Some agrochemicals have unique activities on plant, which modes of actions differ from those of herbicides and plant growth regulators. Because these induce useful and important phenotypic characteristics by activating physiological mechanisms in plant cell, understanding the underlying mechanism of their activities should be crucial for plant physiology and agriculture. As examples of such agrochemicals, studies on agrochemicals that activate the plant immune systems or root elongation, are described. Plant activators, inducers of systemic acquired resistance, were divided into two types, acting on upstream and downstream of salicylic acid (SA) biosynthesis, respectively. They have been useful research tools to clarify the regulation mechanism of SA-mediated disease resistance and to investigate another type of disease resistance mechanism mediated by brassinosteroids. By analyzing the roles of phytohormones in the isoprothiolane-induced root elongation indicated a positive effect of jasmonic acid and ethylene on primary root elongation. These types of research, categorized to one of chemical biology, would provide novel insight into plant physiology, which also contribute to control of crops.

Keywords: phytohormone, plant growth regulator, plant activator, plant immunity.

Introduction

Herbicides and plant growth regulators are the most commonly used agrochemicals that act on plants. Herbicides kill plants or suppress their growth by inhibiting certain physiological systems, preferably in a species-specific mode of action. Most plant growth regulators are either phytohormones and their derivatives or phytohormone inhibitors that target biosynthetic enzymes or signal perception mechanisms, including receptor proteins. Regulation of growth-related signal transduction for various phenotypic characteristics, such as germination, vegetative growth, flowering, fruiting, and pigmentation, enables proper growth and increased yield.

In addition, some agrochemicals stimulate other plant physiological mechanisms and display varying abilities. One such type of agrochemical stimulates the plant immune system to protect plants from pathogen attack. They are called “plant activators”, which were proposed in the mid-1990s. Probenazole (PBZ, 3-allyloxy-1,2-benzisothiazole-1,1-dioxide), a plant activator, has been practically used in Japanese rice fields since the 1970s and is called a "disease resistance inducer" or “resistance inducer.” According to the Japanese Agricultural Chemicals Regulation Law, plant activators are categorized as fungicides because they are mainly used to control rice blast disease in rice fields in Japan.

Another agrochemical is isoprothiolane (IPT, diisopropyl 1,3-dithiolan-2-ylidenemalonate), which is used as a fungicide to control rice blast disease and acts as a plant growth regulator. Its mode of action for plant growth regulation differs from other plant growth regulators that regulate phytohormone-mediated signaling, probably because IPT was developed as a fungicide.

The target of plant activators in the plant cell and the activated signaling is unknown at the time of their development because the action of these agrochemicals on plants differs from that of herbicides and plant growth regulators. In other words, these chemicals may reveal novel mechanisms in plant physiology. Considering that they have interesting and useful activities that can be used widely in the field, the unexplained physiological mechanisms stimulated by them should be crucial. Since the 1990s, the mechanism of plant immune systems has been clarified, with plant activators serving as key research tools for important findings. Here, examples of research conducted on agrochemicals are introduced.
1. Systemic acquired resistance

Systemic acquired resistance (SAR) is an inducible defense mechanism that is activated through the salicylic acid (SA)-mediated signaling pathway after a hypersensitive response (HR), including tissue necrosis caused by avirulent pathogens (Fig. 1).\(^{9,10}\) SAR has been utilized practically for crops, particularly rice, because it is effective against a broad range of pathogens and plays a key role in protecting plants from pathogen attack by exploiting SAR-inducing chemicals, called plant activators. Plant activators are defined as chemicals that are effective against a broad range of diseases, have no or very weak antimicrobial activities, and induce the same biochemical markers as observed in biologically induced SAR, such as the expression of pathogenesis-related (PR) genes in plant cells.\(^{1,3}\)

SA, which is recognized as a phytohormone, positively regulates the plant immune system.\(^{11,12}\) Pathogenic infection in plants enhances the biosynthesis of SA, which occurs in both infected and systemic tissues.\(^{13}\) In infected tissues, SA plays a role in basal resistance against pathogens. Systemically enhanced SA biosynthesis after pathogenic infection contributes to the induction of SAR. SA was considered to be a mobile signal molecule for SAR induction because SAR was not induced in SA-deficient mutants, such as sid1, sid2, and eds5, and NahG transgenic plants that express the bacterium-derived SA-degradation enzyme.\(^{12-14}\) Recently, exogenous application of N-hydroxypippecolic acid (NHP), synthesized in plants upon pathogen infection, was found to induce local and systemic expression of PR genes, SA biosynthesis, and disease resistance.\(^{15-17}\) NHP has been identified as a mobile signaling molecule for systemic disease resistance; however, the contribution of SA to signal transmission has not been completely excluded because SA-signaling through the receptor protein NPR1 is required for the induction of resistance in tissues.

2. Characterization of Plant activators

The ability of PBZ and its active metabolite 1,2-benzisothiazol-3(2H)-one,1,1-dioxide (BIT) to induce SAR was examined in Arabidopsis.\(^{18}\) Treatment with these compounds (0.2 mM), as well as SA, induced the expression of defense-related genes, PR1, PR2 (β-1,3-glucanase), and PR5 (thraumatin-like) in wild-type plants (Col-0). The induction of these genes was detected 6 hr after BIT-treatment and reached a maximum level after 48 hr. The wild-type plants (Col-0) treated with PBZ or BIT exhibited resistance to Pseudomonas syringae pv. tomato DC 3000 (Pst DC3000). Treatment of wild-type plants (Col-0, Nö-0, and Ws-0) with BIT or PBZ induced resistance to the virulent oomycete pathogen Hyaloperonospora arabidopsidis (Hpa; formerly Peronospora parasitica) (downy mildew) Emco5, by reducing hyphal growth to undetectable levels and the percentage of plants containing conidiophores. BIT at concentrations of up to 8 mM did not affect the rate of Pst DC3000 growth in liquid culture, although the direct effect of PBZ or BIT on the growth of Hpa could not be determined because it is an obligate pathogen.

Induction of SAR marker gene expression and enhancement of disease resistance in the absence of antibacterial activity suggest that PBZ and BIT can be considered as plant activators.

Resistance to Hpa was also observed in the jasmonic acid (JA) signal-deficient mutant coi1-1 and the ethylene (ET) signal-deficient mutant etr1-1; however, it was not detected in SA-deficient transgenic NahG plants, suggesting that SA is required for the induction of disease resistance by BIT. Gene expression analysis showed that expression of PR1 by BIT was observed in coi1-1 and etr1-1 mutants, but not in NahG transgenic plants and npr1-1 mutants defective in SA receptor protein. These results suggest that BIT activates PR gene expression and disease resistance via a pathway that is dependent on SA but not on JA or ET. Treatment with BIT increased the levels of free and total SA (free SA and salicylic acid glucoside, SAG) in wild-type plants (Col-0), which is consistent with the failure of PR gene expression in NahG plants. Taken together, PBZ and BIT do not require ET or JA to induce SAR; rather, they activate defense responses via the SA/NPR1 signaling pathway by acting upstream of SA biosynthesis (Fig. 1).\(^{18}\)

Treatment of wild-type tobacco plants with PBZ or BIT induced enhanced resistance to bacterial, fungal, and viral pathogens, enhanced expression of PR genes and increased SA accumulation.\(^{19}\) BIT did not induce disease resistance or PR gene expression in NahG tobacco plants. The ability of BIT to induce SAR was also confirmed in tomato by analyzing resistance against the bacterial pathogen, PR gene expression, and SA accumulation (Fig. 1).\(^{20}\) Thus, BIT can induce SAR by acting upstream of the SA-mediated signaling pathway in tobacco and tomato.

The effects of other possible plant activators on Arabidopsis, tobacco, and rice plants were examined by analyzing disease resistance, PR gene expression, and SA accumulation. Analyses of tobacco and Arabidopsis indicated that N-cyano-methyl-L,2-chloroisonicotinamide (NCI)\(^{21-23}\); 3-chloro-1-methyl-1H-pyrazole-5-carboxylic acid (CMPA)\(^{24,25}\); (3-chloro-4-methylanilinocarbonyl)-4-methyl-1,2,3-thiadiazole-5-carboxylic acid (Hpa)\(^{26,27}\); and 3-nitrobenzylpyrazole (3-NBZ-P)\(^{28}\); treatments induced SAR. The effects of these compounds are summarized in Table 1.
Mechanisms underlying these phenomena is not well understood. 4-methyl-1,2,3-thiadiazole-5-carboxylic acid (SV-03, a derivative of TDL)\(^{26}\); and 4-[3-[(3,5-dichloro-2-hydroxybenzylidene)amino]propyl]-4,5-dihydro-1H-pyrazol-5-one (BAPP)\(^{28}\) are able to induce SAR by acting downstream of SA biosynthesis, the modes of action of which are similar to those of benzo(1,2,3)thiadiazole-7-carboxothioic acid S-methyl ester (BTH) and 2,6-dichloronicotinic acid (INA) (Fig. 1).\(^{1,2}\)

Thus, SAR-activating plant activators are divided into two categories based on their site of action. PBZ and BIT induce SAR through SA-mediated signaling, by acting upstream of SA biosynthesis. In contrast, NCI, CMPA, TDL, SV-03, BTH, and INA induce SAR by acting downstream of SA biosynthesis. The SA receptor protein, NPR1, is required for SAR induction by both types of plant activators. To date, the target sites of PBZ and BIT remain unknown. Considering the signaling pathway after SA biosynthesis to PR gene expression, plant activators acting downstream of SA may act on the same target. Although extensive studies have been conducted, whether these plant activators act on NPR1 has not been determined. Developing more plant activators may reveal their important chemical moiety to activate downstream of SA biosynthesis, which would contribute to identifying their target site.

3. Regulatory mechanism of systemic acquired resistance by abiotic stress

Plant activators are widely used in Japan, but it has been known for years that cooler weather during summer causes significant damage in rice fields by rice blast disease. Although the suppressive effect of low temperature on the resistance to infection by Magnaporthe grisea has been demonstrated,\(^{29}\) the molecular mechanisms underlying these phenomena is not well understood. Furthermore, the reason why the induced resistance by plant activators was ineffective under such conditions remains unknown. Thus, an investigation of the induction and maintenance of SAR under environmental stresses in Arabidopsis was conducted to elucidate the regulatory mechanism of SAR at the molecular level and to help solve the above problem in rice fields.\(^{30}\)

The effects of exogenous abscisic acid (ABA) on SAR were analyzed using different types of plant activators, such as BIT and BTH, which act upstream and downstream of SA biosynthesis, respectively, in the SAR signaling pathway. Treatment with ABA suppressed the resistance to *Pst* DC3000 and expression of *PR* genes by BIT or BTH in wild-type plants, indicating the suppression of SAR induction. The suppression of *ICS1* expression and SA accumulation by treatment with BIT in wild-type plants demonstrated that ABA affects the upstream of SA biosynthesis (Fig. 1). The suppression of SAR induction by BIT in SA biosynthesis-deficient mutants, *sid2* and *eds5*, demonstrated that ABA can affect the downstream of SA biosynthesis (Fig. 1). Analyses of *coi1*-1 and *ein2*-1 mutants indicated that the suppressive effect of ABA on SAR induction is independent of JA and ET. The suppression of SAR induction by NaCl-activated environmental stress response was proved to be ABA-dependent by analyses in CYP707A3ox, the ABA-deficient transgenic plant overexpressing the ABA-degrading enzyme. Furthermore, the enhancement of SAR induction by ABA deficiency, such as enhanced disease resistance, defense gene expression, and SA accumulation, was observed in wild-type plants treated with abamime, a specific ABA biosynthesis inhibitor,\(^{31}\) and in the ABA biosynthesis-deficient mutant *aaos*. Thus, the regulation of SAR induction is dependent on the activation level of ABA-mediated signaling.\(^{30}\) The suppressive effect of ABA on SAR induction was also observed in commercially important crops, such as tobacco and tomato.\(^{32,33}\) This suggests that the regulation of SAR induction by environmental stress responses through the ABA-mediated signaling pathway is a common mechanism in plants (Fig. 1).

Further investigation in Arabidopsis revealed that the activation of SAR conversely suppressed the NaCl-induced expression of ABA biosynthesis-related genes (*ABA1* and *NCED3*) and ABA-responsive genes (*RAB18*, *COR15A*, *MYC2*, and *RD22*).\(^{30}\) The suppressive effects on *ABA1*, *COR15A*, and *RD22* were NPR1-dependent, while others were NPR1-independent. Therefore, antagonistic crosstalk occurs at multiple steps between SA-mediated signaling of SAR induction and ABA-mediated signaling of environmental stress responses.\(^{30}\)

4. Brassinosteroid-mediated disease resistance

Brassinosteroids (BRs) play a significant role in the regulation of stem elongation, leaf bending and unrolling, root inhibition, and cell elongation and have been recognized as a class of phytohormones since the late 1990s.\(^{34}\) Although several reports describing the functions of BRs in cold resistance and induction of ET biosynthesis have suggested that BRs play a role in stress response systems,\(^{35,36}\) the effect of BRs against plant diseases has been indicated only based on evaluations from field trials.\(^{37}\) To elucidate the involvement of BRs in plant immune systems, the protective activity and its mechanism were investigated using brassinolide (BL), the most biologically active BR, in tobacco and rice.\(^{38}\)

Treatment with BL induced enhanced resistance in tobacco to a broad range of pathogens, such as the viral pathogen tobacco mosaic virus (TMV), the bacterial pathogen *Pseudomonas syringae* pv. *tabaci* (*Pst*), and the fungal pathogen *Oidium* sp., which was SA-independent. BL treatment did not induce acidic or basic PR gene expression. Analysis using brassinazole 2001, a specific inhibitor of BR biosynthesis,\(^{39}\) in the SA-deficient NahG transgenic plant indicated the requirement of BR for resistance against TMV. This was confirmed by the increased accumulation of biosynthetic intermediates, castasterone (CS) and 6-deoxo-castasterone, in the TMV-inoculated leaves compared with that of the mock-inoculated leaves. Taken together, brassinosteroid-mediated disease resistance (BDR) is involved in plant defense responses independent of the SA-mediated defense response. Simultaneous induction of SAR and BDR by SAR inducers and BL, respectively, exhibited additive protective effects against...
TMV and Pst, suggesting the absence of crosstalk between SAR signaling and the BDR signaling pathway downstream of BL. In addition to tobacco, BL induced resistance in rice to rice blast disease, caused by the fungal pathogen Magnaporthe grisea, and rice bacterial blight disease, caused by the bacterial pathogen Xanthomonas oryzae pv. oryzae. These results suggest that BDR functions in the innate immune system of higher plants, including dicotyledonous and monocotyledonous species. Many components in the signal transduction of BRs have been identified, and many reports have demonstrated the roles of BRs in biotic and abiotic stress responses.

5. Mechanism of root elongation by an agrochemical

Fuji-one (Nihon Nohyaku Co., Ltd., Japan), containing IPT as the active ingredient, has been used as a control agent for rice blast disease and as a plant growth regulator. It prevents damping-off (Murenace disease), a non-parasitic physiological disorder in rice in the nursery, probably by promoting root development. Extensive studies on the mechanisms of the effect of IPT in rice have demonstrated physiological actions of IPT, promotion of auxin activity by lamina joint analysis, induction of ET production in seedlings and callus, promotion of acid phosphatase activity in roots, and increase in the permeability of root cell membranes. In addition, IPT acts as a biostimulant by promoting root elongation, thus increasing water availability. Nevertheless, the detailed mechanism of action of IPT on root elongation remains unclear. Thus, an investigation using Arabidopsis was conducted to clarify the mechanism of action of IPT in detail.

First, the effects of IPT on root elongation of wild-type Arabidopsis (Col-0) plants were examined by culturing the plants on agar plates with varying concentrations of IPT. The results indicated that root elongation was enhanced by 12.5 µg/mL IPT but strongly suppressed by 75 µg/mL IPT. The positive and negative effects of IPT, at 12.5 µg/mL and 75 µg/mL, respectively, were further examined in terms of the involvement of phytohormones.

Analyses using the inhibitors demonstrated that gibberellin (GA) was not involved in the effect of IPT, but auxin was involved in the positive effect of IPT on the enhancement of root elongation. The promotion of lamina inclination by auxin in rice suggests that IPT activates the BR-mediated signaling pathway. However, the root elongation of det2, a BR biosynthesis-deficient mutant, was greater in the media containing 12.5 µg/mL IPT than in the control, indicating that BR was not involved in the enhancement of root elongation by IPT.

Next, to determine the involvement of stress-related phytohormones in the positive effect of IPT, the root growth of IPT-treated Arabidopsis mutants with defective biosynthesis or signal transduction of ABA, SA, JA, and ET was examined. Analyses using biosynthesis-deficient mutants indicated that SA and ABA were not involved in the effect of IPT. In contrast, IPT failed to enhance root elongation in the jar1-1 mutant, defective in JA signal transduction, and ein2-1 mutant, defective in ET signal transduction. Thus, JA and ET are involved in the effect of IPT on the enhancement of root elongation in Arabidopsis (Fig. 2).

Root cell development consists of three morphologically distinguishable zones: the cell division zone in the meristem, the elongation zone, and the differentiation zone. IPT probably influences cell proliferation in the cell division zone or cell elongation in the elongation zone. Histological analysis of Arabidopsis root cells was performed to clarify the mechanisms underlying the effect of IPT on root development. Analyses of the effect of IPT on the length of trichoblast cells in the lower part of the differentiation zone indicated that the negative effect of IPT was due to the reduction in cell length; however, the positive effect was not due to the increase in cell size (Fig. 2). In contrast, the meristem size of the wild-type plant, estimated by the cell number in the meristem, was enlarged by treatment with 12.5 µg/mL IPT, probably due to increased cell proliferation in the meristem. The increase in cell number by IPT treatment was not observed in the roots of jar1-1 and ein2-1 mutants, indicating that the activation of cell division by IPT treatment was dependent on JA and ET signals. Therefore, the positive effect of IPT (12.5 µg/mL) on primary root elongation in Arabidopsis is due to the JA- and ET-dependent increased cell proliferation in the meristem, and not due to changes in cell elongation (Fig. 2). Generally, JA and ET are known to suppress root growth. However, JA and ET have a positive effect on primary root elongation. Thus, our findings provide insight into identifying the regulatory mechanism of cell proliferation in the root’s apical meristem.
Concluding remarks

During the last two decades, research on chemical biology has been growing, and the techniques are used in various types of research fields today. The development of agrochemicals and elucidation of their action at the physiological or molecular levels, one of the origins of plant chemical biology, have been pursued for decades, which has been accelerated and improved by recent chemical biology techniques with genome-wide, bioinformatics, and bioimaging analyses. As demonstrated in the present study, agrochemicals could be potent research tools for identifying unknown mechanisms in living systems.

Biostimulants, which have been recently exploited in many countries, could be the objects of future research in chemical biology, plant biology, and pesticide science. Biostimulants, consisting of inorganic and organic compounds, biological resources, or their mixtures, are not categorized as pesticides, plant activators, or fertilizers. Biostimulants enable plants to tolerate abiotic stresses, leading to better growth. Their modes of action include different ways, most of which remain unclear.

Elucidation of the mechanism of action of some agents, including biostimulants, will reveal unknown physiological mechanisms. These findings will be important not only for plant biology but also for agriculture, to develop new active chemicals or identify new abilities of plants.

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References

1) B. Vernooij, L. Friedrich, P. A. Goy, T. Staub, H. Kessmann and K. Ryals: 2,6-Dichloroisonicotinic Acid-Induced Resistance to Pathogens Without the Accumulation of Salicylic Acid. Mol. Plant Microbe Interact. 8, 228–234 (1995).
2) K. A. Lawton, L. Friedrich, M. Hunt, K. Weymann, T. Delaney, H. Kessmann, T. Staub and J. Ryals: Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. Plant J. 10, 61–70 (1996).
3) T. Watanabe, H. Igarashi, K. Matsumoto, S. Seki, S. Mase and Y. Sekizawa: The Characteristics of Probenazole (Oryzemate®) for the Control of Rice Blast. J. Pestic. Sci. 2, 291–296 (1977).
4) F. Araki and Y. Miyagi: Effect of isoprothiolane on the infection process of Pyricularia oryzae. Ann. Phytopathol. Soc. Jpn. 42, 401–406 (1976).
5) K. Kakiki and T. Misato: Effect of Isoprothiolane on Fatty Acid Synthesis. J. Pestic. Sci. 4, 305–313 (1979).
6) X. Dong: NPR1, all things considered. Curr. Opin. Plant Biol. 7, 547–552 (2004).
7) K. M. Pajerowska-Mukhtar, D. K. Emerine and M. S. Mukhtar: Tell me more: roles of NPRs in plant immunity. Trends Plant Sci. 18, 402–411 (2013).
8) J. De Kesel, U. Conrath, V. Flors, E. Luna, M. H. Mageroy, B. Mauch-Mani, V. Pastor, M. J. Pozo, C. Pieterse, J. Ton and T. Kyndt: The Induced Resistance Lexicon: Do’s and Don’ts. Trends Plant Sci. 26, 685–691 (2021).
9) K. S. Chester: The Problem of Acquired Physiological Immunity in Plants. Q. Rev. Biol. 8, 275–324 (1933).
10) J. Durner, J. Shah and D. F. Klessig: Salicylic acid and disease resistance in plants. Trends Plant Sci. 2, 266–274 (1997).
11) T. P. Delaney, S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negrotto, T. Gaffney, M. Gut-Rella, H. Kessmann, E. Ward and J. Ryals: A central role of salicylic acid in plant disease resistance. Science 266, 1247–1250 (1994).
12) T. Gaffney, L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Ward, H. Kessmann and J. Ryals: Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261, 754–756 (1993).
13) M. C. Wildermuth, J. Dewdney, G. Wu and F. M. Ausubel: Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414, 562–565 (2001).
14) C. Nawrath, S. Heck, N. Parinthewong and J. P. Métraux: EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. Plant Cell 14, 275–286 (2002).
15) Y. C. Chen, E. C. Holmes, J. Rajniak, J. G. Kim, S. Tang, C. R. Fischer, M. B. Mudgett and E. S. Satelley: N-hydroxy-pipeolic acid is a mobile metabolite that induces systemic disease resistance in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 115, E4920–E4929 (2018).
16) M. Hartmann, T. Zeier, F. Bernsdorff, V. Reichel-Deland, D. Kim, M. Hohmann, N. Scholten, S. Schuck, A. Bräutigam, T. Hölzl, C. Gantner and J. Zeier: Flavin Monoxygenase-Generated N-Hydroxy-pipeolic Acid Is a Critical Element of Plant Systemic Immunity. Cell 173, 456–469 (2018).
17) H. Tian and Y. Zhang: The Emergence of a Mobile Signal for Systemic Acquired Resistance. Plant Cell 31, 1414–1415 (2019).
18) K. Yoshioka, H. Nakashita, D. F. Klessig and I. Yamaguchi: Probena-zole induces systemic acquired resistance in Arabidopsis with a novel type of action. Plant J. 25, 149–157 (2001).
19) H. Nakashita, K. Yoshioka, M. Yasuda, T. Nitta, Y. Arai, S. Yoshida and I. Yamaguchi: Probenazole induces systemic acquired resistance in tobacco through salicylic acid accumulation. Physiol. Mol. Plant Pathol. 61, 197–203 (2002).
20) M. Kusajima, Y. Okumura, M. Fujita and H. Nakashita: Abscisic acid modulates salicylic acid biosynthesis for systemic acquired resistance in tomato. Biosci. Biotechnol. Biochem. 81, 1733–1736 (2017).
21) H. Yoshida, K. Onishi, K. Koike, T. Nakagawa, S. Sekido and I. Yamaguchi: Effect of N-Cyanomethyl-2-chloroisonicotinamide for Control of Rice Blast. J. Pestic. Sci. 15, 413–417 (1990).
31) S. Y. Han, N. Kitahata, K. Sekimata, T. Saito, M. Kobayashi, K. Nakamura, K. Yamaguchi-Shinozaki, K. Hayashi, A. Maruyama-Nakashita, F. S. Che and H. Nakashita: Characterization of plant immunity-activating mechanism by a pyrazole derivative. *Biosci. Biotechnol. Biochem.* **84**, 1427–1435 (2020).

32) M. Kusajima, Y. Yasuda, A. Kawashima, H. Nojiri, H. Yamane, M. Nakajima, K. Akutsu and H. Nakashita: Suppressive effect of abscisic acid on systemic acquired resistance in tobacco plants. *J. Gen. Plant Pathol.* **76**, 161–167 (2010).

33) M. Kusajima, Y. Okumura, M. Fujita and H. Nakashita: Abscisic acid modulates salicylic acid biosynthesis for systemic acquired resistance in tomato. *Biosci. Biotechnol. Biochem.* **81**, 1850–1853 (2017).

34) T. Yokota: The structure, biosynthesis and function of brassinosteroids. *Trends Plant Sci.* **2**, 137–143 (1997).

35) Y. Hotta, T. Tanaka, L. Bingshan, Y. Takeuchi and M. Konnai: Improvement of Cold Resistance in Rice Seedlings by 5-Aminolevulinc Acid. *J. Pestic. Sci.* **23**, 29–33 (1998).

36) H. C. Yi, S. Joo, K. H. Nam, J. S. Lee, B. G. Kang and W. T. Kim: Auxin and brassinosteroid differentially regulate the expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in mung bean (Vigna radiata L.). *Plant Mol. Biol.* **41**, 443–454 (1999).

37) V. Khrisap, V. Zhabinskii and A. D. Groot: Twenty Years of Brassinosteroids: Steroidal Plant Hormones Warrant Better Crops for the XXI Century. *Ann. Bot.* **86**, 441–447 (2000).

38) H. Nakashita, M. Yasuda, T. Nitta, T. Asami, S. Fujioka, Y. Arai, K. Sekimata, S. Takatsuto, I. Yamaguchi and S. Yoshida: brassinosteroid functions in a broad range of disease resistance in tobacco and rice. *Plant J.* **33**, 887–898 (2003).

39) T. Asami and S. Yoshida: brassinosteroid biosynthesis inhibitors. *Trends Plant Sci.* **4**, 348–353 (1999).

40) H. Tong and C. Chu: Functional Specificities of brassinosteroids and Potential Utilization for Crop Improvement. *Trends Plant Sci.* **23**, 1016–1028 (2018).

41) R. Lozano-Durán and C. Zipfel: Trade-off between growth and immunity: role of brassinosteroids. *Trends Plant Sci.* **20**, 12–19 (2015).

42) F. Araki and Y. Miyagi: Effect of isoprothiolane on the infection process of Pyricularia oryzae. *Ann. Phytopathol. Soc. Jpn.* **42**, 401–406 (1976).

43) M. Hikawa and I. Yanai: *Shokubutsu no Kagakushosetsu* 18, 71 (1983) (in Japanese)

44) T. Otsuka and H. Saka: *Ipn. J. Crop. Sci.* **56**, 571–576 (1987) (in Japanese).

45) M. Yoshida and M. Yukimoto: Effects of isoprothiolane on Growth and Membrane Water Permeability of Rice Seedling Root. *J. Pestic. Sci.* **18**, 385–387 (1993).

46) T. Otsuka and H. Saka: *Ipn. J. Crop. Sci.* **57**, 722–727 (1988) (in Japanese).

47) M. Kusajima, M. Nagata, N. Miyashita, Y. Yotagakiuchi, K. Maehara, I. Miyazaki, M. Inoue, M. Fujita and H. Nakashita: *Trends Plant Sci.* **43**, 186–190 (2018).

48) M. Kusajima, M. Inoue, M. Fujita, K. Miyagawa, R. Horita and H. Nakashita: Activation of cell proliferation in Arabidopsis root meristem by isoprothiolane. *J. Pestic. Sci.* **43**, 261–265 (2018).