Induction of resistance against the tobacco cutworm, *Spodoptera litura*, by jasmonic acid in sweet pea.

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Introduction

The sweet pea, *Lathyrus odoratus*, is one of popular ornamental plant and native to Sicily, Cyprus, southern Italy and the Aegean Islands. Its bean shows a toxicity against mammals by beta-aminopropionitril, however, a resistance cue of a sweet pea plant against herbivores is unclear. Actually, a shoot of sweet pea is attacked by insect pests, e.g. armyworms, aphids, spider mites during its cultivation, therefore various pesticides are used for pest control. A chemical cue for inducible resistance caused by an exogenous treatment against insect pests may be useful for a new pest management method. Within this aim, we have conducted a screening of elicitors and an elucidation of its physiological characteristics.

Result and Discussion

Exogenous treatment of plant hormones

For screening to inducible effect of elicitors, sweet pea leaves were treated with various plant hormones (Jasmonic acid: JA, Salicylic acid: SA, Indole 3-acetic acid: IAA, Abscisic acid: ABA, Benzyl adenine: BA) and a heavy metal ($CuCl_2$) by a floating method for 48 hrs. The treated leaves were extracted with 1 mL of methanol, and the methanol solution was analyzed by HPLC. Resultingly, some enhanced peaks were observed in JA and $CuCl_2$ treated leaves (Fig. 1), but not in SA, IAA, and ABA treatment. On the other hand, peak A in BA treated leaves was significantly decreased than that in the control leaves.

Next, the elicitor treated leaves (JA, BA, and $CuCl_2$) were allowed to feed by 2nd instar larvae of tobacco cutworm, *Spodoptera litura,* as follow. Namely, two pieces of the treated leaf, which had been floated on the elicitor solution (100 or 500 μM) for 48 hrs, were put on a moisture filter paper in a plastic Petri dish. And then, five 2nd instar larva of *S. litura* were introduced into the Petri dish, and it was kept at 27℃±3℃ (16L8D). After 24 hrs, the remained leaves were weighted and compared to the weight before the examination. The feeding area of JA treated leaves was significantly smaller than that of the control leaves (Fig. 2), thus it was concluded that an exogenous JA treatment could induce a resistance based on the feeding inhibition activity against a larva of *S. litura*. On the other hand, any significant difference between the control, BA, and $CuCl_2$ was not observed in their feeding area.

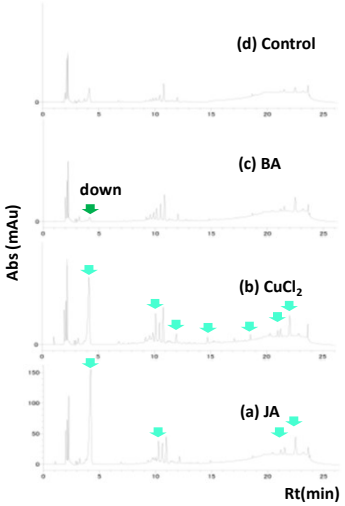

Fig.1 HPLC chromatogram (254 nm) of the extract of leaves treated with 500 μM JA (a), 500 μM $CuCl_2$ (b), 500 μM BA (c), and water (d).

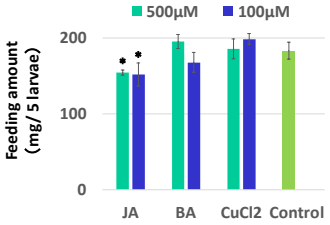

Fig. 2 Feeding deterrent activity of the sweet pea leaves treated with various elicitors.

∗ Significant by t-test at $P < 0.05$

Isolation and identification

As an induce resistance was observed with an accumulation of compound A (peak A) in the JA treated leaves, it was estimated that compound A would be an induced resistance factor. Therefore, it was tried to isolate compound A as follow: Sweet pea plant was cultivated in the green house on the Monobe campus at University of Kochi for 3 months, shoots of sweet pea were extracted with MeOH (800 mL x 2). After filtration, the solvent was removed *in vacuo*, and a residue was dissolved into 200 mL of water. The water solution of crude extract was partitioned with *n*-hexane (70 mL x 3 times), and then followed with ethyl acetate (140 mL x 10 times). Compound A was recovered from ethyl acetate layer. Next, the ethyl acetate layer was purified with an ODS column using a water – methanol stepwise solvent system. After three times repeat of the column separation, compound A (177.6 mg) was isolated from water fraction and 10% methanol-water fraction.

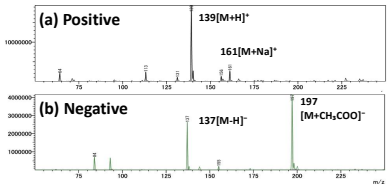

Fig.3 LC-MS spectrum of the compound A in positive ion mode (a), and negative ion mode (b).

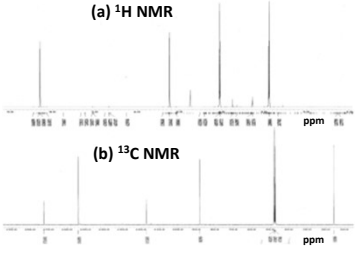

Fig.4 ^{1}H NMR (a: 600 MHz) and ^{13}C NMR (b: 150 MHz) spectrum of the compound A.

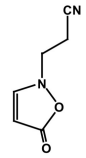

Fig. 5 Compound A 2-cyanoethyl isoxazolin-5-one

The LC-MS spectra of compound A (Fig. 3) gave an $[M+H]^+$ ion at *m/z* = 139 in a positive mode and an $[M-H]^-$ ion at *m/z* = 137 in a negative mode. Thus, it was assumed the molecule weight of compound A to be 138. Furthermore, NMR spectra of compound A were recorded on a Jeol ECA 600 (Jeol, Japan) instrument using with TMS as a standard in methanol-d_6. The ^{13}C NMR spectrum gave six signals and the ^{1}H NMR spectrum gave four signals (Fig. 4), indicating the existence of cyano group and aromatic moiety in the structure. From these data, compound A was identified to be 2-cyanoethylisoxazolin-5-one (Fig. 5).

Feeding inhibition activity of 2-CEIX

A feeding inhibition activity of 2-CEIX against a larva of *S. litura* was confirmed by a dipping test using of an isolated 2-CEIX and sweet pea leaves. Namely, sweet pea leaves were dipped into various concentration of 2-CEIX water solution containing of 1% tween 20 as a surface active agent for 3 sec. After air drying, two pieces of the treated leaf were put on a moisture filter paper in a plastic Petri dish. Later, five 2nd instar larva of *S. litura* were introduced into the Petri dish, and it was kept at 27℃±3℃ (16L8D). After 24 hrs, the remained leaves were weighted and compared to the weight before the examination.

When the larvae were allowed to feed the leaves dipped into 2-CEIX solution (10 mg/ml), the feeding amount was significantly decreased to 7.7 mg/five larvae from 18.4 mg/five larvae in the control leaves. However, the feeding amounts of the leaves treated with 2-CEIX solution at the concentration less than 5 mg/ml was not observed significantly difference between the control leaves. Thus, it was concluded that 2-CEIX was induced by JA and act as a feeding deterrent against the larva of *S. litura* at a high concentration.

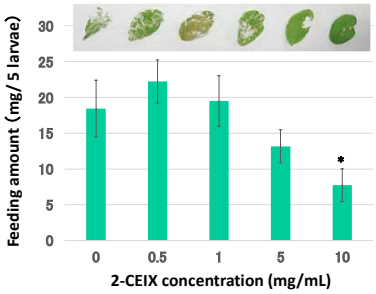

Fig.6 Fig.6 Effects of dipping concentration of 2-CEIX on the resistance against *S. litura*.

∗ Significant by t-test at $P < 0.05$

Effect of the JA concentration on the accumulation of 2-CEIX

The dependency of the 2-CEIX accumulation was next investigated, as a function of various JA concentration (Fig.7). The concentration of 2-CEIX was determined 48 hrs after treatment. The accumulation of 2-CEIX was observed with more than 500 μM JA, but the amount decreased at 1000 μM JA. However, a significantly increase of 2-CEIX was not observed in the leaves treated with less than 100 μM. Thus, it was estimated that a suitable concentration for the 2-CEIX accumulation would be 500 μM.

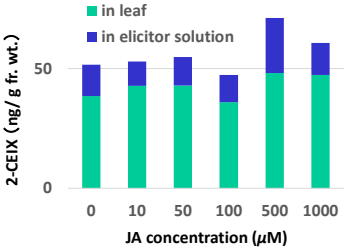

Fig.7 Effect of the JA concentration on the accumulation of 2-CEIX.

Conclusion

The toxicity of 2-CEIX against rat had been reported, however, a role of 2-CEIX in the sweet pea plant against insect pest had been unclear. In this study, it was demonstrated that 2-CEIX could be induced by JA which was a plant hormone related in defensive system. Thus, it was considered that 2-CEIX would play an important role in a defensive system of the sweet pea against herbivores. Although the defensive effects against another insect pests have not yet been investigated, an exogenous JA treatment to a sweet pea may be used for a plant protection against pest insect.