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# Jasmonic acid induced defence responses in *Bt* (*Bacillus thuringiensis*) and non-*Bt* corn (*Zea mays* L.) seedlings

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## ABSTRACT

*Bt* (*Bacillus thuringiensis*) corn is the most commercialized anti-insect transgenic crops. We examined the effects of jasmonic acid (JA) on the changes in contents of defence chemicals and expression of defence-related genes in the treated part (first leaf) and non-treated part (second leaf and root) of *Bt* corn varieties 5422*Bt*1 and 5422CBCL and their conventional corn 5422 (non-*Bt* corn). The JA exposure did not affect the *Bt* protein content of two *Bt* corns. Among the three corn varieties JA-induced effects in the first leaf did not differ. However systemically induced effects in the second leaf and root in *Bt* corns were significant than in non-*Bt* corn. It can be concluded that *Bt* gene introduction and endogenous chemical defence responses of corns act synergistically during the JA systemically induced defence processes.

**Key words:** *Bacillus thuringiensis*, *Bt* corn, defence chemicals, defence-related genes, defence responses, Jasmonic acid, maize, non-Bt corn, systemic induction, *Zea mays*.

## INTRODUCTION

In last few decades, transgenic crops areas have greatly increased i.e. 185.1 million ha in 2016, among which *Bt* (*Bacillus thuringiensis*) corn (*Zea mays* L.) was planted in 53.6 million ha worldwide i.e. covering 26% area of global corn production (15). *Bt* corn is genetically modified to express insecticidal crystal protein from the bacterium *Bacillus thuringiensis* and is now most commercialized anti-insect transgenic crops. On the other hand, a heat debate has been raised on the potential environmental risks, especially on the resistance to target insects caused by single transgenic insecticidal gene during the large-scale commercialization of *Bt* crops (6). Corns have evolved some direct and indirect defence strategies to avoid or defend the diseases and pests during their long co-evolution processes, called "induced defence responses" (16,20,24,25,37). Such defence response can be studied by determining the contents of defence chemicals and the activities of some defensive enzymes, or by analyzing the expression patterns of key genes involved in the synthesis of defence chemicals and enzymes, including the changes of direct defence compounds (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one, DIMBOA; phenolic acids), expression patterns of pathogenesis related protein gene (acidic beta-1,3-glucanase, *PR-2a*), proteinase inhibitor gene (maize proteinase inhibitor, *MPI*) and key genes in terpenoids biosynthesis (farnesyl pyrophosphate synthase, *FPS*; terpene synthase, *TPS*) (5,6,7,37). It is believed that the pest control can be achieved by combining the endogenous defence system with the introduced *Bt* genes, which is promising alternative

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strategy for pest resistance management (1,8). Nowadays, evaluating the difference in induced defence responses between *Bt* corn and conventional corn becomes an important component to assess the ecological risk of transgenic crops (5,6).

Jasmonic acid (JA) is vital signaling substance involved in plant induced defence responses processes (7,31,32,39). It can move within the whole plant (26,29,43), playing important role in the resistance to plant diseases and insect pests (13,22,38). JA application to plants increases the contents or activities of direct defence chemicals, such as nicotine (12,27), proteinase inhibitors (42), DIMBOA (7,37), glucosinolates (9,23) and polyphenol oxidase (30), increases the contents of indirect defence volatiles, which can attract natural enemies of the herbivores (41) and induces expression of defence-related genes in plants (21,38,40). The exogenous JA application to the leaves can systemically induce the defence responses in the non-treated leaves (28,36,40) or roots (7,17,35).

However, few studies on induced defence responses have been done in *Bt* crops so far. This study aimed to investigate the induced defence responses after JA exposure to the leaf in *Bt* corn and to explore the relationship between *Bt* gene introduction and endogenous chemical defence responses during the JA induced defence processes. The findings of our study can be helpful in understanding the defence responses in *Bt* crops against the diseases and pests, also provide useful information for assessment of ecological risk of transgenic crops.

## MATERIALS AND METHODS

### Planting and management of corn

The experiment was conducted in the growth chamber of South China Agricultural University, Guangzhou China (23 °8′ N, 113 °15′ E), tropical monsoon climate. The test crops were *Bt* corns 5422*Bt*1 (*Bt*11) and 5422CBCL (Mon810) and conventional corn 5422 (non-*Bt* corn), these were from Beck's Hybrids Company, USA. One seed was sown in each plastic pot (10 cm height × 15 cm dia) containing 700 g sandy soil. All pots were kept in growth chamber [12 h/12 h (dark/light) photoperiod, 70% relative humidity and 22°C/28°C (dark/light)]. The seeds were watered every other day with 20 ml nutrient solution [2 mM $KNO_3$, 0.5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.75 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM $KH_2PO_4$, 0.25 mM NaCl, 0.25 mM $K_2SO_4$, 60 μM Fe-Na EDTA, 50 μM $H_3BO_3$, 15 μM $MnCl_2 \cdot 4H_2O$, 2 μM $ZnSO_4 \cdot 7H_2O$, 0.25 μM $CuSO_4 \cdot 5H_2O$ and 0.2 μM $Na_2MoO_4 \cdot 2H_2O$].

### Treatment and sampling of corn

We treated the first leaf of *Bt* corns 5422*Bt*1 (*Bt*11) and 5422CBCL (Mon810), and the conventional corn 5422 (non-*Bt* corn) with JA (Jasmonic acid) and determined the content of defence chemicals and expression of defence-related genes in the treated part (first leaf) and non-treated part (second leaf and root).Treatments were applied to corn seedlings with two fully expanded leaves. There were two treatments: 1 $mmol \cdot L^{-1}$ jasmonic acid treatment (JA) and the control (CON) and each treatment was replicated thrice. In the 1 $mmol \cdot L^{-1}$ JA treatment, 50 μL JA solution containing 0.2% ethanol and 0.05% Tween-20 was spread uniformly on the first leaf of seedlings. The same amount of distilled water, which contained 0.2% ethanol and 0.05% Tween-20, was used as control. After the first leaf was treated for 6 h, the first leaf, second leaf and root of each plant were collected separately to determine the contents of *Bt* protein, DIMBOA and total phenolics. RNA in the samples was also extracted to analyze the expression of DIMBOA-mediated

genes (*Bx1*, *Bx6* and *Bx9*), phenolics mediated gene *PAL*, direct defence protein mediated genes (*MPI* and *PR-2a*) and indirect defence volatile mediated genes (*FPS* and *TPS*) in both treated part (first leaf) and non-treated part (second leaf and root) by RT-PCR.

**Analysis of *Bt* protein**

*Bt* protein in the first leaf, second leaf and root was quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the protocol of manufacturer (Agdia Company, USA). Briefly (5), 20 mg sample were ground into powder in liquid nitrogen and immediately transferred into 2 ml centrifugal tube. The samples were extracted by 1 ml PBST (provided with the kit). They were mixed thoroughly and centrifuged under 12000 g at 4 $^{o}$C for 10 min. The supernatant was diluted at a certain ratio (100:1) with PBST for further detection. Diluted sample of 100 µl was added into each well of the plate (supplied with the kit), shaken for 15 min at 200 r·min$^{-1}$ after covering preservative film and followed a 2 h incubation at room temperature. Then the plate was washed for 5 times with PBST and incubated for another 2 h after adding 100 µl enzyme conjugate in each well. The washing was repeated and then 100 µl of TMB substrate was added and shaken for 30 min. After 15 min incubation, 50 µl 3 mol·L$^{-1}$ $H_2SO_4$ was added to stop color development. Absorbance was measured at a wavelength 450 nm with molecular devices microplate reader (Molecular Devices, USA) within 30 min. *Bt* protein concentration was measured by a four-point standard curve of purified Cry1Ab (supplied with the kit).

**Analysis of DIMBOA**

We slightly modified the procedure to prepare samples for DIMBOA concentration from Ni and Quisenberry (19). Samples were weighed and ground into powder with mortar in 10 ml distilled water. Aqueous extracts were incubated for 20 min and samples were diluted with methyl alcohol in a ratio of 1:1. The methanol-diluted extract was centrifuged at 12000 r·min$^{-1}$ for 15 min and filtered. The filtrate was evaporated to dryness under vacuum. The residue was dissolved in 2 ml mixed solution (acetonitrile:0.5% aqueous acetic acid, 1:1, v/v). Extracts were filtered through 0.45 µm membrane filters and then the samples were stored at 20$^{o}$C for further measurement.

DIMBOA concentrations in the samples were quantified by high performance liquid chromatography (HPLC) (Agilent 1100, USA) [column, Hypersil ODS C18 column (250 mm × 4 mm)] with DAD detector by using external standard curves. Gradient elution was performed with a gradient of A (acetonitrile) and B (0.5% aqueous acetic acid), i.e. 25%□45% of A from 0□10 min and 45%□25% of A from 10□15 min. Solvent flow rate was set at 1 mL·min$^{-1}$. The injection volume was 20 µL and the detection wavelength was 262 nm. DIMBOA concentrations in leaves and roots were determined according to the standard calibration curve obtained by peak area of a series of concentrations of DIMBOA standard samples.

**Analysis of total phenolics**

Total phenolics contents were assayed as per Randhir and Shetty (24) and were determined as gallic acid equivalents. Samples were weighed and ground into powder in liquid nitrogen, soaked in 10 ml of 95% ethanol and then kept in freezer for 48 h. The sample was centrifuged at 12000 r·min$^{-1}$ for 10 min and filtered. Filtrate of 1 ml was transferred into a test tube and 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent were added. After an incubation period of 5 min, 1 ml of

5% $Na_2CO_3$ was added, mixed well and kept in dark for 1 h. The samples were vortexed and absorbance was measured at 725 nm using a UV spectrophotometer.

**Analysis of defence-related genes expression**

Previous studies (5,6,7,21,40) showed that expression patterns of defence-related genes could be induced by the herbivore infestation and signal substance, these included three key genes, *Bx1*, *Bx6* and *Bx9* in DIMBOA biosynthesis, a Phenol Ammonia Lyase (*PAL*) gene in phenolics biosynthesis, pathogenesis related protein gene *PR-2a*, proteinase inhibitor gene *MPI* and key genes (*FPS* and *FPS*) in terpenoids biosynthesis (Table 1). Expression patterns of defence-related genes were analyzed using reverse transcription-polymerase chain reaction (RT-PCR). Approximately 150 mg (leaves) or 300 mg (roots) samples were ground into powder in liquid nitrogen and immediately transferred into 2 ml centrifugal tube. Total RNA was isolated from samples using the Trizol Kit according to the protocol provided by manufacturer (GibcoBRL). Total RNA samples were treated with RNase-free DNase (TaKaRa) prior to RT-PCR to ensure the samples were free of contaminating DNA. First-strand cDNA was synthesized from 7 μl (about 6 μg) of total RNA using RNA PCR Kit according to the protocol provided by manufacturer (TaKaRa). The gene-specific primers used in PCR are listed in Table 1 (5).

Table 1. The specific primers used for RT-PCR

| Genes | Accession number | Primers | Size (bp) |
|---|---|---|---|
| Indole synthase (*Bx1*) | AY254103 | F: 5′-ATGGCTTTCGCGCCCAAAACGTCCTC-3′<br>R: 5′-CGTGGACCCCCGCCTCTTTCATCTCG-3′ | 612 |
| 2-oxoglutarate-dependent oxygenase (*Bx6*) | AF540907 | F: 5'-ATCCCGTCCATCTTCCA-3'<br>R: 5'-CTCTGCCCGTTGTTGC-3' | 686 |
| Glucosyltransferase (*Bx9*) | AF331855 | F: 5′-TCGTCACCACGCTGAACGCCAG-3′<br>R: 5′-GGATCCTCCTTGCGCTCCTCTTTC-3′ | 262 |
| Phenylalanine ammonia-lyase (*PAL*) | L77912 | F: 5′-CACAAG CTGAAGCACCACCC-3′<br>R: 5′-GAGTTCACGTCCTGGTTGTG-3′ | 560 |
| Maize proteinase inhibitor (*MPI*) | X78988 | F: 5′-ACAACCAGCAGTGCAACAAG-3′<br>R: 5′-GAAGATGCGGACACGGTTAG-3′ | 370 |
| Acidic beta-1,3-glucanase (*PR-2a*) | M95407 | F: 5′-CCAACGTCTACCCCTACTTC-3′<br>R: 5′-GGGTTGAAGAGGCCGAAGTG-3′ | 394 |
| Farnesyl pyrophosphate synthase (*FPS*) | L39789 | F: 5′-GGCTGGTGCATTGAATGGCT-3′<br>R: 5′-ATGTCCGTTCCAATCTTGCC-3′ | 518 |
| Terpene synthase (*TPS*) | AF529266 | F: 5′-GCCATGCCAGTGAAGCTGACTCCTGC-3′<br>R: 5′-GTAGACGGTCCAATGTGGTGTAGAAG-3′ | 679 |
| Glyceraldehyde-3-phosphate dehydrogenase (*GAPc*) | X07156 | F: 5′-GCTAGCTGCACCACAAACTGCCT-3′<br>R: 5′-TAGCCCCACTCGTTGTCGTACCA-3′ | 512 |

PCR was performed by an MJ Research PTC-100 (Feng et al. 2007; Xu et al. 2005). Optimized PCR conditions were a hot start of 3 min at 95 $^{o}$C followed by 35 cycles of 45 s at 95 $^{o}$C, 60 s at 52.5 $^{o}$C (*PR-2a* and *Bx9*) or 58 $^{o}$C (*GAPc*, *PAL*, *MPI* and *FPS*) or 60.4 $^{o}$C (*Bx1*) or 61.9 $^{o}$C (*Bx6*) or 62.8 $^{o}$C (*TPS*) and 60 s at 72 $^{o}$C and further extended at 72 $^{o}$C for 10 min. PCR product of 5 μL was separated by electrophoresis in 1.5% agarose gels and detected by staining with ethidium bromide. The images were recorded by Bio-Rad ChemiDoc Gel Documentation System.

**Statistical analysis**

The data were expressed as the means ±standard errors of three repeats. Analysis of group *t*-Test was carried out using SAS 8.0 (USA) and significant treatment differences were tested at 0.05 level using group *t*-Test.

## RESULTS AND DISCUSSION

***Bt* protein content**

There was no significant difference in *Bt* protein content between the first (5422*Bt*1: $df$=4, $t$=0.16, $p$=0.8817; 5422CBCL: $df$=4, $t$=-1.80, $p$=0.1467), second leaf (5422*Bt*1: $df$=4, $t$=1.90, $p$=0.1305; 5422CBCL: $df$=4, $t$=-0.75, $p$=0.4940) and root (5422*Bt*1: $df$=4, $t$=-1.80, $p$=0.1469; 5422CBCL: $df$=4, $t$=-1.84, $p$=0.1397) of two *Bt* corns (5422*Bt*1 and 5422CBCL), after the first leaf was exposed to JA for 6 h (Figure. 1).

There was no significant difference in *Bt* protein content in the treated and non-treated parts of *Bt* corns after the first leaf was exposed for 6 h to JA. However, Feng *et al*. (6) found that exogenous JA reduced the *Bt* protein content in the second leaf of *Bt* corn 34B24 (from Pioneer Company, USA) after the corn plants were exposed to JA for 24 h. This can be explained by the difference in *Bt* corn varieties, JA concentration and sampling times used in the experiments. Further researches are still needed to explore the potential reasons for the disagreement of results.

**DIMBOA content and expression of DIMBOA mediated genes**

JA application to the first leaf resulted in a decrease of DIMBOA content of 55.43% in the root of non-*Bt* corn 5422 ($df$=4, $t$=-7.21, $p$=0.0020) and of 17.23% in the second leaf of *Bt* corn 5422CBCL ($df$=4, $t$=-3.58, $p$=0.0232); while it had no obvious effects on DIMBOA content in the first leaf ($df$=4, $t$=-0.73, $p$=0.5379), second leaf ($df$=4, $t$=0.47, $p$=0.6622) and root ($df$=4, $t$=0.77, $p$=0.4821) of *Bt* corn 5422*Bt*1 (Figure. 2).

JA application to the first leaf had some effects on the expression of DIMBOA synthesis mediated genes of *Bt* corns (5422*Bt*1 and 5422CBCL) and non-*Bt* corn 5422 (Figure. 3). JA induced the expression of *Bx9* gene in the first leaf and *Bx6* gene in the second leaf of 5422; it induced the expression of *Bx9* gene in the first leaf and *Bx6* gene in the root, but inhibited the expression of *Bx1* gene in the first leaf of *Bt* corn 5422*Bt*1. JA induced the expression of *Bx9* gene in the first leaf, *Bx6* gene in the second leaf and *Bx1*, *Bx6* and *Bx9* genes in the root of *Bt* corn 5422CBCL, but it inhibited the expression of *Bx6* gene in the first leaf.

JA treatment did not increase the DIMBOA content in the first leaf of three corn varieties, but it showed some induction effects on the expression of DIMBOA mediated genes. It induced the expression of *Bx9* gene in the first leaf, indicating no obvious difference existed in the direct induced effects in the first leaf of three corn varieties and this was in accordance with results of Feng *et al*. (6) who used Bt corn 34B24 and conventional corn 34B23 as experimental materials. Furthermore, the Bt and non-Bt cotton plants also did not differ in their induction levels of terpenoids in response to treatment with JA (11). Nevertheless, for the expression of *Bx6* gene in the second leaf and root, it was induced more significantly in *Bt* corns than in non-*Bt* corn.

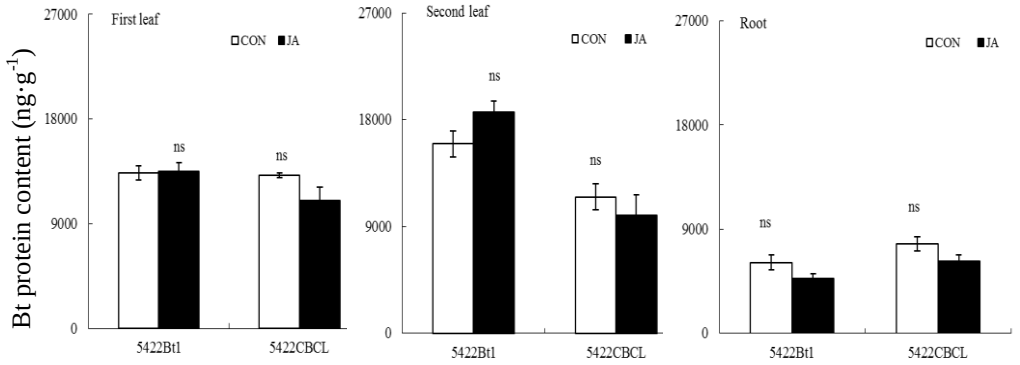


Figure 1**.** Effects of JA treatment to the first leaf of conventional and transgenic corns on the *Bt* protein content. The data in the figure are means ± standard errors. Significance of the data between treatment and control was determined by the paired Student's *t*-test. $P<0.01$ is shown by**; $P<0.05$ is shown by*; $P>0.05$ is shown by ns.

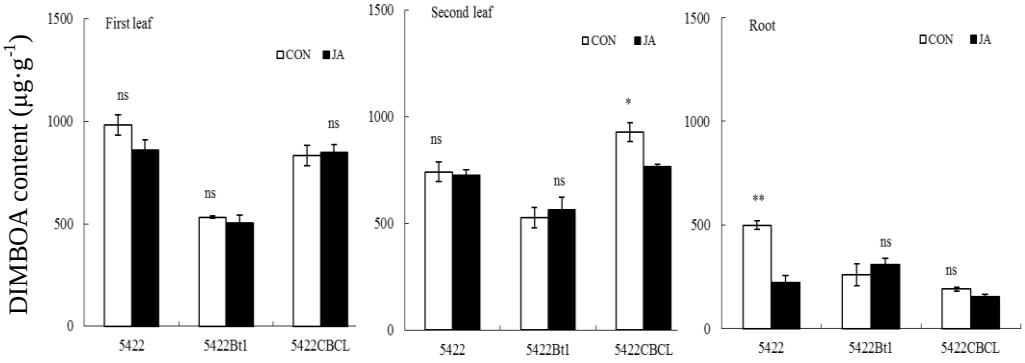


Figure 2**.** Effects of JA treatment to the first leaf of conventional and transgenic corns on the DIMBOA content. The data in the figure are means ± standard errors. Significance of the data between treatment and control was determined by the paired Student's *t*-test. $P<0.01$ is shown by**; $P<0.05$ is shown by*; $P>0.05$ is shown by ns.

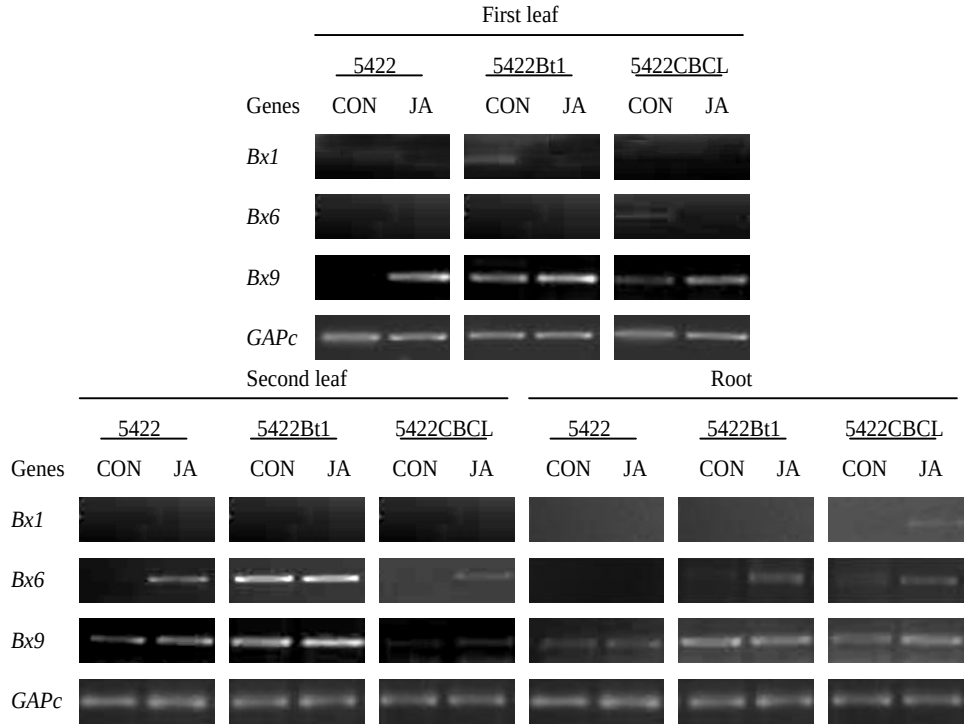


Figure 3**.** Expression of key genes in DIMBOA biosynthesis after JA treatment to the first leaf of conventional and transgenic corns. The figure *GAPc* showed internal standard of RT-PCR.

**Total phenolics content and expression of its mediated genes**

After the first leaf was treated with JA, total phenolics contents in the second leaf of conventional corn 5422 and in the root of *Bt* corn 5422CBCL were increased by 7.72% ($df$=4, $t$=3.82, $p$=0.0188) and 86.73% ($df$=4, $t$=4.51, $p$=0.0107). However, total phenolics content in the first leaf and root of 5422 was decreased by 28.31% ($df$=4, $t$=-7.50, $p$=0.0017) and 45.05% ($df$=4, $t$=-3.39, $p$=0.0275). There was no significant difference of total phenolics content in the first ($df$=4, $t$=1.20, $p$=0.3499), second leaf ($df$=4, $t$=-0.16, $p$=0.8787) and root ($df$=4, $t$=1.81, $p$=0.1446) of *Bt* corn 5422*Bt*1 as compared with control (Figure. 4).

JA exposure to the first leaf could affect the expression of phenolics acid mediated gene *PAL* (Figure. 5). JA induced the expression of *PAL* gene in the first leaf of conventional corn 5422, in the first leaf and root of *Bt* corn 5422*Bt*1 and in the second leaf and root of *Bt* corn 5422CBCL, but it repressed the expression of *PAL* gene in the root of corn 5422 (Figure. 5).

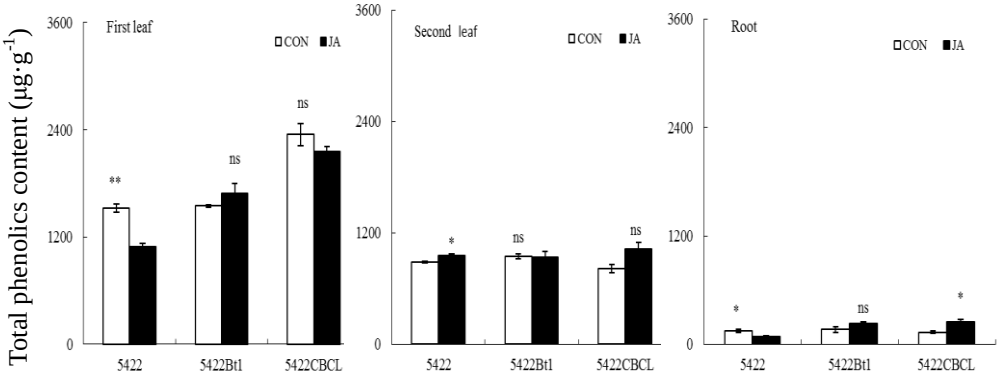


Figure 4**.** Effects of JA treatment to the first leaf of conventional and transgenic corns on total phenolics content. The data in the figure are means ± standard errors. Significance of the data between treatment and control was determined by the paired Student's *t*-test. $P<0.01$ is shown by**; $P<0.05$ is shown by*; $P>0.05$ is shown by ns.

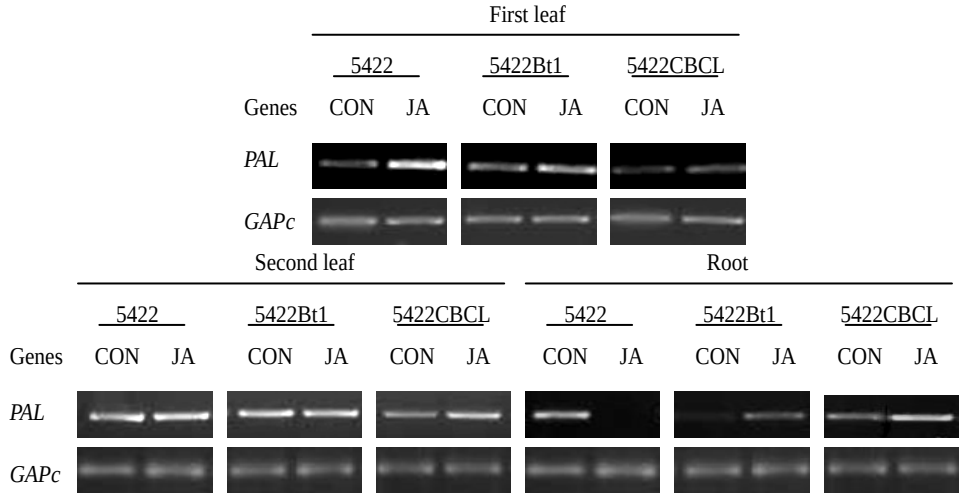


Figure 5**.** Expression of key gene in phenolics biosynthesis after JA treatment to the first leaf of conventional and transgenic corns. The figure *GAPc* showed internal standard of RT-PCR.

After the first leaf was exposed to JA, total phenolics content in the first leaf and root of conventional corn 5422 was decreased, but total phenolics content in the second leaf was increased. For the *Bt* corn variety, 5422*Bt*1, total phenolics content in the second leaf was increased, and the expression of *PAL* gene in the root was systemically induced. For the *Bt* corn 5422CBCL, *PAL* gene in the second leaf and root was systemically induced. Therefore, systemically induced effects in the second leaf and root in *Bt* corns were significant than in non-*Bt* corn, especially for the expression of *PAL* gene. These findings

imply that JA systemically induced the defence processes, there is a synergetic relationship between *Bt* gene introduction and endogenous chemical defence responses, which agrees with the findings of Feng *et al*. (6), who recorded the systemic defence responses in the second leaf of *Bt* corn 34B24 and conventional corn 34B23, when the first leaf was treated with JA.

**Expression patterns of the direct defence protein genes**

JA application to the first leaf had no induced effects on the expression of direct defence protein mediated genes in the first, second leaf and root of conventional corn 5422, however it enhanced the expression of *MPI* and *PR-2a* genes in the second leaf of *Bt* corn 5422*Bt*1 and the expression of *MPI* and *PR-2a* genes in the first, second leaf and root of *Bt* corn 5422CBCL (Figure. 6).

Our results indicated that the differences in expression of direct defence protein mediated genes of *Bt* corn and conventional corn were from the systematic induction of the second leaf and root after first leaf was applied with JA. This led to systemically induced effects in the second leaf and root in *Bt* corns were significant than in non-*Bt* corn. These results were also consistent with the findings of Feng *et al*. (6), who recorded the systemic defence responses in the second leaf of *Bt* corn 34B24 and conventional corn 34B23, when the first leaf was treated with JA.

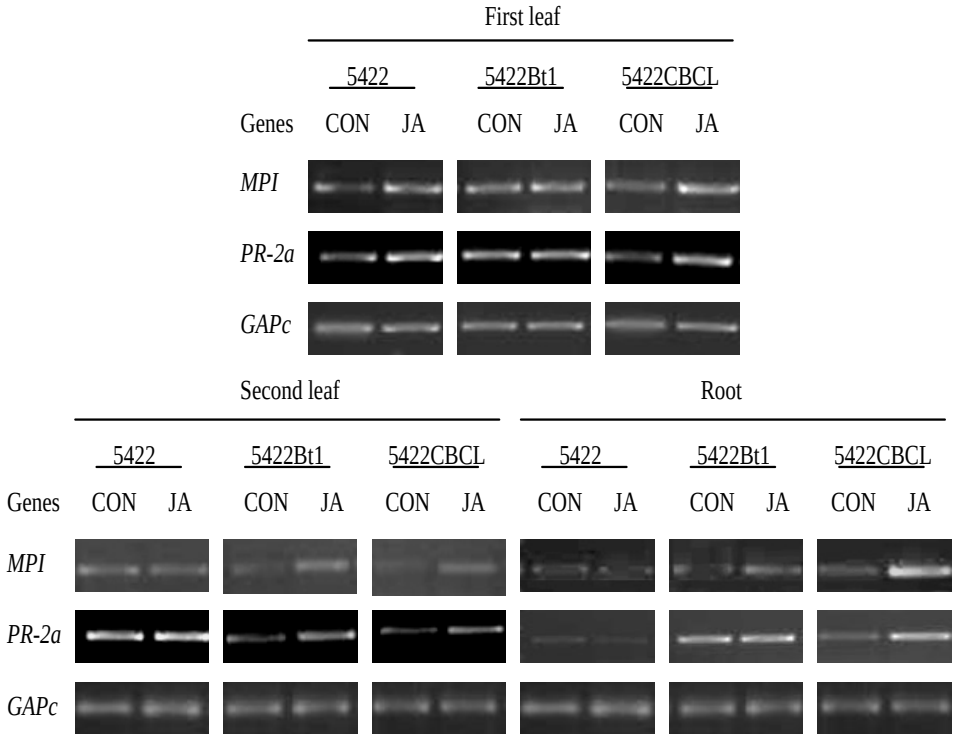


Figure 6**.** Expression of direct defence protein genes after JA treatment to the first leaf of conventional and transgenic corns. The figure *GAPc* showed internal standard of RT-PCR.

**Expression patterns of the key genes in volatile biosynthesis**

There were significant differences in the expression of volatile substance mediated genes after the first leaf was subjected to JA for 6 h (Figure. 7). The expression of *FPS* and *TPS* genes in the first leaf and *TPS* gene in the second leaf of conventional corn 5422 and *Bt* corn 5422*Bt*1 was induced; the expression of *FPS* gene in the second leaf of corn 5422*Bt*1 was suppressed; the expression of *TPS* gene in the first and second leaf, *FPS* gene in the root of *Bt* corn 5422CBCL was induced.

By analyzing the expression patterns of the key genes in volatile biosynthesis, we found that exogenous JA treatment to leaves not only resulted in defence responses in the treated part (leaf), but also caused systemic defence responses in the non-treated part (other leaves and roots) (17,28,34,36,40). The result demonstrated that systemically induced effect in the root in *Bt* corn 5422CBCL was significant than in non-*Bt* corn. On the other hand, only the effects of JA on expression of indirect defence volatiles mediated genes *FPS* and *TPS* were analyzed in the present study, hence future investigations are still needed to explore the effect of JA on volatile quality and quantity, as well as on the tritrophic interactions among corn, herbivore and its natural enemy, so as to provide a scientific base for integrated resistance management practices for *Bt* corns.

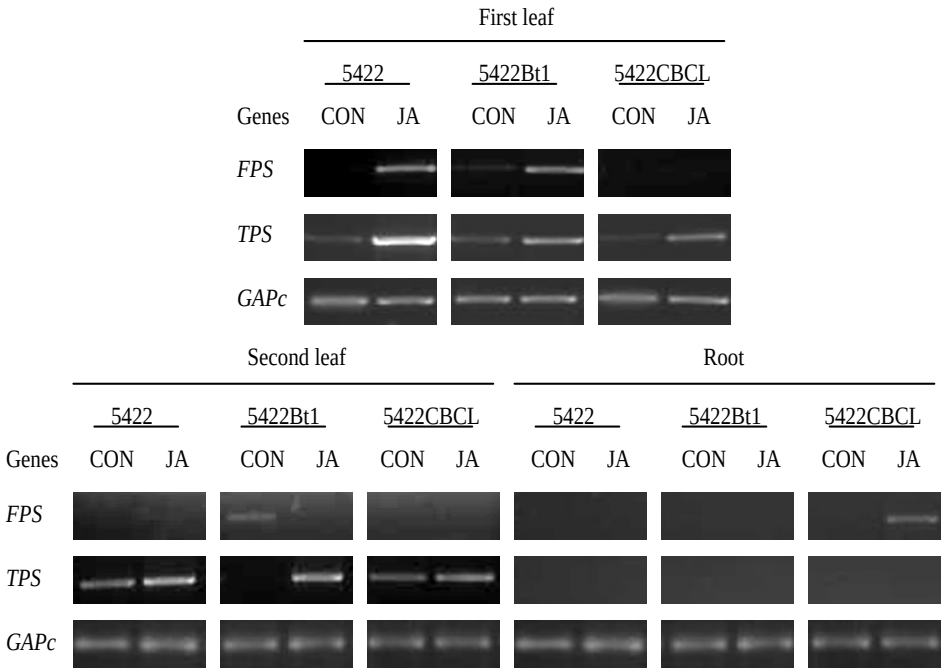


Figure 7**.** Expression of key genes in volatile biosynthesis after JA treatment to the first leaf of conventional and transgenic corns. The figure *GAPc* showed internal standard of RT-PCR.

It has been well documented that plant species possess several direct and indirect defence strategies to protect themselves against insect herbivores or pathogens (2,10,16,23). JA directly induces defence responses in the treated parts (6,28,32,37,40). On the other hand, JA induced defence responses in plants can be systemic (3,31,34). Both direct defence responses and systemic defence responses in *Bt* and conventional corns were examined in our study. Our results show that JA exposure did not affect the *Bt* protein content of two *Bt* corns. Among the three corn varieties JA-induced effects in the first leaf did not differ. However systemically induced effects in the second leaf and root in *Bt* corns were significant than in non-*Bt* corn. It can be concluded that *Bt* gene introduction and endogenous chemical defence responses of corns act synergistically during the JA systemically induced defence processes. It needs to point out that the difference of systemic defence responses may attribute to *Bt* gene introduction or changes of chemicals content resulted from *Bt* gene introduction and more in-depth studies are required to reveal the underlying mechanisms. Mészáros *et al*. (18) assessed the potential interactions between *Bt* protein and jasmonic acid-induced resistance to the fall armyworm, *Spodoptera frugiperda*. They found that exogenous JA application to the leaves induced resistance in cotton plants and acted synergistically with *Bt* protein against *S. frugiperda* (18). Further studies, however, are needed using transgenic corn cultivars to confirm whether there is synergism between *Bt* protein and JA induced resistance to herbivore. On the other hand, further research is still needed to reveal the effects of JA to the aboveground part on the diseases and insect pests, as well as their natural enemies in aboveground and belowground part of the three corn varieties. There is a close relationship between the aboveground parts and belowground parts of plants in the induced defence responses (14,32,33). Exogenous JA exposure to the leaves could systemically induce the defence responses in the roots of plant (7,17,35) and *vice versa* (4,7,23,35). For example, JA exposure to the roots of maize systemically induced the expression of *MPI* and *FPS* genes in the leaves and remarkably increased in the DIMBOA content in the leaves (7). JA application to the roots of *Brassica oleracea* and *Brassica nigra* systemically increased the content of glucosinolates, particularly aliphatic glucosinolates in the leaves (35). JA application to the roots of *Solanum tuberosum* systemically induced the gene expression of cathepsin D inhibitor (*Cdi*) and proteinase inhibitor II (*Pin2*) in the leaves (4). Consequently, future studies should emphasize on the difference in chemical defence responses between JA treated and non-treated parts after JA exposure to the roots of *Bt* and their conventional corns.

## CONCLUSIONS

We found that direct JA-induced effects in the first leaf were not distinctly different among the three corn varieties. But, the systemically induced effects in the second leaf and root in two *Bt* corn varieties were significant than in the conventional corn. It can be concluded that *Bt* gene introduction and endogenous chemical defence responses of corns act synergistically during the JA systemically induced defence processes.

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## REFERENCES

1. Bates, S.L., Zhao, J.Z., Roush, R.T. and Shelton, A.M. (2005). Insect resistance management in GM crops: past, present and future. *Nature Biotechnolgy* **23**: 57-62.
2. Chen, Y.G., Whitehill, J.G.A., Bonello, P. and Poland, T.M. (2011). Feeding by emerald ash borer larvae induces systemic changes in black ash foliar chemistry. *Phytochemistry* **72**: 1990-1998.
3. Creelman, R.A. and John, E.M. (1997). Oligosaccharins, brassinolides and jasmonates: Non-traditional regulators of plant growth, development and gene expression. *Plant Cell* **9**: 1211-1223.
4. Dammann, C., Rojo, E. and Sanchez-Serrano, J.J. (1997). Abscisic acid and jasmonic acid activate wound-inducible genes in potato through separate, organ-specific signal transduction pathways. *Plant Journal* **11**: 773-782.
5. Feng, Y.J., Wang, J.W. and Jin, Q. (2010). Asian corn borer (*Ostrinia furnacalis*). damage induced systemic response in chemical defence in *Bt* corn (*Zea mays* L.). *Allelopathy Journal* **26**: 101-112.
6. Feng, Y.J., Wang, J.W. and Luo, S.M. (2007). Effects of exogenous jasmonic acid on concentrations of direct defence chemicals and expression of related genes in *Bt* (*Bacillus thuringiensis*). corn (*Zea mays*). *Agricultural Sciences in China* **6**: 1456-1462.
7. Feng, Y.J., Wang, J.W., Luo, S.M., Jin, Q., Fan, H.Z., Su, Y.J. and Liu, Y.H. (2010). Effects of exogenous application of jasmonic acid and salicylic acid on the leaf and root induction of chemical defence in maize (*Zea mays* L.). *Allelopathy Journal* **25**: 133-146.
8. Ferry, N., Edwards, M.G., Gatehouse, J., Capell, T., Christou, P. and Gatehouse, A.M. (2006). Transgenic plants for insect pest control: A forward looking scientific perspective. *Transgenic Research* **15**: 13-19.
9. Fritz, V.A., Justen, V.L., Bode, A.M., Schuster, T. and Wang, M. (2010). Glucosinolate enhancement in cabbage induced by jasmonic acid application. *HortScience* **45**: 1188-1191.
10. Glinwood, R., Ninkovic, V. and Pettersson, J. (2011). Chemical interaction between undamaged plants – Effects on herbivores and natural enemies. *Phytochemistry* **72**: 1683-1689.
11. Hagenbucher, S., Wäckers, F.L., Wettstein, F.E., Olson, D.M., Ruberson, J.R. and Romeis, J. (2013). Pest trade-offs in technology: Reduced damage by caterpillars in *Bt* cotton benefits aphids. *Proceedings of the Royal Society B Biological Sciences* **280**: 20130042.
12. Halitschke, R. and Baldwin, I.T. (2003). Antisense LOX expression increases herbivore performance by decreasing defence responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuate*. *Plant Journal* **36**: 794-807.
13. Halitschke, R. and Baldwin, I.T. (2004). Jasmonates and related compounds in plant-insect interactions. *Journal of Plant Growth Regulation* **23**: 238-245.
14. Heil, M. (2011). Plant-mediated interactions between above- and below-ground communities at multiple trophic levels. *Journal of Ecology* **99**: 3-6.
15. ISAAA. (2016). *Global Status of Commercialized Biotech/GM Crops: 2016*. ISAAA Brief No. 52. ISAAA: Ithaca, NY: 1-3.
16. Karban, R. and Baldwin, I.T. (1997). *Induced Responses to Herbivory*. University of Chicago Press, Chicago, USA: 1-11.
17. Ludwig-Müller, J., Schubert, B., Pieper, K., Ihmig, S. and Hilgenberg, W. (1997). Glucosinolate content in susceptible and resistant chinese cabbage varieties during development of clubroot disease. *Phytochemistry* **44**: 407-417.

18. Mészáros, A., Beuzelin, J.M., Stout, M.J., Bommireddy, P.L., Riggio, M.R. and Leonard, R. (2011). Jasmonic acid-induced resistance to the fall armyworm, *Spodoptera frugiperda*, in conventional and transgenic cottons expressing *Bacillus thuringiensis* insecticidal proteins. *Entomologia Experimentalis Et Applicata* **140**: 226-237.
19. Ni, X. and Quisenberry, S.S. (2000). Comparison of DIMBOA concentrations among wheat isolines and corresponding plant introduction lines. *Entomologia Experimentalis et Applicata* **96**: 275-279.
20. Nie, C.R., Luo, S.M., Wang, J.W., Huang, J.H. and Zeng, R.S. (2005). Change in concentration of secondary metabolites-DIMBOA and phenolic acids in leaves of *Bt* corn. *Acta Ecologica Sinica* **25**: 814-823.
21. Pauw, B. and Memelink, J. (2004). Jasmonate-responsive gene expression. *Journal of Plant Growth Regulation* **23**: 200-210.
22. Peña-Cortés, H., Barrios, P., Dorta, F., Polanco, V., Sánchez, C., Sánchez, E. and Ramírez, I. (2004). Involvement of jasmonic acid and derivatives in plant responses to pathogens and insects and in fruit ripening. *Journal of Plant Growth Regulation* **23**: 246-260.
23. Qiu, B.L., Harvey, J.A., Raaijmakers, C.E., Vet, L.E.M. and van Dam, N.M. (2009). Nonlinear effects of plant root and shoot jasmonic acid application on the performance of *Pieris brassicae* andits parasitoid *Cotesia glomerata*. *Functional Ecology* **23**: 496-505.
24. Randhir, R. and Shetty, K. (2005). Developmental stimulation of total phenolics and related antioxidant activity in light- and dark-germinated maize by natural elicitors. *Process Biochemistry* **40**: 1721-1732.
25. Rasmann S. and Turlings T.C.J. (2008). First insights into specificity of belowground tritrophic interactions. *Oikos* **117**: 362-369.
26. Ryan, C.A. (2000). The systemic signaling pathway: Differential activation of plant defensive genes. *Biochimica et Biophysica Acta* **1477**: 112-121.
27. Saedler, R. and Baldwin, I.T. (2004). Virus-induced gene silencing of jasmonate- induced direct defences, nicotine and trypsin proteinase-inhibitors in *Nicotiana attenuate*. *Journal of Experimental Botany* **55**: 151-157.
28. Schenk, P.M., Kazan, K., Rusu, A.G., Manners, J.M. and Maclean, D.J. (2005). The SEN1 gene of Arabidopsis is regulated by signals that link plant defence responses and senescence. *Plant Physiology and Biochemistry* **43**: 997-1005.
29. Stratmann, J.W. (2003). Long distance run in the wound response-jasmonic acid is pulling ahead. *Trends in Plant Science* **8**: 247-250.
30. Thaler, J.S., Fidantsef, A.L. and Bostock, R.M. (2002). Antagonism between jasmonate- and salicylate-mediated induced plant resistance: Effects of concentration and timing of elicitation on defence-related proteins, herbivores and pathogen performace in tomato. *Journal of Chemical Ecology* **28**: 1131-1159.
31. Tian, D., Peiffer, M., De Moraes, C.M. and Felton, G.W. (2014). Roles of ethylene and jasmonic acid in systemic induced defence in tomato (*Solanum lycopersicum*). against *Helicoverpa zea*. *Planta* **239**: 577-589.
32. Tytgat, T.O.G., Verhoeven, K.J.F., Jansen, J.J., Raaijmakers, C.E., Bakx-Schotman, T., McIntyre, L.M., van der Putten, W.H., Biere, A. and van Dam, N.M. (2013). Plants know where it hurts: Root and shoot jasmonic acid induction elicit differential responses in *Brassica oleracea*. *PLoS ONE* **8**: e65502.
33. van Dam, N.M. and Heil, M. (2011). Multitrophic interactions below and above ground: *En route* to the next level. *Journal of Ecology* **99**: 77-88.
34. van Dam, N.M., Horn, M., Mareš, M. and Baldwin, I.T. (2001). Ontogeny constrains the systemic proteinase inhibitor response in *Nicotiana attenuata*. *Journal of Chemical Ecology* **27**: 547-568.
35. van Dam, N.M., Witjes, L. and Svatoš, A. (2004). Interactions between aboveground and belowground induction of lucosinolates in two wild *Brassica* species. *New Phytologist* **161**: 801-810.
36. Walters, D., Cowley, T. and Mitchell, A. (2002). Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley. *Journal of Experimental Botany* **53**: 747-756.
37. Wang, J.W., Xu, T., Zhang, L.W., Zhong, Z.M. and Luo, S.M. (2007). Effects of methyl jasmonate on hydroxamic acid and phenolic acid content in maize and its allelopathic activity to *Echinochloa crusgalli* (L.). *Allelopathy Journal* **19**: 161-170.
38. Wang, X.M. and Ma, Q.H. (2005). Characterization of a jasmonate-regulated wheat protein related to a beta-glucosidase-aggregating factor. *Plant Physiology and Biochemistry* **43**: 185-192.
39. Wasternack, C. (2005). Introductory remarks on biosynthesis and diversity in actions. *Journal of Plant Growth Regulation* **23**: 167-169.
40. Xu, T., Wang, J.W. and Luo, S.M. (2005). Cloning of the key genes in maize oxylipins pathways and their roles in herbivore induced defence. *Chinese Science Bulletin* **50**: 2217-2225.

41. Xu, T., Zhou, Q., Chen, W., Zhang, G.R., He, G.F., Gu, D.X. and Zhang, W.Q. (2003). Involvement of jasmonate-signaling pathway in the herbivore- induced rice plant defence. *Chinese Science Bulletin* **48**: 1982-1987.
42. Zavala, J.A., Patankar, A.G., Gase, K. and Baldwin, I.T. (2004). Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuate*. *Proceedings of the National Academy of Sciences* **101**: 1607-1612.
43. Zhang, Z.P. and Baldwin, I.T. (1997). Transport of (2-$^{14}$C). jasmonic acid from leaves to roots mimics wound-induced changes in endogenous jasmonic acid pools in *Nicotiana sylvestris*. *Planta* **203**: 436-441.