J Gen Plant Pathol (2013) 79:297–306
DOI 10.1007/s10327-013-0456-4

REVIEW FOR THE 100TH ANNIVERSARY

# Pathogenic and genetic diversity in *Plasmodiophora brassicae* (clubroot) from Japan

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Received: 26 December 2012 / Accepted: 12 March 2013 / Published online: 7 June 2013

**Abstract** Clubroot disease, caused by *Plasmodiophora brassicae* Woronin, affects various cruciferous crops. Variations in pathogenicity and virulence are present among field populations of *P. brassicae*. Many races (pathotypes) have been reported in Japan as well as in other countries using various differential systems. Populations can be classified into four pathotypes using two clubroot-resistant (CR) cultivars of Chinese cabbage as differential hosts in Japan. However, it was recently indicated that each population is often heterogenic and composed of multiple genotypes (races or pathotypes). Breakdown in CR cultivars of Chinese cabbage is a problem in some areas of Japan and may contribute to the selective propagation of minor pathogenic genotypes on the CR cultivars. Clubroot has also been recorded on five species of cruciferous weeds in Japan. In particular, clubroot of *Cardamine flexuosa* is widely distributed in Japan. Some populations of *C. flexuosa* are often moderately pathogenic on Chinese cabbage and turnip. Therefore, the epidemiological relationship between clubroot of cruciferous crops and that of the weed has been noted but not thoroughly clarified. The relationship between pathogenic and genetic variations has also been examined among populations from cruciferous crops and weeds in Japan. The result implies an interesting genetic relationship among Williams' races, among pathotypes determined using CR cultivars of Chinese cabbage and among populations from crops and *C. flexuosa*. This review includes an introduction of the status of studies on pathogenic and genetic diversity in *P. brassicae* from Japan.

**Keywords** Epidemiological relationship · Genetic diversity · Pathogenic diversity · *Plasmodiophora brassicae* · Selective propagation · Wild hosts

## Introduction

Clubroot, one of the most destructive diseases of crucifers, is caused by a soil-borne protozoan, *Plasmodiophora brassicae* Wor., and exerts an economically severe impact on the production of cruciferous crops worldwide (Dixon 2009). The disease is spreading rapidly in Asia and has become a problem not only in China, Japan, and Korea but also in tropical countries such as Indonesia, Thailand, and Vietnam (Dixon 2009; Petcharat et al. 1987; Tanaka 1996). In Japan, the disease has been found from Hokkaido (northern Japan) to Kyushu (western Japan), excluding Okinawa (southern Japan). Especially in Kyushu, although clubroot had been considered to be minor (Ikegami 1978), the occurrence has recently been increasing and is becoming a serious problem in many areas where cruciferous vegetables are grown (Shibato et al. 2001; Tanaka 1996; Tanaka et al. 1990, 1997a, 1998).

*Plasmodiophora brassicae* induces the formation of root galls in host plants and produces numerous resting spores within the gall tissues. The resting spores can survive for a long time in the soil and serve as a primary source of inoculum during the next growing season (Karling 1968). Intensive cultivation of crucifers enhances the occurrence of clubroot by increasing the density of resting spores in soil. In the fields, especially where the soil is infested with a high density of resting spores, various control measures,

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including fungicide treatment, frequently lack sufficient efficacy against clubroot (Horiuchi and Hori 1982; Tanaka et al. 1997b). In addition, variations in pathogenicity and virulence (aggressiveness) have been observed among *P. brassicae* populations (Hatakeyama et al. 2004; Kuginuki et al. 1999; Osaki et al. 2008b; Tanaka et al. 1997b, 1998; Yoshikawa 1993). The effectiveness and its levels of clubroot-resistant (CR) cultivars and fungicides often vary among populations. Various races (pathotypes) of the pathogen are present in Japan and other countries as well. Breakdown of resistance in CR cultivars of Chinese cabbage has also become a problem in Japan. Therefore, clubroot is a very troublesome disease that is extremely difficult to control.

Clubroot affects many cruciferous weeds, and an epidemiological relationship has been suggested to exist between the disease on cruciferous crops and weeds (Colhoun 1958; Karling 1968). Susceptible weeds also have been recorded in Japan. In surveys of the distribution of the disease on the weed *Cardamine flexuosa* in Japan, the disease has been found in wide areas (Tanaka et al. 1993, 2006b). Some studies have been conducted on the epidemiological and genetic relationship between *P. brassicae* populations from cruciferous crops and weeds (Murai et al. 1986; Osaki 2008; Osaki et al. 2008b; Tanaka et al. 1994).

This review focuses on studies on pathogenic and genetic diversity in *P. brassicae* from Japan. The epidemiological relationship between clubroot of cruciferous crops and that of weeds is also discussed.

## Terminology

In this review, the term “population” is fundamentally used for a mass of *P. brassicae* resting spores obtained from galls or a gall in a single field, based on a common consideration among clubroot researchers that the mass of the pathogen often includes multiple genotypes (i.e., races or pathotypes) and is genetically heterogenic, as described later. On the other hand, the term “single-spore isolate” means a mass of *P. brassicae* resting spores derived from galls on a host plant inoculated with a single resting spore. The single-spore isolate is considered to be a mass of genetically homogenous resting spores. The term “collection” is also used for a mass of plants grown from seeds of each cruciferous weed collected in a single field.

## Host crops and their CR cultivars

Clubroot affects various cruciferous vegetables of genus *Brassica*, such as Chinese cabbage (*B. rapa* subsp. *pekinennsis*), turnip (*B. rapa* subsp. *rapifera*), cabbage (*B. oleracea* subsp. *capitata*), cauliflower (*B. oleracea* subsp. *botrytis*), broccoli (*B. oleracea* subsp. *italica*), leaf mustard (karashina, *B. juncea* subsp. *cernua*), takana (*B. juncea* subsp. *integlifolia*), oil seed rape (*B. nups*), and leafy greens (some subspp. of *B. rapa*) in Japan (Ikegami 1975; Yoshikawa 1993). In other genera, wasabi (*Eutrema wasabi*) is also highly susceptible to the disease (Suzuki et al. 2006; Tanaka et al. 1994). On the other hand, although most cultivars of Japanese radish (*Raphanus sativus* subsp. *longipinnatus*) had been considered to be highly resistant to the disease (Ikegami 1975; Yoshikawa 1993), a clubroot outbreak in a cultivar of radish grown in a field and the presence of some highly susceptible cultivars in radish has been recently reported in Fukushima Prefecture (Horikoshi and Tairako 2002).

Use of CR cultivars of cruciferous crops is an important measure for control of clubroot. In Japan, CR lines of Chinese cabbage were first bred by introducing a CR gene from a European fodder turnip (Yoshikawa 1981), and thereafter, many commercial CR cultivars of Chinese cabbage, turnip, and other *B. rapa* vegetables have been released and widely grown in Japan. CR cultivars have also been developed in cabbage, broccoli, and nabana (*B. napus*) in Japan (Tanaka et al. 2009; Yoshikawa 1993).

## Resistance mechanisms in CR cultivars of Chinese cabbage

Most CR cultivars (highly resistant cultivars) of crucifers, including Chinese cabbage, suppress the formation of large and/or spindle-form clubroot galls, which affect the growth of plants. Alternatively, small, spheroid, resistant-type galls (SSGs) are often seen on the outer surface of roots of these cultivars (Osaki et al. 2008a; Seaman et al. 1963; Williams 1966). The SSGs have been considered to be a result of a resistant reaction to *P. brassicae* (Seaman et al. 1963).

In roots of the CR cultivars of Chinese cabbage, populations of *P. brassicae*, which are avirulent on these cultivars, can commonly infect at the root hairs, form primary plasmodia within these cells, and produce secondary zoospores (primary infection phase) (Tanaka et al. 2006a; Yamagishi et al. 1986). In addition, although these populations can infect the root cortex and medullary rays and form secondary plasmodia within the infected cells (secondary infection phase), these remain immature plasmodia with a small number of nuclei and cannot develop resting spores in root tissues, with the exception of SSGs (Tanaka et al. 2006a). These populations, however, can develop mature resting spores within SSGs (Osaki et al. 2008a). Populations from these SSGs have characteristics of pathogenicity identical to those of original populations (Osaki et al. 2008a). Thus, populations that are avirulent on CR cultivars of Chinese cabbage can complete their life cycle only within SSGs in these cultivars. The formation of SSGs indicates the restriction of the migration of *P. brassicae* in resistant root tissues (Seaman et al. 1963) and is considered to be one of the resistant mechanisms in the CR cultivars of Chinese cabbage. In addition, suppression of secondary colonization (i.e., suppression of plasmodial development) of *P. brassicae* within infected root cells may also be associated with resistance in the CR cultivars of Chinese cabbage (Tanaka et al. 2006a). Such an incompatible response against *P. brassicae* has also been observed in the CR cultivars of radish (Kroll et al. 1983) and canola (Deora et al. 2012; Hwang et al. 2011) in other countries.

The details of the physiological mechanisms involved in the suppression of secondary colonization by *P. brassicae* in CR cultivars are unknown. However, hardly any starch accumulation is detected within infected root cells of the CR cultivars (Tanaka et al. 2006a). Starch accumulation, which is abnormally stimulated within *P. brassicae*-infected root cells of susceptible plants, is very important for secondary colonization of the pathogen because the starch serves as its carbon source (Keen and Williams 1969; Williams et al. 1968). Morgner (1995) has also reported that starch accumulation is observed in infected root cells of susceptible lines but never in those of CR lines of *Brassica*. Perhaps CR cultivars of some cruciferous crops can selectively regulate starch accumulation in response to different populations of *P. brassicae*.

## Selective propagation of pathogenic genotypes on CR cultivars of Chinese cabbage and breakdown of resistance in the cultivars

Clubroot susceptibility in commercial CR cultivars of Chinese cabbage has become a problem in some areas of Japan. The presence of *P. brassicae* populations pathogenic on these cultivars was first found unexpectedly as a result of an inoculation test in three areas of Yamaguchi Prefecture, where the CR cultivars had not yet been introduced (Tanaka et al. 1991). We presume that race 9 populations were originally pathogenic on the CR cultivars. All three of these populations were identified as Williams' race 9. Because race 9 is a minor race in Japan and might not be one of the objective races (pathotypes) of breeding programs for the selection of CR lines of Chinese cabbage.

Thereafter, populations pathogenic on the CR cultivars have been detected from various areas of Japan (Hatakeyama et al. 2004; Kuginuki et al. 1999; Tanaka et al. 1998; Yoshikawa 1990). At least part of this population has been collected from fields where breakdown in resistance of the CR cultivars had become a problem. Indications are that continuous cropping often leads to a breakdown in resistance of CR cultivars (LeBoldus et al. 2012; Seaman et al. 1963). Such progressive breakdown has been reported for a CR cultivar of Chinese cabbage in Japan. Osaki et al. (2004) have examined the process of breakdown in the CR cultivars in field surveys and inoculation tests in a heavily infested commercial field of Hagi, Yamaguchi Prefecture, where clubroot galls of Chinese cabbage had been collected every year for more than the past 15 years. CR cultivar Daifuku 206 was first introduced and grown in this field in 2001. Relatively small galls (1–3 cm in diameter) were seen at the tips of tap and lateral roots of plants that grew well in their first season (2001), then large gall formed on tap roots of many plants, and their growth was severely inhibited in the second growing season (2002). The inoculation test showed that pathogenicity on many CR cultivars clearly differed between populations obtained before (2000) and after (2001 and 2002) the introduction of the CR cultivar (Daifuku 206) in the same field. A similar progression of resistance breakdown has also been observed for a CR cultivar of hiroshimana (a kind of leafy green, *Brassica rapa* L.) in Hiroshima Prefecture (Oku et al. 2011, 2012). The breakdown may even be preceded by extremely short-term continuous cropping after the CR cultivars are introduced, especially in heavily infested fields.

The breakdown of CR cultivars possibly results from selective propagation of pathogenic genotypes on the cultivars. Each field population of *P. brassicae* is often heterogenic and composed of multiple races (pathotypes, genotypes), as indicated by analyses of pathogenicity of single-spore isolates of *P. brassicae* obtained from a single field (or a gall) population (Fähling et al. 2003; Jones et al. 1982; Manzanares-Dauleux et al. 2001; Somé et al. 1996). Kageyama et al. (1995) have also confirmed such pathogenic and genetic heterogeneity within a *P. brassicae* population from Japan. The population structure of *P. brassicae* is variable on field-grown host plants, and minor pathogenic genotypes, which are masked within the population, emerge and dominate under selection pressure by newly introduced CR cultivars. It is clear that the plant pathogenic organism, *P. brassicae*, has an excellent survival strategy.

A new commercial CR cultivar of Chinese cabbage, Akimeki, bred through selection using multiple DNA markers, was recently released in Japan (Matsumoto 2012). This cultivar is highly resistant to various populations of *P. brassicae*, including those pathogenic on conventional CR cultivars of Chinese cabbage; therefore, the stability and persistence of their usefulness in Japan are widely expected. However, an effective method to avoid a breakdown in resistance should be considered for practical use of this cultivar in fields.

## Variation in pathogenicity of populations from crops

Many field populations of *P. brassicae* vary in their pathogenicity. Such pathogenic diversity among the populations was first alluded to by Honig (1931), and thereafter, various race (or pathotype) classification systems using sets of differential hosts have been proposed (Ayers 1957; Buczacki et al. 1975; Johnston 1968; MacFarlane 1955; Somé et al. 1996; Williams 1966; Wit and van de Weg 1964). At the present time, the systems established by Williams (1966) and Buczacki et al. (1975) are used worldwide. In *P. brassicae* populations from Japan, races 1, 2, 3, 4, 6, 7, 8, and 9 have been identified by the Williams' method, with 1, 2, 3, and 4 as major races (Yoshikawa 1990, 1993). Tanaka et al. (1998) also identified races 1, 4, and 9 using the Williams' method, with race 4 as the most major race. On the other hand, widely varying races in populations were identified using the European Clubroot Differential (ECD) hosts (Buczacki et al. 1975); another report also identified races 16 and 20 using only 01–05 ($n = 10$ group) of ECD hosts, with 16 as the major race (Yoshikawa 1993).

A notable problem, however, with these race differentiation systems is that they are unable to distinguish between pathogenic and nonpathogenic populations on CR cultivars of Chinese cabbage bred in Japan (Hatakeyama et al. 2004; Kuginuki et al. 1999; Tanaka et al. 1998; Yano et al. 1996, 1997; Yoshikawa 1990). For example, all of Williams' race 9 populations tested are pathogenic on CR cultivars of Chinese cabbage, whereas Williams' race 4 populations are divided into two groups pathogenic and nonpathogenic on the cultivars (Tanaka et al. 1998; Yano et al. 1996, 1997). These differential systems involve a fundamental problem in which some differential hosts often yield intermediate and fluctuating responses (disease index) to most populations, and interpretation of the results is often ambiguous (Kuginuki et al. 1999). Therefore, Kuginuki et al. (1999) proposed a new race-differential system using two CR cultivars of Chinese cabbage as hosts. Because these CR cultivars show a clear response (resistant or susceptible) to *P. brassicae* populations from Japan, interpretation of the data obtained is extremely simple and easy. Of these CR cultivars, CR Ryutoku shows population resistance identical to many other CR cultivars of Chinese cabbage (i.e., CR Kukai 65, CR Fukutakara, and CR Kanko), while CR Utage 70 has a unique resistance and clearly differs in resistance from CR Ryutoku and others (Kuginuki et al. 1999; Osaki et al. 2008b). This system classifies *P. brassicae* populations into four groups on the basis of the responses of CR Ryutoku and CR Utage 70.

Osaki (2008) and Osaki et al. (2008b) modified the system of Kuginuki et al. (1999) by using CR Kukai 65 (same response as CR Ryutoku) and CR Utage 70 as differential hosts and assigned four pathotypes (pathotypes A: pathogenic on both cultivars; B: pathogenic on CR Kukai 65 but not on CR Utage 70; C: nonpathogenic on CR Kukai 65 but pathogenic on CR Utage 70; D: nonpathogenic on both cultivars). On the basis of this system, pathotypes A, B, C, and D have been identified in 5, 5, 13, and 12 populations, respectively, from various areas of Japan (Osaki 2008; Osaki et al. 2008b). These differential systems using the CR cultivars of Chinese cabbage are advised for practical use to directly ascertain the effectiveness of the cultivars in the fields, as these cultivars are widely grown in Japan.

Chinese cabbage cv. CR Utage 70 is considered to have a very unique and interesting genetic background (Kuginuki et al. 1999) and is an important determinant in the pathotype classification system. Unfortunately, this cultivar is not currently for sale and cannot be obtained. Therefore, a modified differential system has been proposed using Chinese cabbage cv. Super CR Hiroki as an alternative for CR Utage 70 (Hatakeyama et al. 2004).

## Variations in virulence among populations from crops

The levels of virulence (aggressiveness) on cruciferous crops also often vary with the populations of *P. brassicae*. Highly virulent populations of *P. brassicae* that can severely affect a common cultivar of Chinese cabbage even at a low inoculum density (e.g., $1 \times 10^2$ spores/g dry soil) are known, while weakly virulent populations that can severely affect the cultivar only at a high inoculum density (e.g., $1 \times 10^6$ spores/g dry soil) have also been observed (Tanaka et al. 1990, 1991, 1997b). However, the virulence on common cultivars does not always parallel that on CR cultivars among populations. For example, three populations (Williams' race 9) that are relatively weakly virulent on a common cultivar of Chinese cabbage, can severely affect various CR cultivars of the crop, whereas three other populations (Williams' races 1 and 4), which were relatively highly virulent on the common cultivar, did not affect many CR cultivars (Tanaka et al. 1990, 1991). The levels of effectiveness of fungicides and other various control measures vary not only with the inoculum densities but also with the variations in virulence of populations (Horiuchi and Hori 1982; Tanaka et al. 1997b). The efficacy of fungicides (trichlamide, flusulfamide, and fluazinam) is high against the weakly virulent population but lower against the highly virulent populations (Tanaka et al. 1997b). The effect of day length on clubroot incidence also varies depending on the virulence of populations and is relatively low against highly virulent populations (Horiuchi and Hori 1982; Tanaka et al. 1997b).

Most CR cultivars of cabbage are considered to have partial resistance to *P. brassicae* (Voorrips 1995; Yoshikawa 1993). However, the levels of resistance of the CR cultivars of cabbage and broccoli bred in Japan also vary depending on the populations (Tanaka et al. 1998). Populations may be divided into two groups on the basis of the levels of virulence on cabbage cvs. YCR Rinen and YCR Tae (S Tanaka unpublished data).

## Occurrence and distribution of clubroot on cruciferous weeds

Clubroot affects not only cruciferous crops but also many wild crucifers (weeds). Numerous susceptible cruciferous weeds had earlier been reported from Europe, Canada, and New Zealand (Colhoun 1958; Gibbs 1932; Halsted 1894; Karling 1968; Nowicki 1973; Reyes et al. 1974). The presence of these susceptible weeds in fields has been considered to perpetuate *P. brassicae* in soil and to reduce the effectiveness of crop rotation for clubroot control in cruciferous crop production (Colhoun 1958; Karling 1968). However, the levels of clubroot susceptibility in weeds often differ among collections from various areas and depend on the *P. brassicae* populations (Buczacki and Ockendon 1979; Reyes et al. 1974; Tanaka et al. 1983; Tanina et al. 1985). Heterogeneity in susceptibility has also been indicated within a weed collection from a single field (Buczacki and Ockendon 1979). In fact, clubroot is not always seen on cruciferous weeds even in fields where the cruciferous crops are severely affected. The epidemiological relationship between clubroot on cruciferous crops and that on weeds may not be as simple as indicated by early researchers. In addition, most studies on clubroot susceptibility in cruciferous weeds had been conducted using only populations from cruciferous crops. Not enough detailed information had been available on the actual clubroot incidence on cruciferous weeds in fields, nor had the characteristics of pathogenicity of the populations from the weeds been documented.

In Japan, clubroot has been recorded on five species of cruciferous weeds, *Capsella bursa-pastoris* (Ikegami 1978), *Rorripa indica* (Ikegami 1978), *Rorippa islandica* (Ikegami 1978; Osaki 2008; Umehara and Tamura 1968), *Cardamine flexuosa* (Tanaka et al. 1993, 2006b), and *Cardamine scutata* (Tanaka et al. 1993), based on field surveys (Fig. 1a–c). However, except for *C. flexuosa*, clubroot on these weed species has been recorded only in a few areas (prefectures), and detailed information about their distribution in Japan is lacking, perhaps due to low frequencies of infection or insufficient research on these weeds.

**Fig. 1** Clubroot on cruciferous weeds and Chinese cabbage. **a** *Cardamine flexuosa* (natural infection, typical symptom). **b** *Capsella bursa-pastoris* (natural infection, typical symptom). **c** *Rorippa islandica* inoculated with a population from *R. islandica* (about 45 days after inoculation, typical symptom). **d** Chinese cabbage inoculated with a population from *Cardamine flexuosa* (51 days after inoculation, incomplete swelling)

Clubroot on *C. flexuosa*, a common cruciferous weed in drained paddy fields of Japan, was first found in Nagato, Yamaguchi Prefecture in 1980, and this weed was recorded as a new host species of *P. brassicae* (Tanaka et al. 1993). Thereafter, detailed field studies on the distribution of clubroot of *C. flexuosa* throughout Japan were conducted for more than 20 years (Tanaka et al. 1993, 2006b). Clubroot of this weed has been confirmed to be distributed widely in Japan (Aomori to Kagoshisma Prefectures), excluding Hokkaido and Okinawa prefectures (Fig. 2). Furthermore, the disease is distributed also in major isolated islands, Sado (Niigata Prefecture), Oki (Shimane Prefecture), Mishima (Yamaguchi Prefecture), Tsushima, Iki, Goto (Nagasaki Prefecture), Koshikijima, Yakushima, and Tanegashima (Kagoshima Prefecture). Infected plants of the weed have been recently found on extremely isolated islands, such as Hachijojima of the Izu Islands (Tokyo Prefecture) and Kuchinoshima of the Tokara Islands (Kagoshima Prefecture) (S Tanaka unpublished). Infected plants of *C. flexuosa* are generally highly frequent in western Japan and relatively low in northern Japan. In Yamaguchi Prefecture, in particular, where research is extensive, clubroot of the weed has been observed nearly everywhere (Tanaka et al. 1993). Although clubroot of the weed has not been observed in Hokkaido and Okinawa prefectures, most collections of the weed from these areas have been confirmed with inoculation tests to be susceptible to a *P. brassicae* population isolated from the weed (Tanaka et al. 2006b). Populations of *P. brassicae* pathogenic to *C. flexuosa* may not be present in Hokkaido and Okinawa prefectures.

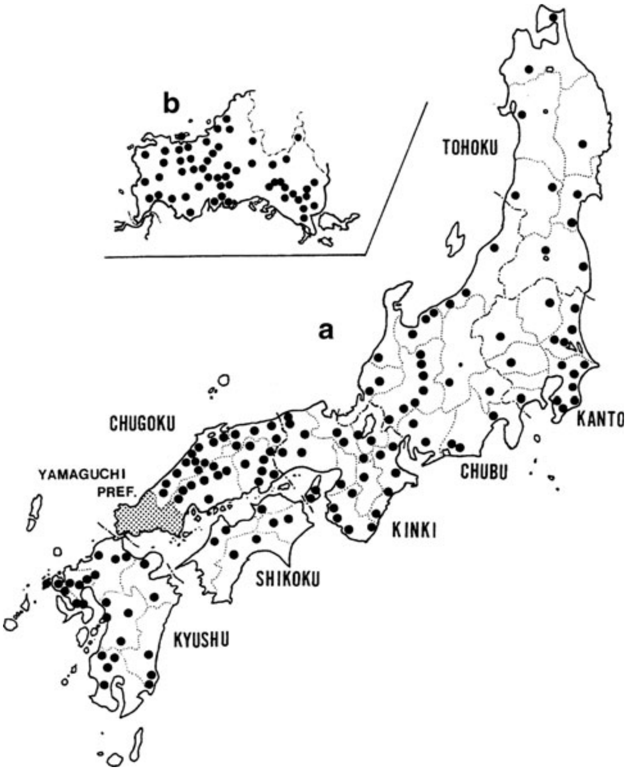

**Fig. 2** Confirmed locations of clubroot on *Cardamine flexuosa* in **a** mainland Japan (Honshu, Shikoku and Kyushu) and **b** Yamaguchi Prefecture (checkered area in **a**)

## Pathogenicity of populations from cruciferous weeds

*Capsella bursa-pastoris* is one of most common cruciferous weeds worldwide and is well known as a wild host of *P. brassicae* in many countries. Although populations from cruciferous crops are often pathogenic on the weed, the level of compatibility varies depending on the specific combination of weed collection and *P. brassicae* population in Japan (Tanaka et al. 1983; Tanina et al. 1985). On the other hand, *P. brassicae* populations from the weed have been confirmed to be highly virulent on cruciferous crops in Fukushima (Tsushima 2003) and Fukuoka prefectures (S Tanaka unpublished). Populations from *C. bursa-pastoris* collected in three fields of Fukuoka, Fukuoka Prefecture were highly virulent on cabbage and Chinese cabbage and were identical in pathotype (determined on the CR cultivars of Chinese cabbage) with populations from cruciferous crops collected in the three fields (S Tanaka unpublished). The presence of *C. bursa-pastoris* in fields possibly perpetuates *P. brassicae* in soil and reduces the effectiveness of crop rotation and decoy crops for clubroot control in cruciferous crop production in certain areas of Japan.

Clubroot of *C. flexuosa* has been recorded exclusively in Japan. Populations from cruciferous crops are nonpathogenic on *C. flexuosa* (Murai et al. 1986; Osaki et al. 2008b; Tanaka et al. 1984, 1994; Umehara and Tamura 1968; Yoshikawa 1993). However, recently, *C. flexuosa* collected from a field of Hiroshima Prefecture was found to be highly susceptible to many populations from cruciferous crops (S Tanaka unpublished). Collections of *C. flexuosa* are also thought to vary in susceptibility to populations from cruciferous crops.

Information on the pathogenicity of *P. brassicae* populations from *C. flexuosa* on cruciferous crops is not yet comprehensive enough. For example, some populations from this weed are pathogenic on Chinese cabbage and turnip (Murai et al. 1986; Osaki et al. 2008b; Tanaka et al. 1984). In many cases, however, although these populations suppress the growth of common cultivars of these crops by affecting their entire taproots, the infected roots frequently have slight or moderate swelling and necrotic root tissues (Fig. 1d). None to very few resting spores develop in infected roots with such symptoms. Therefore, these populations have often been assessed as moderately pathogenic on Chinese cabbage and turnip (Murai et al. 1986; Osaki et al. 2008b; Tanaka et al. 1984), but the symptoms may reflect incomplete resistance of these crops against populations from the weed. However, typical clubroot galls are also sometimes found on a part of their infected plants. Murai et al. (1986) reported that a population obtained primarily from such typical clubroot galls on Chinese cabbages was more virulent than an original population from the weed on Chinese cabbage.

Clubroot on a cruciferous weed, *Rorippa islandica*, has been recorded only in extremely limited areas of Japan (Ikegami 1978; Umehara and Tamura 1968). Therefore, although populations from cruciferous crops have also been reported to be nonpathogenic on *R. islandica* (Osaki 2008; Umehara and Tamura 1968; Yoshikawa 1993), there had been no reports on the pathogenicity of *P. brassicae* from this weed. However, clubroot of *R. islandica* has been recently found in Fukuoka Prefecture, and in a pathogenicity test of a population from this weed, has been examined using inoculation tests (Osaki 2008; S Tanaka unpublished). The results showed that the population from *R. islandica* often induced incomplete (slight or moderate) swelling with necrotic root tissues on most of the infected roots of Chinese cabbage and turnip as did some populations from *C. flexuosa*, but the population was nonpathogenic on a collection of *C. flexuosa* (Osaki 2008; S Tanaka unpublished). The population from *R. islandica* may differ fundamentally in pathogenicity from those from cruciferous crops and *C. flexuosa*.

As already mentioned, the epidemiological relationship between clubroot of cruciferous crops and that of the two cruciferous weeds, *C. flexuosa* and *R. islandica*, has not yet been clarified. However, each population of *P. brassicae* from the weeds may also be heterogenic and includes multiple genotypes (races or pathotypes), as indicated in populations from crops by many researchers (Fähling et al. 2003; Jones et al. 1982; Kageyama et al. 1995; Manzanares-Dauleux et al. 2001; Somé et al. 1996). Different pathotypes within the field populations can be separated by repeated propagation in resistant host plants (Fähling et al. 2003). The process through which minor pathogenic genotypes develop into major ones through selective propagation on the crops may in fact act within each population from the weeds in the fields. The pathogenicity of *P. bassicae* from these weeds has been studied only in a limited number of populations. The study of clubroot susceptibility has been limited to a few collections. Variation in pathogenicity may be present within *P. brassicae* from the weeds as well as within that from crops. In addition, the level of clubroot susceptibility may vary among weed collections and may also be heterogenic even within a collection. Therefore, additional research on more populations of *P. brassicae* from more collections of the weeds is needed to gain a better understanding of the epidemiological relationship among populations of *P. brassicae* from cruciferous crops and weeds in Japan.

## Relationship between pathogenic and genetic variation in *P. brassicae*

Race (or pathotype) identification requires a long time and intensive labor because they must be tested on differential host plants. In addition, the results are easily affected by environmental conditions. Therefore, many workers have used molecular markers to analyze the relationship between genetic variation and pathogenicity or host preference in populations and single-spore isolates of *P. brassicae* (Buhariwalla et al. 1995; Fähling et al. 2003; Ito et al. 1997, 1999; Manzanares-Dauleux et al. 2001; Möller and Harling 1996; Osaki et al. 2008b; Yano et al. 1997). Although information is incomplete, the current findings are contributing to our understanding of the genetic background involved in the pathogenic variation of *P. brassicae* populations.

In Japan, Yano et al. (1996, 1997) have shown that two populations (Williams' race 9) are clearly distinguishable from 14 other populations (Williams' races 1 and 4) by random amplified polymorphic DNAs (RAPDs) and that the result corresponds well with results using isozyme patterns in the same populations. However, their methods could not distinguish between pathogenic and nonpathogenic populations on CR cultivars of Chinese cabbage bred in Japan. Thereafter, Osaki et al. (2008b) analyzed the relationship between RAPDs and pathogenic variation (pathotypes determined using CR cultivars of Chinese cabbage and Williams' races) among additional populations, including those from the weed, *C. flexuosa*, from Japan. The cluster analysis based on RAPD data has indicated that three populations (Williams' race 9) are clearly distinguishable from other populations (Williams' races 1 and 4), as described by Yano et al. (1997) and that partial correspondence exists between pathotypes and genetic variations in populations as follows: all pathotype B and D populations were distinguishable from all pathotype C populations except one (Fig. 3). However, their method could not distinguish pathotype B populations from those of pathotype D or pathotype A populations from others. Pathotype A populations are extremely heterogenic and are presumed to have arisen through plural evolutionary processes (Osaki et al. 2008b).

Genetic variation among populations of *P. brassicae* from Japan is relatively high, but the correlation between pathogenic and genetic variations in these populations is not very high (Yano et al. 1997; Osaki et al. 2008b).

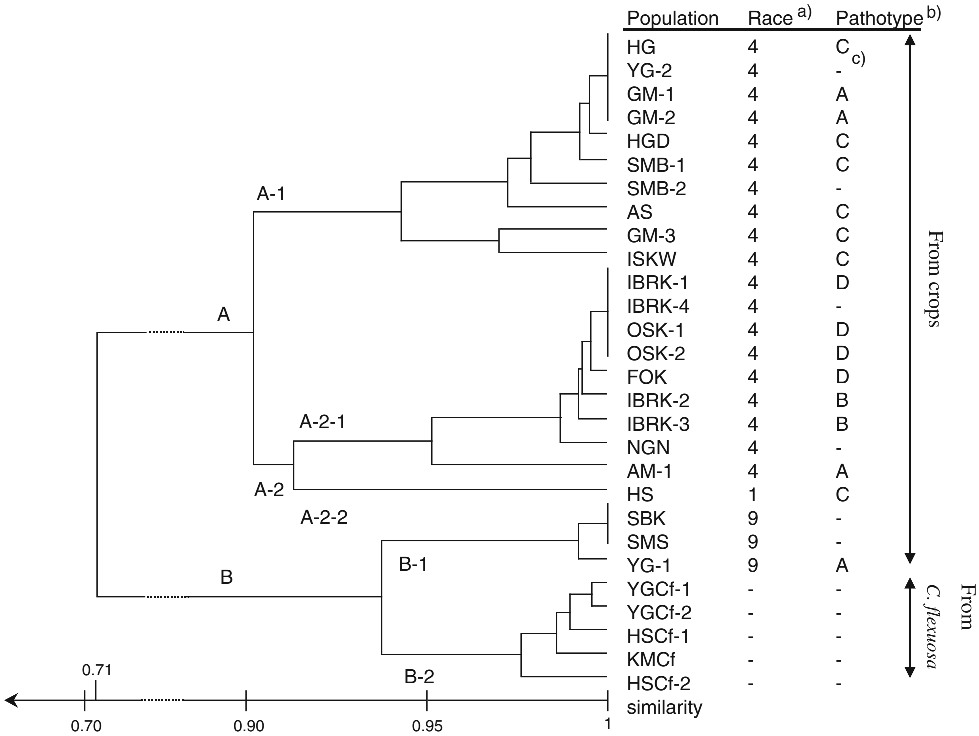

**Fig. 3** Dendrogram based on RAPD data for 28 populations of *Plasmodiophora brassicae* from cruciferous crops and the wild crucifer *Cardamine flexuosa*. The dendrogram was constructed by the unweighted paired group method with arithmetic averaging from the matrix of coefficients of simple matching similarity. **a** Williams' race, **b** pathotype determined on the basis of pathogenicity on two clubroot-resistant cultivars of Chinese cabbage, CR Kukai 65 and CR Utage 70, *pathotype A* pathogenic on both cultivars, *pathotype B* pathogenic on CR Kukai 65 but nonpathogenic on CR Utage 70, *pathotype C* nonpathogenic on CR Kukai 65 but pathogenic on CR Utage 70, *pathotype D* nonpathogenic on both cultivars, **c** not tested

Heterogeneity in each population may have affected the results of the authors' analyses of pathogenicity and DNA polymorphisms. When Manzanares-Dauleux et al. (2001) used single-spore isolates for similar analyses, the correlation between pathogenic and genetic variations was relatively low, even among these isolates. Specialization of races and pathotypes seems to reflect only a limited aspect within the high level of genetic diversity in this organism.

In a dendrogram based on RAPD analysis, *P. brassicae* populations from *C. flexuosa* grouped into a single subcluster and were located in a single cluster with all Williams' race 9 populations from crops (Osaki et al. 2008b) (Fig. 3). The results indicate that these two groups of populations are genetically closely related. The race 9 populations are minor within *P. brassicae* from Japan and have unique pathogenicity (i.e., they are avirulent on many common cultivars of cabbage and have relatively lower virulence than race 4 populations on common cultivars of Chinese cabbage, but they are virulent on many CR cultivars of Chinese cabbage) (Tanaka et al. 1991, 1997a, 1998). The significance of the genetically close relationship between the two groups of populations from crops and *C. flexuosa* is not clear; however, available results may point to an evolutionary process of the pathogen in Japan. *Cardamine flexuosa* is thought to have originated in Eurasia and become naturalized in Japan following the introduction of rice cultivation (Takematsu and Ichizen 1993). On the other hand, clubroot on cruciferous crops was first recognized at the end of the 19th century in Japan (Ikegami 1992), so clubroot on *C. flexuosa* may precede clubroot on cruciferous crops. *Plasmodiophora brassicae*, an obligate parasite, may thus have a long history of coevolution with *C. flexuosa* in Japan.

Because of its wide distribution in Japan (Tanaka et al. 1993, 2006b), clubroot of *C. flexuosa* may be more common than the disease on crops in Japan and may be one source of the pathogenic genotypes on cruciferous crops. Additional studies are thus necessary for a thorough understanding of the origin of *P. brassicae* and the genetic and epidemiological relationship between the populations from *C. flexuosa* and cruciferous crops in Japan.

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