

DL-alanine dodecylester

HClによりイネに誘導されたいもち病抵抗性の世代継続

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	57巻4号
巻号補足	
掲載ページ	p. 522-525
発行年月	1991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



Generational Succession of DL-Alanine Dodecylester HCl-Induced Resistance to Blast Disease in Rice Plants

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Abstract

The outbreak of leaf and panicle blast caused by *Pyricularia oryzae* decreased to 25% (in greenhouse) and 50% (in paddy field) on rice plants by soaking seeds in DL-alanine dodecylester hydrochloride solution (500 ppm). The second and the third generation of the rice plant retained this chemically induced resistance to the disease. The blast severity reduced to 39% in greenhouse test and 48% in field test (2nd generation), and 28% in greenhouse test (3rd generation). When the chemical treatment was repeated on alternate generations of the rice seeds, the induced protection effect of DL-alanine dodecylester hydrochloride reached at high levels of 75% (original), 61% (2nd generation), 87% (3rd generation) and 97% (4th generation) of blast-preventive values. On the rice plant whose blast resistance was chemically induced, conidia of *P. oryzae* germinated and formed appressoria, and then its hyphae penetrated into the host tissues. However, the invading hyphae were restrained in epidermal cells and adjoining cells without subsequent penetration.

(Received October 22, 1990)

Key words: DL-alanine dodecylester hydrochloride, chemically-induced resistance, *Pyricularia oryzae*.

INTRODUCTION

It is known that resistance of plants to diseases is induced by double inoculation of viral pathogens⁷⁾. Initial inoculation with avirulent strains of *Phytophthora infestans* prevented potato from tuber rot disease caused by subsequent inoculation with sporangia of virulent strains of the pathogen⁸⁾. Susceptible cucumbers to *Colletotrichum lagenarium* race 1 was systemically protected from the disease by prior inoculation with the pathogen⁶⁾. Ishiba *et al.* demonstrated that cucumber was protected from *C. lagenarium* by root soaking in a suspension of extract of *Fusarium oxysporum*⁵⁾, indicating that the pathogen does not participate in the resistance induction to plants, and that the disease resistance can be induced by chemicals.

We found that the blast lesions caused by *Pyricularia oryzae* were less numbers on the seedlings from seeds soaked in amino acid derivative solutions¹⁾. The best protection was obtained using DL-alanine dodecylester hydrochloride.

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MATERIALS AND METHODS

Preparation of seedlings. Rice seeds (cultivar: Jukkoku, Nihonbare and Koshihikari) were soaked in 500 ppm DL-alanine dodecylester hydrochloride (1-dodecyloxycarbonyl ethylammonium chloride) solution (treatment: T) or in distilled water (check: C) and incubated at 30°C for 3 days¹⁾. After rinsing with water, both the chemically treated and water-soaked seeds were sown and cultivated in a greenhouse or paddy field until harvest to obtain the second generation seeds. The second generation seeds harvested from the T-seedlings were soaked again either in 500 ppm DL-alanine dodecylester hydrochloride (T-T) or distilled water (T-C) to prepare T-T-seedlings and T-C-seedlings.

These procedures were repeated for preparing the 3rd (T-T-T, T-T-C, T-C-T and T-C-C) and 4th (T-C-T-T, T-C-T-C, T-C-C-T and T-C-C-C) generation seedlings as shown in Fig. 1.

Greenhouse test. At the 4.5-leaf stage, these seedlings (50 seedlings per treatment) were inoculated with spore suspension (4×10^4 spore/ml) of rice blast fungus, *Pyricularia oryzae* (isolate P2) by the method of Homma *et al.*⁴⁾, and incubated in a moist chamber (Temp. 25°C, R.H. 100%) for 4 days. The number of lesions on the fifth leaf of the T-seedlings grown from chemically treated seeds was compared with that of C-seedlings from water-soaked seeds³⁾.

At the 7-leaf stage, the leaf sheaths of both T- and C-seedlings grown in a greenhouse were stripped, and then a spore suspension of *P. oryzae* was inoculated on the inner surface of them. The inoculated leaf sheaths were incubated in a moist chamber. On 100 spores for each treatment, the spore germination, appressorial formation, appressorial penetration and elongation of hyphae invading into the tissue of the T- and C-seedlings were observed through a light microscope 2 and 4 days after inoculation.

Field test. Both the chemically treated (T, T-C) and water-soaked (C, C-C) seedlings were planted in a paddy field, and then cultivated without application of any chemicals to control the rice blast. At harvest time, about 200 panicles in each treatment were used for observation, and the damage by the panicle blast on the plants (T- and T-C-plant) grown from chemically treated seeds was compared with that on the plants (C- and C-C-plant) grown from water-soaked seeds. These experiments were repeated 3 times on three cultivars, Jukkoku, Nihonbare and Koshihikari.

RESULTS AND DISCUSSION

As shown in Fig. 1, on T-seedlings grown in the greenhouse, the number of rice blast lesions was less than that on the C-seedlings with a preventive value of 75%. Several experiments were conducted for the confirmation of this phenomenon, and preventive values of 69, 77, 92, 98 and 98% were obtained. This chemically induced resistance in the T-seedlings was effective without decrease in the 2nd (T-C) and the 3rd (T-C-C) generations of plants, and preventive values were 61% (T-C) and 72% (T-C-C). On the other hand, the fourth generation seedling (T-C-C-C) from seeds whose original-generation seed was treated with the chemical did not show the protective effect against rice blast.

When rice seeds in each generation were treated with the chemical, the preventive value of 75% on the original generation seedlings (T) became 35% in the 2nd (T-T) and 28% in the 3rd (T-T-T) generation.

When rice seeds in alternate generations were treated with the chemical solution, the protective effect remained, without decrease, in all generations from the original to the 4th. These seedlings showed 75 (T), 61 (T-C), 87 (T-C-T), and 97% (T-C-T-C) reduction in disease incidence respectively.

As shown in Table 1, the damage by panicle blast on T-plants in the paddy field was less

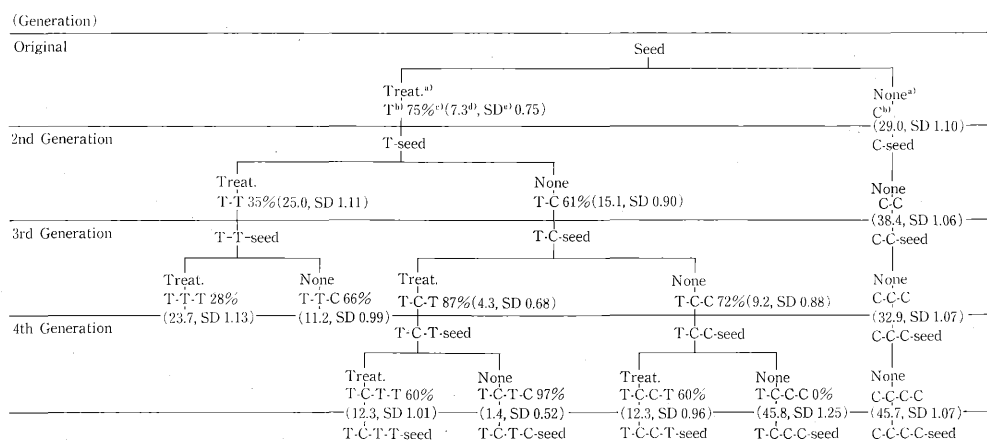


Fig. 1. Effect of seed soaking in DL-alanine dodecylester hydrochloride solution on blast disease of rice seedling (cv. Jukkoku) in greenhouse. At the 4.5-leaf stage, those seedlings (50 seedlings per treatment) were inoculated with spore suspension (4×10^4 spore/ml) of *Pyricularia oryzae* (isolate P2), and incubated in a moist chamber (Temp. 25°C, R.H. 100%) for 4 days. a) rice seeds soaked in 500 ppm of the chemical (Treat.) or in distilled water (None); b) seedlings from the seeds soaked in the chemical (T) or water (C); c) preventive value; d) number of rice blast lesions/leaf; e) standard error.

Table 1. Effect of seed soaking in DL-alanine dodecylester hydrochloride solution on panicle blast of rice in fields^{a)}

Generation	Preventive value (%) ^{b)} on cultivar:		
	Jukkoku	Nihonbare	Koshihikari
Original (T)	31*	57**	67
Second (T-C)	67*	57**	43
Check (C-C)	0	0	0

a) Seedlings from the seeds soaked in the chemical (T, T-C) or water (C-C) were planted in a paddy field, and then cultivated without application of any chemicals to control the rice blast. At harvest time, about 200 panicles in each treatment were used for observation.

b) *, the statistical difference at 10% level. **, the statistical difference at 5% level.

Table 2. Effect of seed soaking in DL-alanine dodecylester hydrochloride solution on the growth stage of *Pyricularia oryzae* by rice sheath method^{a)}

Growth stage	Inhibition (%)
Conidial germination	0.0
Appressorial formation	0.7
Appressorial penetration	8.7
Hypchal elongation in leaf sheath tissue	98.2

a) The inner surface of leaf sheaths of seedlings (7-leaf stage) from the seeds soaked in the chemical or water was inoculated with the spore suspension of *P. oryzae*. After incubation in the moist chamber at 25°C for 2 or 4 days, about 100 spores for each treatment were observed.

than that on C-plants with preventive values of 31, 57 and 67%. The chemically induced resistance of the T-plants was persistent without decrease in the second (T-C) generation of the plant in the paddy field. The T-C-plants showed 57, 67 and 43% reduction in disease incidence.

As shown in Table 2, while spore germination and appressorial formation were not inhibited

on the T-seedlings, the invaded hyphae were restricted in the first penetrated epidermal cells or adjoining cells. No further growth of the invaded hyphae was observed, and small brown spots were formed on sheaths of the T-seedlings for 4 days after inoculation. On the other hand, on the C-seedlings from water-soaked seeds, there was no inhibition at any stages of the fungal growth.

The present experimental results indicate an interesting fact that the chemical induces resistance of rice plant against rice blast caused by *P. oryzae*, resulting in about 70% disease control, and the chemically induced resistance is persistent without decrease, until the third generation, although the mechanism of the resistance persistence is not clear. While the rice blast did not reduced on the first, second and third leaves of the seedlings from seeds soaked in the chemical solution, the disease severity reduced on the 4th leaf or later leaves of the same seedlings¹⁾. Those three leaves, 1st, 2nd and 3rd, had already differentiated in the rice seeds²⁾, and proved that the chemical was unable to induce the resistance in the leaves which had been differentiated before the treatment. On the other hand, the resistance was induced not only in the 4th but also in flag leaves that differentiated after the treatment.

This phenomenon would suggest that the chemical induced the "resistance" in growing point cells of the rice plant, and the "resistance" was able to be persistent in the cells. Therefore, it is thought that the seeds which differentiated from the "resistant" cells also persist in the "resistance." However, more detailed studies will be needed to clarify the mechanisms of the resistance persistence.

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和 文 摘 要

有本 裕・本間保男・吉野嶺一・斎藤司朗：DL-alanine dodecylester HCl によりイネに誘導されたいもち病抵抗性の世代継続

イネ種籾を DL-alanine dodecylester HCl 溶液 (500 ppm) に浸漬し栽培したイネでは、葉いもち病だけでなく穂いもちの発生がそれぞれ約 75% (温室) および 50% (圃場) も少なかった。これはいもち病に対する抵抗性が誘導されたものと考えられた。化学的に誘導された抵抗性は世代を越えて継続され、2 代目では 61% (温室) および 52% (圃場)、3 代目では 72% (温室) いもち病の発生が抑制された。種籾浸漬を 1 年おきに行うと、いもち病の抑制効果は高く保たれた。抵抗性が誘導されたイネにおける *Pyricularia oryzae* の孢子発芽および付着器形成、侵入は無処理イネでのそれらと同じであったが、侵入菌糸は最初に到達した表皮細胞内およびそれに隣接した細胞内に封じ込められた。