

いもち病感染および UV 照射イネ葉における抗菌物質生成

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
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発行元	日本植物病理學會
巻/号	53巻4号
巻号補足	
掲載ページ	p. 449-453
発行年月	1987年10月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター

Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



The Production of Anti-Blast Substances in Blast-infected and UV-irradiated Rice Leaves*

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Abstract

Anti-blast substances (S-1 and its derivatives) produced in blast-infected and UV-irradiated rice leaves of three cultivars showing different horizontal resistance were analyzed quantitatively, in order to clarify the role of these substances in disease resistance. The production of the substance was caused not only in the inoculated leaves, but also in UV-irradiated leaves where brown spots appeared. While, the production was not observed in case of symptomless highly resistance and chemical damages tested. Negative correlation was observed between the amount of the substances formed and the degree of horizontal resistance at 7th day after inoculation. These results seem to indicate that these substances could associate secondarily with the resistance and the production relates closely with browning of host cells in the destruction process. The final conclusion, however, should be drawn after studies at the infection site. UV-damages were severer in aged leaves (lower leaves) and the degree of the damages was different in each cultivar. The amount of the anti-blast substances formed closely correlated with the degree of UV-damages. So far as tested cultivars concerned, the damages were less in horizontally weak cultivar than that of strong cultivar. Further studies on this point with various cultivars were required.

(Received February 12, 1987)

Key words : anti-blast substances, S-1, blast infection, UV irradiation, GLC, horizontal resistance.

Introduction

Formerly, the authors isolated the anti-blast substances (ABS) from rice leaves infected with *Pyricularia oryzae* Cav. during the studies on Momilactones A and B⁵⁾. One of them, S-1 (C₂₀H₃₂O₂, mole. wt. 304) was decided as a novel substance different from Oryzalexin A, B and C^{1,2)} with the results of HRMS, MS and GCMS analyses^{6,7)}. This substance seems to be identical with Oryzalexin D which was reported recently by Sekido *et al.*¹⁴⁾. Endo *et al.*³⁾ reported that these substances may closely associate with the blast resistance as phytoalexin. However, the authors assumed that the production of ABS might closely relate with the destruction of host cells and could not be recognized in symptomless highly resistant interaction. Furthermore, the author supposed that the accumulation of evidence might be required before the final decision on the role of the ABS in blast resistance. To confirm this postulation, the qualitative

* This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan (No. 59480041)

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and quantitative estimations of ABS in physically, chemically damaged and blast-infected rice leaves were conducted by gas-liquid chromatography. The abstract has been partly reported elsewhere⁸⁾ and the details are contributed in present report.

Materials and Methods

Rice cultivars. Three rice cultivars which have single major gene *Pi-i* and are different each other in the horizontal resistance: Inabawase (weak), Fujisaka 5 (medium) and Todorokiwase (strong) were mainly used. While, cultivar Inabawase was used for the studies on the ABS production in rice leaves which were physically and chemically treated. Rice seedlings were raised with the commercial soils (Kumiai Baido) in the plastic pots.

Physical and chemical treatments. At 7th leaf stage, the rice plants were UV-irradiated or chemically treated. The irradiation was conducted with germicidal lamp (10 W Toshiba lamp). Chemical treatments were conducted by spray of 0.1% 1, 1'-dimethyl-4, 4'-bipyridinium-dichloride (paraquat), 2, 2'-bipyridyl and 4, 4'-bipyridine (O_2^- generating agents) and 1% $CuSO_4$ solution with Tween 20, respectively.

Inoculation. Two isolates of *Pyricularia oryzae* Cav.: Naga 69-150 (compatible) and TH 67-22 (incompatible) were spray-inoculated respectively at the stage of full development of uppermost 5th leaves and incubated at 25 C for 17 hr in a moist chamber, and then transferred to green house.

Sampling of leaves. The leaves were harvested at 48 hr after UV-irradiation and chemical treatments. Diseased leaves were collected at 7th day after inoculation.

Extraction. One gram each of the sample leaves was chopped into 200 ml of 70% aqueous methanol with scissors, homogenized with Physcotron homogenizer after keeping in boiling water bath for 3 min and filtered. The filtrate was concentrated *in vacuo* and finally extracted with petroleum ether^{10,11)}. Petroleum ether layer was concentrated and transferred to tiny sharp-pointed silyl-vial. Petroleum ether was eliminated completely by N_2 gas blowing. The extract was re-dissolved carefully into 100 μ l of petroleum ether in tightly sealed vial.

Gas-liquid chromatography. Five μ l of the sample was promptly subjected to GLC (Shimazu GC-7AG) with FID detector. GLC was conducted with OV-17 column (1% loading on Gas Chrom Q, 100-120 mesh, silanized glass column, 150 $\times$ 0.3 cm) under the conditions of 210 C column temperature and 30 ml/min N_2 gas flow rate. Since S-1 and its derivatives appear at Rt 9.9 min as a single peak under this condition⁶⁾, it is convenient to estimate the total amount of the substances. The peak area was automatically integrated with Chromatopac C-R1A (Shimazu Co.). The number in tables represents peak area shown as an electric signal (μ V $\cdot$ sec).

Estimation of UV-damages on various cultivars. At 5th leaf stage the seedlings were UV-irradiated. The irradiation was conducted with germicidal lamp (10 W Toshiba lamp) ca. 15 cm aparted from the top of the seedlings for 1 hr in the incubator at 28 C. Degree of UV damages was graded and designated as 0 (no symptom) to 4

(heavily damaged with brown spots and bronzing).

Results and Discussion

Previously, the authors reported that the degree of blast-resistance clearly increases with the aging of rice leaves and the amount of the anti-blast substances (ABS) (S-1 and its derivatives) formed after blast-infection is higher in aged leaves where resistant brown spots predominantly appeared⁶⁾. These results may indicate that these substances directly play a role as a phytoalexin. However, the possibility that these substances were produced only as a result of the host-cells destruction and may associate secondarily with the resistance⁶⁻⁸⁾ was remaining unsolved. As shown in Table 1, UV-irradiation caused the production of ABS. This result indicates that the production of S-1 and its derivatives is not infection-specific and associates with the destruction of host cells.

In general, it has been well-documented that UV and chemical damages cause a phytoalexin production^{4,11-13,15-17)}. Despite of severe damages, no production was observed in the leaves treated with 1, 1'-dimethyl-4, 4'-bipyridinium-dichloride (paraquat) and CuSO₄. This result may indicate the association of the browning process at the host-cell destruction with the production of the ABS.

Table 1. Effect of physical and chemical injuries on anti-blast substances (ABS) production

Treatment	Symptom	ABS formed ^{a)}
UV irradiation	brown spots	16.9×10 ³
CuSO ₄	gray-green spots	0
paraquat	yellow spots	0
2,2'-bipyridyl	none	0
4,4'-bipyridine	none	0

a) Amount of ABS produced was represented as a peak area on the GLC chromatogram.

Table 2. Production of anti-blast substances (ABS) in blast-infected leaves of rice cultivars of which horizontal resistance are different

Cultivars ^{a)} (<i>Pi-i</i>)	Incompatible		Compatible	
	Symptom	ABS formed ^{b)}	Symptom	ABS formed ^{b)}
Inabawase	b+++	39.5×10 ³	ybg+++	98.0×10 ³
Fujisaka 5	NS.	0	ybg+,bg++	75.8×10 ³
Todorokiawase	NS.	0	bg+,b++	45.2×10 ³

ybg: typical susceptible lesion, bg: intermediate type lesion, b: brown spot, NS.: no symptom. +~###: Severity of the symptoms.

a) Inabawase (weak), Fujisaka 5 (medium) and Todorokiawase (strong in horizontal resistance).

b) Fifth (uppermost) leaves were analyzed.

Table 3. Correlation between UV damage and ABS production in rice cultivars of which horizontal resistance are different

Cultivars ^{a)} (<i>Pi-i</i>)	Degree of damages			Amount of ABS formed in 4th leaves
	5th ^{b)}	4th	3rd	
Inabawase	0	0.94	1.77	4.4×10^3
Fujisaka 5	0.3	1.42	1.57	9.1×10^3
Todorokiwase	0.5	2.05	2.48	93.9×10^3

After full development of 4th leaves, 376mg of ammonium sulfate was additionally amended.

UV irradiation was conducted with the germicidal lamp (10W). Degree of UV damages was graded and designated as 0 (no symptoms) to 4 (heavily damaged with brown spots).

a) See the note of Table 2.

b) Uppermost leaves.

Close correlation between the production of ABS and cell destruction with browning was also observed in leaves of three cultivars infected with incompatible isolate (TH 67-22) (Table 2). ABS were only detected in cv. Inabawase (weak) where brown spots appeared. While, in symptomless resistant leaves of cv. Fujisaka 5 (medium) and Todorokiwase (strong) the substances were not detected. This negative correlation between the amount of the substances formed and the degree of horizontal resistance was also observed in case of the inoculation with compatible isolate (Naga 69-150). These results indicate that the ABS may not associate with the resistance primarily, but secondarily. However, each datum in Table 2 is the result at 7th day after inoculation and a total amount in whole infected area which is larger in weak cultivar. Therefore, the time course of the production in each cultivar and the amount of ABS at unit infected area should be estimated before drawing the final conclusion on a role of the anti-blast substances.

The degree of UV-damages was different with aging of leaves. Young leaves (uppermost leaves) were tolerant to UV irradiation and aged leaves (lower leaves) were heavily damaged showing browning and bronzing⁸⁾. The amount of ABS formed related clearly with the degree of UV-damages. These phenomena will be useful for the marker of aging of leaves. Interesting outcome that the degree of UV-damages is different depending upon cultivars was obviously observed. So far as the tested cultivars concerned, the damages were less in horizontally weak cultivar (Table 3). These results indicate two possibilities. The first one is that it may merely be the result based on the difference in the sensitivity to UV irradiation of cultivars. The second is that the leaves of horizontally weak cultivar remain in physiologically young situation (more susceptible situation) for a while after the development. Further studies with various cultivars will be required to clarify which postulation is probable.

The authors are grateful to Dr. M. Iwano in Hokuriku Natl. Agric. Exp. Stn. for supplying of isolates and Drs. S. Komori, K. Matsuyama and R. Isobe of Fac. Pharmaceutical Sci., Kyushu University for their many advices and kind help.

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和 文 摘 要

松山宣明・脇本 哲：いもち病感染および UV 照射イネ葉における抗菌物質生成

抗いもち病菌物質 S-1 ($C_{20}H_{32}O_2$) とその関連化合物の感染時および物理・化学的障害時における生成程度を主働抵抗性遺伝子 *Pi-i* をもち、圃場抵抗性程度を異にする 3 品種についてガスクロマトグラフィーで比較した。抗菌物質の生成は、UV 照射およびいもち病菌接種葉において認められたが、その生成程度は組織の褐変崩壊程度と密接に相関していた。一方、化学的障害時あるいは褐点を全く生じないような高度抵抗性の場合には全く生成が見られなかった。抗菌物質生成程度と圃場抵抗性程度とは接種 7 日後では逆の相関がみられたが、これらの物質と抵抗性との関連については、経時的観察などさらに詳細な検討が必要と考えられる。UV 照射による障害の程度は若い葉組織（上位葉）で軽く、加齢の進んだ組織（下位葉）で著しかった。また、圃場抵抗性弱品種イナバワセで軽度であった。このことは、圃場抵抗性弱品種では最上葉展開後暫くの期間、生理的により若い状態すなわちより罹病的状態にあることを示しているとも考えられるが、この点についてはさらに検討する予定である。