# 根こぶ病抵抗性ハクサイ品種を侵すアブラナ科野菜根こぶ病菌3系統の病原性の特徴


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# Pathogenicity of Three Isolates of Clubroot Fungus Attacking Clubroot-Resistant Cultivars of Chinese Cabbage

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## ABSTRACT

Clubroot-resistant (CR) cultivars of Chinese cabbage, surmised to possess a single CR gene introduced from European fodder turnip, and some crucifers were grown in soils infected with inoculation by five isolates of the clubroot fungus (*Plasmodiophora brassicae*) from Yamaguchi and Oita Prefectures.

Three isolates from Yamaguchi Pref. showed the high pathogenicity to the CR cultivars and another isolate from Yamaguchi Pref. and one from Oita Pref. were non-pathogenic to these cultivars. All the three pathogenic isolates belonged to race 9, one of the unusual races in Japan, by WILLIAMS' method. Relatively mild virulence to a non-CR Chinese cabbage (cv. Nozaki-nigo) and non-virulence to cabbages were also observed commonly in these three isolates. On the other hand, two isolates non-pathogenic to the CR cultivars were judged to belong to dominant races (races 1 and 4) in Japan. These two isolates were apparently virulent to Nozaki-nigo and cabbages. All five isolates examined did not affect a line of European fodder turnip, 77b, which was one of CR-gene sources used for the breeding of the CR cultivars.

These results suggest that "77b" has multiple CR genes of which the CR cultivars of Chinese cabbage have only one, instead of a single CR gene, and that the clubroot incidence in these CR cultivars is due to missing out part of the CR genes during the breeding.

**Key words** : *Plasmodiophora brassicae*, clubroot-resistant cultivars, race, pathogenicity, Chinese cabbage.

## Introduction

The clubroot incidence on commercial "clubroot-resistant (CR)" cultivars of Chinese cabbage, surmised to possess a single CR gene originated from European fodder turnips[8], has recently become a problem in several regions of Japan[9,10].

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Although a portion of isolates of the clubroot fungus (*Plasmodiophora brassicae*)[9] attacks the CR cultivars, the genetic characteristics of these pathogenic isolates has not been established. The reason why the CR cultivars are unavailable to prevent the clubroot fungus from affecting in several limited regions is also unknown.

Clubroot is one of serious diseases in Chinese cabbage which is commercially grown in Yamaguchi Prefecture. In the prefecture, the CR cultivars, however, have not been introduced to farms and their usefulness has not been confirmed.

The present work describes that three isolates of the clubroot fungus from Yamaguchi Prefecture are pathogenic to the CR cultivars, and also reports the results of investigations on their races, pathogenicity to some crucifers and virulence to a non-CR Chinese cabbage, in connection with a reason of the clubroot incidence in the CR cultivars.

## Materials and Methods

### Fungal isolates

Five isolates of the clubroot fungus were obtained from diseased plants grown in naturally infested fields with the fungus: the Yamaguchi, the Fukue, the Shimonoseki and the Hagi isolates from Chinese cabbage at Yamaguchi, Fukue, Shimonoseki and Hagi in Yamaguchi Prefecture, respectively, and the Kuju isolate from cabbage at Kuju in Oita Prefecture.

### Plant materials

Cultivars of seven CR and one non-CR Chinese cabbage, seven cabbages, and two rutabagas, besides one line of European fodder turnip and two lines[3] of *Brassicoraphanus*, an intergeneric hybrid between cabbage and Japanese radish, were used for inoculation tests (Tables 1 and 2). Two cabbage and two rutabaga cultivars are race-differential hosts in WILLIAMS' method[5] and the turnip is one of CR-gene sources used for the breeding of the CR cultivars.

### Inoculation and disease index

The "insertion method" established by YOSHIKAWA *et al.*[7] was used for the inoculation of clubroot fungus. Six seeds each of host plants were sown on the

artificially-infested soil-peat mixture containing $1 \times 10^7$ or a given number of spores/g dry soil and being set in 10 cm-Jiffy pots, and grown in a plastic film house. Forty days after sowing plants were pulled out of pots and their roots were rinsed with running water. Plant roots were assigned to the following disease rating scales as proposed by SEAMAN *et al.*[3] : 0 = no visible clubbing (healthy); 1 = a few restricted swellings on lateral roots; 2 = moderate swellings on lateral and/or tap roots; 3 = severe swellings on lateral and tap roots. Disease index (DI) for a given lot was calculated by multiplying number of plants in scales 1, 2 and 3 by 10, 60 and 100, respectively, and then dividing the sum of products by the total number of plants.

## Results

### Pathogenicity to CR and non-CR Chinese cabbages and other some crucifers

The results of inoculation tests with Yamaguchi, Hagi and Kuju isolates in disease indices for cultivars are shown in Table 1. The Yamaguchi isolate was highly pathogenic to all cultivars of Chinese cabbage including CR cultivars and to Laurentian, and moderately to Wilhelmsburger. It showed weak and limited pathogenicity to cabbages. Both the Hagi and the Kuju isolates were much less pathogenic to all the CR cultivars tested. The Hagi isolate was highly pathogenic to cabbages, rutabagas and Nozaki-nigo, a non-CR Chinese cabbage. The Kuju isolate was highly pathogenic to Nozaki-nigo, Laurentian and cabbages besides Badger Shipper, and moderately pathogenic to Wilhelmsburger. Two lines of *Brassicoraphanus* showed the high resistance to these three isolates.

The results of inoculation tests with Yamaguchi, Fukue, Shimonoseki and Hagi isolates in disease indices for cultivars are shown in Table 2. The Fukue and the Shimonoseki isolates showed the high pathogenicity to all cultivars of Chinese cabbage including the CR cultivars and to Laurentian, and moderately or weak pathogenicity to Wilhelmsburger. These two isolates showed the weak and limited pathogenicity to cabbages. On the other hand, all isolates including the Yamaguchi and the Hagi ones hardly showed the pathogenictiy to 77b, a European fodder turnip.

Table 1. Disease indices of Chinese cabbage, cabbage and rutabaga cultivars and *Brassicoraphanus* lines inoculated with three isolates of *Plasmodiophora brassicae*.

| Plant | Cultivar or line | Isolate[a] | | |
|---|---|---|---|---|
| | | Yamaguchi | Hagi | Kuju |
| Chinese cabbage | Nozaki-nigo | 100 | 100 | 100 |
| | Kukai 65[b] | 100 | 2.9 | 0 |
| | Kukai 70[b] | 100 | 0.6 | 0.7 |
| | Strong CR 75[b] | 100 | 1.4 | 5.3 |
| | CR Kanki[b] | 100 | 12.6 | 5.5 |
| Cabbage | Natsumaki-riso | 7.9 | 100 | 98.0 |
| | Natsumine | 0 | 100 | 100 |
| | Tenko | 0.5 | 100 | 98.0 |
| | Hogyoku | 0.8 | 98.5 | 98.0 |
| | Jersey Queen[c] | 9.8 | 100 | 98.6 |
| | Badger Shipper[c] | 0.8 | 100 | 16.8 |
| Rutabaga | Laurentian[c] | 100 | 100 | 100 |
| | Wilhelmsburger[c] | 57.3 | 100 | 59.4 |
| *Brassico-raphanus* | K-5 | 0 | 0 | 0 |
| | K-6 | 0 | 0 | 0 |

a) Inoculum density: $1 \times 10^7$ spores / g dry soil.
b) "Clubroot-resistant" cultivars (CR cultivars).
c) Race-differential hosts in WILLIAMS' method.

Table 2. Disease indices of Chinese cabbage, cabbage and turnip cultivars inoculated with four isolates of *Plasmodiophora brassicae*.

| Plant | Cultivar or line | Isolate[a] | | | |
|---|---|---|---|---|---|
| | | Yamaguchi | Fukue | Shimonoseki | Hagi |
| Chinese cabbage | Nozaki-nigo | 98.5 | 100 | 100 | 93.5 |
| | Kukai 70[b] | 100 | 73.5 | 98.6 | 0 |
| | Kukai 75[b] | 98.8 | 95.7 | 97.2 | 5.9 |
| | Strong CR 75[b] | 100 | 100 | 100 | 0 |
| | CR Kanki[b] | 84.7 | 67.9 | 97.1 | 0 |
| | CR Ryoyu[b] | 88.0 | 81.9 | 78.1 | 0 |
| | CR Kaname[b] | 93.5 | 100 | 70.8 | 0 |
| Cabbage | Ushio | — | 5.6 | 0 | 95.9 |
| | Jersey Queen[c] | — | 0 | 0 | 100 |
| | Badger Shipper[c] | — | 0 | 0 | 100 |
| Rutabaga | Laurentian[c] | — | 88.2 | 93.0 | 100 |
| | Wilhelmsburger[c] | — | 36.1 | 6.8 | 94.7 |
| Turnip | 77b[d] | 2.6 | 0 | 1.1 | 0 |

a) Inoculum density: $1 \times 10^7$ spores / g dry soil.
b) "Clubroot-resistant" cultivars (CR cultivars).
c) Race-differential hosts in WILLIAMS' method.
d) One of European fodder turnips used as the clubroot-resistant gene source for the breeding of CR cultivars.

## Races of isolates

The five isolates were determined their races by the method defined by WILLIAMS[5] based on the data described above. A differential host on which disease index exceeded 25 was judged to be susceptible to a given isolate (Table 3). The Yamaguchi and the Fukue isolates were identified as race 9 since they were pathogenic to Laurentian and Wilhelmsburger and non-pathogenic to Jersey Queen and Badger Shipper. The Shimonoseki isolate was pathogenic to three cultivars other than Wilhelmsburger. Although the disease index of Wilhelmsburger inoculated with the Shimonoseki isolate in this experiment was under 25, there was a case where it exceeded this value in another experiment (DI=39.2). Then, the Shimonoseki isolate was also judged to belong to race 9. The Hagi isolate was determined to be race 4 due to its pathogenicity to all the differential hosts. The Kuju isolate was done to be race 1 as it was pathogenic to Laurentian, Wilhelmsburger and Jersey Queen, and not to Badger Shipper.

Table 3. Races of five isolates of *Plasmodiophora brassicae* determined by WILLIAMS' method.

| Differential host | Isolate | | | | |
|---|---|---|---|---|---|
| | Yamaguchi | Hagi | Kuju | Fukue | Shimonoseki |
| Jersey Queen | −[a] | + | + | − | − |
| Badger Shipper | − | + | − | − | − |
| Laurentian | +[b] | + | + | + | + |
| Wilhelmsburger | + | + | + | + | + |
| race number | 9 | 4 | 1 | 9 | 9 |

a) − : resistant (disease index < 25).
b) + : susceptible (disease index ≧ 25).

## Virulence to a non-CR Chinese cabbage

It had been expected that any problems would hardly occur on the usefulness of the CR cultivars in Japan if these cultivars were selected using one of the severest virulent isolates of clubroot fungus without regard to their races[8]. The virulence of the five isolates to Nozaki-nigo, a non-CR cultivar of Chinese cabbage, therefore, was compared using a similar technique at three or eight different densities of inoculum (Table 4 and Fig. 1). Both the Hagi and the Kuju isolates severely attacked Nozaki-nigo even at lower inoculum density such as 1

$\times 10^3$ spores / g dry soil. On the other hand, the disease indices by the Yamaguchi isolate were 6.3, 39.3 and 100 at the inoculum densities $1 \times 10^3$, $1 \times 10^5$ and $1 \times 10^7$ spores / g dry soil, respectively. The virulence of the Fukue and the Shimonoseki isolates was similar to that of the Yamaguchi isolate (Fig. 1). The results indicated that the virulence of the Yamaguchi, the Fukue and the

Table 4. Pathogenicity of three isolates of *Plasmodiophora brassicae* to Nozaki-nigo, a non-CR cultivar of Chinese cabbage.

| Isolate | Inoculum density (spores/g dry soil) | Disease index |
|---|---|---|
| Yamaguchi | $1 \times 10^3$ | 6.3 |
| | $1 \times 10^5$ | 39.3 |
| | $1 \times 10^7$ | 100 |
| Hagi | $1 \times 10^3$ | 100 |
| | $1 \times 10^5$ | 100 |
| | $1 \times 10^7$ | 100 |
| Kuju | $1 \times 10^3$ | 97.1 |
| | $1 \times 10^5$ | 100 |
| | $1 \times 10^7$ | 100 |

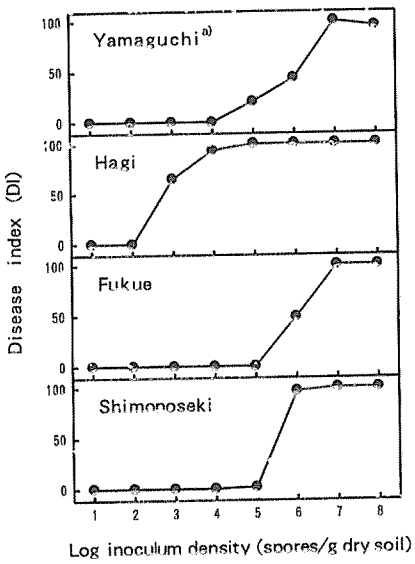


Fig. 1. Relation between spore densities of four isolates of Plasmodiophora brassicae from Yamaguchi Prefecture and disease indices of Chinese cabbage cv. Nozaki-nigo. a) "Yamaguchi", "Hagi", "Fukue" and "Shimonoseki" represent isolates from Yamaguchi, Hagi, Fukue and Shimonoseki in Yamaguchi Prefecture, respectively.

Shimonoseki isolate (race 9) to Nozaki-nigo was clearly lower than that of the Hagi and the Kuju isolates (races 4 and 1, respectively).

## Discussion

The results of the present investigations first indicated the presence of some isolates of clubroot fungus pathogenic to CR cultivars in Yamaguchi Prefecture, and suggested that these CR cultivars might be unable to prevent the clubroot fungus from affecting in several regions of the prefecture.

These results also confirmed that all three isolates pathogenic to CR cultivars were race 9, one of the unusual races in Japan, by WILLIAMS' method[5], and that two isolates non-pathogenic to these cultivars were dominant races (races 1 and 4) in Japan. Mild virulence to non-CR Chinese cabbage (cv. Nozaki-nigo) and non-virulence to cabbages was also observed commonly in these three pathogenic isolates. These data indicated that strongly resistant cultivars to severe virulent races were not always resistant to mild virulent races, against the expectation presented formerly[8]. YOSHIKAWA[10] recently reported that isolates from Awaji Island in Hyogo Prefecture showed relatively mild virulence to some non-CR crucifers contrary to the occurrence of the severest damage to the CR cultivars in this island, and that these isolates were presumed to be unusual races (races 5 and 6). It was suggested that such characteristics of pathogenicity might reflect the genetic backgrounds of several races attacking the CR cultivars in common.

Cultivars of European fodder turnip are known to be highly resistant to dominant races of the clubroot fungus, and they have been presumed to have a single CR gene[8]. These turnips, therefore, were used as CR-gene sources in breeding of the CR cultivars of Chinese cabbage. Although fungal races used for selection of CR cultivars by seed companies in Japan have not been made public, it is presumable that unusual races such as race 9 have been put aside from the objective races during the breeding. If the only restricted races were able to attack CR cultivars, the genetic basis of the clubroot incidence in these cultivars would be readily explained, based on the gene-for-gene theory of resistance.

Dominant races pathogenic to the CR cultivars, however, have been also reported. YOSHIKAWA[10] isolated dominant races (15 isolates) as well as unusual races (5 isolates) from diseased CR cultivars in various fields, and described

that pathogenicity of isolates might be unable to correspond to their races based on the WILLIAMS' method[5]. YOSHIKAWA *et al.*[9] proposed that inhibition of the CR-gene expression by the environmental condition or other complex factors might affect the clubroot occurrence in the CR cultivars. This hypothesis, however, is not always sufficient to explain the presence of the pathogenic and the non-pathogenic isolates to the CR cultivars.

European fodder turnip line 77b, used as one of the CR-gene sources for the breeding of the CR cultivars, was highly resistant to both dominant and unusual races pahtogenic to the CR cultivars[9,10]. The result of the present work also showed that three isolates from Yamaguchi Prefecture, pathogenic to the CR cultivars, were unable to attack 77b. The reaction of the turnip against the unusual races did not coincide with that of the CR cultivars. These results suggest the reason why the CR cultivars are attacked by a portion of isolates. The turnips are considered to have multiple CR genes of which the CR cultivars have only one, as presumed by TANAKA *et al.*[4] and YOSHIKAWA[10], instead of a single CR gene surmised formerly. It is supposed that the clubroot incidence in the CR cultivars may be due to missing out of part of the CR genes during the breeding.

In the present work, the genetically specific relationship was not confirmed between unusual races, or race 9, and the clubroot incidence in the CR cultivars. However, we recently observed that Debra, one of European fodder turnips and known to be highly resistant to dominant races of the clubroot fungus, was attacked severely by the Yamaguchi isolate (race 9) pathogenic to the CR cultivars (unpublished data). This observation implies that unusual races, or race 9, may be genetically different from dominant races in their pathogenicity to the CR cultivars and to some turnips such as Debra which are suggestable to have been used as CR-gene source for the breeding of these cultivars.

*Brassicoraphanus* showed the complete resistance to all fungal isolates examined. MCNAUGHTON[2] and ASHIZAWA *et al.*[1] also reported that *Raphanobrassica* showed the high resistance to clubroot. The $F_1$ hybrids between *Brassicoraphanus* and Chinese cabbage, turnip or cabbage are highly resistant to two isolates of the clubroot fungus[6]. These intergeneric plants between cabbage and Japanese radish may be notable as new CR-gene sources in future.

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# 根こぶ病抵抗性ハクサイ品種を侵すアブラナ科野菜根こぶ病菌3系統の病原性の特徴

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## 要　旨

わが国の根こぶ病菌の主要レースに抵抗性を示す欧州産飼料カブの遺伝子を導入して作出されたハクサイの根こぶ病抵抗性（ＣＲ）品種と他の数種アブラナ科野菜に対する根こぶ病菌（*Plasmodiophora brassicae*）５系統の病原性を，病土挿入法による接種実験によって比較検討した．

ＣＲ品種に対して，山口県と大分県の各１系統はほとんど病原性を示さなかったが，山口県の３系統はいずれも顕著な病原性を示し，山口県にＣＲ品種を侵す根こぶ病菌の存在することが明らかとなった．

ＣＲ品種を侵す山口県の３系統は，ハクサイの野崎２号（非ＣＲ品種）に対する病原力が比較的弱く，キャベツにはほとんど病原性を示さない点でＣＲ品種を侵さない２系統と異なった．また，Williams法によるレース検定の結果，ＣＲ品種を侵さない２系統はわが国では報告例の多いレース４とレース１であったのに対し，ＣＲ品種を侵す３系統はいずれもレース９に属し，わが国では報告例の少ないレースであった．しかし、欧州産飼料カブのひとつである77ｂは山口県の３系統のすべてに抵抗性であったことから，欧州産飼料カブは複数のＣＲ遺伝子を持ち，その内のひとつがＣＲ品種に導入され，他は導入されなかった可能性があることが示唆された．ＣＲ品種の発病は，育種の際の一部ＣＲ遺伝子の欠落に原因の一つがあると推察される．

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