Spermidine, a polyamine, confers resistance to rice blast

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Polyamines are involved not only in fundamental cellular processes such as growth, differentiation, and morphogenesis, but also in various environmental stresses. We demonstrated that spermidine, a polyamine, confers resistance to rice blast accompanied by the up-regulation of marker genes for the salicylic acid-mediated signaling pathway PR1b and PBZ1 and of phytoalexin biosynthesis genes CPS4 and NOMT. This is the first report about the involvement of spermidine in rice disease resistance.

Keywords: polyamines, spermidine, rice blast, disease resistance, salicylic acid.

Electronic supplementary materials: The online version of this article contains supplementary material (Supplemental Table S1), which is available at http://www.jstage.jst.go.jp/browse/pestics/.

Salicylic acid (SA) plays an important role in the induction of systemic acquired resistance (SAR) in plants, which effectively protects plants from the devastating damage caused by pathogens. The study of the mechanisms of SA-induced disease resistance extended our understanding of plant defense mechanisms against pathogens and improved the technology available for agricultural production and stock maintenance.1)

Polyamines, such as putrescine, spermidine, and spermine, are small basic molecules with two or more primary amino groups, as shown in Fig. 1. They are ubiquitous in nature and believed to be important growth regulators in both eukaryotic and prokaryotic cells.2,3) In plants, they are involved not only in fundamental cellular processes such as growth, differentiation and morphogenesis but also in various environmental stresses.4–6) Walter et al. proposed that plant polyamines play an important role in the defense against pathogen attack.7) For example in Arabidopsis, exogenously applied spermine induces gene expression of PR1a that functions in conferring resistance to disease.8) Moreover, leaf tissues infected with tobacco mosaic virus and neighboring tissues form local lesions that accumulate spermine sufficiently high to induce the expression of PR proteins and confer acquired resistance.9,10) In the above reports both putrescine and spermidine induce no significant disease defense in plants, although polyamines, including putrescine and spermidine in addition to spermine, were accumulated with infection of pathogen.11) However, at present, there is no precise report on the effect of spermidine on plant disease defenses.

On the basis of the above background, we studied the effect of spermidine on disease defenses in rice and found that spermidine induces the expression of defense-related genes, perhaps through the SA-signaling pathway, and confers resistance to rice blast. This is the first report to demonstrate the disease defense-inducing activity of spermidine in planta.

Pant materials
Rice (Oryza sativa L. japonica Nipponbare) were grown in greenhouse at 27 to 30°C.

Real-time quantitative RT-PCR
Genes were quantified by real-time quantitative RT-PCR as

Fig. 1. Chemical structures of representative polyamines.
previously described\textsuperscript{(12)} with minor modification. We used PrimeScript RT Reagent Kit with gDNA Eraser (Takara Bio) for cDNA synthesis. The list of primers is shown in Supplemental Table S1.

Quantification of rice phytoalexins
Momilactones, phytocassanes, and sakuranetin were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis as previously described.\textsuperscript{(13)} In this report momilactones and phytocassanes are quantified as a totals of momilactones A and B and phytocassanes A to E, respectively.

Inoculation of rice with rice blast
The resistance-conferring effect of spermidine against rice blast was investigated by the method described previously with a slight modification. In this report 2 mL of spermidine solution (1 mM, 0.01% Tween20) per plant was sprayed on the whole rice (5th leaf stage) 24 hr before spore inoculation. Isolate Kyu89-246 (MAFF101506) of \textit{M. oryzae} was inoculated by spraying.

First, to determine whether spermidine activates defense gene expression, we analyzed the effects of spermidine on the salicylic acid (SA)-mediated signaling pathway by determining the expression levels of PR genes OsPR1b and probenazole-induced protein 1 (PBZ1), which are marker genes for disease resistance in rice, because there is substantial evidence of the involvement of SA with the activation of the disease defense mechanism in rice.\textsuperscript{(14,15)} It has been reported previously that in rice-leaf segments, OsPR1b expression was induced after 24 hr of treatment with SA.\textsuperscript{(16)} PBZ1 is a rice \textit{PR10} that was identified after 72 hr of treatment with probenazole (PBZ, an SA analog).\textsuperscript{(17)} Treatment of the leaf blades with spermidine markedly induced the gene expressions of \textit{OsPR1b} but only slightly induced the gene expressions of \textit{PBZ1} (Fig. 2A and 2B). As phytoalexins are antimicrobial and accumulate rapidly upon pathogen infection,\textsuperscript{(18,19)} we also analyzed the effect of spermidine on the gene expression of phytoalexin biosynthesis genes \textit{CPS2}, \textit{CPS4} and \textit{NOMT}.\textsuperscript{(20–22)} Treating the leaf blades with spermidine induced \textit{CPS4} and \textit{NOMT} gene expressions but did not affect \textit{CPS2} gene expression (Fig. 2C and 2D). On the other hand, salicylic acid (SA) treatment induced almost no change in the level of these gene expressions except \textit{NOMT}.

Based on the result that spermidine treatment induced the gene expressions of \textit{CPS4} and \textit{NOMT}, we measured phytoalexin levels next. Three phytoalexins, momilactones, phytocassanes and sakuranetin are well known in rice.\textsuperscript{(23–25)} Momilactones and phytocassanes are major phytoalexins of rice and show an inhibitory effect on the growth of the blast fungus.\textsuperscript{(24)} Sakuranetin was originally isolated from UV-irradiated rice leaves. It also has an inhibitory effect on the growth of the blast fungus, and it has been observed in blast-infected rice leaves.\textsuperscript{(25)} Spermidine-sprayed leaves were sampled and the momilactones, phytocas-

sanes, and sakuranetin contents were measured. In contrast to in the control leaves, the accumulation of momilactones and phytocassanes increased slightly in spermidine-sprayed leaves (Fig. 3A and 3B), while sakuranetin significantly accumulated in response to treatment with spermidine (Fig. 3C).

Finally, we tested the resistance of spermidine-treated rice to fungal blast. A spore suspension of the compatible blast fungus Magnaporthe oryzae was spray-inoculated onto plants at the 5-leaf-stage. Many gray compatible lesions appeared in the control plants, whereas few gray compatible lesions appeared on the spermidine-treated rice. The numbers of compatible lesions in 4th and 5th leaves were counted and compared between control rice and spermidine-treated rice. The number of compatible lesions in the spermidine-treated rice was much smaller than in the control rice, indicating that the spermidine-treated rice showed blast resistance (Fig. 4).

In this paper, we demonstrated that spermidine treatment induced the expression of disease-resistance marker genes OsPR1b and PBZ1 and phytoalexin biosynthesis genes CSP4 and NOMT, while SA treatment did not induce changes in the expression levels of these genes except NOMT. The elevation of levels of phytoalexin biosynthesis genes is likely, in accordance with the accumulation of phytoalexins. We found only modest increases in the levels of momilactones and phytocassanes, which are not significant. This result could be ascribed to the weaker increases in the expression levels of CPS2 and CPS4 with spermidine treatment than with Cu²⁺ treatment; however, further experiments are required to determine the effect of spermidine on the production of these phytoalexins, while the evident accumulation of sakuranetin was induced by spermidine treatment. This could be consistent with the increase in the expression level of NOMT with spermidine treatment.

OsPR1b is thought to be involved in the SA-mediated signaling pathway. Therefore, on the basis of the above result that spermidine treatment induced the expression of this gene it is possible to think that spermidine treatment should activate SA-mediated signaling and confer resistance to rice blast. Actually, in our in planta assay, spermidine-treated rice showed disease resistance distinct from that of non-treated rice against M. oryzae. However, in our assay condition, SA treatment did not induce the expression of OsPR1b and PBZ1 genes but induced dis-
ease resistance against *M. oryzae* in rice. Shimono *et al.* reported that PR gene expression depends on growth condition: *PR1b* was up-regulated in growth chamber-grown plants but not in greenhouse-grown plants.\(^{16}\) Taking this report into account, we cannot exclude the possibility that our results could be affected by subtle changes in the experimental condition, and, therefore, SA-treated rice did not show the characteristics of SA signal-activated rice. Regardless, it is noteworthy that in our assay condition spermidine acts like SA, only more potently than SA, and confers resistance to rice blast. However, at present, we cannot conclude that this disease resistance results only from the activation of the SA-mediated signaling pathway.

Exogenously supplied spermidine caused no significant changes in the levels of SA and jasmonic acid for at least 2 to 4 days after the treatment (data not shown) although spermidine can activate SA-mediated signaling in rice. This result suggests that spermidine could act downstream of SA biosynthesis or there could be more than two independent pathways for *PR1b* gene induction: SA mediated and spermidine mediated. The possibility remains that spermidine exerts its *in planta* activity by activating signaling pathway other than the SA signaling pathway. Further investigation regarding the action mechanism of spermidine in rice will reveal how this chemical activates and interacts with the SA-mediated signaling pathway.

Plant activators are compounds, such as analogs of the defense hormone salicylic acid (SA), which protect plants from pathogens by activating the plant immune system. As they are useful both for science and for agriculture, a wide variety of compounds have been screened to identify plant activators that are more effective and may be applicable for a broad range of crops.\(^{1}\) In this context, spermidine and its derivatives may be promising new plant activators.

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**References**