# Induced resistance on the root of rice plant by plant hormones against root aphid.

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## Introduction

Induced resistance against pathogens and pest insects has been investigated in many plant species, and research on its application to crop protection has also been conducted. This resistance is caused by accumulation of secondary metabolites, expression of PR proteins and other processes. Among them, a browning is also an important inducible defensive system. Recently, it was demonstrated that a browning on rice plant root was caused by an activation of peroxidase (POX) which could convert serotonin to browning materials (Fig. 1), when a rice plant was attacked by aphids. An exogenous treatment with various chemical regent including plant hormones can induce resistances as well as an attacking by pathogens and pest insects. Particularly,effect of application of jasmonate and salicylate, which were known as hormones related to plant resistance, were well investigated, however resistances induced by abscisic acid (ABA) or auxin (indole-3-acetic acid: IAA) against insect pest was a few reported. Here, we report induced resistance on root of rice plant against rice root aphid by ABA and IAA.

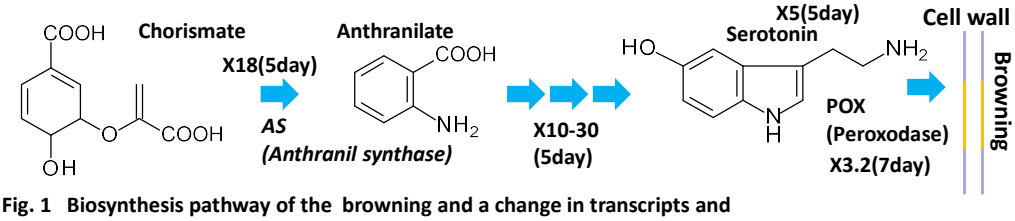
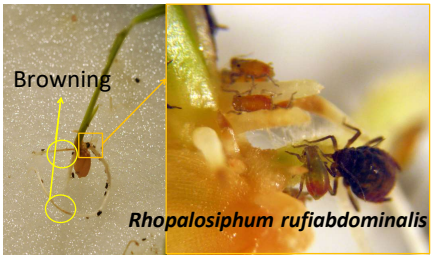



**Fig. 1 Biosynthesis pathway of the browning and a change in transcripts and an expression of enzyme on after aphids inoculation.**




## Result and Discussion

### Serotonin accumulation by exogenous treatment of plant hormones

A resistance inducible effect on rice root by plant hormones were tested by using rice seedlings. Rice plant roots (4 day-old) were treated with plant hormone or CuCl2, and kept at 27° C (16L8D). The seedlings were used for a bioassay or chemical analysis. After 3 days, the roots were harvested and immersed in MeOH (5 mg roots / 0.2 ml MeOH). The methanol extract was analyzed by HPLC to determine the concentration of serotonin, which is a precursor of browning and act as growth inhibitor against aphid. The serotonin concentration in ABA treatment root was significantly increased to 2.3-fold, but JA treatment did not induce a significantly change of serotonin accumulation (Fig. 2). On the other hand, a serotonin concentration on CuCl2, SA or IAA treatment roots was decrease to 0.48, 0.15, 0.49-fold, respectively. Thus, it was assumed that exogenous ABA treatment could induce the resistance caused by accumulation of serotonin. Then, effect of ABA to rice root was further investigated with that of IAA, which might act as an antagonism plant hormone to ABA.

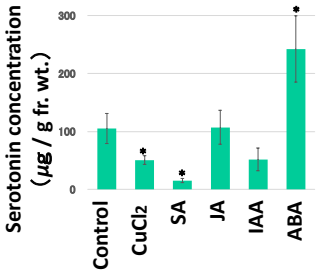



**Fig.2 Serotonin concentration in roots treated with plant hormones.**
∗ Significant by t-test at $P < 0.05$
CuCl2:1mM CuCl2, SA: 0.2mM Salicylate, JA: 0.2mM jasmonate, IAA: 0.02mM indole-3-acetic acid, ABA: 0.5mM abscisic acid

### Induced resistance by ABA treatment

Reproduction of rice root aphid, Rhopalosiphum rufiabdominalis, on the root of rice seedling treated with plant hormones were evaluated. Thirty-three rice seeds were sown on moisten paper towel in plastic cage, and roots of 4-days old seedlings were treated with ABA or IAA (10, 50, 100, 500, 1000 μM). Next day (5th day), five alate aphids were introduced in a cage containing the seedlings. The rearing cages were kept at 27° C (16L8D), and new borne aphid was recorded next 6 days.

The aphid population on root treated with H2O, 10, 50 μM ABA solution steadily increased to 58.0, 56.3, and 51.3 aphids, respectively, at 6 days (Fig. 3a). On the other hand, the aphid population on root treated with 100, 500, and 1000 μM ABA solution temporarily increased to approximately 30 aphids at 2 day, thereafter, their population decrease to approximately 20 aphids at 6 day. Thus, it was estimated that high concentration ABA could induce a resistance against aphid.

Next, the effects of ABA concentration on the induction of serotonin was also investigated. Rice seedling (4 day old) were treated with ABA (10, 50, 100, 500, 1000 μM), and kept at 27° C (16L8D). The roots were immersed in methanol after 5 days, and the extract was analyzed by HPLC. The accumulation of serotonin was enhanced with the increasing concentration of ABA (Fig. 3b). A significantly increasing of serotonin in the root treated with 500 and 1000 μM ABA was detected. Furthermore, a growth inhibition effect against the aphid on the root treated with high concentration (100 - 1000 μM) ABA, which induced the high amount accumulation of serotonin, was observed. Thus, it was assumed that the exogenous ABA treatment on rice seedling induced a resistance against aphid based on accumulation of serotonin.

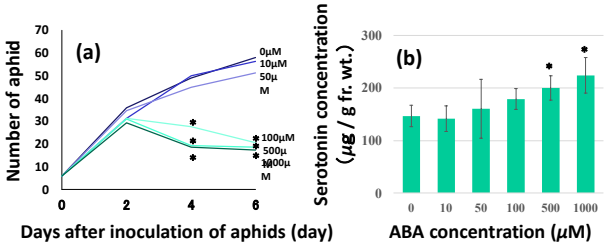



**Fig.3 Dose depend effects of ABA on the population of aphid and the accumulation of serotonin.**
∗ Significant by t-test at $P < 0.05$

### Induced resistance by IAA treatment

Reproduction of aphid and accumulation of serotonin on the root of rice seedling treated with IAA were also evaluated by same manner of the study on ABA effects. It was shown that the change of aphid population on root treated with various concentration of IAA was almost similar to these on ABA treatment root (Fig. 4a). On the other hand, the amount of serotonin in the root decreased to 10 – 35% in the all concentration tested (Fig. 4b). Thus, it was concluded that the exogenous IAA treatment on rice seedling could induce a resistance against aphid, however, it was regard that the resistance would not be based on accumulation of serotonin.

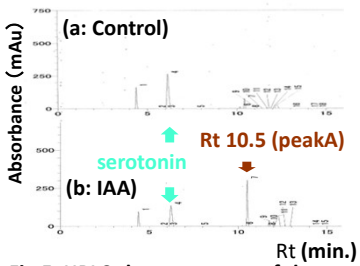
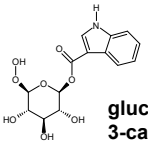



**Fig.5 HPLC chromatogram of the extract of root treated with H2O (a) and 100 μM IAA (b).**

HPLC analysis revealed that a novel peak A (Rt = 10.5 min) was induced by treatment IAA on root of rice seedling (Fig. 5). Furthermore, it was elucidated peak A to be glucosyl indole-3-carboxylate by LC-MSMS analysis, in which the spectra gave following ion peaks: 346 [M+Na]+, 324 [M+H]+, 162 [M+H]2+, 322 [M-H]-. As peak A was enhanced with the increasing concentration of IAA (data not shown), it was assumed that the compound A would be biosynthesized from IAA: IAA was first decarboxylated, and secondly the metabolite was glucosyl conjugated.



**Peak A (Rt 10.5) glucosyl indole-3-carboxylate**

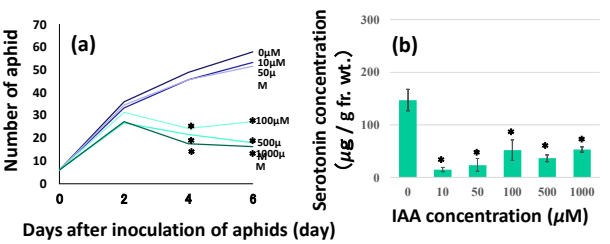



**Fig.4 Dose depend effects of IAA on the population of aphid and the accumulation of serotonin.**
∗ Significant by t-test at $P < 0.05$

## Conclusion

In this study, it was demonstrated that ABA and IAA treatment to rice plant could induce a resistance against aphid. Therefore, ABA, IAA, and their analog compounds might be used for plant protection, although effects of glucosyl indole-3-carboxylate against aphid are not clear and it is needed further studies for elucidation of the resistance mechanisms.