Identification of a clubroot resistance locus conferring resistance to a 
\textit{Plasmodiophora brassicae} classified into pathotype group 3 in Chinese cabbage 
\textit{(Brassica rapa L.)}

Takeyuki Kato$^{1,2}$, Katsunori Hatakeyama$^2$, Nobuko Fukino$^2$ and Satoru Matsumoto*$_{2}^{2}$

$^{1}$ Graduate School of Bioresources, Mie University, 1577 Kurima-machiya, Tsu, Mie 514-8507, Japan 
$^{2}$ NARO Institute of Vegetable and Tea Science, 360 Kusawa, Ano, Tsu, Mie 514-2392, Japan

In Chinese cabbage \textit{(Brassica rapa)}, the clubroot resistance (CR) genes \textit{Crr1} and \textit{Crr2} are effective against the mild \textit{Plasmodiophora brassicae} isolate Ano-01 and the more virulent isolate Wakayama-01, but not against isolate No. 14, classified into pathotype group 3. ‘Akiriso’, a clubroot-resistant F$_1$ cultivar, showed resistance to isolate No. 14. To increase the durability of resistance, we attempted to identify the CR locus in ‘Akiriso’. CR in ‘Akiriso’ segregated as a single dominant gene and was linked to several molecular markers that were also linked to \textit{CRb}, a CR locus from cultivar ‘CR Shinki’. We developed additional markers around \textit{CRb} and constructed partial genetic maps of this region in ‘Akiriso’ and ‘CR Shinki’. The positions and order of markers in the genetic maps of the two cultivars were very similar. The segregation ratios for resistance to isolate No. 14 in F$_2$ populations derived from each of the two cultivars were also very similar. These results suggest that the CR locus in ‘Akiriso’ is \textit{CRb} or a tightly linked locus. The newly developed markers in this study were more closely linked to \textit{CRb} than previously reported markers and will be useful for marker-assisted selection of \textit{CRb} in Chinese cabbage breeding.

Key Words: clubroot, disease resistance, \textit{Plasmodiophora brassicae}, linkage map, \textit{CRb}, simple sequence repeat (SSR) markers, \textit{Brassica rapa}.

Introduction

Clubroot disease, which is caused by the soil-borne plant pathogen \textit{Plasmodiophora brassicae} Woronin, is the most serious disease in cultivated \textit{Brassica} species such as \textit{B. rapa}, \textit{B. oleracea} and \textit{B. napus}. The pathogen causes abnormal cell enlargement and cell division of infected roots that produces characteristic deforming galls (the “clubs”). The disease causes substantial reductions in crop quality and yield. The ability of this pathogen to survive in soil as resting spores for several years makes it difficult to prevent clubroot disease by cultural practices (Voorrips 1995). Although fungicide application is sometimes used as a means of disease control in Chinese cabbage and cabbage grown in Japan, breeding for resistance is the most effective approach to minimize crop losses. The clubroot resistance (CR) genes from European fodder turnip (\textit{B. rapa}) cultivars such as ‘Gelria R’, ‘Siloga’, ‘Debra’ and ‘Milan White’ have been introduced into Chinese cabbage (Yoshikawa 1981), and a number of clubroot-resistant F$_1$ cultivars have been subsequently released. Recently, however, breakdown of the clubroot resistance of several clubroot-resistant cultivars has been reported in some production areas in Japan (Hatakeyama et al. 2004, Kuginuki et al. 1999). Many of the present clubroot-resistant F$_1$ cultivars appear to be heterozygous for a single dominant resistance gene. Because \textit{P. brassicae} is a highly variable pathogen, the likelihood that it could overcome CR controlled by a single gene is high. Therefore, breeding for additional resistant cultivars is needed, and pyramiding of different CR genes into a single line is necessary to confer resistance to the broad spectrum of races of \textit{P. brassicae} (Hirai 2006).

Many mapping studies for CR loci have been reported in \textit{Brassica}, and at least eight CR loci have been identified in \textit{B. rapa} (Diederichsen et al. 2009, Hirai 2006). \textit{Crr1}, \textit{Crr2} and \textit{Crr4} were mapped to \textit{B. rapa} linkage groups R8, R1 and R6, respectively (Suwabe et al. 2006) and \textit{CRc} was mapped to R2 (Sakamoto et al. 2008). Four CR loci, \textit{CRA} (Hayashida et al. 2008), \textit{CRb} (Piao et al. 2004), \textit{CRk} (Sakamoto et al. 2008) and \textit{Crr3} (Saito et al. 2006), were mapped to R3. Although \textit{CRk} and \textit{CRc} have been reported to confer resistance to different races of the pathogen...
Clubroot resistance locus conferring resistance to pathotype group 3

(Sakamoto et al. 2008), knowledge of the relationship between CR loci and pathogenicity is limited. We have developed the CR Chinese cabbage parental line ‘Chukanbohon Nou 9 gôu’ (PL9) containing two CR genes, Crr1 and Crr2, introduced by marker-assisted selection (MAS) (in preparation). We demonstrated that plants having Crr1 conferred resistance to the mild strain Ano-01, whereas both Crr1 and Crr2 were necessary for resistance to the more virulent strain Wakayama-01 (Suwabe et al. 2003, 2006). Four pathotypes (group 1 to group 4) were identified in Japanese field isolates through the use of two commercial CR F1 cultivars of Chinese cabbage (Hatakeyama et al. 2004, Kuginuki et al. 1999). PL9 showed resistance to isolates of pathotype groups 1, 2 and 4, but not group 3. Thus, the resistant cultivars against isolates of pathotype groups 1, 2 and 4, could be developed by introduction of Crr1 and Crr2 with MAS. Although several F1 cultivars have been reported to show complete and partial resistance to pathotype group 3 (Hatakeyama et al. 2004), the genetic basis of CR locus effective to pathotype group 3 is unknown.

To increase the durability of resistance, it is necessary to identify CR gene against this pathotype and develop the useful markers for MAS. ‘Akiriso’, a Verticillium yellows resistant F1 Chinese cabbage cultivar, is resistant to pathotype group 3. Therefore, we performed genetic analysis for the resistance in ‘Akiriso’ and compared the CR locus in this cultivar with previously identified CR loci.

Material and Methods

Plant materials

We used two CR F1 cultivars of Chinese cabbage, ‘Akiriso’ (Nippon Norin Seed Co.) and ‘CR Shinki’ (Takii & Co., Ltd.) and the parental lines of ‘Akiriso’, called T-line and V-line. Populations of 285 and 172 F2 plants were obtained by self-pollinating ‘Akiriso’ and ‘CR Shinki’, respectively. Of these, 189 of the 285 plants in the ‘Akiriso’ population and all of the plants in the ‘CR Shinki’ population were used for CR tests and construction of linkage maps. The remaining 96 F2 plants derived from ‘Akiriso’ were used to analyze the correlation between resistance and the genotypes of previously reported markers linked to CR loci.

Pathogen and CR test

For CR testing, we used Plasmhofla brassicae field isolate No. 14, which is classified into group 3 (Hatakeyama et al. 2004). The CR test was performed as previously described (Suwabe et al. 2003). The disease index (ID) was scored according to Hatakeyama et al. (2004) on a scale of 0 to 3: 0, no symptoms; 1, a few small, separate globular clubs on the lateral roots; 2, no clubs of main root, but moderate clubbing of lateral roots; 3, absence of normal roots, presence of large gall. Plants with ID scores of 0 to 2 were categorized as resistant (which included partial resistance); those with a score of 3 were categorized as susceptible, because healthy roots were observed in the former, but not in the latter.

Developing markers and linkage analysis

DNA markers were designed based on genome sequencing information from the B. rapa Genome Sequencing Project (BrGSP) (http://www.brassica-rapa.org/BRGP/index.jsp) (Mun et al. 2008, 2010). We developed SSR markers based on BAC end sequences of KBr libraries in BrGSP using the read2Marker program (Fukuoka et al. 2005). To investigate the relationship between the CR locus of ‘Akiriso’, here named Crraki, and known CR loci, we used markers reported by Hayashida et al. (2008), Piao et al. (2004), Saito et al. (2006), Sakamoto et al. (2008) and Suwabe et al. (2006). In addition, we converted the CRb-linked CAPS marker TCR02 (Piao et al. 2004) to an SSR marker, designated TCR02-F, based on the sequence of the PCR product of TCR02. The primers designed in this study are listed in Table 1. PCR amplification was carried out in 10-µl reaction mixtures containing 10 ng genomic DNA, 4 pmol of each primer and 2× Quick Taq HS Dye Mix (TOYOBO, Osaka, Japan) in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA). The reaction was performed with the following parameters: 1 cycle of 94°C for 2 min; 35 cycles of 94°C for 30 s, 55°C for 30 s and 68°C for 30 s; and final extension at 68°C for 5 min. Detection of polymorphisms was performed as described previously (Hatakeyama et al. 2010). To construct a linkage map of ‘Akiriso’, markers showing polymorphism between the two parental lines, T-line and V-line, were used for segregation analysis in an F2 population obtained by selfing ‘Akiriso’. Segregation analysis of resistance in an F2 population obtained by selfing ‘CR Shinki’ suggested that ‘CR Shinki’ was heterozygous for a single dominant CR gene (see Results and Discussion), so we concluded that ‘CR Shinki’ was a hybrid of a CR parent and a clubroot-susceptible parent. Because the parental lines of ‘CR Shinki’ are proprietary and not available to us, we could not determine the marker genotypes of the parental lines. Instead, we selected three resistant and three susceptible F2 plants that were homozygous for genotypes of two CRb-linked markers, TCR05 and TCR02-F, to represent the genotypes of the postulated CR parent and clubroot-susceptible parent, respectively. To construct a linkage map of ‘CR Shinki’, we searched for polymorphisms between the selected resistant and susceptible F2 plants. The linkage maps of ‘Akiriso’ and ‘CR Shinki’ were constructed using JoinMap ver. 4.0 (van Ooijen 2006).

Results and Discussion

Association between molecular markers and clubroot resistance

We evaluated the clubroot resistance of the parental lines of ‘Akiriso’ (T-line and V-line), ‘Akiriso’ (F1) and 189 F2 plants by inoculation with P. brassicae isolate No. 14. The frequency distribution of resistance in the F2 population is shown in Fig. 1A and Table 2. The parental lines, T-line and V-line, were resistant (mean ID = 1.08, n = 26) and
susceptible (mean ID = 2.96, n = 25), respectively (Fig. 1A). ‘Akiriso’ showed resistance (mean ID = 1.49, n = 37). In the F2 population, 140 plants (74%) were scored as resistant and 49 (26%) as susceptible (Table 2). This segregation ratio fitted a 3 : 1 Mendelian ratio (P = 0.79 for χ²). These results indicate that the clubroot resistance of ‘Akiriso’ is conferred by a single dominant gene derived from T-line.

To examine whether the CR gene detected in ‘Akiriso’ is an allele of one of the eight known CR genes in B. rapa, we analyzed the correlation between the resistance phenotypes in the F2 population and genotypes of markers linked to each of the eight known CR loci. Among the markers tested, TCR10 and TCR02-F, both of which are linked to CRb (Piao et al. 2004) (Fig. 2A), were also linked to resistance in a subset of the ‘Akiriso’ F2 population (n = 96) (Table 3). F2 plants with a band amplified by the TCR10 primer set showed resistance (mean ID = 1.18), whereas F2 plants with no bands were susceptible (mean ID = 2.88). In addition, plants homozygous for the resistant and susceptible alleles of TCR02-F showed resistance (mean ID = 0.88) and susceptibility (mean ID = 2.86), respectively, and heterozygous plants showed intermediate resistance (mean ID = 1.21). Other CR markers (TCR01, TCR05, TCR08 and TCR09) linked to CRb (Piao et al. 2004) did not show polymorphism between T-line and V-line. There was no correlation between resistance and genotypes of markers linked to other CR loci, including OPC11-2S (Crr3 and CRk) (Saito et al. 2006), BRMS-088 (Crr1) (Suwabe et al. 2006), BRMS-096 (Crr2) (Suwabe et al. 2006) and BRMS-125 (Crr4) (Suwabe et al. 2006) (data not shown), and no polymorphisms were detected between the parental lines for markers HC352b-SCAR (Cra) (Hayashida et al. 2008) and m6R (Crc) (Sakamoto et al. 2008). These results suggest that CRAki is linked to TCR10 and TCR02-F, which are linked to CRb, thus raising the possibility that CRAki and CRb are the same locus.

### Table 1. Primer sequences for detection of genomic SSRs and PCR product sizes in ‘Akiriso’ and ‘CR Shinki’

| Marker name                  | Repeat motif | Forward primer (5’ to 3’) | Reverse primer (5’ to 3’) | Size of PCR product (bp) |
|------------------------------|--------------|---------------------------|---------------------------|-------------------------|
| KBrH129J08Rc                 | TA           | ATGAGATTGTAAGGGAAAAACAAA  | GTTTCAAACTGGAAAACAAAACTCTA | Akiriso: 234, 241, 234, 241<br>CR Shinki: 234, 241, 234, 241 |
| KBrH059N21F                 | CT           | ATGCAGCGGCTTTAGAAGCTCG    | GTTTACACGTCAGTCCTACCT    | Akiriso: 218, 236, 218, 236<br>CR Shinki: 218, 236, 218, 236 |
| KBrH129J18R                 | TC           | AGAGGAGGTTGAAACCCAAACT    | GTTTCAAGTCTACAGTTTTCAG   | Akiriso: 254, 194, 254, 194<br>CR Shinki: 254, 194, 254, 194 |
| KBrB091M11R                 | AG           | ACTTAAAGACAGAAATGC       | GTTGGGAGGCTAGCCTATG       | Akiriso: 750, 177, 750, 177<br>CR Shinki: 750, 177, 750, 177 |
| TCR02-F                     | CTA          | AGTCCATTACGTTAAGCTGTA    | GCTAAAGAAAATGTCGAAGAATT  | Akiriso: 203, 207, 203, 207<br>CR Shinki: 203, 207, 203, 207 |

a SSRs found in genomic sequence information from the B. rapa Genome Sequencing Project (http://www.brassica-rapa.org/BRGP/index.jsp).

b PCR product sizes of T-line and V-line in ‘Akiriso’, and resistant (R) and susceptible alleles (r) detected in homozygous F2 progeny of ‘CR Shinki’.

c This marker was reported by Kakizaki et al. (2011).

### Table 2. Segregation of resistance to isolate No. 14 in F2 populations derived from two F1 cultivars

| Cultivar     | Phenotype | χ² a,b | P-value |
|--------------|-----------|--------|---------|
|              | R         | S      |         |
| Akiriso      | 140       | 49     | 0.07    | 0.79    |
| CR Shinki    | 126       | 46     | 0.24    | 0.62    |

a R, number of resistant plants (ID = 0, 1, 2); S, number of susceptible plants (ID = 3). Fig. 1 shows the data broken down by ID score.

b Test of 3 : 1 expected ratio.

### Table 3. Correlation between genotypes of two CRb-linked markers and resistance to isolate No. 14 in F2 plants derived from ‘Akiriso’ (n = 96)

| Marker | Genotypes | No. of plants | Mean ID |
|--------|-----------|---------------|---------|
| TCR10  | RR, Rr a,b | 79            | 1.18 a  |
|        | rr        | 17            | 2.88 b  |
| TCR02-F| RR        | 29            | 0.88 a  |
|        | Rr        | 45            | 1.21 a  |
|        | rr        | 22            | 2.86 b  |

a RR, genotype of T-line (CR); r, genotype of V-line (clubroot-susceptible); Rr, heterozygous genotype.

b TCR10 is a dominant marker, so the value represents the total number of RR + Rr plants.

c Significant differences of the two (TCR10) and three (TCR02-F) genotype groups were assessed using Mann-Whitney U-test and the Mann-Whitney U-test with Bonferroni’s correction following Kruskal-Wallis analysis, respectively. The same letter is not significantly different by the 5% level.

### Development of DNA markers and construction of linkage maps

To investigate whether CRAki is the same locus as CRb, we attempted to construct detailed linkage maps around CRAki. We assumed that CRb was located in the long arm of chromosome R3 because TCR05-R, which is linked to CRb,
Clubroot resistance locus conferring resistance to pathotype group 3

CRb was originally identified through genetic analysis of a resistant doubled haploid line derived from ‘CR Shinki’ using a single spore isolate with race 4 on the basis of Williams’ (1966) classification (Piao et al. 2004). We performed CR tests with isolate No. 14 on 172 plants from the F2 population derived from ‘CR Shinki’ and compared the results with the results from ‘Akiriso’ (Fig. 1 and Table 2). ‘CR Shinki’ showed resistance (mean ID = 1.25, n = 15). In the F2 population, resistant and susceptible plants were scored in a ratio of 126 (73%) to 46 (27%), respectively, which fit a 3:1 Mendelian ratio (Table 2). Thus, the segregation of resistance observed in the F2 population derived from ‘CR Shinki’ (Fig. 1B) was very similar to that in ‘Akiriso’ (Fig. 1A). These results suggested that the resistance of ‘CR Shinki’ to pathotype group 3 is controlled by a single gene encoded at CRb.

We also demonstrated that the resistance responses of ‘Akiriso’ to four other pathotypes (data not shown) were quite similar to those reported by Hatakeyama et al. (2004) for ‘CR Shinki’. It is unknown whether the pathogenicity of isolate No. 14 used in this study is the same as that of the isolate used in Piao et al. (2004) because we did not perform the CR test using Williams’ hosts (Williams 1966) with No. 14. However, isolate No. 2 classified into pathotype group 3 was found to be race 4 (Hatakeyama et al. 2004), suggesting that the pathogenicity of both isolates was very similar.

CRb was previously reported to be distal to TCR05 on R3 (Piao et al. 2004, Mun et al. 2010), but our mapping data placed CRb proximal to TCR05. This difference may be due to the difference in marker types used for the two analyses. In the present study, we used co-dominant markers to construct the linkage map, whereas three (TCR08, TCR09, TCR10) of the six markers used in Piao et al. (2004) were dominant markers. Co-dominant markers are highly informative: they can discriminate between homozygous and heterozygous genotypes and have greater reproducibility than dominant markers (Jones et al. 1997). In mapping studies, the characteristics of the markers used will influence the precision of the linkage map. Although TCR09 was previously reported to be the closest marker to CRb (Piao et al. 2004) (Fig. 2A), TCR05 has been used as the selection marker for CRb by many researchers (Diederichsen et al. 2009, Nagaoka et al. 2010, Saito et al. 2006, Sakamoto et al. 2008). Fluorescent in situ hybridization (FISH) analysis

T-line (1.08)  V-line (2.96)  Akiriso (1.49)

---

is located between BRMS-124 and BRMS-206 in this chromosome region (Saito et al. 2006; Fig. 2B). These two markers were located at positions 12 cM and 30–40 cM on the long arm of chromosome R3, respectively (Saito et al. 2006, Suwabe et al. 2006). In this region, marker KBrH129J08R, which was developed from the end sequence of BAC clone KBrH129J08 was located on the linkage map reported by Kakizaki et al. (2011) (Fig. 2C). Therefore, we developed three SSR markers based on the sequences of BAC clones assembled around KBrH129J08 by using genome sequence information from BrGSP (Mun et al. 2008, 2010) (Fig. 2). Partial linkage maps were constructed for ‘Akiriso’ (Fig. 2D). Four newly developed markers (KBrH059N21F, KBrH129J18R, KBrB091M11R and TCR02-F) and KBrH129J08R (Table 1) were mapped around CRaki. In particular, KBrH059N21F and KBrH129J18R were localized 0.3 cM distal and 0.4 cM proximal to CRaki, respectively.

We also constructed a linkage map around CRb using an F2 population derived from the selfing of ‘CR Shinki’ (Fig. 2E). Five markers linked to CRaki were also linked to CRb (Fig. 2D, 2E), and the positions of these markers and TCR05 on genome sequence of chromosome R3 were determined (Fig. 2F). Markers KBrH059N21F and KBrH129J18R were localized 0.2 cM distal and 0.4 cM proximal to CRb, respectively. These two markers were closer to CRb than TCR05 (Piao et al. 2004), which was located 1.4 cM from CRb in this experiment. The sizes of the PCR products, positions and order of the five common markers were almost identical in ‘CR Shinki’ and ‘Akiriso’ (Table 1 and Fig. 2D, 2E). These results suggest that the CR loci of these two cultivars are identical and that CRaki is an allele of CRb, but we cannot yet rule out the possibility that CRb and CRaki are distinct but tightly linked loci.

Fig. 1. Frequency distribution of disease index (ID) for clubroot resistance in F2 populations derived from F1 cultivars ‘Akiriso’ (A) and ‘CR Shinki’ (B). 0 = complete resistance; 3 = susceptible. The ID scores of T-line and V-line, the parents of ‘Akiriso’, ‘Akiriso’ and ‘CR Shinki’ are indicated by arrowheads.

---

---
revealed that BAC clones containing TCR09 and TCR05 were localized in different regions of the same chromosome (Piao et al. 2009), indicating that they might not be as closely linked as previously reported (Piao et al. 2004). In this study, the presence of TCR09 did not correlate with resistance in the F2 population derived from ‘CR Shinki’. In addition, TCR10 was located more than 13 cM apart from CRb (data not shown), which was a different position on R3 than was described by Piao et al. (2004). Therefore it is possible that difficulty in using these two markers (TCR09 and TCR10) can affect the apparent order of the markers and the position of CRb. On the other hand, order of 4 markers developed in this study and 2 markers (TCR02-F and TCR05) was consistent with B. rapa genome sequence (Fig. 2F).

In conclusion, we demonstrated that the segregation of resistance to P. brassicae isolate No. 14 in the F2 progeny and the order of SSR markers around the CR loci were quite similar between ‘Akiriso’ and ‘CR Shinki’. This result indicates that the CR locus controlling resistance to isolate No. 14 in ‘Akiriso’ is tightly linked to CRb and is likely to be the same locus. Two SSR markers developed in this study, KBH059N21F and KBH129J18R, were closely linked to CRb and can be scored by agarose gel electrophoresis. These two markers will be useful for introducing CRb into other cultivars using marker-assisted selection.

Acknowledgments
We thank Nippon Norin Seed Co. for providing the parental lines of ‘Akiriso’ and S. Takashita, T. Miyazaki, and T. Kondo for useful comments. We also thank Mss. S. Negoro, T. Yamakawa and K. Takeuchi for technical assistance.

Literature Cited
Diederichsen, E., M. Frauen, E.G.A. Linders, K. Hatakeyama and M. Hirai (2009) Status and perspectives of clubroot resistance breeding in crucifer crops. J. Plant Growth Regul. 28: 265–281.
Fukuoka, H., T. Nunome, Y. Minamiyama, I. Kono, N. Namiki and A. Kojima (2005) Read2Marker: a data processing tool for microsatellite marker development from a large data set. Biotechniques 39: 472–476.
Hatakeyama, K., M. Fujimura, M. Ishida and T. Suzuki (2004) New classification methods for Plasmodiophora brassicae field isolates in Japan based on resistance of F1 cultivars of Chinese cabbage (Brassica rapa L.) to clubroot. Breed. Sci. 54: 197–201.
Hatakeyama, K., A. Horisaki, S. Niikura, Y. Narusaka, H. Abe, H. Yoshiaki, M. Ishida, H. Fukuoka and S. Matsumoto (2010) Mapping of quantitative trait loci for high level of self-incompatibility in Brassica rapa L. Genome 53: 257–265.
Hayashida, N., Y. Takabatake, N. Nakazawa, D. Aruga, H. Nakanishi, G. Taguchi, K. Sakamoto and E. Matsumoto (2008) Construction of a practical SCAR marker linked to clubroot resistance in Chinese cabbage, with intensive analysis of HC352b genes. J. Jpn. Soc.
Clubroot resistance locus conferring resistance to pathotype group 3

Hortic. Sci. 77: 150–154.

Hirai, M. (2006) Genetic analysis of clubroot resistance in Brassica rapa. Breed. Sci. 56: 223–229.

Jones, C.J., K.J. Edwards, S. Castaglione, M.O. Winfield, F. Sala, C. van de Wiel, G. Bredemeijer, B. Vesman, M. Matthes, A. Daly et al. (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed. 3: 381–390.

Kakizaki, T., T. Kato, N. Fukino, M. Ishida, K. Hatakeyama and S. Matsumoto (2011) Identification of quantitative trait loci controlling late bolting in Chinese cabbage (Brassica rapa L.) parental line NOU 6 gous. Breed. Sci. 61: 151–159.

Kuginuki, Y., H. Yoshikawa and M. Hirai (1999) Variation in virulence of Plasmodiophora brassicae in Japan tested with clubroot-resistant cultivars of Chinese cabbage (Brassica rapa ssp. pekinensis). Eur. J. Plant Pathol. 105: 327–332.

Mun, J.H., S.J. Kwon, Y.J. Seol, J.A. Kim, M.H. Lim et al. (2010) Sequence and structure of Brassica rapa chromosome A3. Genome Biol. 11: R94.

Nagaoka, T., M.A. Doullah, S. Matsumoto, S. Kawasaki, T. Ishikawa, H. Hori and K. Okazaki (2010) Identification of QTLs that control clubroot resistance in Brassica oleracea and comparative analysis of clubroot resistance genes between B. rapa and B. oleracea. Theor. Appl. Genet. 120: 1335–1346.

Piao, Z.Y., Y.Q. Deng, S.R. Choi, Y.J. Park and Y.P. Lim (2004) SCAR and CAPS mapping of CRb, a gene conferring resistance to Plasmodiophora brassicae in Chinese cabbage (Brassica rapa ssp. pekinensis). Theor. Appl. Genet. 108: 1458–1465.

Piao, Z.Y., N.Ramhiary and Y.P. Lim (2009) Genetics of clubroot resistance in Brassica species. J. Plant Growth Regul. 28: 252–264.

Saito, M., N. Kubo, S. Matsumoto, K. Suwabe and M. Hirai (2006) Fine mapping of the clubroot resistance gene Crr3 in Brassica rapa. Theor. Appl. Genet. 114: 81–91.

Sakamoto, K., A. Saito, N. Hayashida, G. Taguchi and E. Matsumoto (2008) Mapping of isolate-specific QTL for clubroot resistance in Chinese cabbage (Brassica rapa L. ssp. pekinensis). Theor. Appl. Genet. 117: 759–767.

Suwabe, K., H. Tsukada, H. Iketani, K. Hatakeyama, M. Fujimura, T. Nunome, H. Fukuoka, S. Matsumoto and M. Hirai (2003) Identification of two loci for resistance to clubroot (Plasmodiophora brassicae Worrin) in Brassica rapa L. Theor. Appl. Genet. 107: 997–1002.

Suwabe, K., H. Tsukazaki, H. Iketani, K. Hatakeyama, M. Kondo, M. Fujimura, T. Nunome, H. Fukuoka, M. Hirai and S. Matsumoto (2006) Simple sequence repeat-based comparative genomics between Brassica rapa and Arabidopsis thaliana: the genetic origin of clubroot resistance. Genetics 173: 309–319.

Voorrips, R.E. (1995) Plasmodiophora brassicae: Aspects of pathogenesis and resistance in Brassica oleracea. Euphytica 83: 139–146.

Williams, P.H. (1966) A system for the determination of races of Plasmodiophora brassicae that infect cabbage and rutabaga. Phytopathology 56: 624–626.

Yoshikawa, H. (1981) Breeding for clubroot resistance in Chinese cabbage. In: Taleker, N.S. and T.D. Griggs (eds.) Chinese Cabbage, AVRDC, Shanhua, Tainan, pp. 405–413.