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Black Rot of Cabbage Seeds and Its Disinfection under a Hot-Air Treatment

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Abstract

Among the 85 lots of commercial cabbage seeds that were produced by 30 seed companies in Japan in 1988, three lots from three companies were found infested with s pathogen of black rot. The pathogen of black rot was effectively disinfected under a hot-air treatment: i.e. at 75°C for 7 days for artificially infested seeds, and at 70°C for 6 days or 75°C for 2 days for naturally infested ones. No adverse effect on seed germination was observed under the treatment of 70°C for 7 days, while the treatment of 75°C even for 1 day prevented the germination. When the seeds were dried in advance at 40-70°C for 24 hr, the germination rate was not lowered, even if those seeds were subsequently exposed to the temperature of 75°C for 7 days. Pre-drying of seeds at 40°C for 24 hr followed by an air treatment of 75°C for 5-7 days is an effective method to disinfect cabbage seeds infested by black rot without any seed damage.

Discipline: Plant diseases
Additional key words: seedborne disease, *Xanthomonas campestris pv. campestris*

Introduction

Black rot is one of the most influential worldwide diseases of cruciferous crops. *Xanthomonas campestris* pv. *campestris*, the pathogen of black rot of cabbage and other cruciferous crops is seedborne and its infested seeds are a primary source of that disease. Several methods have been proposed to disinfect the pathogen from infested seeds: i.e. hot water soaks[1,2], various kinds of antibiotics[6,7], sodium hypochlorite[6], a slurry treatment of calcium hypochlorite[17], nyolate[5], and hot acidified cupric acetate soaks[15]. Hot water soak under 50°C for 20-30 min. is a well-accepted treatment at present for eradicating the pathogen from seeds. This treatment, however, is often detrimental to seed viability and not always effective in disinfecting the pathogen[6,16]. A seed treatment with chemicals is effective, but it is occasionally phytotoxic[7,15]. In the circumstance, an effective, reliable, and non-phytotoxic seed treatment is still to be developed yet. Some papers reported that a hot-air treatment was effective to some bacterial seedborne diseases[8,19]. This paper reviews the results of the study that was conducted to examine effectiveness of the hot-air treatment in disinfecting the pathogen of black rot of cabbage seeds.

Materials and methods

(1) Cabbage seeds

The cabbage seeds used for detecting a pathogen of black rot were all obtained from commercial companies which provided the seeds produced in Japan in 1988. Two to four cabbage seed lots were sampled in each seed company and a total of 84 seed lots were collected from 30 seed companies.

(2) Detection of the pathogen of black rot

In order to detect a pathogen of black rot a seed

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washing liquid method[14] was employed. One thousand seeds per a seed lot were soaked in 20 ml of a phosphate saline solution containing 0.02% tween 20 and incubated at 30°C for 6 hr. After that, the solution was placed on an NSCAA medium[12,14], which was selective for the pathogen of black rot. After the plates were incubated at 30°C for 2 days, all of the bacterial colonies, and suspected ones, of the pathogen on the NSCAA medium were counted.

(3) Identification of suspected colonies of the pathogen

Suspected colonies of the pathogen, which were yellowish colonies surrounded by a zone of starch hydrolysis on the NSCAA medium, were transferred to the PSA slant medium[20] for testing their pathogenicity and the bacteriological characteristics. The pathogenicity test was undertaken by a clip inoculation method developed by Ohata et al. in cabbage seedlings[11]. Investigations on major bacteriological characteristics were made according to the method proposed by Schaad[13] and Cowan[3].

(4) Hot-air treatment

Two types of seeds, i.e. artificially and naturally infested seeds, were subjected to the tests. The artificially infested seeds were prepared with the following method: cabbage seeds were soaked in bacterial suspension with a concentration of about 10^9 CFU/ml for 60 min. and then dried under incubation in circulating air at 30°C for 12 hr.

Those seeds, in which a pathogen was detected by the seed washing liquid method as mentioned earlier, were used as naturally infested seeds.

Electric ovens with a forced air-circulation system were used for the hot-air treatment. One hundred seeds were treated at 60–80°C for a period of 1–7 days. The detection of a pathogen of black rot from the seeds treated with hot-air was conducted by the same method as that from the commercial seed lots.

(5) Seed testing

Germination rate and germination energy of those seeds treated with hot-air were examined according to the prescription for seed testing provided by National Center for Seeds and Seedlings, MAFF in 1988[10].

Results

1) Pathogen of black rot from commercial cabbage seeds

Most of the commercial cabbage seeds collected were contaminated with bacteria with a population rate of 10^4–10^5 CFU/ml on the NSCAA medium, except in the four seed lots which had been treated commercially with a fungicide for seed disinfection in advance. The results obtained on the suspected colonies and their pathogenicity are shown in Table 1. The suspected colonies were obtained from nine lots of the cabbage seeds of eight companies with a population rate of 10–10^4 CFU/ml. Seven bacterial isolates from three seed lots produced by three companies were pathogenic to cabbage through clip inoculation. Those bacterial isolates showed symptoms of V-shaped chlorotic lesions on cabbage leaves under spray inoculation. Those isolates were also pathogenic to broccoli, Chinese cabbage, turnip, Japanese radish, and cauliflower, while they were

Table 1. Isolation test of the pathogen of black rot from commercial cabbage seed lots in Japan[a]

Seed lot used	Total bacteria (CFU/ml)	Suspected Xanthomonas-like bacteria (CFU/ml)	Pathogenicity[b] test to cabbage
Co.A-1	54×10^3	23×10	5 / 5
Co.B-1	24×10^3	2×10	1 / 5
Co.C-1	700×10^3	36×10^3	1 / 5
Co.D-1	73×10^3	19×10^3	0 / 5
Co.E-1	220×10^3	8×10^2	0 / 5
Co.E-2	94×10^3	4×10^3	0 / 5
Co.F-1	102×10^3	10×10^2	0 / 5
Co.G-1	109×10^3	2×10	0 / 5
Co.H-1	17×10^3	1×10	0 / 5

a): 1,000 seeds produced in 1988 were used in each lot.
b): Pathogenic bacterial isolates / Inoculated bacterial isolates.

Table 2. Bacteriological characteristics of pathogenic bacteria isolated from commercial cabbage seed lots

Bacterial character	Tested bacteria (bacterial isolates used)			
	Co.A(5)	Co.B(1)	Co.C(1)	*X.camp.*pv. *campestris*[a)]
Gram positive	−	−	−	−
Yellowish colony on YDC medium	+	+	+	+
0-F test	0	0	0	0
Fluorescent pigment on KB	−	−	−	−
Motility	+	+	+	+
Growth on D1 agar	−	−	−	
Growth at 35°C	+	+	+	
Aesculin hydrolysis	+	+	+	+
Mucoid growth	+	+	+	
Urease	−	−	−	
Gelatin liquefaction	+	+	+	V[b)]
Casein hydrolysis	+	+	+	
Acid from:				
Arabinose	+	+	+	+
Glucose	+	+	+	+
Mannose	+	+	+	+

a): Cited from Dye (1962)[4)].
b): Reactions differ.

not pathogenic to lettuce, chrysanthemum, tomato, and sweet pepper. Main bacteriological characteristics are presented in Table 2. Seven pathogenic bacterial isolates were identical with each other in regard to their bacteriological characteristics. These isolates almost coincided in their bacteriological characteristics with *Xanthomonas campestris* pv. *campestris*[4)]. From these results, the pathogenic bacterial isolates derived in this study were identified as the pathogen of black rot, i.e. *X. campestris* pv. *campestris*.

2) Effects of hot-air treatment on pathogen survival

(1) Artificially infested seeds

Suitable temperature and duration of hot-air treatment for testing were different among the crops studied. Under the pathogen survival test on the artificially infested cabbage seeds, it was observed that when the tested seeds had a pathogen concentration rate of 66×10^2 CFU/m*l*, no pathogen was detected after the treatment either of 65°C for 4 days or 70–75°C for 2 days. However, when the seeds were infested at a concentration rate of 146×10^3 CFU/m*l*, the pathogen was observed after the treatment either of 70°C for 7 days or 75°C for 5 days (Table 3).

(2) Naturally infested seeds

It is very likely that the location of the pathogenic bacteria in artificially infested seeds is different from that of naturally infested seeds, since the pathogen may systemically invade inner part of the seed tissues in the latter case. The naturally infested seeds were contaminated by a pathogen with a population rate of 19×10^2 CFU/m*l*. The pathogen survived after the treatment of 65–70°C for 5 days, but not after the treatment of 75°C for 7 days (Table 4).

3) Effects of hot-air treatment on germination

One hundred seeds each treated with hot-air at 65, 70, 75 and 80°C were examined regarding their germination abilities. The germination rate and germination energy of the control seeds were 98 and 92%, respectively. When the seeds were subjected to 65 and 70°C for 7 days, the germination rate decreased to the level of 85 and 82%, and germination energy decreased to 72 and 76%, respectively. A drastic decrease took place when the seeds were treated at 75°C. The germination energy was 48, 34 and approximately 10% after the treatment for 1 day, 5 days, and 6–7 days, respectively (Table 5).

Table 3. Effects of hot-air treatment on survival of the pathogen of black rot (artificially infested seeds)[a)]

	Temp. of treatment	Duration of treatment and survived bacteria (CFU/ml)							
		0	1	2	3	4	5	6	7 days
Exp. 1	65°C	53×10^2	1×10	0×10	1×10	0×10	0×10	–	–
	70°C	–	0×10	0×10	0×10	0×10	–	–	–
	75°C	74×10^2	3×10	0×10	0×10	–	–	–	–
	80°C	–	0×10	0×10	0×10	–	–	–	–
Exp. 2	60°C	146×10^3	28×10^3	–	26×10^2	–	12×10^2	–	18×10^2
	65°C	–	28×10^2	–	29×10	–	3×10	–	16×10
	70°C	–	29×10^2	–	2×10	–	2×10	–	22×10
	75°C	–	45×10	–	1×10	–	3×10	–	0×10

a): 100 seeds were used for each temperature treatment.

Table 4. Effects of hot-air treatment on survival of the pathogen of black rot (naturally infested seeds)[a)]

Temp. of treatment	Duration of treatment and survived bacteria (CFU/ml)							
	0	1	2	3	4	5	6	7 days
60°C	19×10^2	3×10^3	–	7×10^2	–	0×10	–	0×10
65°C	–	2×10^3	–	9×10^2	–	2×10	–	0×10
70°C	–	8×10	2×10	1×10	0×10	1×10	0×10	0×10
75°C	–	4×10	0×10	0×10	0×10	0×10	0×10	0×10

a): 100 seeds were used for each temperature treatment.

Table 5. Effects of hot-air treatment on germination of cabbage seeds

Temp. of treatment	Duration of treatment and germination rate (germination energy)[a)]							
	0	1	2	3	4	5	6	7 days
65°C	98(92)	91(86)	93(85)	94(80)	84(73)	85(73)	86(79)	85(72)
70°C	–	91(80)	96(86)	90(82)	89(77)	88(80)	89(72)	82(76)
75°C	–	92(48)	73(37)	81(34)	75(24)	77(34)	46(12)	31(7)
80°C	–	8(0)	1(0)	–	–	–	–	–

a): 100 seeds were used for each temperature treatment.

Table 6. Effects of pre-drying on germination of cabbage seeds

Temp. at pre-drying	Duration of hot-air treatment at 75°C and germination rate (germination energy)[a)]				
	0	1	3	5	7 days
40°C	99(99)	98(98)	97(97)	97(97)	97(92)
50°C	99(96)	96(95)	96(96)	98(98)	94(86)
60°C	100(100)	99(99)	97(94)	97(96)	94(86)
70°C	99(97)	99(97)	99(98)	98(96)	97(92)

a): 100 seeds were used for each temperature treatment.

4) Germination of pre-dried cabbage seeds

It is recognized that pre-dried seeds are not very sensitive to a hot-air treatment with high temperature[9]. A series of tests were undertaken to confirm the effectiveness of pre-drying treatment for cabbage seeds infested with black rot pathogen. The pre-drying treatments included four plots under 40, 50, 60, and 70°C for 24 hr. The pre-dried seeds were subsequently treated with hot-air at 75°C for 1–7 days. The germination rate and germination energy of cabbage seeds that were pre-dried at 40–70°C were not affected, even when those seeds were subsequently treated at 75°C for 7 days for disinfestation (Table 6).

Discussion

Sugiyama[18] reported that in Japan a rate of the commercial cabbage seeds infested with black rot was 4–7%. The present study has indicated that cabbage seeds produced in Japan are infested with that disease with an infestation rate of 3.6% on a lot-basis. However, none of the information on infestation rates on a seed-basis is available yet at present.

The effects of the hot-air treatment on artificially infested seeds varied in accordance with the degree of seed infestation. The pathogen was disinfested almost completely for the artificially infested seeds through the hot-air treatment of 75°C for 7 days. The germination rate of the cabbage seeds treated with hot-ir at 70°C for 7 days was about 80%. The germination energy which was about 70% under the treatment of 70°C for 7 days declined to the level of less than 50% with the hot-air treatment of 75°C even for one day. However, the germination of abbage seeds which were pre-dried at 40–70°C for 24 hr was not adversely affected, even though the seeds were subsequently treated at 75°C for 7 days. Nakamura et al.[9] reported that heat tolerance of seeds varied among the vegetable crops, the water contents of seeds, the kinds of varieties, and the ages of seeds. They also reported that the germination rates of seeds treated with hot-air were different, depending on the duration of storage after the treatment. For the purpose of establishing a suitable method of hot-air treatment for cabbage seeds, dditional data of the effects of the treatment particularly after a long-period storage on germination abilities are required.

References

1) Clayton, E. E. (1924): Control of black rot and black leg of cruciferous crops by seed and seed bed treatments. *Phytopathology,* **14**, 24–25.
2) Clayton, E. E. (1925): Second progress report on seed treatment for black leg (*Phoma lingam*) and black rot (*Pseudomonas campestris*) of cruciferous crops. *Phytopathology,* **15**, 49.
3) Cowan, S. T. (1974): Cowan and Steel's manual for the identification of medicinal bacteria, 2nd. ed. Cambridge University Press, pp. 335.
4) Dye, D. W. (1962): The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. *N. Z. J. Sci.*, **5**, 393–416.
5) Harman, G. E., Norton, J. M. & Stasz, T. E. (1987): Nyolate seed treatment of *Brassica* spp. to eradicate or reduce black rot caused by *Xanthomonas campestris* pv. *campestris. Plant Dis.*, **71**, 27–30.
6) Humaydan, H. S. et al. (1980): Eradication of *Xanthomonas campestris*, the causal agent of black rot, from *brassica* seeds with antibiotics and sodium hypochlorite. *Phytopathology,* **70**, 127–131.
7) Klisiewicz, J. M. & Pound, G. S. (1961): Studies on control of black rot of crucifers by treating seeds with antibiotics. *Phytopathology,* **51**, 495–500.
8) Murata, A. & Numata, I. (1970): Resistance of *Corynebacterium michiganense* and tomato seeds to dry heating. *Proc. Kanto Pl. Prot. Soc.,* **17**, 55–56 [In Japanese].
9) Nakamura, H., Kobayashi, K. & Yamada, H. (1981): Heat resistance of seeds in various vegetable crops. *Bull. Veg. Ornam. Crops Res. Sta. Jpn.*, Ser. A **8**, 33–51 [In Japanese with English summary].
10) National Center for Seeds and Seedlings (1988): The rule for seed testing. 1–52 [In Japanese].
11) Ohata, K., Azegami, K. & Tsuchiya, Y. (1982): Clip inoculation, a brief evaluation method for the black rot resistance of cabbage varieties. *Bull. Nat. Inst. Agr. Sci.*, Ser. C **36**, 89–96 [In Japanese with English summary].
12) Randhawa, P. S. & Schaad, N. W. (1984): Selective isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. *Phytopathology,* **74**, 268–272.
13) Schaad, N. W. (1980): Laboratory guide for identification of plant pathogenic bacteria. The American Phytopathology Society, St. Paul, Minnesota, 1–72.
14) Schaad, N. W. & Donaldson, R. C. (1980): Comparison of two methods for detection of *Xanthomonas campestris* in infested crucifer seeds. *Seed Sci. Technol.*, **8**, 383–391.
15) Schaad, N. W., Gabrielson, R. L. & Mulanax, M. W. (1980): Hot acidified cupric acetate soaks for eradication of *Xanthomonas campestris* from crucifer seeds. *Appl. Environ. Microbiol.*, **39**, 803–807.
16) Schaad, N. W., Sitterly, W. R. & Humaydon, H. (1980): Relationship of seedborne *Xanthomonas*

campestris to black rot of crucifers. *Plant Dis.*, **64**, 91–92.
17) Schultz, T. (1986): Control of *Xanthomonas campestris* pv. *campestris* in crucifer seed with slurry treatments of calcium hypochlorite. *Plant Dis.*, **70**, 1027–1030.
18) Sugiyama, M. (1977): Ecology of black rot of cabbage. *Plant Protection*, **31**, 175–179 [In Japanese].
19) Umekawa, M. & Watanabe, Y. (1978): Dry heat and hot water treatment of cucumber seeds for control of angular leaf spot. *Bull. Veg. Ornam. Crops Res. Sta. Jpn.*, Ser. B **2**, 55–61 [In Japanese with English summary].
20) Wakimoto, S. (1955): Studies on the multiplication of OP_1 phage (*Xanthomonas oryzae* bacteriophage). 1. One-step growth experiment under various conditions. *Sci. Bull. Fac. Agr. Kyushu Univ.*, **15**, 151–160 [In Japanese with English Summary].

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