Characteristics of *Alternaria alternata* Strawberry Pathotype Isolated in New Zealand and the Resistance of the 'Akita Berry' Strawberry to the Fungus

Harumi Takahashi, Hiromitsu Furuya, Takatsugu Takai and Tsutomu Matsumoto

Akita Prefectural College of Agriculture, Ohgata, Akita 010-04

Summary

Cultural characteristics, morphology, and pathogenicities of ICMP-2487, a New Zealand isolate of *Alternaria alternata* strawberry pathotype were compared with 5 Japanese isolates (OH-5, 90-12, MO-1, SN-3, and MA-2). Concurrently, the resistance of a new strawberry cultivar designated as 'Akita Berry', a somaclonal variant to a New Zealand isolate (ICMP-2487) was examined. The following results were obtained.

1. No differences between the New Zealand and Japanese isolates were found in the growth and colors of colonies, shapes, sizes, and septa of conidia.

2. Pathogenicities of ICMP-2487 to 58 strawberry cultivars and strains, 4 pear cultivars, 3 cabbage cultivars, 3 tomato cultivars, 3 Welsh onion cultivars, 2 carrot cultivars, and 2 sunflower cultivars were similar to those of Japanese isolates.

3. No lesions on 'Akita Berry' were formed by inoculation of ICMP-2487 and Japanese isolates. This cultivar is, therefore, resistant to the isolates of *Alternaria alternata* strawberry pathotype occurring in Japan and in New Zealand.

Introduction

'Morioka-16', a strawberry cultivar, is specifically susceptible to *Alternaria* black spot of strawberry caused by *Alternaria alternata* strawberry pathotype. To solve this problem, the authors bred 'Akita Berry', a resistant strawberry cultivar without any loss of the superior horticultural characters of 'Morioka-16', the parental cultivar through tissue culture (Takahashi, 1993; Takahashi *et al.*, 1992, 1993b, 1994). This cultivar showed resistance to all the isolates of the pathogen collected from 8 municipalities in Tohoku and Hokkaido, Japan (Takahashi *et al.*, 1993a).

Comparisons among isolates reported in Japan, New Zealand and Korea showed some differences on host ranges and pathogenicities to strawberry cultivars (Cho and Moon, 1980; Dingley, 1970; Maekawa *et al.*, 1984; Takahashi *et al.*, 1990, 1993a; Watanabe and Umekawa, 1977). These reports suggest variations in pathogenicities of this

fungus and the possibilities that resistant cultivars such as 'Akita Berry' may become susceptible by the invasion of different races of the fungus or by mutation. Therefore, when 'Akita Berry' was released, the authors used it to assay the pathogenicities of isolates collected in Japan and from other countries.

Comparisons on morphological characteristics and pathogenicities between the New Zealand isolate by Dingley (1970) and those of Japanese were made and the resistance of 'Akita Berry' to these isolates was examined.

The authors found no morphological and pathogenic differences between the isolates from both countries and the clear-cut resistance of 'Akita Berry' to the New Zealand isolate.

Materials and Methods

Observation of cultural characteristics and morphology of New Zealand and Japanese isolates

Six isolates, which included a New Zealand isolate (ICMP-2487) provided from Crown Research Centre (Auckland, New Zealand) and 5 Japanese isolates: OH-5 from Ohgata-Mura, Akita Pref., 90-12 from Gonohe-Cho, Aomori Pref., MO-1

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Breeding of strawberry cultivars resistant to *Alternaria* black spot of strawberry. part 7. The outline of this study was presented at the 1992 Spring Meeting of Japan. Soc. Hort. Sci.

from Morioka-Shi, Iwate Pref., SN-3 from Shinjyo-Shi, Yamagata Pref., and MA-2 from Mashike-Cho, Hokkaido were studied. These isolates were cultured on potato dextrose agar medium (PDA) plates containing 20 g·liter⁻¹ of glucose and 15 g·liter⁻¹ of powdered agar at 25 °C in the dark.

During the culture period, colors of the colonies and the presence of the zonation were observed on alternate days. Two right-angled diameters of colonies in 10 plates were measured 2, 4, and 6 days after culturing and their averages calculated. After, 100 conidia were randomly selected from colonies 14 days after culturing, the lengths, widths, and numbers of their longitudinal and transverse septa were measured under a microscope.

Pathogenicity test of the isolates

Pathogenicities of ICMP-2487 and Japanese isolates OH-5, 90-12, MO-1, SN-3, and MA-2 were tested on 58 domestic and foreign strawberry cultivars and strains including 'Akita Berry' and also 'Talisman' and 'Redgauntlet' which are reported to

be susceptible to ICMP-2487.

Besides strawberries, the pear 'Nijisseiki', 'Shinsui', 'Chojuro', and 'Hosui'; the cabbage 'Aurora No. 1', 'Echo', and 'Kitahikari'; the tomato 'Momotaro', 'Homare 114', and 'Ohgata-zuiko'; the Welsh onion 'Ishikura', 'Yoshikura', and 'Kaga-nebuka'; the carrot 'Natsumaki senko-gosun' and 'Natsumaki kinko-gosun', and the sunflower 'Kagayaki' and 'Sun Gold' were tested. These were reported as crop plants affected by strawberry pathotype or other pathotypes of *Alternaria alternata* (Cho and Moon, 1980; Maekawa *et al.*, 1984).

A pathogenicity test was performed by the methods reported previously (Takahashi *et al.*, 1993a) in which a 2 cm × 2 cm square on the undersurface of the leaf blade of each crop plant was inoculated with one drop (ca. 0.05 ml) of a spore suspension (5 × 10⁵ spores·ml⁻¹) from each isolate. After inoculation, leaves were kept in a moist chamber at 26 °C for 72 hr, after which the presence of the disease was judged. The experiments were replicated three times with 5 leaf samples per examination.

Table 1. Comparisons of cultural characteristics^z and morphology of conidia between ICMP-2487^y and Japanese isolates^x.

		Isolate	
		ICMP-2487	Japanese
Cultural characteristics			
Diameter of colony after culture (cm)	2nd day	1.6~1.7	1.5~2.0
	4th day	4.0~4.3	4.2~4.9
	6th day	6.1~6.5	5.8~6.8
Color		Gray~Dark Gray	Gray~Dark Gray
Aerial mycelium		+ ^w	+~#
Zonation		- ^v	-
Measurement of Conidia			
Length (μm)		22.4~44.0(32.2) ^u	20.7~41.2(29.1)
Width (μm)		12.3~16.4(14.4)	10.6~17.4(13.8)
Beak (μm)		4.0~24.4(8.4)	4.0~22.6(7.0)
Numbers of longitudinal septa		1~3	1~3
Numbers of transverse septa		2~5	2~5

^z ICMP-2487 and Japanese isolates were cultured on potato dextrose agar medium (PDA) plates containing 20g·liter⁻¹ of glucose and 15g·liter⁻¹ of powdered agar.

^y New Zealand's isolate.

^x Five isolates including OH-5 from Ohgata-Mura, Akita Pref., 90-12 from Gonohe-Cho, Aomori Pref., MO-1 from Morioka-Shi, Iwate Pref., SN-3 from Shinjyo-Shi, Yamagata Pref. and MA-2 from Mashike-Cho, Hokkaido which showed no differences on pathogenicities among them.

^w +: Poor, #: Rich.

^v Not formed.

^u Values in parentheses are the arithmetic mean.

Results

Comparisons of cultural characteristics and morphology of conidia between ICMP 2487 and the Japanese isolates

Comparisons of cultural characteristics and morphology of conidia in ICMP-2487 with those

of Japanese isolates revealed no significant differences on the growth of colonies among these isolates (Table 1). Only a few aerial mycelia on the surface of colonies in ICMP-2487 and Japanese isolates were recognized and no zonation was found. The color of colonies of all isolates was gray or dark gray.

Many of the conidia were dark yellow, spindly,

Table 2. Disease reactions of various strawberry cultivars and starins to ICMP-2487^z and Japanese isolates^y.

Cultivar and strain	Isolate		Cultivar and strain	Isolate	
	ICMP-2487	Japanese		ICMP-2487	Japanese
1 Akita Berry ^x	— ^w	—	30 Kurume 103	—	—
2 Aiberry	—	—	31 M16-AR 1	—	—
3 Aga	—	—	32 M16-AR 3	—	—
4 Berry Star	—	—	33 America	—	—
5 Belle Rouge	—	—	34 Apollo	—	—
6 Chiyoda	—	—	35 Badgerglow	—	—
7 Harunoka	—	—	36 Cascade	—	—
8 Himiko	—	—	37 Catskill	—	—
9 Hokowase	—	—	38 Cavalier	—	—
			39 Cambridge Favourite		
10 Meiho	—	—		—	—
11 Nyoho	—	—	40 Donner	—	—
12 Ohishishikinari	—	—	41 Earlimore	—	—
No.2			42 Elista	—	—
13 Reiko	—	—	43 Fairfax	—	—
14 Toyonoka	—	—	44 Fresno	—	—
15 The Sun	—	—	45 Gorella	—	—
16 Ever Berry	—	—	46 Holiday	—	—
17 Morioka 16 ^y	+	+	47 Merton Princess	—	—
18 EB-172 ^u	—	—	48 North West	—	—
19 EB-174	—	—	49 Premier	—	—
20 EB-175	—	—	50 Profession	—	—
21 41-4	—	—	51 Redgauntlet ^t	—	—
22 Morioka 17	—	—	52 Redgauntlet ^s	—	—
23 Morioka 19	—	—	53 Regina	—	—
24 Morioka 20	—	—	54 Revada	—	—
25 Morioka 21	—	—	55 Robinson	+	+
26 Morioka 22	—	—	56 Senga Gigana	—	—
27 S-10	—	—	57 Tahoe	—	—
28 S-20	—	—	58 Trumpeter	—	—
29 Saitama 58	—	—	59 Talisman	+	+

^z See Table 1.

^y See Table 1.

^x Alternaria black spot-resistant cultivar bred by authors.

^w —: Resistant, +: Susceptible. Data were taken 72 hr after artificial inoculation with spore suspension (5×10^5 spores·ml⁻¹).

^v Parental cultivar of 'Akita Berry'.

^u No. 18~28 strains were bred in Morioka Branch Natl. Res. Inst. Veg. Ornam. Plants and Tea, Japan. No. 29 strain was bred in Saitama Hort. Exp. Sta, Japan. No. 30 strain was bred in Kurume Branch Natl. Res. Inst. Veg. Ornam., Japan. No. 31 and 32 strains were bred in Akita Pref. Coll. Agri., Japan.

^{t,s} Cultivars reported as Alternaria black spot-susceptible cultivar in New Zealand.

^s Cultivar reserved in Morioka Branch Natl. Res. Inst. Veg. Ornam. Plants and Tea, Japan.

and some, very close to ellipsoid. No significant differences on the sizes of spores were recognized among the isolates, the average value being ca. $30\mu\text{m}$ long and ca. $14\mu\text{m}$ wide. The numbers of longitudinal septa and transverse septa were 1–3 and 2–5, per conidium respectively, of all the isolates.

Pathogenicities of ICMP 2487 and the Japanese isolates to various strawberry cultivars, especially 'Akita Berry' and other crops

When 58 cultivars and strains of strawberry were inoculated with ICMP-2487 and the Japanese isolates, typical lesions of the disease were formed on 'Morioka16', 'Robinson', and 'Talisman'; no infections appeared on the other cultivars (Table 2). Furthermore, the six isolates produced lesions on the Japanese pear 'Nijisseiki'

and 'Shinsui' (Table 3). These results reveal that no difference on pathogenicities exists between ICMP-2487 and the Japanese isolates.

'Akita Berry', bred through tissue culture, was found to be resistant not only to isolates of *Alternaria alternata* strawberry pathotype occurring in Japan, but also to ICMP-2487.

Discussion

Some characteristics of the *Alternaria alternata* strawberry pathotype isolated in Japan were described in detail by Watanabe and Umekawa (1978), Maekawa, *et al.* (1984), Takahashi (1993), and Takahashi *et al.* (1993a); no apparent differences among the isolates on morphology and pathogenicities of this fungus were recognized. Therefore, the differentiation of pathogenicity of the fungus is contradictory in Japan. But some differences between reports from New Zealand and Japan on the pathogenicities of the fungal isolates were found. That is, 'Redgauntlet' and 'Talisman' were reported as susceptible to ICMP-2487 by Dingley (1970), whereas 'Redgauntlet' was reported as being resistant to Japanese isolates (Watanabe and Umekawa, 1977; Takahashi *et al.*, 1990). 'Redgauntlet', reserved at our college and at the Morioka Branch, National Research Institute of Vegetable Ornamental Plants and Tea, was resistant to ICMP-2487 and the five Japanese isolates. We can not explain the difference between our results and those of Dingley (1970). 'Talisman', susceptible to ICMP-2487, was also susceptible to the Japanese isolates. Those results revealed that ICMP-2487 showed the same pathogenicity as the Japanese isolates. In addition, the resistance of 'Akita Berry' to ICMP-2487 was proven by this study.

Diseases caused by *Alternaria alternata* are known on 7 crops, such as the *Alternaria* black spot of strawberry, the *Alternaria* black spot of Japanese pears, the *Alternaria* leaf blotch of apples (Otani and Kohmoto, 1990; Tsuge and Nishimura, 1986). However, the differentiation of pathogenicity as races has not been reported on any diseases of *Alternaria alternata* including that in strawberry (Narita *et al.*, 1996; Saito *et al.*, 1983; Tsuge and Nishimura, 1986). The new strawberry 'Akita Berry' has not been affected by the disease under field conditions for seven years in Japan and, therefore, it is presumed that *Alter-*

Table 3. Disease reactions of various crops to ICMP-2487^z and Japanese isolates^y.

Cultivar	Isolate	
	ICMP-2487	Japanese
Japanese Pear cv.		
Nijisseiki	+	+
Shinsui	+	+
Chojuro	—	—
Hosui	—	—
Cabbage cv.		
Aurora No.1	—	—
Echo	—	—
Kitahikari	—	—
Tomato cv.		
Momotaro	—	—
Homare 114	—	—
Ohgata-zuiko	—	—
Welsh onion cv.		
Ishikura	—	—
Yoshikura	—	—
Kaga-nebuka	—	—
Carrot cv.		
Natsumaki	—	—
senko-gosun	—	—
Natsumaki	—	—
kinko-gosun	—	—
Sunflower cv.		
Kagayaki	—	—
Sun Gold	—	—

^z See Table 1.

^y See Table 1.

^x See Table 2.

naria black spot-resistant strawberry 'Akita Berry' has the low possibility on the break down of resistance.

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ニュージーランドで分離されたイチゴ黒斑病菌の諸性質とニュージーランド菌株に対するイチゴ‘アキタベリー’の抵抗性

高橋春實・古屋廣光・高井隆次・松本 勤

秋田県立農業短期大学 010-04 南秋田郡大潟村南 2

摘 要

ニュージーランドで分離されたイチゴ黒斑病菌の菌株 ICMP-2487 の培養性質、形態、病原性などの諸性質をわが国の東北および北海道で分離された菌株 (OH-5, 90-12, MO-1, SN-3, MA-2 の 5 菌株) と比較するとともに、本菌株に対するイチゴ‘アキタベリー’の抵抗性について試験した結果、以下のことが明らかとなった。

1. ICMP-2487 はわが国の菌株と比較して、菌その色、菌の培養性質、分生胞子の形、大きさ、隔膜

数などにはほとんど違いが認められなかった。

2. イチゴ (58 品種・系統)、ナシ (3 品種)、キャベツ (3 品種)、トマト (3 品種)、ネギ (3 品種)、ニンジン (2 品種)、ヒマワリ (2 品種) の 7 作物に対する ICMP-2487 の宿主範囲および病原性は、わが国の菌株のそれと同じであった。

3. イチゴ‘アキタベリー’はわが国の菌株だけでなくニュージーランドの菌株 (ICMP-2487) に対しても病斑を形成せず、抵抗性を示した。