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Isolation and characterization of RAPD-based markers linked to the beet cyst nematode resistance locus (*Hs1*$^{pat-1}$) on chromosome 1 of *B. patellaris*

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Abstract A beet cyst nematode (BCN)-resistant telosomic addition of *B. patellaris* chromosome 1 in *B. vulgaris* was used to isolate 6 RAPD markers linked to the BCN resistance locus *Hs1*$^{pat-1}$. Southern analysis showed that the analyzed RAPD products contain either low-, middle- or high-repetitive DNA. The relative positions of the random amplified polymorphic DNA (RAPD) markers and of the restriction fragment length polymorphism (RFLP) loci corresponding to the low-repetitive RAPD products were determined by deletion mapping using a panel of seven nematode-resistant *B. patellaris* chromosome-1 fragment additions. One RAPD marker, OPB11$_{800}$, was found to be present in two copies on the long arm telosome of *B. patellaris* chromosome 1. These copies are closely linked to the BCN resistance gene and flank the gene on both sides. On the basis of the nucleotide sequence of OPB11$_{800}$, sequence-tagged site (STS) primers were developed that amplify specific fragments derived from the two OPB11$_{800}$ loci. These STS markers can be used in the map-based cloning of the BCN gene, as they define start and finishing points of a chromosomal walk towards the *Hs1*$^{pat-1}$ locus. Two copies of the middle-repetitive OPX2$_{1100}$ marker were mapped in the same interval of the deletion mapping panel as the resistance gene locus and thereby belong to the nearest markers as yet found for the BCN gene in *B. patellaris*.

Key words Randomly amplified polymorphic DNA (RAPD) · Deletion mapping · Sequence Tagged Site (STS) · Monosomic fragment additions · Beet cyst nematode resistance · *Heterodera schachtii* Schm · *Beta patellaris*

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Introduction

Beet cyst nematodes (*Heterodera schachtii* Schm.) are an important pest in sugar beet (*Beta vulgaris* L.) cultivation and can cause crop losses exceeding 30% of the maximum root yield (Cooke 1991). In *B. vulgaris* no useful level of nematode resistance is present, but complete resistance against *H. schachtii* has been found in wild beets of the section *Procumbentes* (=*Patellaris*) of the genus *Beta*, *Beta procumbens* Chr. Sm, *B. webbiana* Moq. and *B. patellaris* Moq. (Hijner 1952; Yu 1984; Coons 1975). As attempts to introduce nematode resistance into *B. vulgaris* through interspecific hybridization have not resulted in the stable insertion of the transferred genes for resistance (Lange et al. 1990), the isolation of a gene for beet cyst nematode (BCN) resistance and the subsequent introduction of the gene into *B. vulgaris* seems to be the way of choice to obtain resistant sugar beet varieties. As no products of the resistance gene are known at present, the most promising approach to isolate the BCN resistance gene is by positional cloning (Wicking and Williamson 1991). A prerequisite for this cloning technique is the saturation with molecular markers of the chromosomal region that harbors the gene of interest. For this purpose restriction fragment length polymorphism (RFLP) markers already have proven their value as many plant resistance genes directed against various pathogens have been tagged with these markers (see for instance Young et al. 1988; Sarfatti et al. 1989; Barone et al. 1990; Klein-Lankhorst et al. 1991a; Schüller et al. 1992; Kreike et al. 1993). Two beet cyst nematode resistance loci present on chromosomes 1 of *B. patellaris* (*Hs1*$^{pat-1}$) and *B. procumbens* (*HS1*$^{pro-1}$) (nomenclature according to Lange et al. 1993) have also been tagged with RFLP markers (Salentijn et al. 1992; Jung et al. 1992; Salentijn et al. 1994).

The use of molecular markers based on the random amplification of DNA, such as random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) and arbitrary primed (AP) -polymerase chain reaction (PCR) markers (Welsh and McClelland 1990) forms a powerful alternative to RFLP markers. An advantage of these markers above RFLP markers is the speed and easiness of the detection procedure and, more important, RAPD markers may be obtained from genomic regions that are inaccessible to RFLP analysis (Paran et al. 1991). Several resistance genes have also been tagged with this kind of markers, including the *Pto* gene conferring resistance to *Pseudomonas syringae* pv. tomato in tomato (Martin et al. 1991), several *Dm* genes conferring resistance against downy mildew in lettuce (Paran et al. 1991; Michelmore et al. 1991) and the tomato *Mi* gene directed against the root knot nematode *Meloidogyne incognita* (Klein-Lankhorst et al. 1991b). Unfortunately, in addition to the advantages of RAPDs mentioned above, several handicaps are also adherent to RAPD markers: a typical RAPD reaction always reveals several non-specific loci and the RAPD technique is sensitive to changes in the experimental conditions, which hamper, for instance, the screening of DNA libraries with RAPD markers. To be able to use RAPD markers for screening purposes they first have to be converted into more "robust" kinds of markers like RFLP markers or PCR-based markers that can reliably amplify single loci, e.g. sequence-tagged site markers (STSs) (Olson et al. 1989) or sequence characterized amplified regions (SCARs) (Paran and Michelmore 1993).

In the present study the isolation of 6 RAPD markers linked to the $Hs1^{pat-1}$ locus in *B. patellaris* is described. The RAPDs were mapped relative to the resistance locus by deletion mapping, and 4 of the markers were converted into RFLP markers. All RFLP markers are shown to detect multiple loci in *B. patellaris*, some of which are tightly linked to the $Hs1^{pat-1}$ locus. One RAPD marker was converted into an STS marker that reliably amplifies two loci flanking the resistance gene on both sides. This STS marker will be of potential importance in the positional cloning of the BCN gene.

Materials and methods

Plant material

AN5 is a telosomic addition carrying the long arm of *B. patellaris* chromosome 1 (Speckmann et al. 1985; Lange et al. 1990). Backcrossing with diploid *B. vulgaris* yielded seven BCN resistant monosomic fragment additions: AN5-8, AN5-72, AN5-90, AN5-109, AN5-120, AN5-203b and (MS2-3 × AN5)-2, each carrying a fragment of the long arm telosome of chromosome pat-1 in a heterogenous diploid *B. vulgaris* background. Other plant materials, viz. the nematode-resistant introgressions B883 (Heijbroek et al. 1988) and AN1-65-2 (Lange et al. 1993) and the fragment addition AN1-89 (De Jong et al. 1986), all containing the *B. procumbens* $Hs1^{pro-1}$ locus, *B. patellaris* and the plants of *B. vulgaris* used in the backcrosses, were obtained from the CPRO-DLO *Beta* Collection.

Testing for nematode resistance

Plants were grown under greenhouse conditions and tested twice for BCN resistance according to Toxopeus and Lubberts (1979). The first test was carried out in 36-ml containers, using 300 larvae per plant. Plants with 0–5 cysts were considered to be resistant and re-tested in 96-ml containers with 900 larvae per plant. In the second test plants having 0–13 cysts were considered to be resistant. In both tests the susceptible plants usually carried more than 50 cysts.

Chromosome studies

All resistant plants were studied cytologically to verify the presence of the extra chromosomes or chromosome fragments. Root tips were pre-treated in aqueous 8-hydroxyquinoline (0.002 *M*, 5 h at 6 °C), fixed in acetic-ethanol (1:3), hydrolized in 1 *M* hydrochloric acid (7 min at 60 °C) and squashed in 45% acetic acid. The preparations were stained by carefully lifting the cover-slip and adding a drop of 1% aqueous crystal violet. The size of the chromosome fragments was estimated through microscopical measurements on mitotic metaphase chromosomes (De Jong et al. 1986) as a percentage of the total genome of 750 Mbp (Arumuganathan and Earle 1991).

DNA methodology

All standard DNA methodologies were carried out according to Maniatis et al. (1982). Total DNA was isolated from leaf tissue using an upscaled version of the method of Dellaporta et al. (1983) that yielded about 50 µg of DNA per gram of leaf tissue.

DNA sequencing was performed by the dideoxytriphosphate method (Sanger et al. 1977) with double-stranded DNA and fluorescent dyes attached to the oligonucleotide primers. Analysis was automated using an Applied Biosystems 370A nucleotide sequence analyzer.

Primers and PCR conditions

Ten-mer oligonucleotides were obtained from Operon Technologies, Alameda, Calif. Relevant primer sequences are: OPB11 5′GTAGACCCGT 3′, OPX2 5′TTCCGCCACC 3′, OPX15 5′CAGACAAGCC 3′, OPY10 5′CAAACGTGGG 3′, OPZ16 5′TCCCCATCAC 3′ and OPZ18 5′AGGGTCTGTG 3′. RAPD amplifications were performed in 40 µl of 10 m*M* Tris-HCl (pH 9.0), 50 m*M* KCl, 0.01% (w/v) gelatin, 1.5 m*M* $MgCl_2$, 0.01% Triton X-100, 0.1 m*M* dATP, 0.1 m*M* TTP, 0.1 m*M* dCTP, 0.1 m*M* dGTP, 50 ng primer (10-mer), 300 ng template and 0.2 U *Taq* (Boehringer) polymerase. Amplification was carried out in a DNA Thermal Cycler 480 (Perkin Elmer Cetus). Amplification parameters were: 92 °C for 7 min followed by 25 cycles of 1 min at 92 °C, 2 min at 35 °C, 2 min at 72 °C. After these cycles, a further 0.2 U *Taq* polymerase was added and the amplification was continued for another 25 cycles followed by a 5-min incubation at 72 °C. The RAPD products were separated by agarose gel electrophoresis in 1.5% agarose gels and visualized by ethidium bromide staining.

The oligonucleotides 254 (5′CCTAATCCGTCGGGGTATTAG 3′), 255 (5′GTAGACCCGTCAAAATTGAG 3′) and 256 (5′GAGTTTGATGAGGTGTGGTGG 3′) amplifying the $OPB11_{800}$ RFLP loci were synthesized using β-cyanoethyl phosphoramidites in a ABI 381A DNA synthesizer (Applied Biosystems). Amplification was carried out on 100 ng template DNA using 50 ng of each of the primers 254 and 255 during 25 cycles at an annealing temperature of 55 °C. Subsequently, 10% of the amplified DNA was used as template DNA in a second round of amplification of 25 cycles using the primers 254 and 256 under the same conditions.

Molecular cloning of RAPD products

RAPD bands (ca. 50 ng) were isolated from the agarose gels by freeze squeezing and dissolved in 10 µl TE (10 m*M* Tris-HCl pH 8.0, 1 m*M*

EDTA). An aliquot of 2–3 µl was re-amplified using the original RAPD primers and conditions for RAPD amplification. The amplified DNA was separated on a agarose gel (1.5%), isolated by freeze-squeezing and purified by phenol/chloroform extraction. After ethanol precipitation the RAPD product was dissolved in 40 µl TE buffer. The 3'-ends were filled in using Klenow polymerase, whereafter the blunt fragments were cloned into the *Eco*RV site of plasmid pBluescript SK^+, (Stratagene) and transformed to *E-coli* DH5α.

Results

Composition of the deletion mapping panel

The nematode-resistant telosomic addition AN5 carries the long arm of chromosome 1 of *B. patellaris* in a heterogenous background of 18 *B. vulgaris* chromosomes. The backcrossing of AN5 with diploid *B. vulgaris*, which is susceptible to the beet cyst nematode, produced seven BCN-resistant fragment additions. The gametophytic transmission of these monosomic fragments is low, and the incidence of nematode resistance was used to determine the transmission rates (Table 1). The telosomic fragment of AN5 shows a female transmission rate of 18.9%, which is similar to the transmission rate of the entire chromosome (Speckmann et al. 1985; Lange et al. 1990). The smaller fragments, however, have a considerable lower female transmission rate. The fragment additions AN5-90 and AN5-109 have been described previously (Salentijn et al. 1992), while in this study five new BCN-resistant monosomic fragment additions are presented (AN5-72, AN5 × MS, AN5-203b, AN5-120 and AN5-8). The sizes of the alien chromosome fragments were cytologically estimated to be respectively 28 Mbp, 15 Mbp, 12 Mbp, 12 Mbp and 10 Mbp. A rather good correlation between the size of the *B. patellaris* fragments and their transmission frequency could be inferred. Only for AN5-203b and AN5-120 were the transmission rates too high relative to their estimated sizes.

If the assumption is made that all addition fragments have resulted from breakage of the telosomic fragment in AN5 and no major rearrangements have occurred, the *B. patellaris* fragments are collinear and thus can be used as a deletion mapping panel. The fragments were aligned by RFLP analysis using a set of five single-copy markers, CPRO101 to CPRO105 (see Table 1), which are specific for the *B. patellaris* telosome in AN5 (Salentijn et al. 1992). The hypothesized RFLP map resulting from this alignment served as a frame work for the mapping of the obtained RAPD markers (see Fig. 1).

Table 1 The female transmission rates of the beet cyst nematode resistance in monosomic fragment addition material as determined after the second nematode test. The size of the alien chromosome fragments is given in megabase pairs (Mbp) (*n* the number of plants tested for nematode resistance, *101*, *102*, *103*, *104* or *105* presence of AN5-specific RFLP markers CPRO101-105)

Material	Fragment size (Mbp)	*n*	Transmission rate (%)	RFLP markers[a]
AN5	Telosomic (ca.42)	15196[b]	18.9[b]	101-105
AN5-72	28	1343	13.0	101;102 104;105
AN5 × MS	15	361	5.8	101;102 105
AN5-109	13[a]	758[a]	8.8[a]	102
AN5-203b	12	154	12.3	101;102 105
AN5-120	12	292	12.3	101;102 105
AN5-8	10	174	5.2	101;102 105
AN5-90	8[a]	3051[a]	5.1[a]	101

[a] Source: Salentijn et al. 1992
[b] Source: Speckmann et al. 1985; Lange et al. 1990

Isolation and mapping of RAPD markers linked to the $Hs1^{pat-1}$ locus

RAPD markers for the $Hs1^{pat-1}$ locus were isolated by screening *B. patellaris*, the nematode-resistant telosomic addition AN5 and susceptible sibs of AN5 for RAPD loci that are present both in *B. patellaris* and in AN5 but absent in the susceptible sibs. Using 180 random primers, we scored approximately 1260 RAPD loci. Out of this first screening 17 primers (9.4%) were selected. In the next screening the selected primers were used to amplify DNA from each of the seven fragment additions of the deletion mapping panel. The occurrence of the specific RAPD product was determined, as exemplified for a 700-bp product ($OPX15_{700}$) amplified by primer OPX15 (Fig. 2), whereafter the position of the RAPD marker relative to the BCN gene could be deduced. Of the initially selected 17 RAPD primers, 6 gave raise to reproducible amplification patterns when used on the different fragment additions, resulting in the positioning of the markers $OPX2_{1100}$, $OPX15_{700}$, $OPY10_{400}$, $OPZ16_{1300}$, $OPZ18_{600}$ on the map for the $Hs1^{pat-1}$ locus (Fig. 1). As marker $OPB11_{800}$ appeard to be amplified from two different loci it was not added to the map as a RAPD marker, but only as a RFLP marker and STS marker (see below).

Characterization of the RAPD markers

To analyze the nature of the DNA amplified by the RAPD primers, attempts were made to clone all identified RAPD markers. However, 2 of the markers, $OPY10_{400}$ and $OPX15_{700}$, consistently resisted molecular cloning. The other markers were sucessfully cloned, and the nucleotide sequences of the termini of the RAPD markers were determined. In all cases, the nucleotide sequence of the 10-mer primers used to amplify the markers was present at both ends of the cloned fragments (not shown).

The cloned RAPDs were used to probe Southern blots containing *Eco*RV-digested or *Eco*RI-digested DNA from, respectively, *B. vulgaris*, a nematode-suscep-

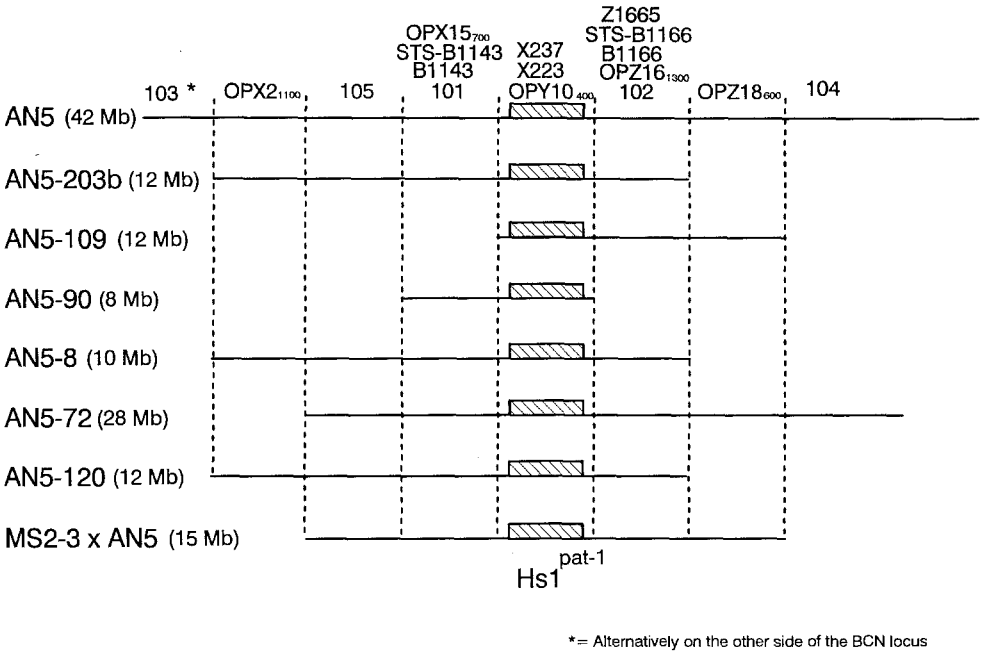

Fig. 1 Map of the $Hs1^{pat-1}$ locus in *B. patellaris*. RAPD markers (*OP*...), STS markers (*STS*-...) and RFLP (CPRO101 to CPRO105, *B1143*, *B1166*, *X237*, *X223*, *Z1665*) markers were mapped relative to the $Hs1^{pat-1}$ locus using a deletion mapping panel consisting of seven monosomic fragment additions

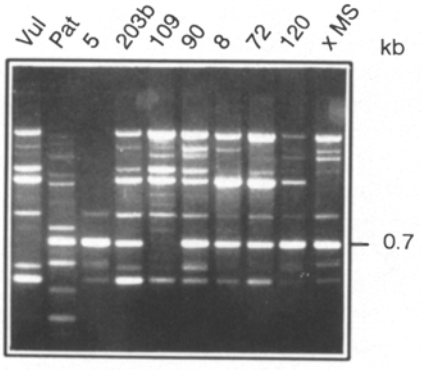

Fig. 2 Differential amplification of RAPD marker $OPX15_{700}$ on DNA from seven resistant monosomic fragment additions (AN5-203b, AN5-109, AN5-90, AN5-8, AN5-72, AN5-120, and AN5 × MS) on DNA from the telosomic addition AN5 and on DNA from *B. vulgaris* and *B. patellaris*

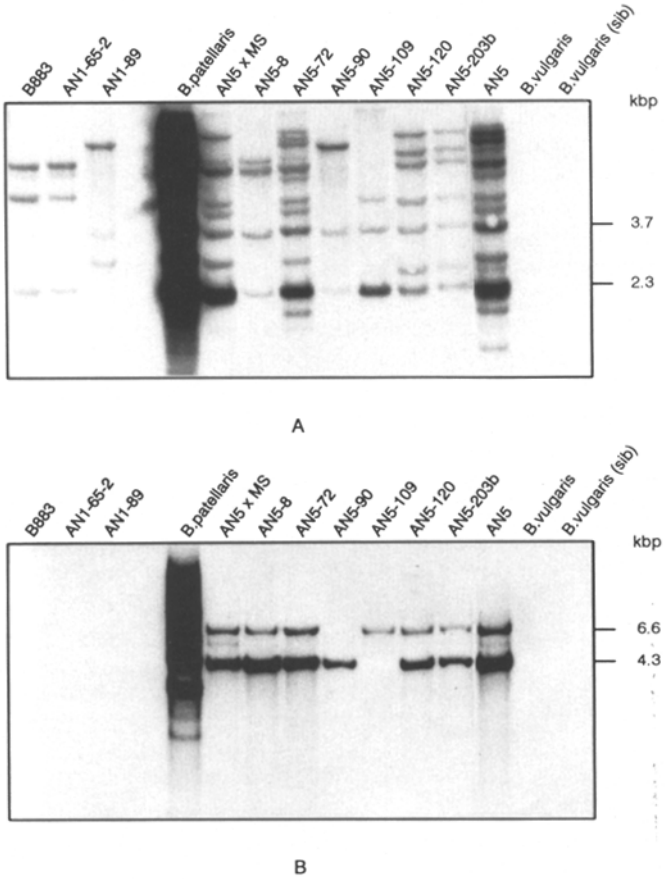

Fig. 3 A,B RFLP patterns detected among *B. patellaris*, *B. vulgaris* and the BCN-resistant monosomic fragment additions and introgressions by cloned RAPD products. **A** $OPX2_{1100}$, **B** $OPB11_{800}$

tible sibling of AN5-203b, *B. patellaris*, the telosomic addition AN5 and the fragment additions forming the deletion mapping panel (Fig. 3 and results not shown). The 4 RAPDs appeared to be *B. patellaris*-specific as none of the cloned fragments hybridized to *B. vulgaris* DNA. All of the cloned RAPDs appeared to contain repetitive DNA when hybridized to *B. patellaris* DNA, ranging from high-repetitive DNA to low-copy DNA; $OPZ18_{600}$ consists of very high-repetitive DNA and is present in an estimated 1000–5000 copies in *B. patellaris*. Also, the telosomic addition and fragment additions contain several hundreds of copies of the $OPZ18_{600}$ sequence (not shown). $OPX2_{1100}$ appears to be a middle-repetitive sequence with an estimated 100–500 copies in *B. patellaris* (Fig. 3A). The telosomic addition AN5 harbors approximately 30 copies of $OPX2_{1100}$, whereas up to 15 different hybridizing *Eco*RI fragments are discernable in the different fragment additions. Two of these fragments with sizes of 2.3 and 3.7 kb were found to be present in all seven fragment additions. The RFLP loci detected by these fragments,

X223 and X237, therefore have to be positioned at the same interval of the mapping panel as the $Hs1^{pat-1}$ locus (see Fig. 1). Copies of OPX_{2100} were also found to be present in nematode-resistant introgressions B883 and AN1-65-2 and the fragment addition AN1-89 carrying the $Hs1^{pro-1}$ locus from *B. procumbens* (Fig. 3A).

Marker $OPB11_{800}$ contains middle-repetitive DNA with an estimated 50–100 copies in *B. patellaris* (Fig. 3B). In the telosomic addition and in the deletion mapping panel only 2 major fragments of 4.3 and 6.6 kb hybridized. From the hybridization profile in the mapping panel it was deduced that these 2 fragments are flanking the BCN gene at short range, and the fragments were consequently added to the map of the $Hs1^{pat-1}$ locus as individual RFLP markers B1143 and B1166 (see Fig. 1). No hybridization of $OPB11_{800}$ with the material carrying $Hs1^{pro-1}$ was observed (Fig. 3B, lanes 1–3).

RAPD $OPZ16_{1300}$ appeared to be a low-copy RFLP marker with 5–10 hybridizing fragments in *B. patellaris*. In the mapping panel only 1 hybridizing 6.5-kb *Eco*RV fragment was present (not shown), which was mapped at the same locus as RAPD $OPZ16_{1300}$. This RFLP locus is referred to as Z1665 (Fig. 1).

Conversion of RAPD $OPB11_{800}$ into a sequence-tagged site (STS) marker

The location of the two RFLP loci B1143 and B1166 as detected by the cloned RAPD product $OPB11_{800}$ makes them attractive candidates for the start and finish of a chromosomal walk towards the BCN resistance gene. Such a walk requires the screening of YAC libraries with markers flanking the BCN gene and the subsequent building of a YAC contig till the two markers have been connected. Because YAC libraries are most conveniently screened with STS markers, primers were designed according to the nucleotide sequence of $OPB11_{800}$ to convert the cloned RAPD marker into an STS marker. In Fig. 4 panel A, the banding profiles obtained after RAPD PCR using primer OPB11 on DNA from *B. vulgaris*, *B. patellaris* and AN5 show the 800-bp $OPB11_{800}$ product that is only amplified from *B. patellaris* and AN5 DNA. From panel B it is evident that the designed STS primers specifically amplify this 800-bp fragment from *B. patellaris* DNA and from DNA from all the fragment additions constituting the mapping panel, whereas no product is amplified from *B. vulgaris* DNA. The amplification of the 800-bp product from AN5-109 DNA was found to be much less efficient than that of the other fragment additions, resulting in only a very faint band on the agarose gels. This phenomenon was observed in at least three independent experiments. As AN5-109 is the sole fragment addition that contains only the B1166 locus and not the B1143 locus (see Fig. 1 and Fig. 3) it can be concluded that amplification of the 800-bp fragment from the B1166 locus is less efficient than amplification from the B1143 locus, probably caused by an imperfect binding of (one of) the used primers to the target DNA in the B1166 locus. The two STS loci corresponding to RFLP loci B1166 and B1143 are referred to as STS-B1166 and STS-B1143, respectively (see Fig. 1).

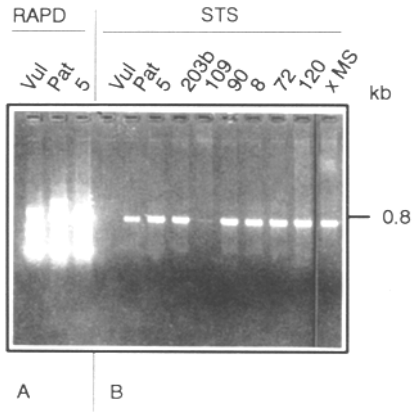

Fig. 4 A RAPD profile of primer OPB11 on *B. vulgaris* (*Vul*), *B. patellaris* (*Pat*) and AN5 (*5*). **B** Amplification of the RFLP loci B1143 and B1166 with STS primers. Template DNA: *Vul B.vulgaris*, *Pat B. patellaris*, *5* AN5, *203b* AN5-203b, *109* AN5-109, *90* AN5-90, *8* AN5-8, *72* AN5-72, *120* AN5-120, *xMS* AN5 × MS

Discussion

In this paper the isolation and characterization of RAPD markers linked to the beet cyst nematode resistance locus $Hs1^{pat-1}$ of the wild beet *B. patellaris* is described. From a screening of *B. patellaris*, *B. vulgaris* and the nematode-resistant telosomic addition AN5 with a total of 180 random 10-mer primers, generating approximately 1260 RAPD loci, 17 primers were selected that amplify a *B. patellaris*-specific RAPD locus from AN5. With the estimated size of the *B. vulgaris* genome as 750 Mbp (Arumuganathan and Earle 1991) and a size of 42 Mbp for the telosomic *B. patellaris* chromosome-1 fragment in AN5, the expected number of RAPDs specific for the telosomic fragment is approximately 70. The fact that only 17 RAPDs resulted from the first screening might indicate that the RAPD loci are not randomly distributed over the *B. vulgaris* and *B. patellaris* genomes. The shortage in RAPDs cannot be attributed to a possible low degree of polymorphism between *B. vulgaris* and *B. patellaris* as almost every 10-mer primer tested produced totally different patterns of amplification products for the two beet species (not shown).

Since no mapping population segregating for the beet cyst nematode resistance is available, due to the instable insertion of the *B. patellaris* chromosomal fragments in a *B. vulgaris* genomic background, the positions of the obtained RAPD markers relative to the $Hs1^{pat-1}$ locus was determined by deletion mapping (Overhauser et al. 1993; Vollrath et al. 1992; Werner et al. 1992) using a

mapping panel consisting of seven nematode-resistant monosomic fragment additions of *B. patellaris* chromosome 1 in *B. vulgaris*. With this panel the telosomic addition fragment in AN5 was divided into eight different intervals. Of the 17 RAPDs resulting from the first screening, 6 could be placed on the map for the *Hs1* locus. The remaining RAPD markers had to be omitted from the map as the profiles of amplified DNA fragments appeared to be poorly reproducible in the second screening.

To gain more insight into the nature of the amplified DNA by the RAPD primers, we analyzed 4 of the mapped RAPDs on Southern blots. All 4 RAPDs were found to consist of repetitive *B. patellaris* DNA, varying from low-repetitive DNA for RAPD $OPZ16_{1300}$ to high-repetitive DNA for RAPD $OPZ18_{600}$. An explanation for the repetitive character of the RAPD markers might be sought in the fact that the amplification of a specific DNA fragment by a single 10-mer RAPD primer is dependent on the occurrence of two identical primer binding sites within approximately 2000 bp, one of which must be in an inverted orientation. This requirement probably is met more frequently in chromosomal regions containing repetitive DNA than in regions containing non-repetitive DNA. The predominant repetitive character of RAPDs has also been observed for several other crop species like wheat (Devos and Gale 1992), potato (Baird et al. 1992) and lettuce (Paran and Michelmore 1993).

The repetitive nature of the isolated RAPD markers proved to be a source of additional markers for the $Hs1^{pat-1}$ locus. For example, the RAPD marker $OPX2_{1100}$ was mapped in an interval on the telosomic fragment relatively far away from the $Hs1^{pat-1}$ locus. However, by using the cloned $OPX2_{1100}$ fragment as a hybridization probe, and thus converting the RAPD marker into an RFLP marker, approximately 15 polymorphic loci were discernable in the mapping panel. Two of these loci were mapped at the same interval as the $Hs1^{pat-1}$ locus. Although the exact size of this interval is not known, the two RFLP loci are estimated to be within 2 Mb from the resistance gene. The hybridization of $OPX2_{1100}$ with the *B. procumbens* introgression lines, which harbor only small segments of introgressed DNA (Salentijn et al. 1994), shows that also in *B. procumbens* copies of this marker are closely linked to a nematode resistance locus ($Hs1^{pro-1}$).

Two other RFLP loci, B1143 and B1166, were shown to flank the $Hs1^{pat-1}$ locus on both sides. As these loci can be used in a chromosomal walk encompassing the resistance gene, STS primers were developed based on the nucleotide sequence of RAPD $OPB11_{800}$. These primers were shown to amplify DNA fragments derived from both the RFLP loci and will be used to isolate YAC clones from a library constructed of the fragment addition AN5-203b (Klein-Lankhorst et al. 1994).

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