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Jasmonate-induced defense in tomato and cabbage deterred *Spodoptera litura* (Noctuidae) growth

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ABSTRACT

Methyl jasmonate (MeJA) could trigger plant induction response. However, the induction response may vary among plant species. In this study, we assessed the effects of MeJA and host plant species on plant-induction responses and performance of the subsequent insect herbivore *Spodoptera litura*. Two plant species (cabbage and tomato) were sprayed with MeJA and foliage from different treated plants was fed to a third-instar *S. litura* larvae. In addition, the foliage was also collected during the bioassay to assess the foliar chemistry. The results indicated that the MeJA application and plant species exerted various influences on the foliar nutrient and anti-nutrient compounds, and affected the relative growth rate of *S. litura*. Moreover, the MeJA employed a marked effect on the plant's induction response. In summary, applying MeJA could induce the similar plant-induction response in various plant species and subsequently deter the insect growth performance.

Introduction

Nearly all crop plants suffer a certain degree of pest problems that can affect crop survival and production (Karban and Baldwin, 1997; Harrison, 2005; Johnson and Agrawal, 2005). For example, Common cutworm, *Spodoptera litura* is a polyphagous insect and the host plant range of this caterpillar include over 290 crop plant species (Wu et al., 2004; Patil et al., 2014). Damage caused by *S. litura* could reduce the yield and make heavy loss in many crop plants (Dhir et al., 1992; Patnaik, 1998; Lau et al., 2012). To protect crop plant loss from *S. litura*, chemical control has been used most of the time and the chemicals most applied include such as carbaryl, organophosphates, and endosulfan etc. (Ramakrishnan et al., 1983; Kamala Jayanthi and Padmavathamma, 2001; Kranthi et al., 2001: Janarthanan et al., 2003; Lau et al., 2012). In addition to pesticide management, ecologically friendly alternatives are being developed to assist crops in defending themselves. Previous studies have revealed that induced systemic resistance (ISR) might be an option for controlling insect infestation (Tuzun and Kuć, 1985; Hunt et al., 1996; Sticher et al., 1997). Certain inducers assist plants in resisting pest invasion through physical or chemical mechanisms (Green and Ryan, 1972; Haukioja, 1990; Karban and Baldwin, 1997; Traw and Dawson, 2002; Howe and Schaller, 2008; Poelman et al., 2008). Although the evolution of such induced defensive traits can be genetically fixed (Berenbaum et al., 1986; Adler et al., 1995; Hwang and Lindroth, 1997), the outcome of such traits might be affected by other factors (Bryant et al., 1983; Herms and Mattson, 1992).

Methyl jasmonate (MeJA) and jasmonic acid (JA) are primary signals leading to plant-induction resistance against insect attack (Wasternack and Parthier, 1997; Rohwer and Erwin, 2008; Schaller and Stintzi, 2008). Exogenous MeJA application induces endogenous signals in wound-induced gene expression after insect damage (Wasternack et al., 2006). Previous studies have indicated that exogenous MeJA application might able to affect insect-feeding performance and induce production of various defensive substances, such as proteinase inhibitors (PIs), polyphenol oxidase (PPO), and lipoxygenase (LOX) (Green and Ryan, 1972; Mayer and Harel, 1979; Farmer and Ryan, 1990; Thaler et al., 1996; Constabel and Ryan, 1998; Stout et al., 1998; Tan et al., 2011, 2012). Hence, endogenous accumulation and exogenous application of MeJA could play important roles in a plant's induced defense response and such induced responses may vary across plant species (Tan et al., 2011).

The past studies regarding such jasmonate-induced plant responses were major restrained to single plant species and only limited studies have evaluated the possible interaction between MeJA and plant species on the induction responses and herbivorous insects (Tan et al., 2011). In addition, the result revealed a markedly different response (Tan et al., 2011). However, the jasmonate-induction pathway supposed to be quite conserved and should occur for most angiosperm species. Therefore, it become very important to first clarify the species-specific jasmonate-induction response before further consider using jasmonate as one of the pest control agents. This study was conducted to assess the jasmonate-induction responses on two crop species, tomato

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(*Lycopersicon esculentum* Mill.) and cabbage (*Brassica oleracea* L.), and its subsequent effect on relative growth rates of *S. litura* caterpillars.

Materials and methods

Plants

Two plant species were used in this study; cabbage (*Brassica oleracea* L. var. *capitata* L.) and tomato (*Lycopersican esculentum* Mill. Var. *cerasiforme* (Dunal) Alef.). Seeds of each plant species were sown in soil (Know-You Seed Company, Taiwan) in 104-well plates and watered daily. Sixteen days after sowing, the seedlings were transplanted to 5-in. pots with field soil, and the plants were subsequently placed in a greenhouse (25 ± 2 °C, 16:8 h light: dark photoperiod) and watered daily. The soil properties were analyzed by the Soil Survey and Testing Center, National Chung Hsing University, Taichung, Taiwan. After 39 d, the plants were used for the insect feeding assay and foliar chemistry analysis.

Insects

The eggs of cutworm, *Spodoptera litura* L., were collected from a field in Taichung Province, Taiwan and kept in a 250-mL rearing cup with a moistened cotton ball for maintaining moisture. The rearing cup was put in the growth chamber (25 ± 2 °C, 16:8 h light: dark photoperiod). After hatching, the larvae were kept in the rearing cup and fed with an artificial diet (Yadav et al., 2010). Male and female pupa were separated and kept in a 250-mL rearing cup. After emerging, adults (10 pairs) were kept in a plastic cylinder (21 cm height × 14.9 cm diameter) and tissue papers were stacked inside the plastic cylinder on which adults could deposit eggs. A sugar solution was added to feed the adults (Yadav et al., 2010) and the plastic cylinder was kept in a laboratory conditions. We maintained the insect from first generation until next generation, and then we used this insect for our experiment.

MeJA and plant species treatments

This study was conducted to evaluate the effect of two factors, methyl jasmonate (no MeJA and MeJA) and plant species (cabbage and tomato) on foliar chemistry and insect-feeding performance. Four different treatments were used in this experiment: (1) cabbage plant only (2) cabbage plant treated with MeJA (3) tomato plant only, and (4) tomato plant treated with MeJA. The plants for the MeJA treatment were treated with the MeJA when they were 36 d old. Methyl jasmonate (MeJA) (BioWorld, USA) was first dissolved in 95% alcohol (1:10) and diluted with water to reach a concentration of 1.5 mM. The MeJA solution was subsequently mixed with the surfactant Break-Thru S-240 (1/10,000) (Evonik Goldschmidt GmbH, Germany) before use. The prepared MeJA and surfactant-mixed solution were sprayed on the foliage 3–5 times until the solution began to drip from the leaf. For the control treatment, only the surfactant solution was used to spray. Three days after spraying, foliage (fourth leaf) was collected from each plant for chemical analysis and an insect growth bioassay.

Foliar chemistry

We measured foliar protein content, polyphenol oxidase (PPO) activity, and trypsin inhibitor (TI) activity. The foliage (fourth leaf) was collected during the bioassay for foliar chemical analysis. During the period of insect bioassay, plants contained about 8 leaves. The fourth leaf was the middle leaf of whole plant and fully expanded. For consistence, we used the fourth leaf for both insect feeding and chemical analysis. Five plants were used for each treatment. Leaf samples were ground using liquid nitrogen and homogenized in 7% grinding buffer (polyvinylpolypyrrolidone in potassium phosphate buffer, pH 7). The leaf extract (1 mL) was mixed with 100 μL of 10% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) in a microtube. The crude extract solution was centrifuged at 4 °C, 10,000 rpm for 15 min, after which the resulting supernatant was used for determining enzyme activity. To quantify the amount of protein, a standard curve was prepared using bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA). Ten microliters of the sample were mixed with 40 μL of a K—P buffer. Each tube of the sample and standard was loaded with 5 μL of 0.1 N HCL, 40 μL of double-distilled water, and 1750 μL of 20% protein assay dry reagent (Bio—Red, Hercules, CA, USA) into the Eppendorf tube (Axygen Scientific Inc., Union City, CA, USA). After coloring for 5 min, a 200-μL solution from each tube was loaded into a 96-microwell plate (Sterile, 25805-96) and an absorbance value below 570 nm was detected using an ELISA reader (Kelowna Intl. Scientific Inc., San Chung, Taipei, Taiwan). Polyphenol oxidase (PPO) activity was measured based on the procedures of Stout et al. (1999) to calculate the formation rate of melanin-like material from catechol. For this assay, 15 μL of a supernatant liquid was mixed with catechol (0.1 M potassium phosphate buffer, pH 8). After mixing for 1 min, an absorbance value below 470 nm was recorded (Ryan et al., 1982; Thaler et al., 1996, 2001; Cipollini et al., 2004).

For the trypsin inhibitor (TI) assay, we collected and measured the foliage sample (fourth leaf). The leaf sample was ground with liquid nitrogen and homogenized in the extraction buffer (phosphate buffer pH 7.8, 1% PVP, 1% ascorbic acid, 1 mM potassium chloride, 10 mM magnesium chloride, 50 mM EDTA-Na_2) and loaded into the 1.7-mL Eppendorf tube. The crude extract solution was centrifuged at 4 °C and at 12,000 rpm for 20 min after which the resulting supernatant was used for determining enzyme activity. The TI assay was conducted for three groups; sample, blank, and standard. Each tube of the sample and blank groups were loaded with 50 μL of double distilled water, 75 μL of crude extract, and 250 μL of 2% heated casein solution with 10 mM phosphate buffer (pH 7.6), and incubated at 37 °C for 20 min. After incubation, each tube of the sample group was loaded with 250 μL of trypsin solution and 250 μL of double distilled water into the blank group, and incubated at 37 °C for 20 min. Each tube of the standard group was loaded with 125 μL of double distilled water, 250 μL of trypsin solution, and 250 μL of 2% heated casein solution with a 10 mM buffer (pH 7.6), and incubated at 37 °C for 20 min. Each sample, blank, and standard group was subsequently loaded with 750 μL of 10% TCA and maintained at room temperature for 2 h. Finally, each tube was centrifuged at 4 °C, 12,000 rpm for 10 min to detect the absorbance value using a spectrophotometer at 280 nm. The TI activity was calculated using the following equation: ((OD280 of standard + OD280 of blank − OD280 of sample)/OD280 of standard) × 100% (Lee and Lin, 1995; Tan et al., 2011).

Insect growth bioassay

The insect-feeding performance was conducted to evaluate the effect of MeJA and plant species on the relative growth rate of *S. litura*. The fourth leaf of the plant was removed from the base of the plant by using surgical scissors. The petioles of the leaves were kept in a 2-mL Eppendorf tube with reverse osmosis (RO) water to maintain freshness, after which the leaves were placed individually into petri dishes (9 cm in diameter). Third instar larvae of *S. litura* were weighed and individually placed on various treated leaves, and kept in the growth chamber (25 ± 2 °C, 16:8 h light: dark photoperiod). The larvae were allowed to feed on the foliage for 48 h before being subsequently separated, frozen, oven-dried, and weighed. Fourteen replications (larvae) were conducted for each treatment. At the time of the bioassay, fresh weights of 15 third instar larvae were measured individually and oven-dried at 45 °C. After 1 wk., the dry weights of the larvae were measured again. The average water content of the larvae was used to calculate the initial larval dry weight used in the feeding study. The relative growth rate was calculated using the following equation: ((final dry weight