Theor Appl Genet (2009) 118:227–234
DOI 10.1007/s00122-008-0891-3

ORIGINAL PAPER

Quantitative trait locus responsible for resistance to Aphanomyces root rot (black root) caused by *Aphanomyces cochlioides* Drechs. in sugar beet

Kazunori Taguchi · Naoki Ogata · Tomohiko Kubo · Shinji Kawasaki · Tetsuo Mikami

Received: 31 July 2007 / Accepted: 9 September 2008 / Published online: 24 September 2008

Abstract Aphanomyces root rot, caused by *Aphanomyces cochlioides* Drechs., is one of the most serious diseases of sugar beet (*Beta vulgaris* L.). Identification and characterization of resistance genes is a major task in sugar beet breeding. To ensure the effectiveness of marker-assisted screening for Aphanomyces root rot resistance, genetic analysis of mature plants' phenotypic and molecular markers' segregation was carried out. At a highly infested field site, some 187 F_2 and 66 F_3 individuals, derived from a cross between lines 'NK-310mm-O' (highly resistant) and 'NK-184mm-O' (susceptible), were tested, over two seasons, for their level of resistance to Aphanomyces root rot. This resistance was classified into six categories according to the extent and intensity of whole plant symptoms. Simultaneously, two selected RAPD and 159 'NK-310mm-O'-coupled AFLP were used in the construction of a linkage map of 695.7 cM. Each of nine resultant linkage groups was successfully anchored to one of nine sugar beet chromosomes by incorporating 16 STS markers. Combining data for phenotype and molecular marker segregation, a single QTL was identified on chromosome III. This QTL explained 20% of the variance in F_2 population (in the year 2002) and 65% in F_3 lines (2003), indicating that this QTL plays a major role in the Aphanomyces root rot resistance. This is the first report of the genetic mapping of resistance to *Aphanomyces*-caused diseases in sugar beet.

Communicated by F. van Eeuwijk.

K. Taguchi (✉) · N. Ogata
Memuro Upland Farming Research Station,
National Agricultural Research Center for Hokkaido Region
(NARCH), Hokkaido, Japan
e-mail: ktaguchi@naro.affrc.go.jp

T. Kubo · T. Mikami
Laboratory of Genetic Engineering,
Research Faculty of Agriculture,
Hokkaido University, Hokkaido, Japan

S. Kawasaki
Plant Science
Division, National Institute of Agrobiological Sciences (NIAS),
Tsukuba, Japan

Introduction

In most sugar beet growing regions, including those in North America, Europe and Asia, the oomycete *Aphanomyces cochlioides* Drechs. is a serious soil-borne pathogen. In the United States, losses equivalent to 1% of the entire crop have been attributed to *A. cochlioides* (Luterbacher et al. 2005; Papavizas and Ayers 1974), making it one of the major targets of pathogen control under sugar beet production. *A. cochlioides* can be the causative agent of two diseases: acute seedling disease, also known as damping off, and chronic rot of mature roots (Aphanomyces root rot), also known as black root or black leg (Duffus and Ruppel 1993; Panella 2005).

Typically, the first symptom of seedling damping off can be seen one to 3 weeks after germination, as a dark gray, water-soaked lesion on the hypocotyl (Duffus and Ruppel 1993). The lesion rapidly expands, reducing the seedling's vigor, and the infected seedlings may fall over and die (Duffus and Ruppel 1993). Chemical fungicides, such as hymexazol and thiram, applied to the seed balls at planting, are effective in controlling this disease (Duffus and Ruppel 1993; Francis and Luterbacher 2003). In Japan, sugar beet is grown exclusively in the northern Hokkaido prefecture, where, by virtue of fungicides and cultivation practices, including the transplanting of nursery grown plantlets,

seedling damping off is now completely suppressed. However, it should be noted that continuous application of chemical agents could result in their accumulation in the soil and the emergence of tolerant races.

Black root can also emerge on the survivors of damping off or on mature roots located in infectious fields. The severity of black root is influenced by soil moisture and temperature: wet, warm conditions enhancing the disease's prevalence, increasing its intensity, and rendering it of special concern in such areas (Duffus and Ruppel 1993; Panella 2005). Root rot, mainly caused by *A. cochlioides*, remains a serious problem in Hokkaido, as, on a year to year basis, 20% of sugar beet fields suffer more or less from this disease (Statistical report by Hokkaido Plant Protection Office. 1999–2006). Symptoms include rotting of the root, wilting and poor top growth, and in severe cases, the formation of abundant lateral roots (Duffus and Ruppel 1993), all leading to a significant loss in yield. Because of the need to protect the enlarging root throughout its development, single application chemical control of Aphanomyces root rot is inadequate (Luterbacher et al. 2005; Asher 1993). Field rotations have been proposed as one means to control black root (Duffus and Ruppel 1993); however, once black root emerges, even in the absence of a sugar beet crop, the oomycete can persist in the soil for at least 3 years (Beal et al. 2002). Thus, the identification of resistant sources and the introduction of resistance into breeding lines will clearly lead to a more definitive solution to this issue.

Since the early 1940s, attempts have been made to identify sources of *Aphanomyces* resistance (Panella 2005). While screening of breeding lines under greenhouse conditions (Coe and Schneider 1966) provided current commercial hybrids' sources of resistance (cited in Panella 2005), such screening programmes continue. A recent survey revealed that wild relatives of beet (e.g., *Beta vulgaris* ssp. *maritima*) are potential sources of resistance (Francis and Luterbacher 2003; Luterbacher et al. 2005). However, such screening was largely done at the seedling level, with few mature plants ever having been screened. Greenhouse seedling-stage resistance does not necessarily guarantee resistance at the field level (but see Schneider 1978). In a series of laborious and time-consuming trials undertaken in *Aphanomyces*-infested fields, mature pea (*Pisum sativum* L.) plant phenotypes were assessed for their resistance to the root rot caused by *Aphanomyces euteiches* Drechs. (Pilet-Nayel et al. 2002). In a similar manner, we have succeeded in screening resistance sources from a number of sugar beet lines (Taguchi et al. 1999), indicating that field trials are also feasible in sugar beet.

Little is known regarding the genetics of the resistance to *Aphanomyces*-caused diseases. Bochstahler et al. (1950) indicated that resistance to *Aphanomyces* was expressed in a dominant manner; however, details regarding number, map position and products encoded by the resistance genes are still unclear (Panella 2005). Largely due to environmental factors that affect screening at the field level, mass selection for resistant phenotypes in infested-field trials has led to little progress. Thus, if one intends to employ marker assisted selection (MAS), detailed genetic information, as outlined above, will be essential (Panella 2005). To avoid the ambiguity of field tests, line tests, in combination with quantitative trait analysis, must be implemented.

In order to introduce resistance into Japanese sugar beets lines, we have launched a multifaceted research program on sugar beet resistance to *Aphanomyces*-caused diseases. Our ultimate goal is to establish a disease resistance MAS system within our breeding program. While, in an initial step, field trials identified a sugar beet line with a high level of resistance to *Aphanomyces* root rot (Taguchi et al. 1999), we now seek to characterize the genetic nature of this resistance. For the past decade, genetic mapping of sugar beet has been performed using molecular markers such as RFLP, AFLP, RAPD and SSR (reviewed in Skaracis 2005). However, in spite of these achievements, widely used markers applicable to fine scale mapping remained insufficient; therefore, we deemed it more expedient to construct a molecular genetic map on our own, using the progeny of hybrids derived from crosses between the resistant and susceptible lines. A combination of phenotypic and mapping data would reveal the genetic loci involved in conferring resistance. Here, we report the first QTL responsible black root resistance in sugar beet.

Materials and methods

Plant materials

'NK-310mm-O', a sugar beet maintainer line (O-type), was used as a donor parent with a high level of resistance to black root (Taguchi et al. 2004). A single 'NK-310mm-O' was crossed with a single 'NK-184mm-O', a sugar beet line showing susceptibility to black root. 'NK-310mm-O' is self-incompatible whereas 'NK-184mm-O' is self-fertile. The F_2 population was derived from a single F_1 plant of 'NK-310mm-O' × 'NK-184mm-O'. To produce F_3 lines, each of the F_2 plants was physically isolated to prevent cross-pollination.

Evaluation of resistance to black root

Seeds were sown in paper pots in early April, and the germinated seedlings allowed to grow 40 days in a green house under natural day length in from 5 to 15°C. Seedlings were transplanted into an *Aphanomyces*-infested field in Ikeda, Hokkaido, Japan. For at least 5 years before our

study this field had shown consistent and typical evidence of black root infestation. An area of high density in *Aphanomyces* oomycetes was chosen. In 2002, the two parental lines, F_1 plants and 187 F_2 plants were tested in a single plot. Parental lines and F_1 with four replications consisted of ten plants. In 2003, a complete randomized block with four replications was implemented for parental lines, F_1 plants and 66 F_3 lines with 120 F_2 plants (same population in 2002). Each of the parental lines, F_1 and F_3 lines with four replications consisted of ten plants. Sugar beets were harvested by hand in early October and whole plants assessed for disease symptoms according to an index of root rot severity ranging from 0 (no symptoms) to 5 (fully decayed). The typical appearance of plants in each class is illustrated in Fig. 1. Data for F_3 lines were averaged across replications.

DNA isolation and genotyping with molecular markers

Total cellular DNA was extracted from fresh leaves according to the procedure described by Roger and Bendich (1988). Amplified fragment length polymorphism (AFLP) was detected by using an AFLP Analysis System I (Invitrogen). The restriction endonucleases *Eco*RI and *Mse*I were used in this analysis. The adapter-ligated DNA was preamplified with primers having a single selective nucleotide. For selective amplification, *Eco*RI-NNN and *Mse*I-NNN primers were employed. The amplified products were electrophoresed in a high efficiency genome scanning (HEGS) system (Kawaguchi et al. 2001; Hori et al. 2003; Kikuchi et al. 2003) using discontinuous non-denatured acrylamide gel and TBE buffer. The gels were scanned after staining with Vistra Green I (GE Healthcare) and photographed under a UV transilluminator (ATTO). Procedures for random amplified polymorphic DNA (RAPD) were basically the same as described in Uphoff and Wricke (1992), in which a pair of 10-mer primers was used. The cycling parameters were 40 cycles of 94°C for one min, 35°C for one min and 72°C for one min, followed by one cycle at 72°C for 10 min. The amplified products were electrophoresed in a 2% agarose gel. Cleaved amplified polymorphic sequence (CAPS) markers were developed. Using primers of single nucleotide polymorphism (SNP) marker sets (Möhring et al. 2004), PCR products were generated from 'NK-310mm-O' and 'NK-184mm-O', then digested with one of thirteen restriction endonucleases: *Hae*III, *Hha*I, *Taq*I, *Hap*II, *Mbo*I, *Afa*I, *Xsp*I, *Alu*I, *Acc*II (Takara Bio, Ohtsu, Japan), *TspE*I (TOYOBO, Osaka, Japan), *Mse*I, *HpyCh4*IV, *Nla*III (New England BioLabs). The resultant fragments were electrophoresed in a 2% agarose gel to check for polymorphism.

Linkage map construction and QTL mapping

Segregation of the polymorphic bands in the F_2 population was checked for its coupling to 'NK-310mm-O' based on the banding pattern of the parental lines. The multiple segregation data were manually scored using an MS-Excel spreadsheet (Microsoft Japan, Tokyo, Japan), then analyzed using MAPL98 (Ukai et al. 1995). The derived AFLP, RAPD and CAPS markers were grouped at a logarithm of odds (LOD) threshold of 3.0 and a maximum distance of 25 cM. Marker order in each of the linkage groups was verified by using MAPMAKER/EXP ver 3.0 (Lander et al. 1987). The Kosambi mapping function was used to calculate the map distance. QTL analysis was carried out by using composite interval mapping (CIM) methods, with Win QTL Cartographer ver 2.5 (Basten et al. 2005). Using the permutation test with 1,000 permutations, a mean LOD threshold was chosen to declare a putative QTL significant.

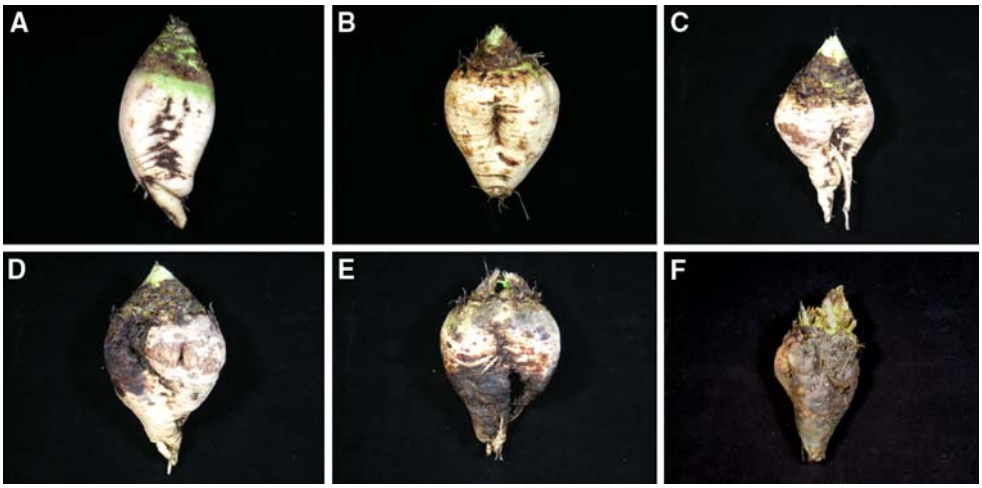

Fig. 1 Visual symptoms of Aphanomyces root rot resistance. **a** Index 0 (no symptoms), **b** index 1 (symptoms apparent), **c** index 2 (root browning observed), **d** index 3 (root rot apparent), **e** index 4 (>50% root rot), **f** index 5 (near complete root rot, death of root)

Results

Phenotype of black root resistance

For the genetic characterization of the black root resistance harbored by 'NK-310mm-O', a single resistant 'NK-310mm-O' plant was crossed with a single susceptible 'NK-184mm-O' plant, and their progeny were generated. We employed field trials to assess whole plant symptoms in mature beets. In the infested field, 'NK-310mm-O' exhibited elevated resistance to black root, scoring (mean ± standard errors) 0.06 ± 0.23 (2002) and 0.21 ± 0.04 (2003) on the root-rot intensity index. Comparatively, 'NK-184mm-O' scored 4.84 ± 0.42 (2002) and 4.81 ± 0.05 (2003) (Fig. 2). Their F_1 offspring had resistance scores of 1.0 ± 0.48 and 0.94 ± 0.10 for 2002 and 2003, respectively. Therefore, resistance appeared to behave in a dominant manner. Resistance index values for the combined total F_2 population were 0.83 ± 0.80 (2002) and 1.59 ± 1.37 (2003) and F_3 lines were 1.73 ± 0.87 (2003).

DNA polymorphism coupling to the resistant line and construction of a linkage map

As a second step toward the genetic analysis of resistance to black root, a linkage map was constructed using DNA markers such as AFLP, RAPD and CAPS. In our AFLP analysis with HEGS, we detected, on average, 13 fragments per single primer combination, of which five were polymorphic between the parental lines. A total of 312 primer combinations, generating more than 4,000 DNA fragments, were tested for polymorphism. As a result, 159 polymorphic bands from 97 primer combinations were subjected to further study. In our RAPD analysis, we tested 50 primer combinations, resulting in the identification of two polymorphic bands from two primer combinations.

Next, of all the polymorphic bands obtained from AFLP and RAPD analysis, we selected the DNA bands specifically amplified from the resistant parent 'NK-310mm-O' and their segregation in F_2 population was investigated. The segregation data was used for the construction of a linkage map, including 159 AFLP and two RAPD markers. As a result, all the markers were classified into nine linkage groups in a linear array (Fig. 3).

We next sought to assign each of the resultant linkage groups to one of the nine sugar beet chromosomes (Butterfass 1964; Schondelmaier and Jung 1997). Based on the primer sequences of the SNP marker sets (Möhring et al. 2004), we generated CAPS markers for genetic analysis. Of 22 primer combinations with 13 restriction endonucleases, 16 were polymorphic between the parental lines (Table 1). Segregation data of the 16 CAPS markers was successfully incorporated into the data set of the F_2 population. In the resultant linkage map, arrangement of CAPS markers generally agreed with a previous report (Möhring et al. 2004), indicating the fidelity of our map. Considering these CAPS markers as sequence tagged sites (STS), it was assumed that each of the linkage groups was assigned to one of the nine sugar beet chromosomes (Fig. 3). The map covers 695.7 cM and the mean distance between loci is 4.6 cM. However, the density of the markers was uneven: clusters of markers were observed on chromosomes II, III, IV, V, VII and VIII, whereas markers were scarce on chromosome VI. We detected significant ($P \leq 0.05$) segregation distortion of 18 markers, all located on chromosome V.

QTL for Aphanomyces root rot resistance

To identify the genomic region responsible for Aphanomyces root rot resistance, we sought correlations between root-rot intensity index and genotype by CIM methods. Figure 4 shows the profiles of LOD score obtained through CIM analysis. In both years a peak LOD score was consistently

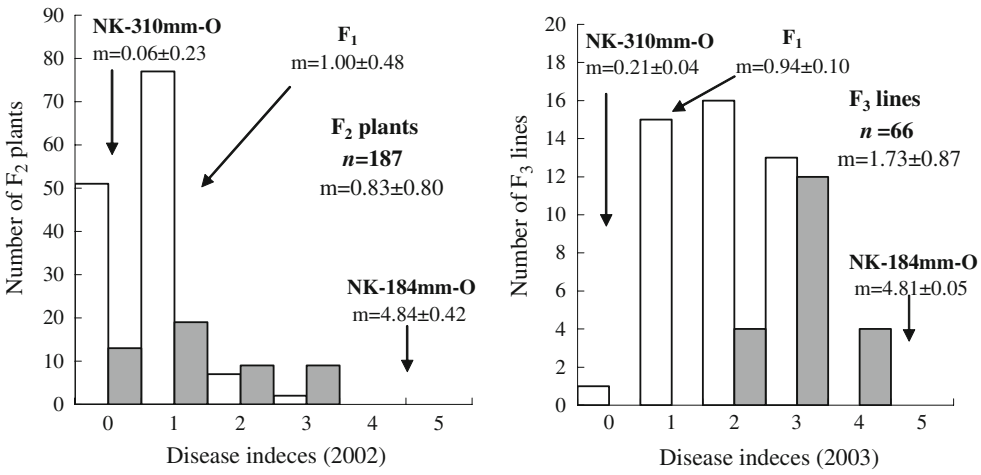

Fig. 2 Frequency distribution for Aphanomyces root rot indices in F_2 population (**a**) and F_3 lines (**b**) of 'NK-310mm-O' × 'NK-184mm-O'. Different genotypic classes in F_2 population and F_3 lines, as defined at the nearest marker locus for the QTL peak on chromosome III. *White* represents individuals homozygous or heterozygous for the allele from the resistant parent and *gray* represents individuals homozygous for allele from the susceptible parent

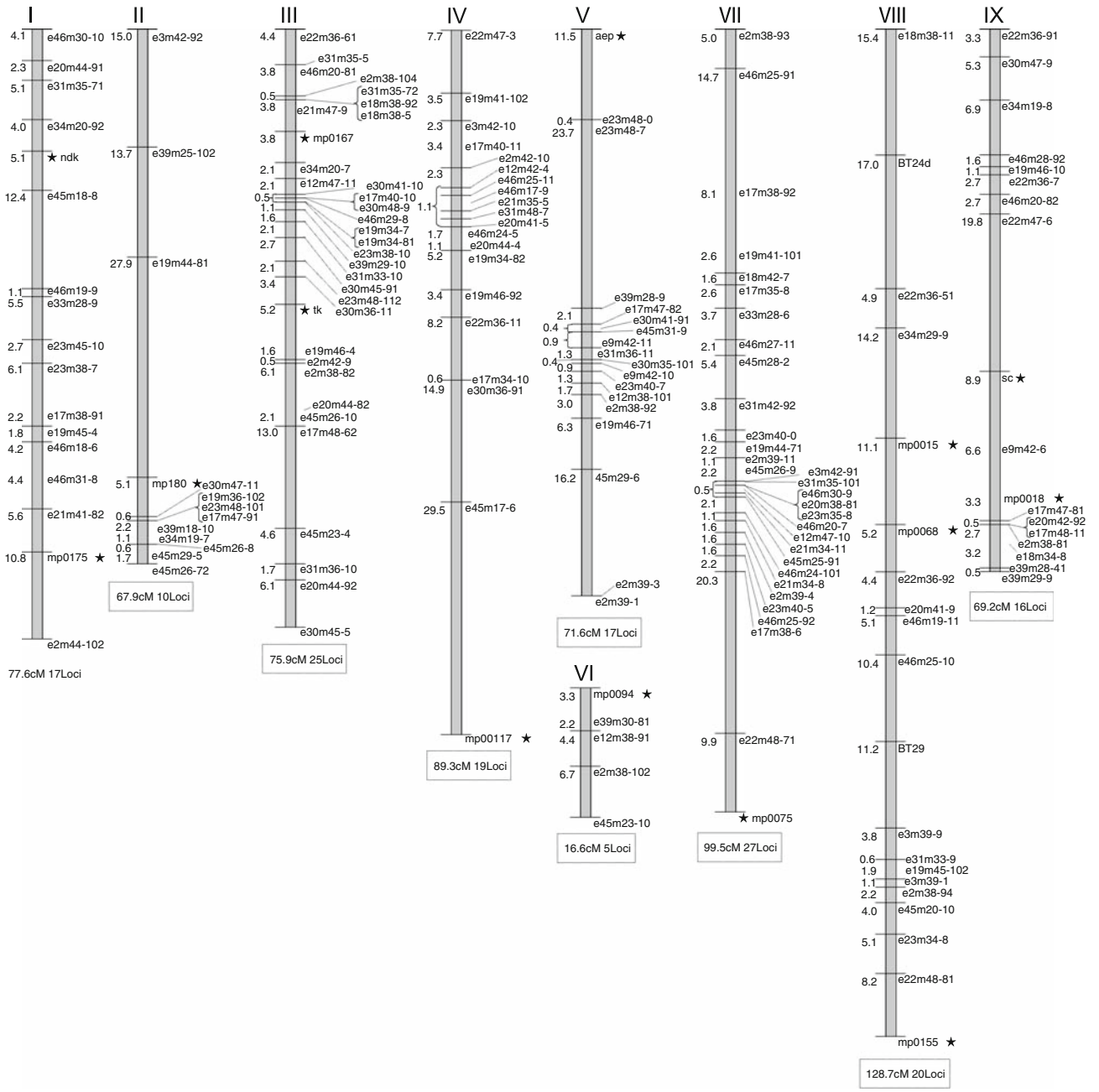

Fig. 3 Linkage map based on F_2 population (NK-310mm-O/NK-184mm-O). Markers with the prefix "*stars*" were STS-CAPS markers supplied by Möhring et al. (2004) and "BT" were RAPD markers. Markers described "e**M**-**" were AFLP markers. Marker intervals are indicated in cM. The total map length is 695.7 cM

detected on chromosome III, indicating the presence of a QTL for Aphanomyces root rot resistance (details shown in Table 2). When an alternative SIM method was used, the LOD score peak was detected at the same position of chromosome III. According to both these methods, the resistant allele was derived from 'NK-310mm-O'. Based on these observations we conclude that there is a QTL for Aphanomyces root rot resistance on chromosome III, and have designated it *qAcr1* (for *A. cochlioides* resistance 1). The *qAcr1* is confined between two markers, *e45m23-4* and *e19m46-4*, and is 5.2 cM away from the CAPS marker *tk*, and 26.7 cM away from the CAPS marker *mp0167* (Fig. 3). The phenotypic variance explained by *qAcr1* is 20% in the 2002 F_2 population and 65% in the 2003 F_3 lines.

Discussion

In the present study, we mapped 177 markers distributed throughout the nine sugar beet chromosomes as a first step

Table 1 Primer design for RAPD markers and STS markers (Möhring et al. 2004)

Marker	Primer sequence		
RAPD			
BT29	CAACAAGGAC		
BT24d	CAACAACTGC		
Marker	**Primer forward (5′ → 3′)**	**Primer reverse (5′ → 3′)**	**Enzyme**
STS-CAPS			
ndk	GTTGTTGCTATGGTCTGGGA	ATGAGTGWAGGCTGCTYTGC	*Hap*II
mp0175	ATACCACAACTTGCGGTTGC	GGCAATTCTTGAACACGCAG	*Hae*III
mp0180	AAAGGCTCCAACTAACCTCC	ACAGGTTCATCGTGCTACAC	*HpyCH4*V
mp0167	GAGAATGTAGGATCAGCGAAG	TGCAGACGTAAACAGTGTG	*Afa*I
tk	GGTTTTGGSTCTCCTAACAAG	GAGCATMAGAATGTTGGGCAT	*Xsp*I
mp0117	GCAGTCATCACATTTCACAATC	AGGATCGACAAATTGATGGAAG	*Hae*III
aep	GAATCAAGGACGGGAAGTTC	AGCGAGATTGACTGGAGTTG	*Afa*I
mp0094	AGTCACAGCAAGAGGGGATAAG	TGTGGGGCTGATAGAATCGTC	*Acc*II
mp0075	ACCTTTATTACAGCCAAGTGCC	ATCTTATACCCAGCCCAGCAAC	*Hap*II
mp0015	CTGCTTTCAGAGGCAAGAAG	CTCCTCTTCACAATATCTTGC	*Afa*I
mp0068	AGCTTCTCTCTGCTTCAAGC	CTCCATCCTCTAGTTTCTCC	*Acc*II
mp0155	GCGATAAGAACAAGCACCAAC	TGATGATGCTGACAGGATCAC	*Hap*II
sc	CAGCTGGTAGAACATCCGAT	TCCAGCACTCTGAAAGATCC	*HpyCH4*V
mp0018	AAGCAAACACAGCATTAGCC	GTATGCAAAGTCCAGACAGAAG	*Hae*III

STS-CAPS markers indicate the restriction enzyme employed in digestion

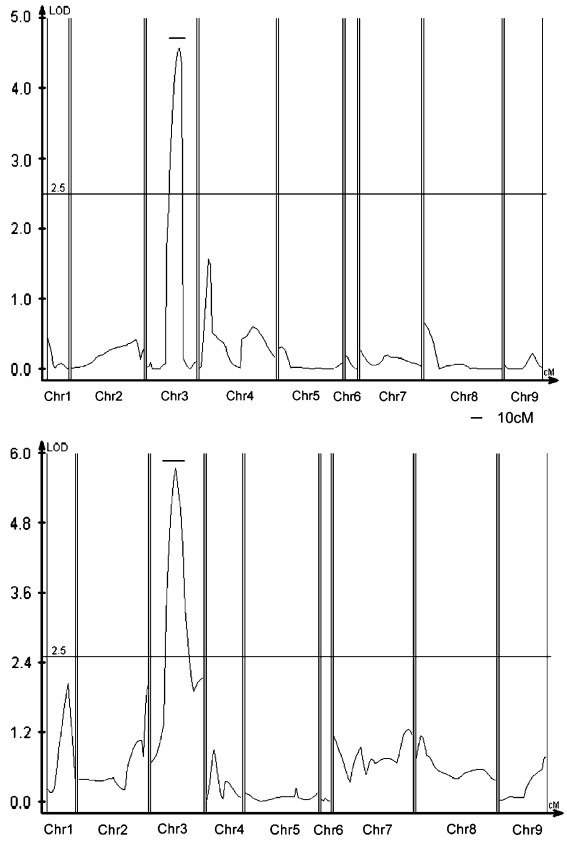

Fig. 4 Putative QTLs for Aphanomyces root rot detected in F_2 population (**a**) and F_3 lines (**b**) by CIM method. A significant region was detected on chromosome III

to identify a QTL for Aphanomyces root rot resistance. This process was necessary because both the resistant strain 'NK-310mm-O' and susceptible strain 'NK-184mm-O' had never been subjected to a mapping study. Molecular markers were mainly obtained from the combination of AFLP and HEGS. Although the number of discrete bands detected in single electrophoresis is smaller than that of a system using fluorescent- or radio-labeled primers (Hansen et al. 1999; El-Mazawy et al. 2002; Hagihara et al. 2005), we proceeded to map the genome in such a manner, due to the rather high proportion of polymorphic bands (38%). In an outbreeding crop such as sugar beet, combination of AFLP and HEGS can provide an alternative method to obtain a linkage map without labeled primers.

Our map includes 16 CAPS markers which are shared with that reported by Möhring et al. (2004). Considering the 16 markers as STS, each of our linkage groups was successfully assigned to one of the nine sugar beet chromosomes. All the markers on the map segregated in Mendelian manner except those on chromosome V: these showed significant segregation distortion. This phenomenon is frequently observed in mapping studies of sugar beet. Various loci throughout the nine chromosomes showed aberrant segregation in different cross-combinations (Wanger et al. 1992; Pillen et al. 1993; Schumacher et al. 1997). It is assumed that segregation distortion is caused by lethal loci, abnormal chromosome organization, or gametic selection. Crossing barriers caused by these factors would be a problem in sugar beet breeding if the transmission of desired

Table 2 QTL associated with resistance to Aphanomyces root rot identified in the F_2 and F_3 generations of 'NK-310mm-O' × 'NK-184mm-O'

Material	Chromosome	LOD[a]	Significant marker region[b]	R^2 (%)[c]	Additive	Dominance
F_2 population (2002)	3	4.56	e45m26-10–e45m23-4	20.0	−0.24	−0.65
F_3 lines (2003)	3	5.74	e19m46-4–e45m23-4	65.1	−0.50	−1.37

[a] Log of the odd probability of detecting a QTL in a particular place

[b] Position of the significant LOD peak of the QTL in relation to the first marker of given interval

[c] % of explainable variation

trait was hampered by aberrant segregation, as is the case with nematode resistance (Kleine et al. 1998).

The present study represents the first QTL mapping of Aphanomyces root rot resistance in sugar beet. The map position of the LOD score's peak was consistent across all experimental methods and years, showing the QTL, designated as *qAcr1*, to really exist. Since the explained variance of *qAcr1* was 65% in 2003, it seems likely that a major portion of the resistance is controlled by *qAcr1*. Although such QTLs remained unidentified in our present study, we can not exclude the possibility that other minor QTLs exist and confer additive effects, given that *qAcr1* does not explain full resistance. To investigate whether such minor QTLs, if any, exist, we are now conducting an analysis using recombinant inbred lines.

Because the examination area was not able to be maintained, the numbers of F_3 lines was decreased with 1/3 of F_2 at random. It should be noted that the explained variance is lower in the F_2 population than in the F_3 lines. Two reasons for this suggest themselves. First, the emergence of Aphanomyces root rot is influenced by environmental factors such as soil humidity and temperature (Harveson and Rush 1993), which would affect the efficiency of field trial. In fact, disease intensity in 2002 was lower than in 2003, perhaps due to the lesser amount of rainfall and lower temperatures in that year (data not shown). Second, we employed F_3 line test in 2003 compared to an F_2 individual test in 2002, making the error variation in 2003 smaller than that in 2002.

Together with the resistant line 'NK-310mm-O', identification of *qAcr1* will accelerate the breeding program for Aphanomyces root rot resistance. We first crossed 'NK-310mm-O' with nine different CMS lines and tested for black root resistance in the F_1 generation. These showed nearly complete resistance, indicating the substantial potential of 'NK-310mm-O' as a source of resistance (K. Taguchi et al., manuscript in preparation). Flanking the QTL region, there are six AFLP markers coupled to resistance, which may allow us to conduct MAS for Aphanomyces root rot resistance. To date, such information concerning the genetics of *Aphanomyces* resistance has only come to fruition in pea (Pilet-Nayel et al. 2002).

Although *qAcr1* was discovered through the field trial, we have noticed that it also confers resistance to seedling damping off in the greenhouse. This raises a question about the relationship between the nature of resistance borne by 'NK-310mm-O' and that of other sources screened by greenhouse methods. Although details of genetic aspects are unknown, there are some genetic resources for resistance to *Aphanomyces*-caused diseases which are derived from breeding lines (Taguchi et al. 2004) or wild relatives such as *B. vulgaris* ssp. *maritima* (Luterbacher et al. 2005). Although the whole picture of the resistance, including the number of involved loci and their roles, is unclear, our mapping study of *qAcr1* might shed some light on the genetic mechanism of resistance to *Aphanomyces*.

Acknowledgments The authors wish to thank Chiho Tanaka and Hidetaka Tadano for technical assistance. This work was supported in part by the grant from the Ministry of Agriculture, Forestry and Fisheries, Japan (Research project for utilizing advanced technologies in agriculture, forestry and fisheries No. 1423).

References

Asher MJC (1993) Rhizomania. In: Cooke DA, Scott RK (eds) The sugar beet crop (science into practice). Chapman and Hall, Tokyo, pp 311–346

Basten CJ, Weir BS, Zeng ZB (2005) QTL Cartographer. A reference manual and tutorial for QTL mapping. North Carolina State University, Raleigh, pp 30–87

Beal JW, Windels CE, Kinkel LL (2002) Spatial distribution of *Aphanomyces cochlioides* and root rot in sugar beet fields. Plant Dis 86:547–551

Bochstahler HW, Hogaboam GJ, Schneider CL (1950) Further studies on the inheritance of black root resistance in sugar beets. Proc Am Soc Sugar Beet Technol 6:104–107

Butterfass H (1964) Die Chloroplastenzahlen in verschiednartigen Zellen trisomer Zuckerrruben (*Beta vulgaris* L.). Z Bottanik 52:46–77

Coe GE, Schneider CL (1966) Selecting sugar beet seedlings for resistance to *Aphanomyces cochlioides*. J Am Soc Sugar Beet Technol 14:164–167

Duffus JE, Ruppel EG (1993) Aphanomyces seedling diseases and root rot. In: Cooke DA, Scott RK (eds) The sugar beet crop (science into practice). Chapman and Hall, Tokyo, pp 369–372

El-Mazawy A, Dreyer F, Jacobs G, Jung C (2002) High-resolution mapping of the bolting gene B of sugar beet. Theor Appl Genet 105:100–105

Francis SA, Luterbacher MC (2003) Identification and exploitation of novel disease resistance genes in sugar beet. Pest Manag Sci 59:225–230

Hansen M, Kraft T, Christiansson M, Nilsson NO (1999) Evaluation of AFLP in Beta. Theor Appl Genet 98:845–852

Hagihara E, Itchoda N, Habu Y, Iida S, Mikami T, Kubo T (2005) Molecular mapping of a fertility restorer gene for Owen cytoplasmic male sterility in sugar beet. Theor Appl Genet 111:250–255

Harveson RM, Rush CM (1993) An environmentally controlled experiment to monitor the effect of Aphanomyces root rot and Rhizomania in sugar beet. Phytopathology 83:1220–1223

Hori K, Kobayashi T, Shimizu A, Sato K, Takeda K, Kawasaki S (2003) Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theor Appl Genet 107:806–813

Kawaguchi M, Motomura T, Imaizumi-Anraku H, Akao S, Kawasaki S (2001) Providing the basis for genomics in Lotus japonicus: the accessions Miyakojima and Gifu are appropriate crossing partners for genetic analyses. Mol Genet Genomics 266:157–166

Kikuchi S, Taketa S, Ichii M, Kawasaki S (2003) Efficient fine mapping of the naked caryopsis gene (nud) by HEGS (high efficiency genome scanning)/AFLP in barley. Theor Appl Genet 108:73–78

Kleine M, Voss H, Cai D, Jung C (1998) Evaluation of nematode-resistant sugar beet (*Beta vulgaris* L.) lines by molecular analysis. Theor Appl Genet 97:896–904

Lander ES, Green P, Abrahamson J, Barlow A, Daly M (1987) MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

Luterbacher MC, Asher MJC, Beyer W, Mandolino G, Scholten OE, Frese L, Biancardi E, Stevanato P, Mechelke W, Slyvchenko O (2005) Sources of resistance to diseases of sugar beet in related Beta germplasm: II. Soil-borne diseases. Euphytica 141:49–63

Möhring S, Salamini F, Schneider K (2004) Multiplexed, linkage group-specific SNP marker sets for rapid genetic mapping and fingerprinting of sugar beet (*Beta vulgaris* L.). Mol Breed 14:475–488

Panella L (2005) Black root. In: Biancardi E, Campbell L, De Biaggi M, Skaracis GN (eds) Genetics and breeding of sugar beet. Science Publishers, Enfield, pp 101–102

Papavizas GC, Ayers WA (1974) Aphanomyces species and their root diseases in pea and sugar beet—a review. USDA Technical Bulletin 1845, Washington DC

Pilet-Nayel ML, Muehlbauer FJ, McGee RJ, Kraft JM, Baranger A, Coyne CJ (2002) Quantitative trait loci for partial resistance to Aphanomyces root rot in pea. Theor Appl Genet 106:28–39

Pillen K, Steinrucken G, Herrmann RG, Jung C (1993) An extended linkage map of sugar beet (*Beta vulgaris* L.) including nine putative lethal genes and the restorer gene X. Plant Breed 111:265–271

Roger SO, Bendich AJ (1988) Extraction of DNA from plant tissues. Plant Mol Biol Manual A6:1–10

Schneider CL (1978) Use of oospore inoculum of *Aphanomyces cochlioides* to initiate blackroot disease in sugarbeet seedlings. J Am Soc Sugar Beet Technol 20:55–62

Schondelmaier J, Jung C (1997) Chromosomal assignment of the nine linkage groups of sugar beet (*Beta vulgaris* L.) using primary trisomics. Theor Appl Genet 95:590–596

Schumacher K, Schondelmaier J, Barzen E, Steinrucken G, Borchardt D, Weber WE, Jung C, Salamini F (1997) Combining different linkage maps in sugar beet (*Beta vulgaris* L.) to make one map. Plant Breed 116:23–38

Skaracis GN (2005) Genomics in genetic improvement. In: Biancardi E, Campbell L, De Biaggi M, Skaracis GN (eds) Genetics and breeding of sugar beet. Science Publishers, Enfield, pp 234–238

Taguchi K, Ogata N, Takahashi H, Kuranouchi T, Kawakatu M, Tanaka M (1999) Breeding Aphanomyces root rot resistance in sugar beet 1. On site screening for resistance to Aphanomyces root rot in disease-infested field. Proc Jpn Soc Sugar Beet Technol 42:52–58

Taguchi K, Ogata N, Okazaki K, Nakatsuka K (2004) Breeding Aphanomyces root rot resistance in sugar beet 4. Genetic effect of the highly Aphanomyces root rot resistant line "NK-310mm-O". Rep Hokkaido Branch Jpn Soc Breed 45:43–44

Ukai Y, Ohsawa R, Saito A, Hayashi T (1995) MAPL: a package of computer programs for construction of DNA polymorphism linkage maps and analysis of QTLs. Breed Sci 45:139–142

Uphoff H, Wricke G (1992) Random amplified polymorphic DNA (RAPD) markers in sugar beet (*Beta vulgaris* L.): mapping the genes for nematode resistance and hypocotyl colour. Plant Breed 109:168–171

Wanger H, Weber WE, Wricke G (1992) Estimation linkage relationship of isozyme markers in sugar beet including families distorted segregations. Plant Breed 108:88–96