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FULL PAPER

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Distribution of clubroot disease of a cruciferous weed, *Cardamine flexuosa*, in major isolated islands, Hokkaido and Okinawa in Japan

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Abstract To investigate the distribution of clubroot of a cruciferous weed, *Cardamine flexuosa*, caused by *Plasmodiophora brassicae*, field surveys were conducted in Hokkaido, Aomori, and Okinawa, and major isolated islands in Japan during 1993–2004. The disease was newly recorded in Aomori and nine islands in five different prefectures, including Sado (Niigata), Oki (Shimane), Mishima (Yamaguchi), Tsushima, Iki and Goto (Nagasaki), and Koshiki, Yakushima, and Tanegashima (Kagoshima). The diseased plants were not found in Hokkaido and Okinawa (islands of Okinawa, Kumejima, Ishigaki, Iriomote, and Kohama). However, inoculation tests showed that most *C. flexuosa* collected from Hokkaido and Okinawa included many susceptible plants. The result suggests that resistance of the plants is not the reason that the disease was not found in these areas.

Key words *Cardamine flexuosa* · Clubroot · Cruciferous weed · Distribution · *Plasmodiophora brassicae*

Introduction

Clubroot disease caused by *Plasmodiophora brassicae* Wor. affects not only cruciferous crops but also wild crucifers (Halsted 1894; Gibbs 1932; Colhoun 1958; Karling 1968; Nowicki 1973; Reyes et al. 1974). The disease has been recorded on five species of cruciferous weeds in Japan (Umehara and Tamura 1968; Ikegami 1978; Tanaka et al. 1993). A common cruciferous weed, *Cardamine flexuosa* With., growing in drained paddy fields of Japan, is susceptible to the disease (Tanaka et al. 1993) as well. Clubroot of the plant is most commonly distributed in areas of Japan, including 43 prefectures from Tohoku to Kyushu (Tanaka et al. 1993). For example, the disease of the plant has been confirmed to be distributed in the whole area of Yamaguchi prefecture whereas distribution of clubroot of cruciferous crops is limited in a small number of the locations (Tanaka et al. 1993). Therefore, *C. flexuosa* is considered to be the most suitable plant species to investigate the distribution of the pathogen.

However, occurrence of clubroot on *C. flexuosa* is not still clear in some locations including Hokkaido, Aomori, Okinawa, and many isolated (solitary) islands in Japan. In this work, we conducted a field survey to investigate the distribution of clubbed *C. flexuosa* in these areas during 1993–2004. In addition, inoculation tests were conducted under pot-cultured condition.

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Materials and methods

Field surveys on the distribution of *C. flexuosa* clubroot

Cardamine flexuosa growing in drained paddy fields in Hokkaido, Aomori, and Okinawa, and other major isolated islands in Japan was investigated during 1993–2004 (Fig. 1). One to three fields were selected in each location. In most fields, at least 100 plants were uprooted and examined for their clubroot gall formation, and rates of diseased plants were recorded. The investigation, based on visible symptoms, was carried out mainly from late autumn to spring in each year.

Inoculation tests

For the inoculation tests, the seeds of *C. flexuosa* were collected from 27 different locations including Hokkaido, Okinawa (Okinawa, Kumejima, Ishigaki, and Iriomote Island), Nagasaki (Tsushima Islands), and Yamaguchi (Yamaguchi city) during 2002–2004. The highly susceptible

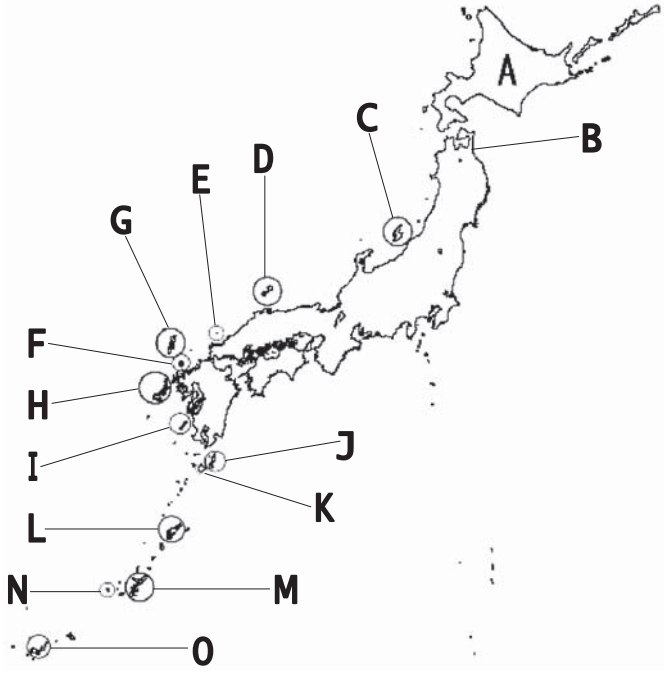

Fig. 1. Surveyed area map on *Cardamine flexuosa* clubroot in Japan (1993 to 2004). Details of these surveyed locations (*A*–*O*) are shown in Figs. 2 and 3

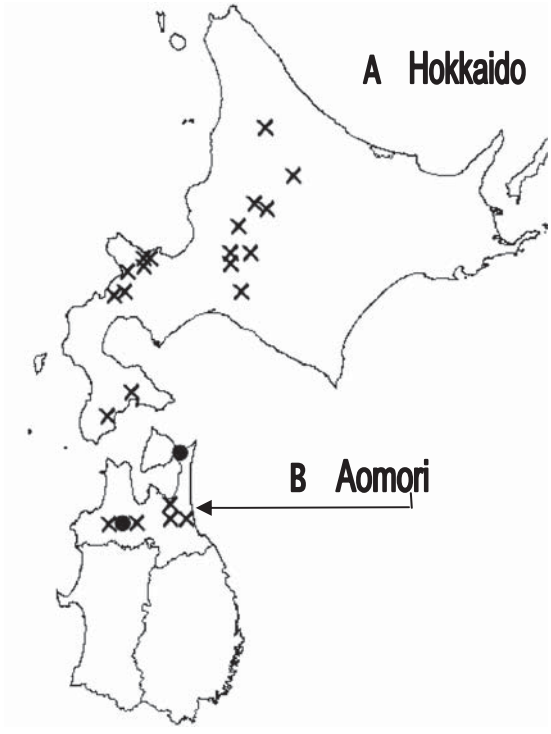

Fig. 2. Enlargement of map of Hokkaido (*A*) and Aomori (*B*). ●, Clubroot was confirmed; ×, clubroot was not confirmed

host seeds were collected from Yamaguchi city and used as control. Resting spores of *P. brassicae* were obtained from clubroot galls on field-grown *C. flexuosa* (in Kamitono, Akiota, Hiroshima) and stored at –40°C. The galls were macerated in a mixer with deionized water and filtered through three layers of cheesecloth. The filtrate was centrifuged at 300 rpm for 5 min (Timely-sp; Tomy Seiko, Tokyo, Japan) to remove plant tissue debris, and the supernatant was centrifuged at 1500 rpm for 15 min. The pellet obtained was resuspended in deionized water and centrifuged at 1500 rpm for 15 min. After an additional centrifugation, the concentration of resting spores in the suspension was determined using a hemocytometer.

Before use, seeds of *C. flexuosa* were disinfected with sodium hypochloride (active chlorine concentration, 0.25%) for 30 min followed by washing with tap water for 1 h and then treated with 100 µg/ml gibberellin for 2 days to break their dormancy. The inoculation tests were conducted in artificial soil [a mixture of one part clay powder, three parts perlite (Ube, Tokyo, Japan), and one part peat moss (Sunshine Canadian Sphagnum Peat Moss; Sun Gro Horticulture, Bellevue, WA, USA) in dry weight ratio] according to the method of Yoshikawa et al. (1981). Clay powder was autoclaved before use. Twenty seeds were sown in a pot (Jiffy pot, 10 × 8 cm; Jiffy AS, Ryomgaard, Denmark) containing the mixed soil infested with *P. brassicae* at 1×10^7 spores/g dry soil. Three to six pot replicates were used for each treatment. Seedlings were grown for 40–50 days after sowing in the greenhouse condition, and thereafter the symptom severity was assessed and the data were converted to disease index (Seaman et al. 1963).

Results

Geographical distribution of clubroot

Clubroot of *C. flexuosa* was confirmed at two locations including Hirosaki and Mutsu cities in Aomori (Fig. 2B). However, occurrence of clubroot on the plant was not confirmed at 16 locations in Hokkaido (Fig. 2A).

Clubroot of *C. flexuosa* was found in all nine major isolated islands from Niigata to Kagoshima, excluding one, Amamioshima Island, in Kagoshima (Fig. 3). Frequencies of the disease in these areas varied from 17.1% (Tsushima Islands, Nagasaki) to 90.9% (Koshiki Island, Kagoshima). The maximal rate of diseased plants was 85% at Iki Island (Nagasaki), 60% at Koshiki Islands (Kagoshima), 47% at Oki Islands (Shimane), and 36% at Goto Islands (Nagasaki) (Table 1). Especially in Morisato, Tsuma, of Oki Islands, diseased plants with extremely severe symptoms (Tanaka et al. 1993) were often found, at a frequency of 49% (Fig. 4A). Such diseased plants were seen in other islands as well, but the frequencies were relatively low. Clubbed plants were found only in the northeastern area of Yakushima Island in Kagoshima (Fig. 3K) but not found in the northern area of Tsushima Island (Fig. 3G). Clubroot of *C. flexuosa* was not found in the subtropical region, including Amamioshima Island (Kagoshima) and five islands of Okinawa (Fig. 3L–O).

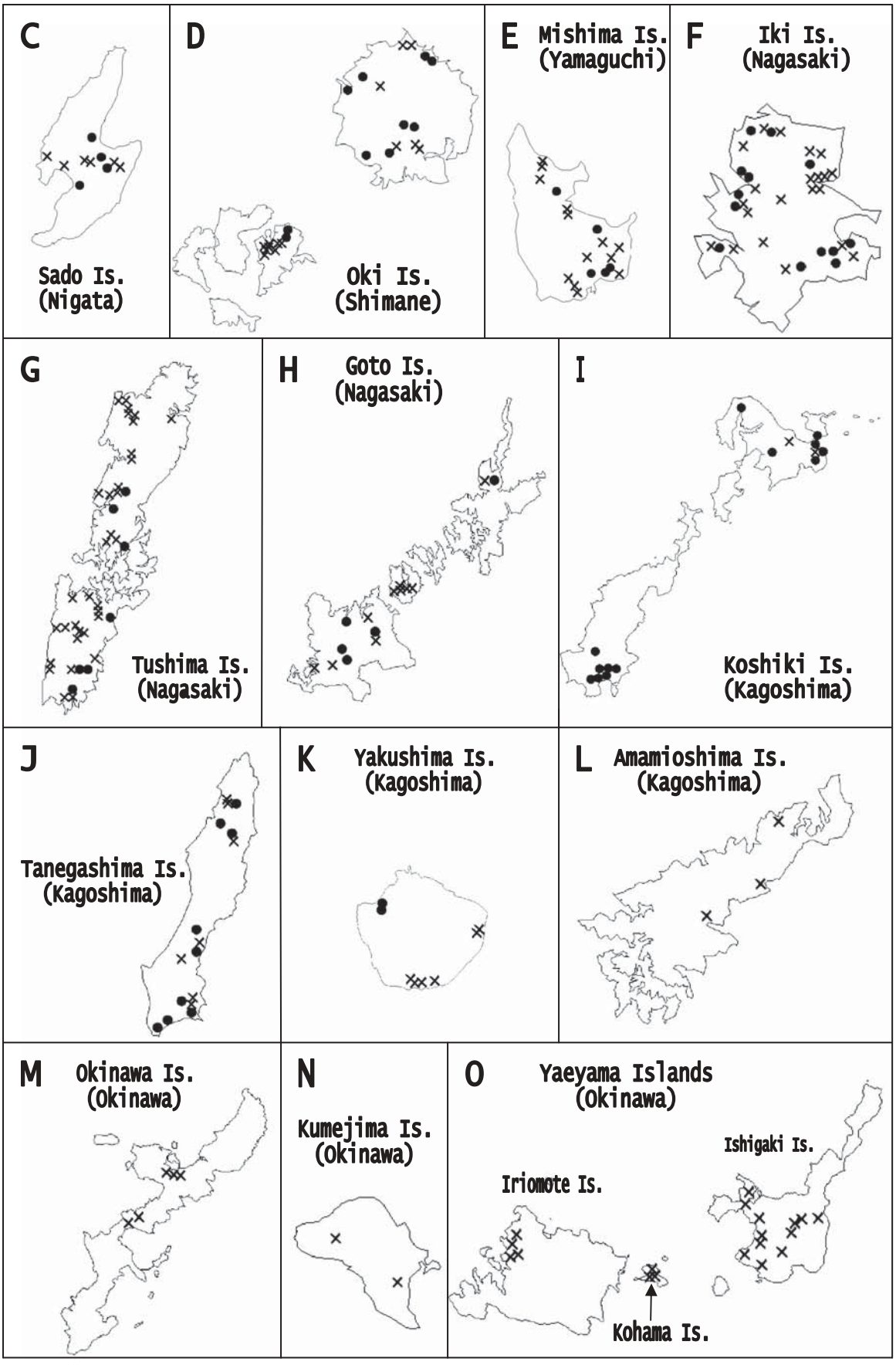

Fig. 3. Enlarged map of major isolated islands of Japan. ●, Clubroot was confirmed; ×, clubroot was not confirmed

Table 1. Frequencies and incidences (%) of diseased host plants growing in Hokkaido, Aomori, and major isolated islands in Japan (1993–2004)

Prefecture	Island	Locations where diseased plants were confirmed (%)[a]	Incidence range of diseased plants (%)[b]
Hokkaido	Hokkaido	0	–[c]
Aomori	Honshu	50.0	1–3
Niigata	Sado	40.0	1–10
Shimane	Oki	43.5	1–47
Yamaguchi	Mishima	27.8	1–14
Nagasaki	Tsushima	17.1	1–10
	Iki	48.1	1–85
	Goto	33.3	1–36
Kagoshima	Koshiki	90.9	2–60
	Tanegashima	60.0	1–31
	Yakushima	25.0	5–16
	Amamioshima	0	–
Okinawa	Okinawa	0	–
	Kumejima	0	–
	Ishigaki	0	–
	Iriomote	0	–
	Kohama	0	–

[a] Number of confirmed locations/number of surveyed locations ×100
[b] Incidence (%) = number of diseased plants/number of surveyed plants ×100
[c] No diseased plant was confirmed in any locations surveyed

Fig. 4. Clubroot of *Cardamine flexuosa*. **A** Diseased field-grown plants with severe symptoms collected from Tsuma, Iki Islands, in Shimane. **B** Diseased pot-cultured plants collected from the northern area of Tsushima Islands in Nagasaki. These plants were inoculated with resting spores of the pathogen under greenhouse condition. *H*, plant without disease (control)

Table 2. Clubroot susceptibility of collected *Cardamine flexuosa* from Hokkaido and Nagasaki

Prefecture	Code	Collected location	Disease index[a]
Hokkaido	H-A	Toyohoro, Ebetsu	24
	H-B	Nakahoro, Iwamizawa	69
	H-C	Kurisawa	97
	H-D	Nanporo	0.1
Nagasaki (Tsushima Islands)	T-1	Okubo, Mine	100
	T-2	Nitanouchi, Kamiagata	100
	T-3	Nitanouchi, Kamiagata	100
	T-4	Naiin, Izuhara	97
	T-5	Kuda, Izuhara	100
	T-6	Kashi, Mitsushima	95
	T-7	Tamazuke, Mitsushima	100
Yamaguchi	H-2	Hirakawa, Yamaguchi	77

[a] Disease index = $(n_0 \times 0 + n_1 \times 10 + n_2 \times 60 + n_3 \times 100)/N_t$, where n_0–n_3 is the number of plants in each disease class and N_t is the total number of plants tested: disease class 0, no clubs; 1, a few small clubs on the lateral roots; 2, a moderate clubbing on lateral and/or taproots; 3, severe clubbing on the taproot

Table 3. Clubroot susceptibility of collected *Cardamine flexuosa* from Okinawa

Prefecture	Code	Collected location	Disease index[a]
Okinawa	O-2-1	Yaga, Kimu	13
	O-2-2	Yaga, Kimu	20
	O-5	Kawakami, Nago	100
	O-6	Gabeso, Nago	72
	K-8B	Kamiezu, Kumejima	30
	K-9A	Magari, Kumejima	54
	Y-2	Nagura, Ishigaki	94
	Y-3	Nagura, Ishigaki	100
	Y-4	Motonagura, Ishigaki	86
	Y-5	Kabira (Ootaka), Ishigaki	89
	Y-6	Kabira, Ishigaki	100
	Y-9	Hiratoku, Ishigaki	45
	Y-10	Omoto, Ishigaki	55
	Y-11	Osato, Ishigaki	100
	Y-14	Iriomote, Taketomi	46
Yamaguchi	H-2	Hirakawa, Yamaguchi	85

[a] Defined in Table 2

Clubroot susceptibility of *C. flexuosa* collections

Four collections of *C. flexuosa* from Hokkaido showed different levels of susceptibility to clubroot. Collections H-C, H-B, and H-A were highly [disease index (DI) = 97], moderately (DI = 69), and weakly (DI = 24) susceptible to the disease, respectively, and collection H-D was resistant (DI = 0.1) to the disease (Table 2).

On the other hand, all seven collections of *C. flexuosa* from Tsushima Islands were highly susceptible (DI = 95–100) to the disease. Of these collections, T-1, T-2, and T-3 plants were completely diseased and could hardly grow (Fig. 4B). Collections T-2 and T-3 were collected from the northern area (Nitanouchi, Kamiagata) where no diseased plants had been found.

Fifteen collections of *C. flexuosa* from Okinawa also showed various levels of susceptibility to clubroot. Of these, collections O-5, Y-2, Y-3, Y-4, Y-5, Y-6, and Y-11 were highly susceptible (DI = 86–100), O-6, K-9A, and Y-10 were moderately susceptible (DI = 54–72), and O-2-1, O-2-2, K-8B, Y-9, and Y-14 were weakly susceptible (DI = 13–46) to the disease. The presence of susceptible plants was confirmed in all collections (Table 3).

Discussion

Occurrence of *C. flexuosa* clubroot was newly recorded in Aomori and many major isolated islands from Niigata to Kagoshima in Japan. These diseased plants were found even in Mishima Island (Hagi, Yamaguchi), which is a small island on the Japan Sea located at a distance of 45 km from the mainland (Honshu). These results indicate that the disease is distributed widely in many isolated islands as well as in the mainland (Honshu, Shikoku, and Kyushu) in Japan (Tanaka et al. 1993).

On the other hand, no diseased plants of *C. flexuosa* were found in Hokkaido and Okinawa. However, most plant collections from these areas were susceptible to clubroot. In addition, the plant collections from the northern area of Tsushima Islands where no clubbed plants were found were

also highly susceptible to the disease. Occurrence of clubroot on crucifers is affected by not only various environmental factors such as soil moisture and soil pH (Ikegami 1978) but also pathogen density or pathogenic races, as described by previous workers (Williams 1966; Tanaka et al. 1991, 1997, 1998; Kuginuki et al. 1999). The entire reason why the clubbed plants were not found in these areas such as Hokkaido and Okinawa remains unknown and requires further study. However, from our observations and previous findings, it became clear that resistant plants of *C. flexuosa* were scarcely distributed in these areas.

Cardamine flexuosa has been considered to be a weed originally from Eurasia and became naturalized in Japan following the introduction of rice cultivation (Takematsu and Ichizen 1993). Clubroot of cruciferous crops has been presumed to be first recognized in the end of the 19th century in Japan (Ikegami 1992). The history of clubroot on *C. flexuosa* is possibly older than that on commercial crops. Therefore, *P. brassicae*, an obligate parasite, may have a long history of coevolution with *C. flexuosa* in Japan. Additional study is needed for the origin of *P. brassicae* and the genetic and epidemiological relationship between the populations from *C. flexuosa* and cruciferous crops.

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