

イチゴ黒斑病菌 (*Alternaria alternata* strawberry pathotype)の生成する宿主特異的毒素(AF - 毒素)に関する研究 (3)

イチゴ品種盛岡16号における黒斑病感受性の遺伝解析へのAF - 毒素の利用

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Studies on Host-Specific AF-toxins Produced by *Alternaria alternata* Strawberry Pathotype Causing Alternaria Black Spot of Strawberry (3) Use of Toxin for Determining Inheritance of Disease Reaction in Strawberry Cultivar Morioka-16*

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Abstract

The inheritance was determined for the reaction of strawberry to *Alternaria alternata* strawberry pathotype and its host-specific toxin (AF-toxin). Susceptible cv. Morioka-16 and resistant cv. Hōkowase were used for the crosses. Susceptibility to the pathogen and sensitivity to the toxin were both inherited as a single locus with two alleles expressing incomplete dominance when heterozygous. Susceptible cv. Morioka-16 was found to be susceptible heterozygote and resistant cv. Hōkowase was found to be resistant homozygote. Furthermore, susceptible homozygote selected from F₁ progenies was more susceptible to the pathogen and also more sensitive to AF-toxin than cv. Morioka-16, susceptible heterozygote.

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Key Words : *Alternaria alternata* strawberry pathotype, AF-toxin, inheritance.

Introduction

Alternaria black spot disease of strawberry was first reported in 1977⁵⁾. The causal pathogen was identified as *Alternaria alternata* (Fries) Keissler, but differed in pathogenicity and host-specific toxin production from typical strains of *A. alternata* reported so far; it could be therefore characterized as a new pathotype of *A. alternata* (*A. alternata* strawberry pathotype)²⁾.

Newly bred and introduced cultivar, "Morioka-16", which was mainly cultivated in Tohoku district of Japan, was observed to be highly susceptible to the disease, and the rest cultivars were resistant^{2,9)}. However, genetic control of the host reaction to the pathogen remained to be elucidated.

In an attempt to understand the susceptibility mechanism of cv. Morioka-16 strawberry, it seems to be important to ascertain inheritance of the host reaction. Since the causal *Alternaria* produces a host-specific toxin (AF-toxin) and the toxin is a potent candidate for a pathogenicity factor³⁾, it is assumed that the toxin can be readily used

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as a surrogate of the pathogen for identifying host reaction.

In the present study, therefore, the inheritance of the disease reaction of strawberry was determined with responses to AF-toxin as well as with symptoms to the pathogen inoculation. The results presented in this paper have also been provided as an example of practical use of host-specific toxin in agriculture^{4,6)}.

Brief reports of portion of this study have been published previously^{7,8)}.

Materials and Methods

Highly pathogenic isolate O-187 of *A. alternata* strawberry pathotype was used for spore inoculation and AF-toxin preparation. Cultures for toxin production were grown in potato sucrose broth for 14 days at 26 C, and partially purified toxins were prepared by solvent extractions, followed by silicic acid column, Sephadex LH-20 column and thin layer chromatographies, as previously described²⁾. Among three AF-toxins isolated, AF-toxin I was used through all experiments.

For seedling bioassay of toxin sensitivity, seeds of F_1 progenies were treated with 0.1% methylthiophanate for 12 hr at 26 C. The treated seeds were then placed on moist filter papers in Petri plates. Seeds were then allowed to germinate for 5 to 7 days and uniformly germinated seeds with radicles less than 2 mm long were selected for the bioassay. Twenty seedlings were placed in each plate and treated with AF-toxin I solution (0.2 $\mu\text{g/ml}$). After 24 hr incubation at 26 C, viability of the seedlings were observed. Further, inhibition of root growth of seedlings was examined by the treatment with toxin concentrations less than 0.2 $\mu\text{g/ml}$ for 6 days in the light. Inhibition of rate on the root growth was calculated against control treated with distilled water.

To determine disease reaction of F_1 progenies, the seedlings were allowed to grow for 3 months in a greenhouse. Two leaves were detached from each plant. One was inoculated with spore suspension (5×10^5 spores/ml) of the pathogen by an atomizer. The other leaf was used for leaf-necrosis bioassay with two different toxin concentrations (0.1 and 1.0 $\mu\text{g/ml}$). Then the inoculated and toxin-treated leaves were incubated in a moist chamber for 24 hr at 26 C to observe the host reactions. The leaf-necrosis bioassay was done as previously reported²⁾.

To compare the host reactions to the pathogen and AF-toxin I among susceptible homozygote, susceptible heterozygote and resistant homozygote in strawberry plants, four characters of the host reaction were observed; diameter of lesion area and number of lesions per cm^2 leaf by spore inoculation, and toxin-induced black necrosis and potassium leakage from the leaf tissues by the treatment with AF-toxin I.

Results and Discussion

Inheritance of the host reaction to the pathogen and to AF-toxin I

There were two types of host reaction when the seedlings of susceptible strawberry were exposed to toxin solution (0.2 $\mu\text{g/ml}$) for 24 hr. One was water-soaking necrosis of seedlings (sensitive) and the other was only inhibition of root growth without any

visible damage (resistant).

Of the F_1 seedlings from the crosses, percentages of toxin-sensitive seedlings were as follows; 72.0% of susceptible Morioka-16 $\times$ susceptible Morioka-16, 44.5% and 52.1 % of susceptible Morioka-16 $\times$ resistant Hōkowase and reverse cross, respectively, and 0.0% of resistant Hōkowase $\times$ resistant Hōkowase. Data from reciprocal crosses were combined since statistically both segregation suggested a 1:1 ratio (Table 1). These data suggested that, among the parental cultivars used, one locus controlled the response to AF-toxin I, and sensitivity to the toxin could be expressed as dominance. Susceptible cv. Morioka-16 was heterozygously susceptible and resistant cv. Hōkowase was homozygously resistant.

On the other hand, three types of host reaction were observed when the detached leaves of 3-month-old strawberry plants were inoculated with the pathogen and treated with the toxin. Leaves of highly susceptible strawberry were severely damaged by spore inoculation and the black necrotic symptom was expressed as fused lesion. These leaves also responded clearly to the toxin even at lower concentration of 0.1 $\mu\text{g/ml}$. Another group of susceptible strawberry was allowed to induce characteristic black spot following inoculation with the pathogen and a slight veinal necrosis with the toxin (0.1

Table 1. Disease reaction of F_1 strawberry seedlings to AF-toxin I

Cross ^{a)}	Seedling reaction		
	Sensitive	Resistant	Probability ^{b)}
cv. Morioka-16 $\times$ cv. Morioka-16	143	55	$0.25 < P < 0.50^c)$
cv. Morioka-16 $\times$ cv. Hōkowase	99	106	$0.50 < P < 0.75^d)$
cv. Hōkowase $\times$ cv. Hōkowase	0	52	$P > 0.90^e)$

a) cv. Morioka-16 is susceptible and cv. Hōkowase is resistant.

Data from reciprocal crosses between cv. Morioka-16 $\times$ cv. Hōkowase were combined.

b) χ square test.

c) Probability for 3:1 segregation.

d) Probability for 1:1 segregation.

e) Probability for 0:1 segregation.

Table 2. Disease reaction of F_1 strawberry plants to the pathogen and AF-toxin I

Cross ^{a)}	Host reaction to the pathogen and AF-toxin I			
	Highly susceptible	Susceptible	Resistant	Probability ^{b)}
cv. Morioka-16 $\times$ cv. Morioka-16	17	32	22	$0.25 < P < 0.50^c)$
cv. Morioka-16 $\times$ cv. Hōkowase	0	14	11	$0.75 < P < 0.90^d)$
cv. Hōkowase $\times$ cv. Hōkowase	0	0	16	$P > 0.90^e)$

a) See in Table 1.

b) χ square test.

c) Probability for 1:2:1 segregation.

d) Probability for 0:1:1 segregation.

e) Probability for 0:0:1 segregation.

$\mu\text{g/ml}$), although such leaves were covered with severe necrosis at the higher concentration ($1.0 \mu\text{g/ml}$). The rests were completely resistant to both the pathogen and the toxin (Table 2).

All progenies of resistant $\times$ resistant were resistant. Of 25 plants from reciprocal crosses of susceptible $\times$ resistant, 14 plants were susceptible, and of 71 plants from susceptible $\times$ susceptible, 49 plants were susceptible. Furthermore, these 49 susceptible plants were assorted to 17 highly susceptible and 32 susceptible plants; totally 17 highly susceptible, 32 susceptible and 22 resistant. No evidence was obtained for independent assortment of the phenotypes exhibiting susceptibility to the pathogen and sensitivity to the toxin.

Since F_1 progenies from a cross between the heterozygotes segregated into three discrete classes with a good fitness to a 1:2:1 ratio, the data from the present studies on genetic control support the conclusion that, among the parental cultivars used, susceptibility to the *Alternaria alternata* strawberry pathotype and sensitivity to its host-specific AF-toxin I was controlled by a single locus with two alleles expressing incomplete dominance^{1,4}. Since F_2 seedlings from highly susceptible $\times$ highly susceptible were all highly susceptible, these 17 strawberry plants were used as a susceptible homozygote in the following experiments.

Comparison of host reaction to the pathogen and to AF-toxin I among susceptible homozygote, susceptible heterozygote and resistant homozygote

To compare the host reaction in detail, highly susceptible F_1 plants, susceptible cv. Morioka-16 and resistant cv. Hōkowase were inoculated with the pathogen and treated with the toxin on their detached leaves. Diameter and number of lesions following inoculation were observed after 24 hr and 36 hr incubation, respectively. Relative necrotic area on the leaves in 24 hr and potassium leakage from leaf tissues in 3 hr were assessed to evaluate the toxin sensitivity.

Both diameter of lesion and number of lesions were greater on highly susceptible plants than susceptible cv. Morioka-16 (Fig. 1). Apparent severe symptom on the highly susceptible leaves which was observed with the experiment on the inheritance of the disease reaction was derived from the increased and fused lesions. On the leaves of resistant cv. Hōkowase, no lesions were observed.

Highly susceptible plants were more

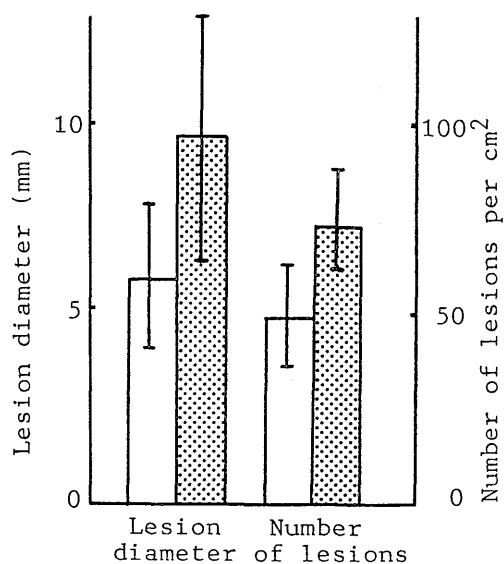


Fig. 1. Comparison of induced lesions following inoculation of the pathogen between highly susceptible and susceptible leaves. Six leaves of each highly susceptible ($\square$) and susceptible (stippled) were inoculated with spores of pathogen. Fifty lesions were totally measured for lesion diameter. Each value represents mean $\pm$ S. E.

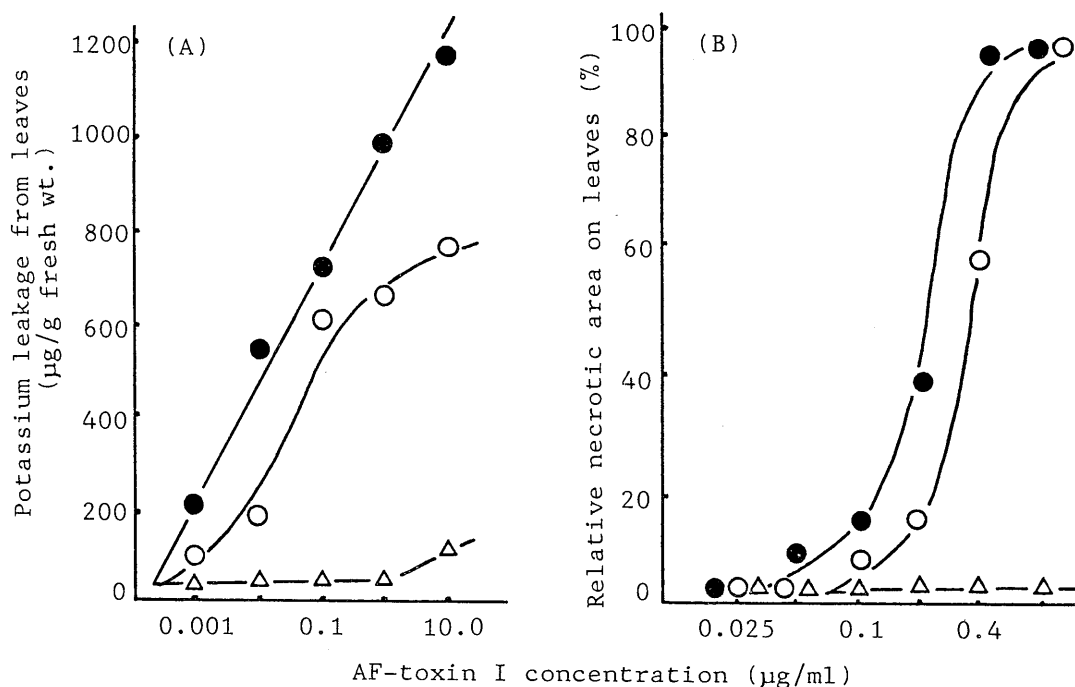


Fig. 2. Comparison of sensitivity to AF-toxin I among three types of strawberry plants. Leaves of highly susceptible (●), susceptible (○) and resistant (△) strawberry plants were treated with AF-toxin I solution. Potassium leakage from leaves (A) and formation of necrotic area (B) were determined, respectively. Each value represents mean of two separate experiments.

sensitive than susceptible cv. Morioka-16 in both bioassays of necrosis formation and potassium leakage (Fig. 2). Such leaves were approximately 20-100% more sensitive in quantity than susceptible leaves. On the resistant leaves, necrosis and potassium leakage by toxin treatment did not occur.

Effect of toxin on root growth was examined with highly susceptible and resistant seedlings. The root growth of highly susceptible strawberry was slightly inhibited (16.7 % decreased to distilled water control) even at the toxin concentration as low as 0.003 µg/ml and completely inhibited at 0.1 µg/ml. In the case of resistant seedlings, root growth was reduced (8.5%) at 0.05 µg/ml and 81.3% of growth was inhibited at 0.1 µg/ml. Although, in this experiment, heterozygous seedlings could not be investigated, F₁ seedlings from the cross between susceptible plants showed the intermediate response between highly susceptible and resistant seedlings.

The above mentioned results suggested the clear correlation between genotypes and phenotypes in susceptibility of strawberry to the pathogen and sensitivity to AF-toxin I.

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和 文 摘 要

山本幹博・並木史郎・西村正暘・甲元啓介：イチゴ黒斑病菌 (*Alternaria alternata* strawberry pathotype) の生成する宿主特異的毒素 (AF-毒素) に関する研究 (3) イチゴ品種盛岡16号における黒斑病感受性の遺伝解析への AF-毒素の利用

イチゴ黒斑病菌 (*A. alternata* strawberry pathotype) はイチゴの栽培品種中では盛岡16号のみを宿主とし、感染時に宿主特異的毒素 (AF-毒素 I, II および III) を生成する。今回は、AF-毒素感受性を指標として、盛岡16号の黒斑病に対する感受性の遺伝子分析を行った。感受性品種として盛岡16号、抵抗性品種として宝交早生を用い、交配によって種子を得た。実生と AF-毒素を用いた感受性検定の結果、感受性と抵抗性は 3 : 1 に分離し、感受性が優性であった。しかし、実生を育成し、幼葉に病原菌および AF-毒素を処理すると、感受性遺伝子をホモに有する個体がヘテロの個体に比べ、病原菌と AF-毒素に高い感受性を示した。感受性遺伝子を持たない劣性個体は、すべての検定において無反応であるか極めて低い感受性しか示さなかった。以上の結果および表現型の分離比から、イチゴ品種盛岡16号における黒斑病感受性は AF-毒素感受性と同一の一对の対立遺伝子に支配される不完全優性として遺伝するものと考えた。なお、今回交配親に用いた、盛岡16号は感受性遺伝子に関してヘテロ、宝交早生は劣性ホモであった。