Studies on the mechanism of agricultural chemicals focused on plant hormone signals

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In recent years, it has become clear that the crosstalk of various plant hormones controls plant growth and disease resistance. Plant hormone signals may also be involved in the actions of a variety of pesticides and disease control techniques used for crop protection. From this point of view, we have focused on plant hormones to analyze the mode of action of pesticides that function in plants. Disease resistance inducers are pesticides that induce systemic acquired resistance (SAR) by activating the salicylic acid (SA)-mediated signaling pathway. However, when under unfavorable climate conditions, such as cold and cloudy weather, the resistance inducers are not sufficiently effective. Since the environmental stress response mediated by abscisic acid (ABA) may affect disease resistance, extensive studies of tobacco and tomato plants were performed, which clarified that SAR induction was suppressed by ABA. On the other hand, it was shown that transient high temperature treatment enhanced disease resistance via SA biosynthesis. These results suggest that changes in temperature due to climate change have an impact on disease resistance. The mode of action of a plant-growth regulator was analyzed by focusing on plant hormones. Isoprothiolane (IPT), an active ingredient of Fuji-one, is used as a plant-growth regulator and a fungicide. In Arabidopsis thaliana, we demonstrated that jasmonic acid and ethylene are required for the root elongation-promoting effect of IPT. As shown above, mode-of-action studies on pesticides in relation to plant hormones will lead to the development of new techniques for the better cultivation and protection of crops.

Keywords: plant hormone, disease resistance, systemic acquired resistance, plant growth regulator.

Introduction

In the future, the increase in food demand due to population growth and the decrease in crop yield due to climate change will be problems.1) The importance of crop-protection technology for the stable supply of food will increase. In June 2019, Food and Agriculture Organization (FAO) organized World Food Safety Day (WFSD) to highlight the important role of safe food in promoting health and ending hunger.

In order to achieve the United Nations-proposed SDGs 2030 agenda, safe food must be universally accessible.2) The role of agriculture and pesticides in achieving this goal is considered to be significant. However, pesticides are not the perfect tools for this purpose, because use of pesticide always brings the potential for resistant pathogen emergence, leading to unexpected damages. In addition, breeding disease-resistant varieties and developing new chemicals take a fairly long time. Therefore, it is considered necessary to effectively exploit the existing crop-protection technology.

As a continuous method for controlling plant diseases, resistance inducers that activate plant immunity can be mentioned. In Japan, Oryzemate (Meiji Seika Pharma, Tokyo), developed as a rice blast disease control agent, has been used for many years as a low environmental impact pesticide.3) It has been revealed that treatment with probenazole (PBZ), an active ingredient of Oryzemate, induces the expression of defense-related genes such as PBZ1 in rice.4) In Arabidopsis thaliana, we demonstrated that jasmonic acid and ethylene are required for the root elongation-promoting effect of IPT. As shown above, mode-of-action studies on pesticides in relation to plant hormones will lead to the development of new techniques for the better cultivation and protection of crops.

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tomato plants. Previous studies have suggested that HS treatment activates the SA-mediated disease resistance mechanism. To clarify the detailed mechanism, the effects of HS treatment were analyzed using Arabidopsis.

In recent years, biostimulants for agricultural materials have also attracted attention. Biostimulation is a technology that imparts abiotic stress resistance to plants and makes them healthy. In order to use these technologies effectively for crop protection, scientific evidence of their mechanisms is important.

Isoprothiolane (IPT) is an active ingredient of the rice blast disease control agent Fuji-one developed by Nihon Nohyaku Co., Ltd., and is also used as a plant-growth regulator. The mechanism by which Fuji-one prevents damping-off (Murrenae disease), a nonparasitic physiological disorder in rice in the nursery, is thought to be the promotion of rice root development, but the detailed mechanisms have not been clarified. Analyses in rice indicated that IPT’s effects are the promotion of auxin activity by lamina joint analysis, the induction of ethylene (ET) production in seedlings and callus, the promotion of acid auxin activity by lamina joint analysis, the induction of ethylene Analyses in rice indicated that IPT’s effects are the promotion of auxin activity by lamina joint analysis, the induction of ethylene (ET) production in seedlings and callus, the promotion of acid phosphatase activity in roots, and an increase in the permeability of root cell membranes. However, the direct contribution of IPT’s action to root elongation has not been clarified. In addition, IPT may act as a biostimulant to help obtain water during drying by promoting root elongation.

1. Suppression of SAR induction

Extensive studies on the regulation mechanism of SAR, a model plant pathosystem in Arabidopsis, have shown that the ABA-mediated signaling pathway, activated by environmental stresses, suppresses SAR induction. To confirm that the suppressive effect is common to plants and important in agricultural systems, we analyzed the influence of ABA on SAR in practical crops, tobacco and tomato.

1.1. Suppression of SAR by ABA in tobacco

We first analyzed the mechanism of ABA’s suppressive regulation of SAR in tobacco plants because it is a practical crop that has been studied as a model SAR plant. Nicotiana tabacum cv. Xanthi nc possesses the N gene that confers resistance to tobacco mosaic virus (TMV); consequently, its defense response to TMV infection results in an HR lesion. SAR inducers enhance this resistance and reduce the size of lesions. For SAR induction, we used 1,2-benzisothiazole-3(2H)-one 1,1-dioxide (BIT) and benzo[1,2,3]thiadiazole-7-carboxylic acid S-methyl ester (BTH), which activate upstream and downstream of SA in the SAR signaling pathway, respectively. Wild-type tobacco plants treated with BIT or BTH exhibited enhanced disease resistance against TMV and the tobacco wildfire bacterium Pseudomonas syringae pv. tabaci (Pst), which was suppressed by pretreatment of plants with ABA. Pretreatment with ABA also suppressed the expression of SAR marker genes by BIT and BTH, indicating that ABA suppressed the induction of SAR. ABA suppressed BTH-induced disease resistance and pathogenesis-related (PR) gene expression in NahG transgenic plants that are unable to accumulate SA. The accumulation of SA in wild-type plants after BIT treatment was also suppressed by pretreatment with ABA. These data suggest that ABA suppresses both upstream and downstream of SA in the SAR signaling pathway in tobacco (Fig. 1).

1.2. Suppression of SAR by ABA in tomato plants

To obtain more examples of the SAR regulation mechanism, we investigated SAR in tomato, a commercially important solanaceous crop. In order to analyze SAR in tomato plants, we first constructed a pathosystem with a commercial tomato cultivar, Solanum lycopersicum cv. Momotaro, and Pseudomonas syringae pv. tomato DC3000 (Pst DC3000), which causes bacterial leaf spot. This bacterial strain is compatible with tomato plants and grows faster in the tomato than in Arabidopsis, enabling us to estimate the bacterial growth in leaf tissues in 2 days post inoculation.

Tomato plants were prepared by culturing them in sterilized potting soil. Plants were treated with BIT by soil drenching 3 days prior to challenge inoculation with Pst DC3000. Pretreatment with ABA by soil drenching was performed 1 day before the BIT treatment. By 2 days after inoculation with Pst DC3000, BIT-treated plants contained more than tenfold lower bacterial titers than untreated control plants. However, BIT-treated plants pretreated with ABA contained only about twofold lower titers than control plants. Because BIT’s effects on disease resistance were suppressed by ABA pretreatment, either the induction of resistance or the effect of resistance against the pathogen was reduced by ABA-mediated signaling in the tomato.

Some PR genes are coordinately expressed during the induction of SAR in tomato plants. Whereas treatment with BIT induced the expression of PR genes in plants, pretreatment with ABA suppressed the BIT-induced expression of PR genes. This result indicated that at least the induction of SAR was sup-
pressed by ABA in the tomato.

Next, we examined the effect of BIT on SA accumulation in tomato plants. The application of BIT, which activates the SAR signaling pathway upstream of SA, gradually increased the levels of SA in leaf tissues. However, in ABA-pretreated plants, the accumulation of SA was dramatically suppressed after BIT application. Thus, the use of BIT, which is capable of activating SA biosynthesis, clearly indicated that ABA suppresses SAR induction by inhibiting SA biosynthesis in the tomato (Fig. 1).

2. Induction of SAR by heat shock treatment in Arabidopsis

In the greenhouse horticulture of tomato and cucumber plants, high-temperature treatment at 45°C for 1–2 hr has been developed to protect plants from insects and diseases.7–10 Analyses of those plants indicated that the activation of their defense mechanisms, in addition to the direct action of high temperature on insects and microbes, takes part in the protective effect. Elevated levels of SA and the expression of defense-related genes after heat shock (HS) treatment have been observed in cucumber and tomato plants; however, the detailed mechanisms remained to be clarified.

Hence, we investigated the mechanism of HS treatment of Arabidopsis, a model plant for the analysis of plant defense mechanisms. First, we optimized the HS treatment conditions for Arabidopsis by hot water dipping, with dipping at 45°C for 2.5 min as the optimal experimental condition.

With HS treatment, a significant induction of PR genes was detected 2 days post treatment. Next, to confirm the induction of disease resistance by HS treatment, challenge inoculation with Pst DC3000 was performed. The bacterial growth 2 days post inoculation in the tissues of HS-treated plants was about tenfold lower than in the control plants. These results suggested that HS treatment induced SAR in Arabidopsis.

To clarify whether SA biosynthesis was involved in the induction of disease resistance by HS treatment, SA levels and the expression of SA-related genes after HS treatment were analyzed. Transient induction of the SA biosynthesis gene ICS1 was only observed at 2 days post HS treatment. The levels of both free SA and total SA increased from 2 days post treatment and reached a peak at 3 days post treatment. The level of free SA then rapidly decreased in the next day, whereas the level of total SA gradually decreased. We next examined the effects of HS treatment on the SA signal-deficient mutant sid2, a mutant defective in SA biosynthesis, and a NahG transgenic plant that is unable to accumulate SA due to expression at the SA hydrolase. In the HS-treated sid2 and NahG plants, no enhancement of PR-1 expression or resistance to Pst DC3000 was detected. These results indicated that HS treatment induced SAR by the activation of SA biosynthesis; however, different from pathogen-triggered SAR, the activation of SAR by HS was transient and ceased in 5 days (Fig. 2).25

3. Analysis of the effect of IPT on root elongation in Arabidopsis

IPT is used as an agent in “Fuji-one” to control rice blast disease.26,27 IPT also has a plant-growth regulating activity and has been used to protect rice seedlings cultured in nursery flats from non-parasitic damping-off (Murenae disease).27 Previous studies have suggested the unique plant-growth regulating activity of IPT as a kind of biostimulant and the existence of unidentified physiological mechanisms in the development of seedlings, especially root system development.16–19 To gain a better understanding of IPT’s activity, we investigated its effects on root elongation in relation to the roles of plant hormones using Arabidopsis.

3.1. Involvement of plant hormones in the root elongation activity of IPT in Arabidopsis

To understand the enhancement mechanism of root elongation by IPT, the effects of IPT treatment on the root growth of Arabidopsis were examined. First, we examined various concentrations of IPT on root elongation activity in wild-type Arabidopsis (Col-0) plants by culturing the plants on agar plates. Measurement of the root length indicated that root elongation was enhanced by 12.5 µg/mL IPT but strongly suppressed by 75 µg/mL IPT. To clarify the mechanism of the positive effect of IPT (12.5 µg/mL) on root elongation in Arabidopsis, the involvement of growth-related plant hormones in IPT’s effect was examined.

The involvement of gibberellin (GA) and auxin in IPT’s effect was analyzed using inhibitors. The results indicated that GA was not required for IPT’s effect; however, auxin was at least involved in the effect of IPT on the enhancement of root elongation. The promotion of lamina inclination by auxin suggested the possibility that IPT can activate the brassinosteroid (BR)-mediated signaling pathway.18 To determine the involvement of BR in IPT’s effect, the root elongation was examined using a BR biosynthesis-deficient mutant, det2. The root elongation of the det2 mutant was greater in the media containing 12.5 µg/mL IPT than in the control, indicating that BR was not involved in the
length of trichoblast cells in the lower part of the differentiation zone, we first examined whether IPT influences the elongation in the root. To determine IPT’s effect on the primary root elongation, IPT probably influences cell proliferation or cell division in the root. To enhance the positive effect, distinguishable zones, the cell division zone in the meristem, are known to suppress root elongation.

ET, important plant hormones functioning in stress responses, indicated that JA and ET are involved in IPT’s effect on the enhancement of root elongation in Arabidopsis (Fig. 3).

3.2. Activation of cell proliferation in the Arabidopsis root meristem by IPT
To understand the mechanisms of IPT’s effect on root development, its effect on Arabidopsis root cells was investigated histologically. During plant growth under normal conditions, endogenous auxin is known to function in root elongation by activating cell division in the root apical meristem, whereas JA and ET, important plant hormones functioning in stress responses, are known to suppress root elongation.

The root cell development consists of three morphologically distinguishable zones, the cell division zone in the meristem, the elongation zone, and the differentiation zone. To enhance root elongation, IPT probably influences cell proliferation or cell elongation in the root. To determine IPT’s effect on the primary root elongation, we first examined whether IPT influences the length of trichoblast cells in the lower part of the differentiation zone. The result suggests that the negative effect of IPT on root elongation is due to the reduction in cell length; however, the positive effect is not due to the increase in cell size. Next, to determine whether the positive effect of IPT is due to enhanced cell proliferation in the meristem, the root meristem sizes were analyzed. Meristem size was enlarged by treatment with 12.5 µg/mL IPT, probably due to increased cell proliferation in the meristem, stem. To understand the detailed mechanism of the increase in meristem size, the effect of 12.5 µg/mL IPT on the cell number in the meristem was analyzed. The cell number of the wild-type root treated with 12.5 µg/mL IPT was ca. 17% higher than that of the untreated control root, indicating that IPT activated the cell division in the meristem. The increase in cell number by IPT treatment was not observed in the root of jar1 or ein2 mutants, indicating that the activation of cell division by IPT treatment was dependent on JA and ET signals. Our results demonstrate that the positive effect of 12.5 µg/mL IPT on primary root elongation in Arabidopsis is due to the JA- and ET-dependent increased cell proliferation in the meristem but not due to changes in cell elongation. Generally, JA and ET are known to suppress root growth; however, JA and ET take part in IPT’s positive effect on primary root elongation. Thus, our finding offers an important clue to identifying the regulation mechanism of cell proliferation in the root apical meristem.

Concluding remarks
The development of plant-protection technology is necessary for sustainable agricultural production. Therefore, we analyzed the action mechanism of the existing technology in order to establish a more effective use. New genome editing technology would accelerate the breeding of disease-resistant plants in the near future. However, plant-protection technologies with pesticides are effective for many plant species and varieties and can be used promptly. To respond to unexpected crises, it will be more and more important to gain scientific knowledge about disease control for a sustainable agriculture system.

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