Browning on the roots of rice plant infested by rice root aphid, *Rhopalosiphum rufiabdominalis*

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Introduction

Studies on the resistance to pests of rice plant have been well investigated. A browning on rice plant foliages caused by attacking pathogens is one of important defensive systems. Ishihara *et al.* (2004) had demonstrated that the browning on rice plant leaf acted as a defense against pathogens and the browning material would be accumulated by polymerization of serotonin. However the role and the mechanism of a browning on a rice plant root attacked by insects is unclear.

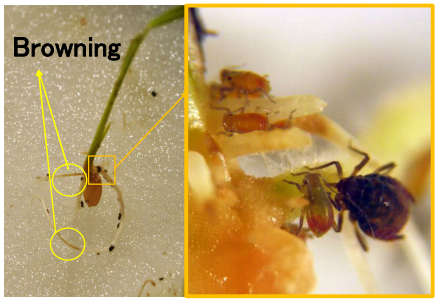


Fig.1 *Rhopalosiphum rufiabdominalis*

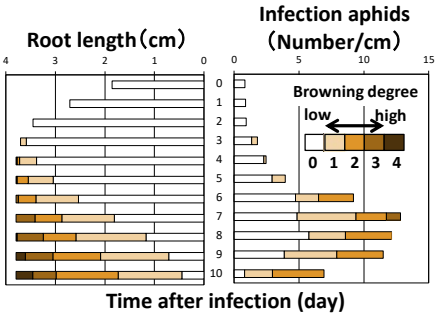


Fig.2 Change in browning degree and aphid populations on the roots.

Material and Method

Insects and plants: Rice seeds were sterilized with a 0.6% triflumizole water solution for 1 day, and then were forced sprouting for 3 days at 25 ± 3 ℃. The sprouting seeds were placed on a paper towel and incubated at 27 ± 3 ℃ (16L: 8D). A root of rice seeding at 4 days old was infested by a winged aphid and they were kept under the same condition. A length of the root, a color of the root, and number of the aphid was measured. The color was compared to color swatches.

Chemical analysis: Plant material (ca. 20 mg) was extracted in 200 μL MeOH, and the solvent was removed under reduce pressure. The extract was separated by liquid–liquid partition between hexane and water. The water layer was analyzed by CE–TOFMS, which was performed using an Agilent CE capillary electrophoresis system (Agilent Technologies, Waldbronn, Germany).

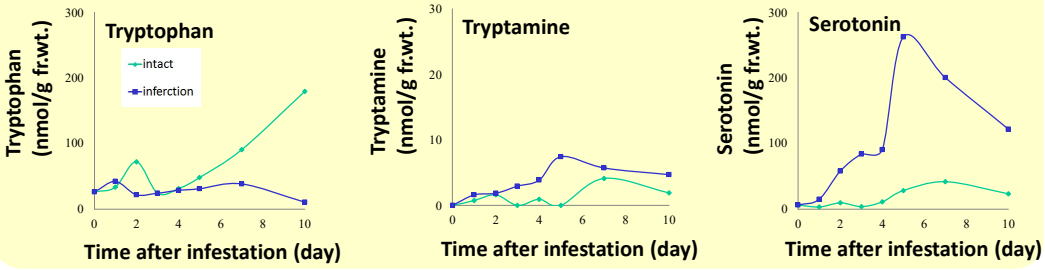
Quantitative RT–PCR: Total RNA was extracted from the rice leaves (200 mg) using an RNeasy Plant Mini Kit (Qiagen). The RNA was treated with DNase (RQ1, RNase–free DNase, Proomega), and then purified further using an RNeasy column. Single–strand cDNA was synthesized from the total RNA by reverse transcription using a Hight Capacity RNA–to–cDNA Kit (ABI). Quantitative real–time PCR was performed using a Step One Plus (ABI) with specific primer sets and a realtime PCR master mix (Thunderbird, TOYOBO).

Peroxidase activities: Peroxidase activity was measured by using a crude enzyme solution and serotonin as substrate. Roots (10 mg) were homogenized with sea sand and 1.5 mL of 50 mM K–Pi buffer (pH 6.9). The homogenate was centrifuged twice (12,000g, 10 min) and the supernatant was used in the assay for peroxidase. The reaction mixture, which consist of 100 mM McIlvain buffer (pH 6.0), 7 mM H_2O_2, and 4.8 mM serotonin, was incubated at 30℃ for 5 min. The reaction was stopped by 25 μL of 6 M HCl and 225 μL of MeOH, and the reaction mixture was analyzed by HPLC.

Result and discussion

The browning was induced by an infestation of aphid (Fig.2) and the aphids avoided the deep browning area (e.g. 3 or 4) on the roots. Thus it was thought that rice roots could induce any defensive system with browning when they were attached by pests. The metabolome analyses of the root extract was revealed that an infestation of an aphid on rice roots induced a high accumulation of serotonin with an accumulation of tryptamine but not tryptophan. Furthermore a high concentration of serotonin inhibited the survival of nymph aphids but not affected the fertilities (Table) on rearing test using an artificial diet. Therefore it was concluded that rice plants could induced a chemical defense system against insect pests.

Fig.3 Change in concentrations of browning related compounds after infestation with an aphid.



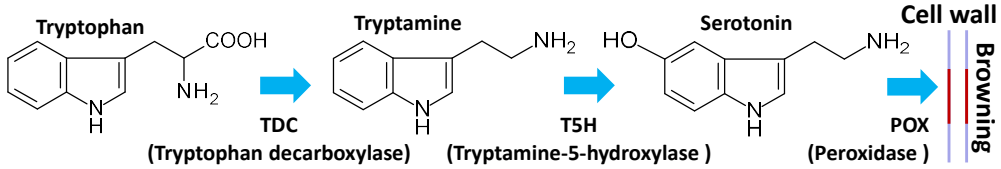


Table Effects of serotonin on the rearing test using an artificial diet (*n* = 4).

Serotonin (ppm)	No. of Total Fertility	No. of Survival
0	19.3	7.0
500	17.8	5.3
1000	22.0	10.3
5000	18.5	0.5

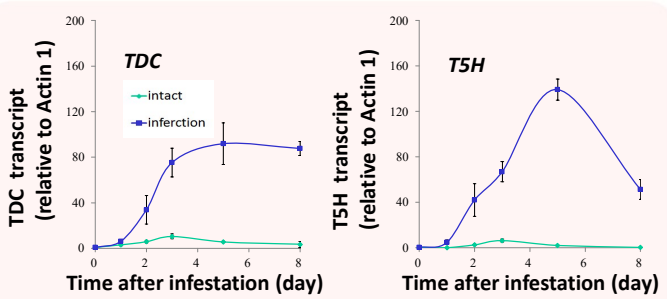


Fig.4 Change in transcripts of TDC and T5H after infestation.

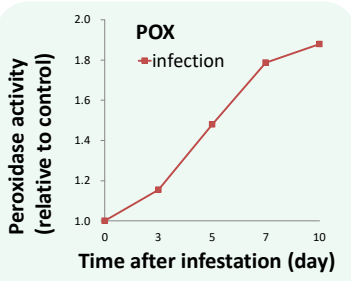


Fig.5 Change of peroxidase activities after infestation.

Next, an accumulation mechanism of serotonin on a roots was also investigated. When roots was attacked by aphids, the transcript of *tryptophan decarboxylase* (*TDC*)and *tryptamine–5–hydroxylase* (*T5H*) was also induced (Fig.4). The transcript of *TDC* started to increase after 1 day lag period and reached a plateau after 3 days, being ca. 50 times greater than in control roots. *T5H* also started to increase after 1 day lag period and a maximum 5 days after infestation, being ca. 100 times greater than in control roots. Thereafter, the transcript of *T5H* decrease to 40% of the maximum after 8 days. The expression of POX, which metabolizes serotonin, is delayed than the expression of T5H, which biosynthesizes of serotonin. Thus this deviation of the expression timing would induce an accumulation of serotonin, and then make a chemical defense system.