BROWNING MECHANISM ON THE ROOTS OF RICE PLANT INFESTED BY RICE ROOT APHID, *RHOPALOSIPHUM RUFIABDOMINALIS*, AND EFFECTS OF SALICYLIC ACID

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Introduction

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. A browning on a root is also occurred by attacking of aphids (Fig.1). However a role and a mechanism of a browning on a rice plant root is unclear.

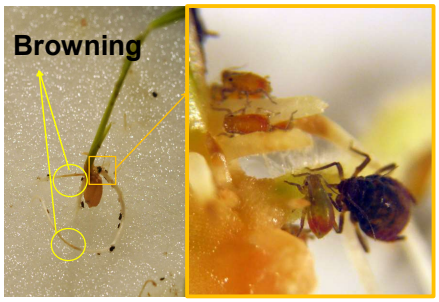


Fig.1 ***Rhopalosiphum rufiabdominalis***

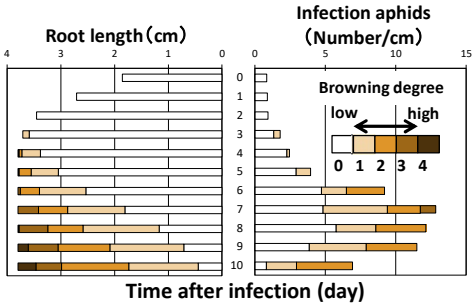


Fig.2 Change in browning degree and aphid populations on the roots.

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.

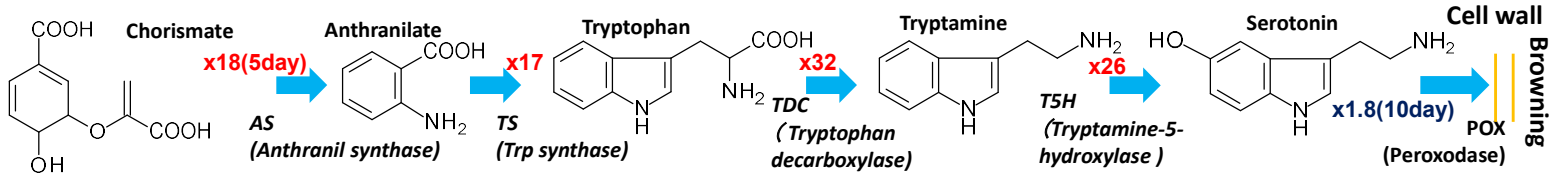
Chemical analysis: Plant material (ca. 20 mg) was extracted in 200 μL MeOH, and the solvent was removed under reduce pressure. The extract was separated by liquid-liquid partition between hexane and water. The water layer was analyzed by CE-TOFMS, which was performed using an Agilent CE capillary electrophoresis 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 real time 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 (12,000*g*, 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 225μL of MeOH, and the reaction mixture was analyzed by HPLC.

Result and Discussion

The browning was induced by an infestation of aphids (Fig.2) and the aphids avoided the deep browning area (e.g. 3 or 4) on roots. Thus, it was thought that rice roots could induce a defensive system(s) with a browning when they were attached by pests. The metabolome analyses of the root extract was revealed that an infestation of aphids induced a high accumulation of serotonin but not another metabolites to associate with serotonin biosynthesis pathway (Fig.3). 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 induce a chemical defense system against insect pests.



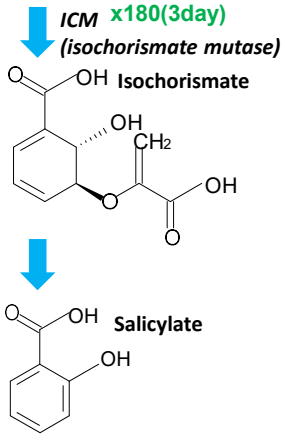


Fig.3 Change in transcripts of genes which associated to biosynthesis of browning and salicylate

Table Effects of serotonin on a 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

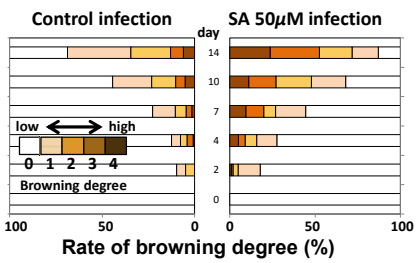


Fig.4 Change in browning degree after treatment of 50 μM SA.

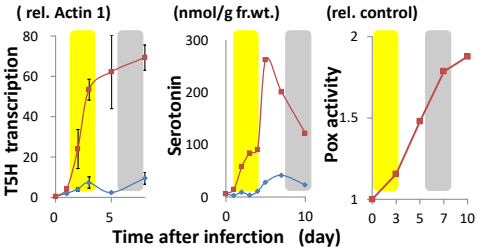


Fig.5 Change of transcreption of *T5H*, acummulation of serotonin, and Pox activity after aphids infestation.

Next, an accumulation mechanism of serotonin on a root was also investigated. When a root was attacked by aphids, the transcript of serotonin biosynthesis-related genes (*Anthranil synthase* (*AS*), *Tryptophan synthase* (*TS*), *Tryptophan decarboxylase* (*TDC*), and *Tryptamine-5-hydroxylase* (*T5H*)) was induced. On the other hand, an activation of POX activity, which metabolizes serotonin, is delayed than the expression of *T5H*, which biosynthesis of serotonin. Thus, this deviation of the expression timings would induce the accumulation of serotonin (Fig.5).

Finally, an effect of salicylate of browning on a root was examined. The expression of salicylate biosynthesis-related gene, *Isochorismate mutase* (*ICM*), was increased by an infestation of aphids. Furthermore, when the roots were infested by aphids, the root treated with salicylate was browning deeper than the control root early on (Fig.4). Then it was assumed that the browning on rice roots was regulated by salicylate.