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Research Article

MAMP-triggered resistance induced by elicitor protein PeBA1 derived from *Bacillus amyloliquefaciens* NC6 in common bean (*Phaseolus vulgaris* L.) against green peach aphid (*Myzus persicae* Sulzer)

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Abstract

Bacterial microbe-associated molecular patterns (MAMPs) play an important role in innate plant immunity. This *in vitro* study evaluated the putative role of protein elicitor PeBA1 derived from *Bacillus amyloliquefaciens* NC6 strain in eliciting induced resistance type responses in common bean (*Phaseolus vulgaris*) plants against green peach aphid *Myzus persicae*. Nymphal developmental time of aphids was significantly prolonged and the fecundity was significantly reduced by different concentrations of PeBA1 elicitor (*i.e.* 40.51, 24.91 and 16.38 μg mL^{-1}) applied at three different temperature regimes (*i.e.* 21, 27 and 30 °C). Moreover, foliar application of PeBA1 elicitor protein strongly up-regulated the expression levels of salicylic acid (SA) pathway-associated genes, while the expression levels of jasmonic acid (JA) pathway-associated genes exhibited a moderate induction. Quantification by LC/MSMS revealed a linear increase of both SA and JA plant defense hormones along with the time of exposure. Our findings suggest that the bacterial elicitor protein PeBA1 could be used as an effective biological pest management tool against phloem-feeding insect pests such as green peach aphids *M. persicae*.

Keywords: elicitor protein; jasmonic acid pathway; induced resistance; life-history traits; *Myzus persicae*; PeBA1; plant defense hormones; salicylic acid pathway

Introduction

In nature, plants are confronted by a wide range of pathogens and herbivore pests. In response to these biotic stresses, they have evolved different physiological and molecular defense mechanisms including hypersensitive response (HR), defense signaling pathways, reactive oxygen species (ROS) and reactive nitrogen

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species (RNS) synthesis (Delledonne *et al*., 2001; Garcia-Brugger *et al*., 2006). These plant defense pathways are regulated by transcriptional and metabolic changes through physiological reactions, and are often directly or indirectly activated by different signaling molecules such as nitric oxide (NO) and ROSs produced by the activation of plasma membrane proteins or protein phosphorylation (Garcia-Brugger *et al*., 2006).

Apart from plant-derived compounds, plant defense reactions are also elicited by a number of pathogenic microbe- or herbivore-derived molecular patterns (MAMPs or HAMPs) which ultimately induce systemic and local resistance against the pests (Durrant and Dong, 2004; Maffei *et al*., 2012). Various MAMP type elicitors have been extracted from different microorganisms such as fungi, bacteria and viruses and are usually glycoproteins, oligosaccharide, lipids and proteins (Boller and He, 2009; Ellis *et al*., 2009; Maffei *et al*., 2012). Most of these elicitor molecules are perceived by plants under attack as signal molecules to defend themselves through inducing resistance against attacking herbivore pests and/or pathogens via activation of different plant defense pathways (Bostock *et al*., 2001).

Plant hormones play a major role in triggering host plant defenses against different abiotic and biotic stresses such as cold, heat and drought stresses and herbivore infestation or pathogenic infection. For instance, salicylic acid (SA) and jasmonic acid (JA) are two most important signaling molecules that elicit and enhance various plant defense responses (Thaler *et al*., 2012). Commonly, SA has been found associated with resistance to biotrophic pathogens and herbivory by phloem-feeding insect pests, while JA is usually induced in plants stressed by necrotrophic pathogens and chewing insect pests (Glazebrook *et al*., 2005; Smith *et al*., 2009). Moreover, expression studies of classical JA- and JA-associated marker genes have shown the antagonistic biosynthesis and signaling of both these plant hormones (Mur *et al*., 2006; Loake and Grant, 2007).

Although, entomopathogenic fungi constitute an important biological control strategy against different insect pests (Vega *et al*., 2012), their ability to grow endophytically within different plant portions has suggested their new ecological functions (Quesada-Moraga *et al*., 2014; Nazir *et al*., 2019). For instance, these fungi may induce systemic resistance in plants against different biotic and abiotic stresses (Waller *et al*., 2005; Sánchez-Rodríguez *et al*., 2018), suggesting their new perspective to develop novel plant protection strategies (Sánchez-Rodríguez *et al*., 2016). Recently, a number of elicitor proteins have been isolated from different biotrophic and necrotrophic fungal species exhibiting induced resistance against herbivore pests and pathogens. For instance, the elicitor proteins PeBC1 and PeBA1 cloned respectively from a necrotrophic fungus *Botrytis cinerea* and bacterial strain *Bacillus amyloliquefaciens* NC6, induced resistance against diseases in *Arabidopsis* (Zhang *et al*., 2014) and in tobacco (Wang *et al*., 2016).

Keeping in view these novel ecological aspects of microbes, this *in-vitro* study was carried out to determine the biological activity of an elicitor protein PeBA1 derived from the gram-positive bacteria *B. amyloliquefaciens* NC6 strain against green peach aphids *M. persicae*. This elicitor protein consists of 285 amino acid residues and is capable of inducing systemic resistance features in tobacco plant including transcription of JA and SA related defense genes, cell death, HR necrosis and ROS synthesis (Wang *et al*., 2016). Moreover, as the abiotic factors such as temperature may influence the JA and SA signaling pathways (Zhu *et al*., 2010), bioassays were carried out at three different temperature regimes in order to assess the response of aphids to PeBA1 elicitor protein.

Materials and Methods

Insect and plant culture

In a growth chamber, a colony of green peach aphid *M. persicae* was maintained on common bean (*Phaseolus vulgaris* L.) plants in isolation cages. In order to ensure that aphids have been properly adapted to the chemistry of bean plants, they were maintained in a controlled chamber at 65 to 75% relative humidity and

21 ± 2 °C temperature on the bean plants for at least three months prior to the start of actual bioassays. Bean plants were grown in plastic pots with sterile commercial potting mix and were raised in a greenhouse under natural light with day and night temperature fluctuating between 21 to 33 °C (from June to September). Prior to experimentation, short-term feeding bioassays were conducted in order to observe the suitability of foliage from the control and elicitor-treated bean plants for aphid growth and development. Experimental bean plants were grown in 15 cm plastic pots filled with sterile commercial potting mix (manure and soil in 1:5 ratio). Ten days old plants at 3-leaf stage were used in bioassays.

Protein purification

The elicitor protein PeBA1 was extracted from the colony of bacterial strain *Bacillus amyloliquefaciens* NC6 cultured in 1 L of LB medium for 12 h at 16 °C at 200 rpm. The pellets were then collected, broken and re-suspended by sonication. Then, centrifugation of the solution was performed for 15 min at 12000 rpm followed by the collection and filtration of supernatant through a 0.22 μm filter paper. Final purification of elicitor protein was carried out with help of affinity chromatography using a His-trap HP column and A, B, C and D loading buffers. Buffer A (50 mM Tris-HCl, pH 8.0) was used to wash the column, while buffer B (50 mM Tris-HCl, 200 mM NaCl; pH 8.0) was used to stabilize the column. Buffer C (50 mM Tris-HCl, 200 mM NaCl and 20 mM imidazole; pH 8.0) and buffer D (50 mM Tris-HCl, 200 mM NaCl and 500 mM imidazole; pH 8.0) were used to elute the elicitor protein. Elicitor protein was then desalted by desalting tubes and by centrifugation at 4 °C at 5000 rpm. The molecular mass of purified recombinant elicitor protein was determined by 10% SDS PAGE gel and by using a protein marker.

Bioassays with PeBA1 elicitor protein against aphids

Different concentrations of purified PeBA1 elicitor protein were bio-assayed against *M. persicae* aphids on bean plants. Treatments included three different concentrations of elicitor protein (*i.e.* 40.51, 24.90 and 16.38 μg mL^{-1}) and the control in which plants were treated only with buffer A (50 mM Tris-HCl, pH 8.0). Protein concentrations were determined by Bradford assay using bovine serum albumin (BSA) as standard. Elicitor treatments were applied on bean plants at 3-leaf stage with a precise spray bottle until the bean plants were thoroughly covered and began to drain. Three milliliters of each elicitor solution were applied on each plant. Control plants were sprayed with buffer only. Plants were allowed to dry overnight and next day five freshly molted (0-6 h old) adult winged aphids per leaf were released on these plants. Nymphal development time for each instar and total number of offspring produced by these F1 aphid progeny were recorded by consecutive observations at 3 h intervals until the completion of bioassays (in approximately 3 weeks). Each treatment was replicated 10 times and the bioassay was repeated thrice at three different temperature regimes (i.e. at 21, 27 and 30 °C) independently.

Isolation of RNA and cDNA synthesis

For the molecular characterization of PeBA1 elicitor-induced resistance in plants against *M. persicae* aphids, bean plants were treated with the highest elicitor concentration (i.e. 40.51 μg mL^{-1}), and were allowed to be infested by aphids as described in previous bioassay. Leaf samples were then collected from both treated and un-treated plants and RNA was extracted from these leaves using plant RNA kit Trans ER 301-01(All Style Gold Tech. Co. Ltd., Beijing, China) following manufacturer's protocol. Quantity and quality of the extracted RNA were measured with Nano Photometer (NP80-Touch, Implen Inc., NP West Burg, USA). Later on, this extracted RNA was reverse transcribed to cDNA using one step gDNA and cDNA kit Trans AT 341-01 (All Style Gold Tech. Co. Ltd., Beijing, China) following manufacturer's protocol.

RT-qPCR analysis

Using RT-qPCR, the relative expression of genes putatively involved in JA and SA plant defense pathways were determined in the elicitor-treated and buffer-treated bean plants. Marker genes involved in the JA pathway included phvul.001G000800g, phvul.001G001300g, phvul.001G017800g, phvul.002G175500g, phvul.002G06700g, phvul.003G011600g, phvul.003G111500g and phvul.003G096400g. Similarly, the SA-associated marker genes used in the study were phvul.006G048600g, phvul.008G272800g, phvul.008G057700g, phvul.009G022200g and phvul.011G176100g. Actin was used as the reference gene (internal control). Ten folds diluted cDNA was used as a template. Primer pairs used for the RT-qPCR amplifications of these genes are given in Table S1. The efficacy and specificity of all primer pairs were validated through qPCR and gel electrophoresis. Thermocycler ABI 7500 (Applied Biosystems, USA) was used for RT-qPCR amplifications. Each reaction mixture was 20 µL holding 2 µL of cDNA template, 0.5 µL of each 10 µmol L^{-1} forward and reverse primer, 10 µL of 2× SYBR® Premix Ex Taq (Takara, Dalian, China) and 7 µL of ddH_2O). PCR thermal program was constituted of preheating at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 30 s and annealing at 60 °C for 40 s and elongation at 72 °C for 60 s. There were three biological and three technical replicates for each sample.

LC-MS/MS analysis

Apart from the expression analysis of potential JA- and SA-associated genes, we quantified these plant hormones in the leaves of aphid infested elicitor treated and buffer-treated plants using LC-MS/MS chromatography. For this determination, leaves of bean plants were collected at different time intervals (i.e. 6, 12, 18 and 24 h) and were frozen dried using liquid nitrogen. Leaf samples were then placed into 2 mL micro-centrifuge tubes and were ground in a bead-beater using 2 mm tungsten-carbide beads with 25 Hz/s for 4 min. About 20 mg of powdered tissue (equivalent to almost 200 mg fresh weight) was extracted with 400 µL of 10% methanol and 1% acetic acid. Extraction was repeated twice recovering about 90-95% of the sample. Both plant hormones were determined using QTRAP® 5500 LC-MS/MS System (Applied Biosystems, Foster City, CA, USA) equipped with a 1290 Series HPLC System (Agilent Technologies, Foster City, CA, USA) and Turbo Ion Spray Electrospray Ionization (ESI) source. Quantifications were performed using multiple reactions monitoring (MRM) method in a negative ion detection mode (m/z 209.1/59.2 for JA and 137.0/65.1 for SA). The retention times for JA and SA were 5.02 and 4.47 min, respectively.

Statistical analysis

The experiments were repeated three times independently and the mean values of all parameters are presented in figures along with standard errors. Statistix Version 8.1 (Analytical Software, Tallahassee, FL, USA) was used for statistical analyses of data. Some data, for instance of fecundity of aphids, was subjected to square-root transformation prior to analysis. One-way analysis of variance was run to find out significant differences among treatment factors followed by Tukey's HSD test at 95% level of probability. The RT-qPCR expressions of genes were measured using comparative CT ($2^{-\Delta\Delta CT}$) method. The fold change in the elicitor-treated and buffer-treated (control) plant samples was compared by Student's *t*-test at $\alpha = 0.05$.LC-MS/MS data acquisition and processing were carried out using AB Sciex (V 1.6.2) software and were analyzed by regression analysis.

Results

Influence of PeBA1 elicitor on nymphal development time of aphids

Analysis of variance showed a significant impact of different concentrations of PeBA1 elicitor protein ($F_{3, 468} = 12.71$; $p < 0.001$), temperature regimes ($F_{2, 468} = 274.27$; $p < 0.001$) and of their interaction ($F_{6, 468} = 5.61$; $p < 0.001$) on the overall developmental time of *M. persicae* (Table S2). The effect of protein elicitor on

nymphal development time of aphids was found different at different temperature regimes. The developmental time of each nymphal instar was prolonged along with the concentration of PeBA1 elicitor. Maximum nymphal developmental time was recorded as 4.8 days for 1st instar to 6.4 days for 3rd instar nymphs for high concentration (40.51 µg mL^{-1}) at low temperature (21 °C). While minimum nymphal development time (1.8 days) was recorded for 1st instar for low elicitor concentration (16.38 µg mL^{-1}) at high temperature regime (30 °C). In buffer-treated (control) plants, the nymphal development time varied from maximum (4.2 days) for 3rd instar at 21 °C to minimum (1.7 days) for 1st instar at 30 °C (Figure 1).

Overall, the effect of PeBA1 elicitor concentrations appeared to be highly significant for the 1st instar ($F_{3, 108} = 2.84$; $p = 0.04$), 2nd instar ($F_{3, 108} = 6.17$; $p < 0.01$), 3rd instar ($F_{3, 108} = 6.91$; $p = 0.003$) and 4th instar ($F_{3, 108} = 6.26$; $p < 0.001$). Nevertheless, the effect of PeBA1 elicitor on nymphal development time of all aphid instars was more significant at low temperature (21 °C) than at medium or high temperatures (Figure 1).

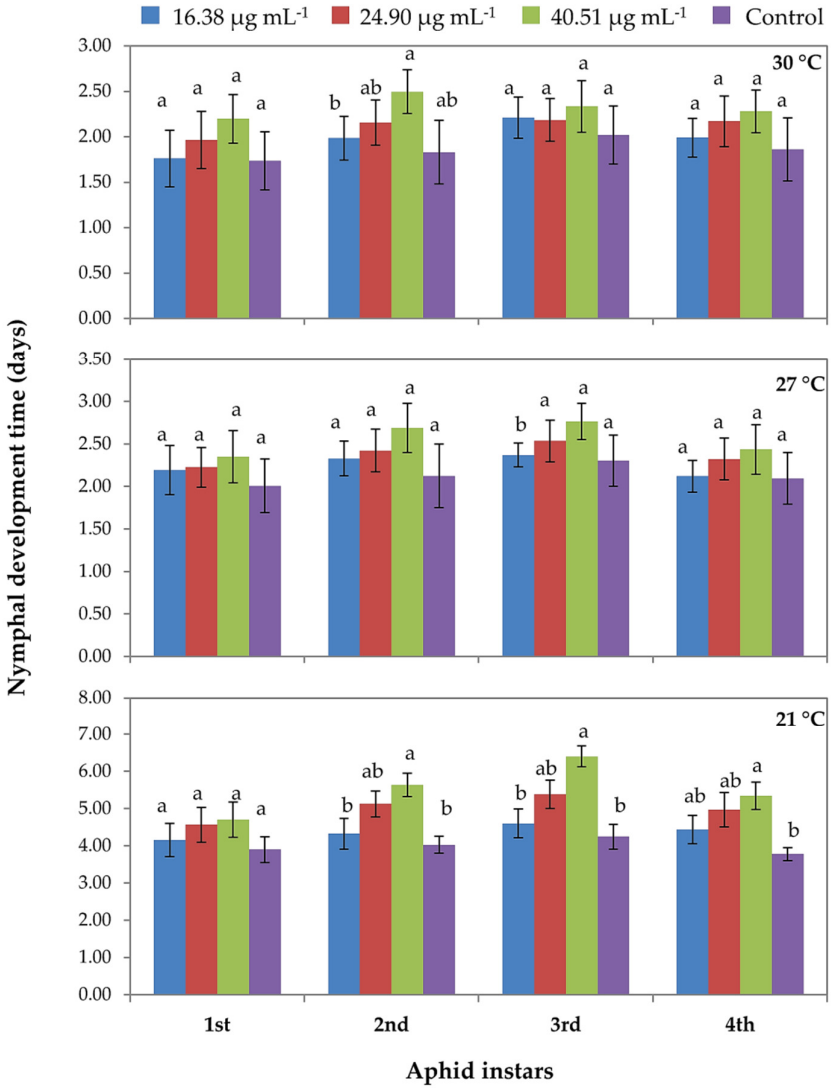

Figure 1. Mean development time (± SE) of different nymphal instars of green peach aphid (*Myzus persicae*) on common bean plants in response to the application of PeBA1 elicitor protein at different temperature regimes (n = 10). For each temperature regime, different alphabets above bar tops indicate significant differences between treatments (one-way ANOVA; Tukey's HSD at $\alpha = 0.05$)

Effect of Elicitor PeBA1 on the fecundity of aphids

Results showed that different concentrations of PeBA1 elicitor ($F_{3, 108}$ = 23.08; $p \leq 0.001$) and temperature regimes ($F_{2, 108}$ = 225.72; $p \leq 0.001$) significantly influenced the fecundity *of M. persicae* adults (Table S3). Aphid individuals fed on the elicitor-treated plants produced significantly less number of offspring during their lifespan than those fed on the control (buffer-treated) plants. Moreover, minimum fecundity was observed at high temperature (30 °C), while the maximum fecundity was observed at low temperature (21 °C) (Figure 2).

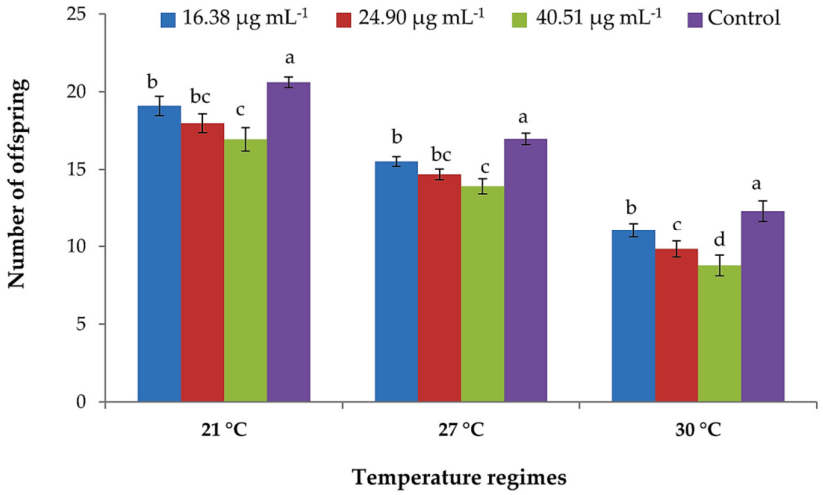

Figure 2. Mean fecundity (± SE) of green peach aphids (*Myzus persicae*) on common bean plants in response to different concentrations of PeBA1 elicitor protein at different temperature regimes (n = 10). For each temperature regime, different alphabets above bar tops indicate significant differences between treatments (one-way ANOVA; Tukey's HSD at $\alpha = 0.05$)

Effect of PeBA1 elicitor on expression of JA and SA pathway-associated genes

Results revealed differential expression levels of different key genes associated with JA and SA pathways in elicitor-treated bean plants as compared to control ones. All SA-associated genes were highly up-regulated and were significantly different from the control samples for all observation times (Figure 3). Although relatively moderate but a similar trend of expression levels was observed for all JA-associated genes. Most of these genes were up-regulated at all observation times. However, one gene (phvul.002G175500g) initially showed no up-regulation till 6 h post aphid infestation and then was up-regulated from 12 h onward (Figure 4). Moreover, maximum gene expression levels for both types of key genes were recorded at 18 and 24 h of the elicitor application and aphid infestation.

Quantification of JA and SA hormones

Results of LC-MS/MS quantification of plant defense hormones have shown that levels of both JA and SA were gradually and significantly increased along with time. Maximum hormone amount was recorded after 24 h of elicitor treatment, while minimum was recorded at 6 h. In control plants, no significant amount of JA was recorded, while a considerable quantity (2.30 µg g^{-1}) of SA was observed in control plants (Figure 5). A positive correlation was found between these two plant hormones and time of exposure to protein elicitor PeBA1 (R^2 = 0.98 for SA and 0.82 for JA).

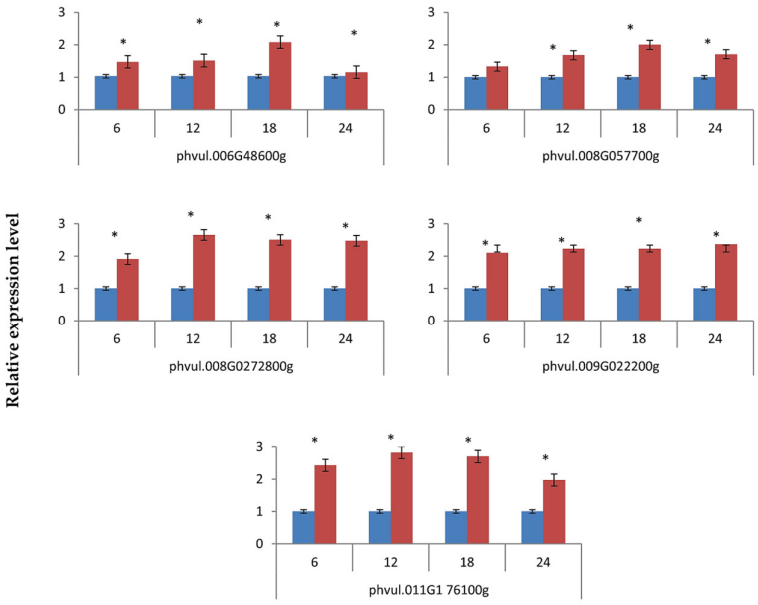

Figure 3. Relative expression levels (± SE) of SA-associated plant defense pathway determined at different time intervals after the application of elicitor PeBA1 and aphid infestation. Red bars indicate the result of PeBA1 elicitor treatments, while blue bars show the result of buffer-treated (control) treatments. For each gene, asterisk symbols above bar tops indicate the significant difference among treatments (Student's t-test; $p < 0.05$)

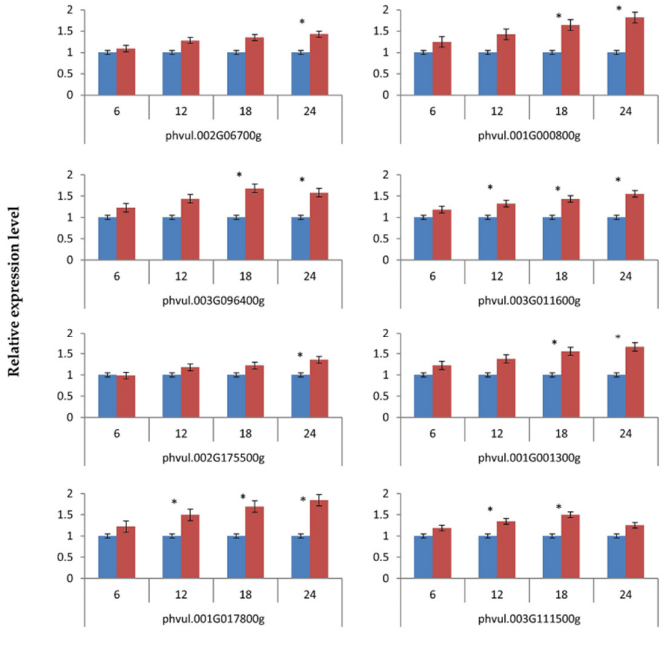

Figure 4. Relative expression levels (± SE) of JA-associated plant defense pathway determined at different time intervals after the application of elicitor PeBA1 and aphid infestation. Red bars indicate the result of PeBA1 elicitor treatments, while blue bars show the result of buffer-treated (control) treatments. For each gene, asterisk symbols above bar tops indicate the significant difference among treatments (Student's t-test; $p < 0.05$)

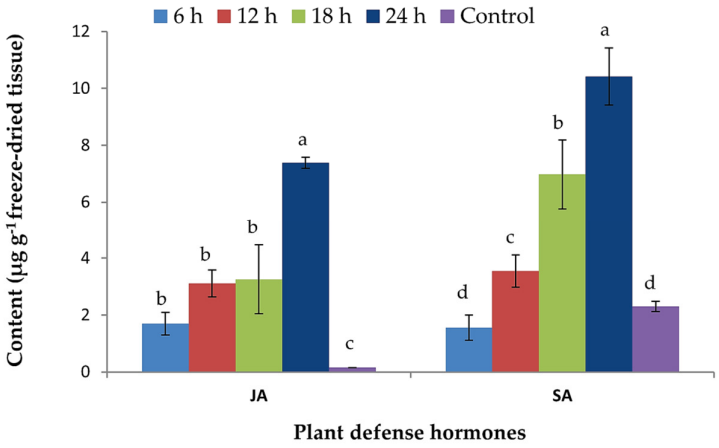

Figure 5. JA and SA plant hormone contents (± SE) as determined by LC-MS/MS in one day old leaves of common bean (*Phaseolus vulgaris*) after the treatment of PeBA1 elicitor. For each plant hormone, different alphabets above bar tops indicate significant differences between time intervals (one-way ANOVA; Tukey's HSD at $\alpha = 0.05$)

Discussion

Elicitors play a vital role in signaling defense mechanisms in plants under attack and are considered as a novel biological pest management approach. Pathogenic fungi, either biotrophic or neurotropic, constitute a major source for microbes-derived elicitors such as PAMPs or MAMPs (Zhang *et al*., 2014; Wang *et al*., 2016). This *in-vitro* study elucidated the activity of an elicitor protein PeBA1 derived from *B. amyloliquefaciens* NC6 strain for the control of aphid *M. persicae*. The results of our bioassays have demonstrated that aphid population developed significantly slower on elicitor-treated plants than on the buffer-treated control plants. Our results are consistent with the previous studies demonstrating the negative impact of exogenous applications of different plant defense inducing chemicals including JA, benzothiadiazole (BTH) and methyl jasmonate (MJ) on the population growth and fitness parameters of different aphid species (Cooper and Goggin, 2005; Boughton *et al*., 2006). Similarly, our results corroborate the findings of Mallinger *et al*. (2011) who documented that methyl salicylate elicitor reduced the population of soybean aphid *Aphis glycines* up to 40%. Similarly, some studies have shown that the activity of herbivore insect pests was significantly reduced after the application of plant defense inducing molecules such as MJ and BTH and other plant defense proteins such as proteinase inhibitors in tomato crop (Bostock *et al*., 2001; Bale *et al*., 2002). Exogenous application of elicitor molecules usually induce systemic or local resistance in plants against herbivore insect pests most probably by activating the plant SA and JA associated pathways resulting in the induction of different plant defense proteins such as peroxidase (POD), polyphenol oxidase (PPO), PR proteins, lipoxygenase and protein inhibitors etc.is well known (Bostock *et al*., 2001; Maffei *et al*., 2012).

Although PeBA1 elicitor application caused reduced nymphal developmental time and fecundity of aphids, the impact is statistically more significant and pronounced at low temperature (21 °C). Maximum nymphal development time was observed at lower temperature (21 °C) confirming the fact that with the increase of one degree in temperature, insect life cycle would become shorter (Bale *et al*., 2002). Similarly, the application of PeBA1 elicitor reduced the aphid fecundity. Aphids reproduced much lower on the elicitor treated plants as compared to buffer treated control plants. These results are in line with the previous studies

evidencing a lower mean lifetime fecundity of aphids induced by the exogenous application of SA (Mahmoud and Mahfouz, 2015) and MJ (Boughton *et al*., 2006).

Our study revealed the potential of PeBA1 elicitor on the suppression of population performance and growth parameters of sap-feeding herbivores. However, as different chemical elicitors such as JA and MJ may induce the production of various proteinase inhibitors in plants as described in tomato plants (Farmer *et al*., 1992), more studies are needed to better comprehend the underlying mechanisms induced in bean plants by the PeBA1 elicitor protein which actually influenced the nymphal developmental time and fecundity of aphid individuals.

Moreover, SA and JA hormones play an essential role in signaling plant defense pathways and in the regulation of defense associated genes (Moran and Thompson, 2001; Thaler *et al*., 2012). These molecules enhance plant defense responses including resistance against insect herbivory and pathogenic attacks (Ali and Agrawal, 2012). In this study, we found that elicitor PeBA1 produced a significant and strong up-regulation of all key genes potentially associated with SA pathway, while a moderate up-regulation was observed in case of JA pathway associated genes. Our findings are in agreement with the fact that phloem-feeding insect pests such as aphids trigger more strongly the expression of SA pathway associated genes than those related to JA pathway (Smith *et al*., 2009; Ali and Agrawal, 2012; Coppola *et al*., 2013). This is further supported by the results of liquid chromatography-mass spectrometry which showed some quantity of SA contents in aphid infested control plants as compared to the negligible JA contents. This corroborates the fact that herbivory by sap-feeding pests such as aphids usually triggers more strongly the key genes associated with SA plant defense pathway rather than those involved in the JA pathway (Boughton *et al*., 2006; Coppola *et al*., 2013). Moreover, our results are in line with our recent study (Basit *et al*., 2019) which demonstrated a significant sub-lethal effect of PeBC1 protein elicitor, derived from the fungus *Botrytis cinerea*, on *M. persicae* concomitantly with a pronounced up-regulation of the expression level of genes related to SA and JA pathway.

Conclusions

In this study, we postulated that the application of PeBA1 elicitor would prolong the nymphal development time and reduce the fecundity of *M. persicae* on common bean plants. Bioassays performed with different concentrations of PeBA1 applied at three different temperature regimes revealed that aphids developed and reproduced slower on elicitor-treated plants than on control plants. Furthermore, functional characterization of key genes potentially involved in JA and SA plant defense pathways indicated that elicitor PeBA1 induced a significant and differential expression of genes in bean plants concomitantly with an enhanced production of both hormones. Based on overall results of the study, it is concluded that MAMPs such as PeBA1 could be effectively used as novel biological pest control tool against aphids and other phloem-feeding insect pests.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Supplementary Tables

Table S1. Primer pairs of key genes potentially involved in the plant defense JA and SA pathways

Target Gene	Forward Sequence (5′ → 3′)	Reverse Sequence (5′ → 3′)
PHAVU_002G175500g	GAAAAGCGTGGAAAGCTACG	AGCCATGAACGATGATCTCC
PHAVU_001G017800g	GGGAGAAGCTGCTGAAACAC	CCGACCTGAATATCGAAGGA
PHAVU_003G111500g	GAATTTCCCTGCTGCTCTTG	CTGGCTTAGCCTCAGGAATG
PHAVU_001G000800g	AGCCGCATGCTGTTCTCTAT	TTTTCATGAACAGCGCTCAC
PHAVU_001G001300g	TGAAATGGCCAAGAAGGAAC	GGCGACGAGACCGTATATGT
PHAVU_002G06700g	CTGATGAGCAGCAGCAGAAG	AAACGGGCATAAACAACAGC
PHAVU_003G096400g	ACGACCATGGGTTGCTAGTC	AATGCTTCAGCTTCCTTCCA
PHAVU_003G011600g	TAGTGATGGTGCAGGAGCTG	GATGCAAAGGCCTCATTGAT
PHAVU_006G048600g	CAGGATGCTTGGGATGATCT	CAAGGGCCTTTCCTACTTCC
PHAVU_008G057700g	TGCTTCACATGAATGGTGGT	CAACCCAAGTCTGCCACTTT
PHAVU_008G272800g	TCCTTGTTGATGCCCACATA	CAAAGAAAAAGGGGGAGAGG
PHAVU_011G176100g	CCCATGCACAGTGTACCAAG	ACCAATTAACCCCCAAGGAG
PHAVU_011G17200g	GCTGATTTGGGATGCTCTTC	CGTTTCCCTTGTTGAGTGGT
β-actin	GGAAAATCAGTCTCGGTTCAG	TCATACAGCAGCAAGCAC

Table S2. ANOVA table for the effect of PeBC1elicitor protein and temperature on the nymphal development time of green peach aphids *Myzus persicae*

SOV	df	1st Instar			2nd Instar			3rd Instar			4th Instar			Overall		
		MS	F-Value	P-value	MS	F-Value	P-value	MS	F-Value	P-value	MS	F-Value	P-value	MS	F-Value	P-value
Conc.	3	4.06	2.84	0.04	7.09	6.17	<0.01	6.94	6.91	0.003	7.12	6.26	<0.001	15.91	12.71	<0.001
Temp.	2	74.53	52.27	<0.001	104.36	90.89	<0.001	123.23	122.67	<0.001	94.68	83.26	<0.001	343.15	274.27	<0.001
Conc.× Temp.	6	0.52	0.37	0.89	1.25	1.09	0.38	3.00	2.99	0.01	1.69	1.49	0.19	7.02	5.61	<0.001
Error	108	1.43			1.15			1.01			1.14			1.25		
Total	119															
Mean / CV		2.88 / 41.41			3.27 / 32.80			3.46 / 28.98			3.23 / 35.18			3.21 / 34.80		

Table S3. ANOVA table for the impact of PeBA1 elicitor protein and temperature on the fecundity of green peach aphid *Myzus persicae*

SOV	DF	SS	MS	F-Value	P-value
Conc.	3	188.83	62.942	23.08	< 0.001
Temp.	2	1402.52	701.258	225.72	< 0.001
Conc. × Temp.	6	14.75	22.258	0.90	0.4967
Error	108	2294.50	2.727		
Total	119	1900.59			
Grand Mean / CV	15.058 / 10.97				