* gene in response to the fungal pathogen, *Sclerotinia sclerotiorum* and to the

oomycetous pathogen, *Pythium ultimum* was reported as an indicator of the activation of SA pathway during induced systemic resistance of lettuce plants [36].

Other genes related to SAR pathway, chitinases (*Chi3*, and *Chi9*) were up-regulated as the result of the inoculation by *B. thuringiensis* strain IBRC-M11096. These genes are usually expressed in reaction to the occurrence of pathogens and during the incidence of SAR [37]. On the other hand, the induced expression of type-2 chitinase gene (*Chi3*) in the roots of tomato plants treated with the biological control fungus, *Trichoderma harzianum*, illustrates the importance of the product of this gene during the incidence of induced systemic resistance [38]. Also, the accumulation of *Chi3* transcripts in the leaves of tomato plants treated with *F. oxysporum* Fo47 (as a biological control agent) has been reported, while foliar accumulation of *Chi9* transcripts was not observed [39]. Furthermore, the induction of chitinase genes has been reported during the incidence of induced systemic resistance caused by the application by the bacterial biological control agent such as *Bacillus* sp. JS [40]. These studies indicate the recognition of molecular patterns of the beneficial microbes and the subsequent activation of chitinase genes of plant. So, it can be concluded that SAR and ISR pathways share common parts in the transduction of defensive signals [40]. The reason for the capability of the up-regulation of *chi* gene transcription may be in the chemical composition of at least some bacteria, especially of gram-positive bacteria. The cell walls of the bacteria, especially gram-positive bacteria, has composed of peptidoglycan (also called murein), a hetero-polymer consisted of alternating residues of beta-1, 4-linked *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM), where the latter is attached to a peptide chain of 3-5 amino acids. *N*-acetylglucosamine (NAG) is also found in fungal cell wall homopolymer, chitin consisted of *N*-acetylglucosamine [41]. The effect of *N*-acetylglucosamine (NAG) in the up-regulation of *chi* genes has previously been reported [42]. As *Bacillus* spp. [43-45] as well as plants [46-47, 41] are known to produce and secrete chitinases, therefore, it is expectable that the *N*-acetylglucosamine released from these chitinolytic activities can increase the transcription of *chi* genes in plants like tomatoes. The synergism among chitinases has previously been reported [48], and the enzymes produced as the result of synchronous up-regulated expression of *chi3*, and *chi9* genes may also act synergistically. Additionally, the cell wall degrading enzymes produced by plant can have synergistic effects

on the activity of the enzymes and toxins produced by the biological control agent and improve its biological control yield.

CONCLUSION

B. thuringiensis strain IBRC-M11096 is able to induce all three hormonal pathways of plant defensive reactions, therefore, it can be very useful in the integrated management of plant diseases and pests. It seems that the positive interactions between plant and the bacterial strain can lead to further and better control of plant diseases and pests.

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REFERENCES

- [1] Petatan-Sagahon, I., Anducho-Reyes, M.A., Silva-Rojas, H.V., Arana-Cuenca, A., Tellez-Jurado, A., Cárdenas-Álvarez, I.O. and Mercado-Flores, Y. 2011. Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora*. Int J Mol Sci, 12(9): 5522-5537.
- [2] Van der Ent, S., Van Wees, S. C. and Pieterse, C. M. 2009. Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry, 70: 1581-1588.
- [3] Durrant, W.E. and Dong, X. 2004. Systemic acquired resistance. Annu Rev Phytopathol, 42:185-209.
- [4] Conrath, U., Beckers, G.J., Langenbach, C.J. and Jaskiewicz M.R. 2015. Priming for enhanced defence. Annu Rev Phytopathol, 53: 97-119.
- [5] Van der Ent, S., Van Hulten, M.H.A., Pozo, M.J., Czechowski, T., Udvardi, M.K. 2009. Priming of plant innate immunity by rhizobacteria and β -aminobutyric acid: differences and similarities in regulation. New Phytol, 183:419-31.
- [6] Pozo, M.J., Van der Ent, S., Van Loon, L.C., Pieterse, C.M.J. 2008. Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. New Phytol, 180:511-23.
- [7] Verhagen, B.W.M., Glazebrook, J., Zhu, T., Chang, H.S., Van Loon, L.C., Pieterse, C.M.J. 2004. The transcriptome

- of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Mol Plant Microbe Interact*, 17:895–908
- [8] Conrath, U. 2011. Molecular aspects of defence priming. *Trends Plant Sci*, 16 (10):524-531
- [9] van de Mortel, J. E., de Vos, R. C., Dekkers, E., Pineda, A., Guillod, L., Bouwmeester, K. 2012. Metabolic and transcriptomic changes induced in *Arabidopsis* by the rhizobacterium *Pseudomonas fluorescens* SS101. *Plant Physiol*, 160: 2173–2188.
- [10] Van Oosten, V. R., Bodenhausen, N., Reymond, P., Van Pelt, J. A., Van Loon, L. C., Dicke, M. 2008. Differential effectiveness of microbially induced resistance against herbivorous insects in *Arabidopsis*. *Mol. Plant Microbe Interact*, 21: 919–930.
- [11] Pieterse, C. M. J., Van Wees, S. C. M., Van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P. J., Van Loon, L. C. 1998. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell*, 10(9): 1571–1580.
- [12] Niu, D. D., Liu, H. X., Jiang, C. H., Wang, Y. P., Wang, Q. Y., Jin, H. L., et al. (2011). The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces systemic resistance in *Arabidopsis thaliana* by simultaneously activating salicylate- and jasmonate/ethylene-dependent signaling pathways. *Mol Plant Microbe Interact*, 24: 533–542.
- [13] Rosas-García, N.M. 2009. Biopesticide production from *Bacillus thuringiensis*: an environmentally friendly alternative. *Recent Pat Biotechnol*, 3(1): 28-36.
- [14] Kamenek, L., Kamenek, D., Terpilowski, M. and Gouli, V. 2012. Antifungal action of *Bacillus thuringiensis* delta-endotoxin against pathogenic fungi related to Phytophthora and Fusarium. *Int J Agric Technol*, 8(1): 191-203.
- [15] Radhakrishnan, R., Hashem, A. and Abd_Allah, E.F. 2017. Bacillus: a biological tool for crop improvement through bio-molecular changes in adverse environments. *Front Physiol*, 8: 667
- [16] García Robles, I., Ochoa Campuzano, C., Fernández Crespo, E., Camañes, G., Martínez Ramírez, A., González Bosch, C., García Agustín, P., Rausell, C. and Real, M. 2013. Combining hexanoic acid plant priming with *Bacillus thuringiensis* insecticidal activity against Colorado potato beetle. *Int J Mol Sci*, 14(6): 12138-12156.
- [17] Akram, W., Mahboob, A. and Javed, A.A. 2013. *Bacillus thuringiensis* strain 199 can induce systemic resistance in tomato against Fusarium wilt. *Eur J Microbiol Immunol*, 3(4): 275-280.
- [18] Hyakumachi, M., Nishimura, M., Arakawa, T., Asano, S., Yoshida, S., Tsushima, S. and Takahashi, H. 2013. *Bacillus thuringiensis* suppresses bacterial wilt disease caused by *Ralstonia solanacearum* with systemic induction of defense-related gene expression in tomato. *Microbes Environ*, 28(1): 128-134.
- [19] Akköprü, A. and Demir, S. 2005. Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. lycopersici by AMF *Glomus intraradices* and some rhizobacteria. *J Phytopathol*, 153(9): 544-550.
- [20] Lacy, G.H. and Lukezic, F.L. 2008. Plant pathology concepts and laboratory exercises. 2nd edn, In Trigiano, R.N., Windham, M.T. and Windham, A.S. (Eds), *Laboratory Exercises for Plant pathogenic bacteria* pages. pp: 57-63. CRC press, NewYork.
- [21] Livak, K.J. and Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *methods*, 25(4): 402-408.
- [22] Pfaffl, M.W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res*, 29(9): e45-e45.
- [23] Kwon, Y.S., Ryu, C.-M., Lee, S., Park, H.B., Han, K.S., Lee, J.H., Lee, K., Chung, W.S., Jeong, M.-J. and Kim, H.K. 2010. Proteome analysis of *Arabidopsis* seedlings exposed to bacterial volatiles. *Planta*, 232(6): 1355-1370.
- [24] Timmermann, T., Armijo, G., Donoso, R., Seguel, A., Holuigue, L. and González, B. 2017. *Paraburkholderia phytofirmans* PsJN protects *Arabidopsis thaliana* against a virulent strain of *Pseudomonas syringae* through the activation of induced resistance. *Mol Plant Microbe Interact*, 30(3): 215-230
- [25] Pozo, M.J., Van Der Ent, S., Van Loon, L. and Pieterse, C.M. 2008. Transcription factor MYC2 is involved in priming for enhanced defense during rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *New Phytol*, 180(2): 511-523.
- [26] Howe, G.A. 2004. Jasmonates as signals in the wound response. *J Plant Growth Regul*, 23(3): 223-237.
- [27] Bakshi, M. and Oelmüller, R. 2014. WRKY transcription factors: Jack of many trades in plants. *Plant Signal Behav*, 9(2): e27700.
- [28] Dong, J., Chen, C. and Chen, Z. 2003. Expression profiles of the *Arabidopsis* WRKY gene superfamily during plant defense response. *Plant Mol Biol*, 51(1): 21-37
- [29] Zheng, Z., Qamar, S.A., Chen, Z. and Mengiste, T. 2006. Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. *Plant J*, 48(4): 592-605.
- [30] Fan, W. and Dong, X. 2002. In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in *Arabidopsis*. *Plant Cell*, 14(6): 1377-1389.
- [31] Niu, D. D., Wang, C. J., Guo, Y. H., Jiang, C. H., Zhang, W. Z., Wang, Y. P. and Guo, J. H. 2012. The plant growth-promoting rhizobacterium *Bacillus cereus* AR156 induces resistance in tomato with induction and priming of

- defence response. *Biocontrol Sci Technol*, 22(9): 991-1004.
- [32] Ahn, I. P., Lee, S. W. and Suh, S. C. 2007. Rhizobacteria-induced priming in *Arabidopsis* is dependent on ethylene, jasmonic acid, and NPR1. *MPMI*, 20(7): 759-768.
- [33] Ongena, M., Duby, F., Jourdan, E., Beaudry, T., Jadin, V., Dommes, J. and Thonart, P. 2005. *Bacillus subtilis* M4 decreases plant susceptibility towards fungal pathogens by increasing host resistance associated with differential gene expression. *Appl Microbiol Biotechnol*, 67(5): 692-698.
- [34] Pieterse, C.M., Van Wees, S.C., Van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J. and Van Loon, L.C. 1998. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell*, 10(9): 1571-1580.
- [35] Park, K.S. and Kloepper, J.W. 2000. Activation of PR-1a promoter by rhizobacteria that induce systemic resistance in tobacco against *Pseudomonas syringae* pv. tabaci. *Biol Control*, 18(1): 2-9.
- [36] Fatouros, G., Gkizi, D., Fragkogeorgi, G., Paplomatas, E. and Tjamos, S. 2018. Biological control of Pythium, Rhizoctonia and Sclerotinia in lettuce: association of the plant protective activity of the bacterium *Paenibacillus alvei* K165 with the induction of systemic resistance. *Plant pathol*, 67(2): 418-425.
- [37] Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science*, 261(5122): 754-756
- [38] Ene, M., Alexandru, M., NEGUȚ, D.C. and ȘESAN, T.E. 2013. Class II acidic chitinase expression in tomatoes treated with *Trichoderma* and AM Fungi. *Rom Biotechnol Lett*, 18(5): 8645.
- [39] Aime, S., Cordier, C., Alabouvette, C. and Olivain, C. 2008. Comparative analysis of PR gene expression in tomato inoculated with virulent *Fusarium oxysporum* f. sp. *lycopersici* and the biocontrol strain *F. oxysporum* Fo47. *Physiol. Mol. Plant Pathol.*, 73(1-3): 9-15.
- [40] Kim, J. S., Lee, J., Lee, C. H., Woo, S.Y., Kang, H., Seo, S. G. and Kim, S.-H. 2015. Activation of pathogenesis-related genes by the rhizobacterium, *Bacillus* sp. JS, which induces systemic resistance in tobacco plants. *Plant Pathol J*, 31(2): 195.
- [41] Rathore, A.S. and Gupta, R.D. 2015. Chitinases from bacteria to human: properties, applications, and future perspectives. *Enzyme Res*, 2015.
- [42] Haran, S., Schickler, H., Oppenheim, A. and Chet, I. 1996. Differential expression of *Trichoderma harzianum* chitinases during mycoparasitism. *Phytopathology*, 86(9): 980-985
- [43] Bressan, W. and Figueiredo, J.F. 2010. Chitinolytic *Bacillus* spp. isolates antagonistic to *Fusarium moniliforme* in maize. *J Phytopathol*, 92 (2): 343-347.
- [44] El Hamshary, O., Salem, H.H. and Soliman, N.A. 2008. Molecular screening of chitinase gene in *Bacillus* spp. *J Appl Sci Res*, 4: 1118-1123.
- [45] Usharani, T. and Gowda, T. 2011. Cloning of chitinase gene from *Bacillus thuringiensis*. *Indian J Biotechnol*, 10:264-269.
- [46] Collinge, D.B., Kragh, K.M., Mikkelsen, J.D., Nielsen, K.K., Rasmussen, U. and Vadl, K. 1993. Plant chitinases. *Plant J*, 3(1): 31-40.
- [47] Punja, Z.K. and Zhang, Y.Y. 1993. Plant chitinases and their roles in resistance to fungal diseases. *J Nematol*, 25(4): 526.
- [48] Suzuki, K., SuGAWARA, N., Suzuki, M., Uchiyama, T., Katouno, F., Nikaidou, N. and Watanabe, T. 2002. Chitinases A, B, and C1 of *Serratia marcescens* 2170 produced by recombinant *Escherichia coli*: enzymatic properties and synergism on chitin degradation. *Biosci Biotechnol Biochem*, 66(5): 1075-1083.

پرایمینگ حاصل از باکتری باسیلوس تورینجینسیس (*Bacillus thuringiensis*) ژن‌های مسیر دفاعی جاسمونات/اتیلن و اسید سالیسیلیک را در گیاه گوجه فرنگی القاء می‌کند.

معصومه دزآباد^۱، هنگامه طاهری^{۱*}، بابک پاکدامن سردرد^۲

^۱ گروه مهندسی تولید و ژنتیک گیاهی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، خوزستان، ایران

^۲ گروه گیاه‌پزشکی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، خوزستان، ایران

* نویسنده مسئول: taheri@asnruk.ac.ir

چکیده

باکتری باسیلوس تورینجینسیس (*Bacillus thuringiensis*) به عنوان عامل کنترل بیولوژیک نقش مهمی در مدیریت تلفیقی طیف وسیعی از آفات و بیماری‌های گیاهی ایفاء می‌کند. انتظار بر این است که این باکتری پاسخ دفاعی گیاه را از طریق شناسایی الگوهای مولکولی مرتبط با میکروب (MAMPs) القاء نماید. با این وجود اطلاعات بسیار کمی در ارتباط با اساس مولکولی مقاومت سیستمیک در گیاه گوجه فرنگی در دست است. در این مطالعه میزان رونویسی ژن‌های پاسخ دهنده به اسید سالیسیلیک (*Chi3*، *PR1* و *Chi9*)، ژن پاسخ دهنده به جاسمونیک اسید (*Pin2*) و ژن‌های تنظیمی پاسخ دهنده به سیگنال هورمون‌های جاسمونیک اسید و اتیلن (*MYC2*، *ERF1* و *WRKY33*) در فواصل زمانی ۶، ۱۲، ۲۴، ۴۸، ۷۲ و ۹۶ ساعت پس از القاء گیاهچه‌های گوجه‌فرنگی با باکتری محرک رشد *B. thuringiensis* نژاد IBRC-M 110960 با استفاده از تکنیک qRT-PCR اندازه‌گیری شد. این نژاد باکتریایی هر سه مسیر سیگنالی هورمونی (سالیسیلیک اسید، جاسمونات و اتیلن) را که در القاء مقاومت بر علیه طیف وسیعی از بیمارگرهای گیاهی نکروتروف و بیوتروف سهیم هستند را القاء نمود. اما بیان بیشتر *WRKY33*، *ERF1*، *MYC2* و *Pin2* در گیاهان تلقیح شده با باکتری نشان داد که اثر پرایمینگ بیشتر به مسیر سیگنالی جاسمونات/اتیلن وابسته است. این یافته‌ها حاکی از پتانسیل بالای این نژاد باکتریایی در مدیریت زراعی گیاه گوجه فرنگی می‌باشد.

کلمات کلیدی: باکتری‌های مفید خاک، مسیر سیگنالی هورمونی، مقاومت سیستمیک القاء شده، مقاومت اکتسابی سیستمیک