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Long-range organization of a satellite DNA family flanking the beet cyst nematode resistance locus (*Hs1*) on chromosome-1 of *B. patellaris* and *B. procumbens*

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Abstract New members of a satellite DNA family (Sat-121), specific for wild beets of the section *Procumbentes* of the genus *Beta*, were isolated. Sequence analysis showed that the members of Sat-121 fall into two distinct classes. The organization of Sat-121 in the vicinity of a beet cyst nematode (*Heterodera schachtii* Schm.) resistance locus (*Hs1*) in *B. patellaris* and *B. procumbens* was investigated by pulsed-field gel electrophoresis (PFGE) using DNA from a series of resistant monosomic fragment additions, each containing an extra chromosome fragment of *B. patellaris* chromosome-1 (pat-1) in *B. vulgaris*. In this way several clusters of Sat-121 flanking the $Hs1^{pat\text{-}1}$ locus were identified. In nematode resistant diploid introgressions (2n=18), which contain small segments of *B. procumbens* chromosome-1 (pro-1) in *B. vulgaris*, only two major Sat-121 clusters were detected near the $Hs1^{pro\text{-}1}$ locus.

Key words Molecular markers
Beet cyst nematode resistance · *Hs1*
Heterodera schachtii Schm. · Satellite DNA
Monosomic fragment additions
Beta · Pulsed-field gel electrophoresis

Introduction

Repetitive DNA sequences constitute a large fraction of plant genomes varying from 14% in the genome of *Arabidopsis* (Leutwiler et al. 1984) to as much as 60–80% in the genomes of maize and wheat (Hake and Walbot 1980; Flavell 1986) and 63% in species of the genus *Beta* (Flavell et al. 1974). These repetitive DNA sequences are arranged in tandem arrays (i.e., satellites, rDNA, 5*s* DNA, and telomeric repeats) or are interspersed with unrelated repetitive-or unique-DNA sequences (Flavell 1986).

In the genus *Beta* several repetitive DNA sequences have been identified. Three satellite DNA families were isolated from sugar beet (*B. vulgaris*) consisting of, respectively, two *Eco*RI monomers of 157–160 basepairs (bp) and a *Bam*HI monomer of 327–360 bp (Schmidt and Metzlaff 1991; Schmidt et al. 1991; Santoni and Bervillé 1992). Both *Eco*RI satellite DNA families are found in the sections *Beta* and *Procumbentes*. In addition, one of the *Eco*RI satellites is present in the section *Corollinae* (Schmidt et al. 1991; Santoni and Bervillé 1992); the *Bam*HI satellite DNA family is restricted to the section *Beta* (Schmidt and Metzlaff 1991; Santoni and Bervillé 1992). Members of yet-another satellite DNA family specific for wild beets of the section *Procumbentes* were isolated from *B. procumbens* (Jung et al. 1990; Schmidt et al. 1990; Jung and Herrmann 1991; Jung et al. 1992) and *B. patellaris* (Salentijn et al. 1992). The *Procumbentes* -specific satellite DNAs were also detected in different nematode-resistant monosomic additions and introgressions of *B. patellaris*, *B. procumbens* and *B. webbiana*, which contain a beet cyst nematode (*Heterodera schachtii* Schm.) resistance locus (*Hs1* locus) present on the introduced wild beet DNA (Jung et al. 1992; Salentijn et al. 1992). This *Hs1* locus is considered to be a single dominant locus (Savitsky and Price 1965; Yu 1978).

The present study describes the isolation and analysis of new members of the *Procumbentes* -specific satellite DNA family (Sat-121). The long-range organization of Sat-121 in the vicinity of the *Hs1* locus on chromosome-1 of *B. patellaris* ($Hs1^{pat\text{-}1}$) was investigated by PFGE . Using a series of related monosomic fragment additions of chromosome pat-1 (AN5, AN5-90, AN5-109 and AN5-203b) several large clusters of Sat-121 could be mapped relative to the $Hs1^{pat\text{-}1}$ locus. In addition, the long range

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organization of Sat-121 close to the *Hs1*-locus on *B. procumbens* chromosome-1 ($Hs1^{pro\text{-}1}$) was studied using a beet cyst nematode (BCN)-resistant monosomic fragment addition, AN1-89, and two resistant diploid introgressions, B883 and AN1-65-2, containing small segments of chromosome pro-1 (nomenclature according to Lange et al. 1993).

Materials and methods

Plant material

The beet cyst nematode (BCN)-resistant plant material consisted of monosomic fragment additions of *B. vulgaris* carrying a chromosome fragment of either chromosome pro-1 or chromosome pat-1. The *B. patellaris* fragment additions AN5-90, AN5-109 and AN5-203b, with alien chromosome fragments of respectively 8, 13 and 12 megabasepairs in size, were recovered from backcrossing AN5, a telosomic addition carrying the long arm of chromosome pat-1, with diploid *B. vulgaris* (Speckmann et al. 1985; Lange et al. 1990; Salentijn et al. 1992). The size of the chromosome fragments was estimated by microscopical measurements on mitotic metaphase chromosomes (De Jong et al. 1986) as a percentage of the total haploid genome, which is 750 Mbp (Arumuganathan and Earle 1991). The *B. procumbens* fragment addition, AN1-89, was recovered from backcrossing AN1, a monosomic addition carrying chromosome pro-1, with *B. vulgaris* (De Jong et al. 1986). The resistant diploid introgression B883 contains a segment of chromosome pro-1 harboring the $Hs1^{pro\text{-}1}$ resistance locus incorporated into the *B. vulgaris* genome. This homozygous introgression shows 90–100% stability for transmission of the nematode resistance (Heijbroek et al. 1988; Lange et al. 1990). AN1-65-2 is a diploid introgression recovered from backcrossing AN1 with *B. vulgaris*. This homozygous introgression shows 64–100% transmission of BCN-resistance. Other plant material, viz., the BCN-resistant wild *Beta* species, as well as the *B. vulgaris* plants used in the backcrosses, were obtained from the CPRO-DLO *Beta* collection. The satellite DNA probes 121, 208, 342 and 551 were isolated from the *B. patellaris* fragment addition AN5-90 as described previously (Salentijn et al. 1992).

PCR

PCR was carried out with the primers REP: CGTAAGAGACTATGA and REP.INV: TGAACACCTTTCAAAT. Thirty rounds were performed with denaturation at 92 °C for 1 min in the first round and for 0.5 min. in the following rounds; annealing was for 1 min at 35 or 37 °C and elongation for 2 min at 72 °C. The products were run on agarose gels. This resulted in a ladder of products, each step corresponding to a specific number of repeat units. For cloning, kinated oligos were used and the products were filled out with Klenow enzyme and subcloned into pUC19.

B883 genomic library

A B883 genomic library was constructed in the λ DASHII vector by partial *Sau*3A digestion of leaf DNA and cloning into the *Bam*H1 site of the vector.

Isolation of genomic clones

Approximately 2×10^6 recombinants were screened by plaque hybridization to a ^{32}P-labelled insert of clone B3-2 isolated with PCR from B883 DNA. *Eco*RI fragments of the genomic clones were subcloned into PUC19.

DNA-sequence analysis

DNA sequence analysis was carried out using the dideoxy chain-termination method according to Sanger et al. (1977).

DNA-isolation

Total DNA was isolated from leaf tissue, sampled from at least five individual plants, using an upscaled version of the method of Dellaporta (1983).

High-molecular-weight DNA from *Beta* was obtained basically as described by Schwartz and Cantor (1984) for the preparation of yeast chromosomal DNA. Mesophyll protoplasts (pps) were isolated from young leaves (20–50 mm) taken from at least five individuals kept under greenhouse conditions. The pps were isolated according to a method described by Krens et al. (1990) with the following modifications. No preplasmolysis was performed and n-propylgallate was omitted from the enzyme mixtures [1% (w/v) cellulase R-10 and 1.5% (w/v) macerozyme R-10]. After an incubation time of 3 h the pps were collected from the enzyme mixture and washed once with CPW salts containing 9% mannitol. After isolation the pps were concentrated by centrifugation (800 rpm) to a final concentration of 8×10^6 pps per 100 μl, mixed (1:1) with 1.5% Incert agarose (FMC) in 0.25 M EDTA, and quickly poured into molds (V=100 μl). The final concentration in the agarose plugs of 4×10^6 pps is equivalent to 6.3 μg of DNA based on a DNA content of 1.57 picograms for the diploid *Beta* genome (Arumuganathan and Earle 1991). After solidification the plugs were immediately incubated twice for 24 h in 0.5 M EDTA supplemented with 1 mg/ml of proteinase K and 0.02 M sodium bisulphite (V=15 ml per eight plugs) at 50 °C, until the green color had disappeared. For subsequent enzyme treatment, the proteinase K activity was inhibited by incubation in TE-buffer (10 Mm Tris, 10 mM EDTA, pH 7.5) supplemented with 1 mM of PMSF (V=15 ml per eight plugs) during 12 h at 50 °C. Restriction enzyme digestion of high-molecular-weight DNA was performed according to Van Daelen et al. (1989), using 30 units of restriction enzyme per plug. The enzyme was added in three portions during a total incubation time of 6 h.

Pulsed-field gel electrophoresis

Pulsed-field separations of high-molecular-weight DNA were performed using a Rotaphor (type IV, Biometra). Restriction fragments in the size range of 100–1,000 kbp were separated using a linear increasing pulse time of 50–70 s and a field strength of 180 V (6 V/cm) during a run of 20 h. The angle between the two fields was 120°. The agarose gels (1%, SeaKem UltraPure, FMC) were run in 0.25×TBE buffer at 14 °C.

Southern analysis

For Southern analysis cloned DNA fragments were purified by agarose-gel electrophoresis (SeaKem GTG, FMC), isolated and labelled by random priming (Random primed DNA labelling kit, USB) with [^{32}P]αdATP. Prior to Southern blotting the DNA in the agarose gels was nicked by UV-treatment (5 s, UV-crosslinker, Hoefer). Washing stringencies used after hybridization were respectively 1×SSC, 0.1% SDS at 65 °C for analysis of the PFGE gels, and up to 0.1×SSC, 0.1% SDS at 65 °C for the DNA separated by conventional electrophoresis.

Results

Identification of new members of a satellite DNA family (Sat-121) in *B. procumbens*

In a previous study, a member (121–3) of a *Procumbentes*-specific satellite DNA family, Sat-121, was isolated from a genomic subtraction library of AN5-90, a BCN-resistant fragment addition of chromosome pat-1 (Salentijn et al. 1992). Here the DNA-sequences of three additional members of Sat-121 (208 and 551, each containing two repeat units, and 342, containing three repeat units) are presented

```
                    10         20         30         40         50         60         70         80
                                                                           ▼  ▼   C             ▼
Sat-121.1   GATCCAAGGG CTTCATATGC TTTACATATA CCTAATACCT ATTAAAGGAA TAAAAAAxCA ATAGGTAATT AAGCACATCx
551(1)      ---------- ---G------ ---------- ---------- ---------- -TG------- --T-G----- -----T----
551(2)      A--------- -A-------- ---------- ---------- -AA------- ---------- ----C----- ----------
208(1)      ------T--- ---------A ----A----- ---------- ---G---T-G -C-------- ----GA---- ----------
208(2)      T---T----- A--------- --AG------ -A---C---- ---------- -C-------- ----C----- ----------
342(1)      ---------- ---------- C--------- ---------- --G------- ---------- ----G----- -----T----
342(2)      A-----x--C -A-------- ---------- ---------- --A------- ---------- ----C----- ----------
342(3)      --C--T---- ---------- ---------- -----C-G-- --A------- ---------- ----C----- ----------
121-3       ---------- ---------A ----A----- -------A-- ---C-----G -C-------- ----GA---- ----------

                                                                           ▼  ▼             T   ▼
Sat-121.2   GATCCAAGGG CTTCATATGC TTTAAATATA TCTAATACCT ATTCAAGGAG TCAAAAAxTA TTTGGTAATT ATGCACAACA
B 3-2       -C-------- T--------A --G----T-- -------AA- ------T--- ------C--- -A-------- -AG-----A-
B 3-4       T--------- ---------- ---------- -A-------- ---------- ---T------ ---T----A- --T-------
B 2-5       ----T----- --------C- ------G--- ---------- ---------- ---------- --------C- -AG------x
B 2-6       T--------- --A------- ---------- --------G- ---------- ---T--G--- ---------- --T--A--A-
B 2-7       ---------- A--------- ---------- ---------C ---------- -------A-- -A---A---- --x-G-----
B 2-8       ---------A --------T- ------A-G- ------CT-- ---------C -------A-- ---TT----- --T-C-----
B 2-10      ----T----- --------C- ------G--- ---------- ---------- ---------- --------C- -AG------x

                    90        100        110        120        130        140        150        160
                                                                                                                                     ▼    ▼                 ▼       ▼
Sat-121.1   AAATGATTTG AAAGGTGTTC ATACACCACA AAATCTCGTA AGAGACTATG ATAGTTTTAA CCTTTGATTT GAAATGAGTT T
551(1)      ---------- ---------- -----T---- -----A---- ---------- -----A---- ---------- -------A-- -
551(2)      ---------- ---------- ---------- ---------- -C-------- ---------- ---------- ---------- -GATC
208(1)      ---------- ---------- ------A--- ---------- ---------- ----A----- GT--C----- ---------- -
208(2)      ----A----- ---------- ---------- ---------- ---------- -------A-- A--------- --------A- -GATC
342(1)      ---------- ---------- -----T---- ---------- ---------- ----A---C- -------A-- -------A-- -
342(2)      ---------- ---------- ------T--- ---------- ---------- ---------- ---------- ---------- -
342(3)      x-------A- -G-------- ----C----- ---------- ---------- ---------- ---------- ---------- -GATC
121-3       ---------- ------C--- ------A--- G--------- ---------- ---------- -T--C----- ---C------ -GATC

                                             ▼    ▼                 ▼       ▼
Sat-121.2   AAATAATTTG AAAGGTGTTC ATACACCACA xAATCGCCTA AGAGACTATG ACGGTTTTAC CCTTTGATTT GAAATAAGTT T
B 3-2       ---G-x---- --T------A ---------- x--------- ---------- ---------- -A-------- ---------- -
B 3-4       --------x- ---------- ---------G x--------G ---------- ---A------ ---------- ---------- A
B 2-5       C---G----- ---------- -------C-- x------G-- ---------- -------x-- ---------- ---TCG---- -
B 2-6       T--------- ---------- ----G----- x---T---G- -T-------- --AA------ ---------- ---------- -
B 2-7       ---------- ---------- ---------- x-T------- ----T----- ---A------ ---C------ ---------- -
B 2-8       ----G----- ---------- --T------- x-------A- ---------- --A------- ----G---A- ---T-G---- -
B 2-10      C---G----- ---------- -------C-- x------A-- ---------- ---------- ---A------ ---T-T---- -
Primers   ◄(INV)TAAAC TTTCCACAAG T                                                  CGTA AGAGACTATG A►
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Fig. 1 Sequence alignment of satellite DNA family Sat-121. Only nucleotides differing from the consensus sequences of subfamilies Sat-121.1 and Sat-121.2 are shown. Identical sequences are represented by *dashes* (–); × indicates positions with deletions. The direct and inverted (*INV*) primer sequences used for the isolation of Sat-121 elements from B883 are given below. ▼ indicates basepair changes used to discriminate between the subfamilies. (*1*), (*2*) and (*3*) indicate first, second, and third repeat unit from the indicated Sat-121 clones. In the Sat-121.2 subfamily only one repeat unit of the isolated clones is shown

(Fig. 1). Using PCR with primers constructed according to conserved regions of Sat-121, new members of Sat-121 (B3-2; B3-4; B2-5; B2-6; B2-7; B2-8; and B2-10) were cloned from B883, a nematode-resistant diploid introgression of chromosome pro-1 (Fig. 1). The approximately 159-bp monomeric units of Sat-121 show a mutual sequence homology of 70%-90%. Based on basepair changes at positions 59, 61, 78, 111, 116, 132 and 140 two subfamilies can be discriminated within Sat-121 (Fig. 1).

Southern analysis confirmed the specificity of Sat-121 for the section *Procumbentes* within the genus *Beta*. No hybridization was found with *B. lomatogona*, *B. corolliflora*, *B. intermedia* and *B. trigyna* of the section *Corollinae* or with the *B. vulgaris* subspecies *maritima* accession BMH, the sugar beet variety 'Hilde' or the heterogenous *B. vulgaris* material that was used for breeding the monosomic addition material (data not shown).

Organization of Sat-121 near the $Hs1^{pat\text{-}1}$ locus

The organization of Sat-121 on chromosome pat-1 near the $Hs1^{pat\text{-}1}$-locus was examined by Southern-blot analysis of DNA from a series of nematode-resistant monosomic fragment additions (AN5, AN5-203b, AN5-109 and AN5-90) each containing a fragment of chromosome pat-1 of respectively 42, 12, 13 and 8 megabasepairs. Using *Sau*3A and *Nco*I, two different types of hybridization patterns specific for elements arranged in tandem arrays were observed. A hybridization profile (*type A* ; Hörtz and Zachau 1977) starting with a basic repeat unit of 160 bp was obtained with *Sau3*A (Fig. 2A) while a profile starting with a 640-bp fragment (*type B*) results as a consequence of *Nco*I digestion. The *Nco*I ladder, with repeats of 160 bp, increases up to a size of 16 repeat units (2,560 bp) whereafter the ladder pattern becomes less pronounced (Fig. 2B). Digestion with *Eco*RI generates yet another hybridization profile since it does not result in a typical ladder pattern. Interestingly, hybridization of Sat-121 with AN5-90 is confined to a single *Eco*RI restriction fragment in the high-molecular-weight region (>50 kbp) (Fig. 2C).

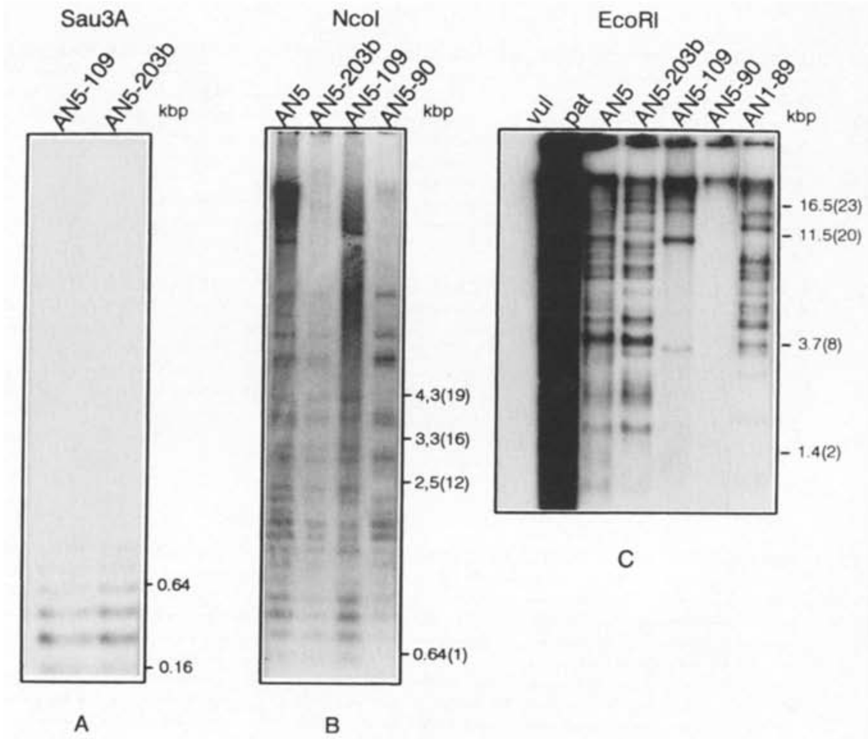

Fig. 2 A–C Southern analysis of DNA from the different resistant fragment additions, AN5, AN5-203b, AN5-109, AN5-90 and AN1-89, and from *B. vulgaris* and *B. patellaris* digested with *Sau*3A (**A**) *Nco*I (**B**) or *Eco*RI (**C**). Hybridization was with Sat-121

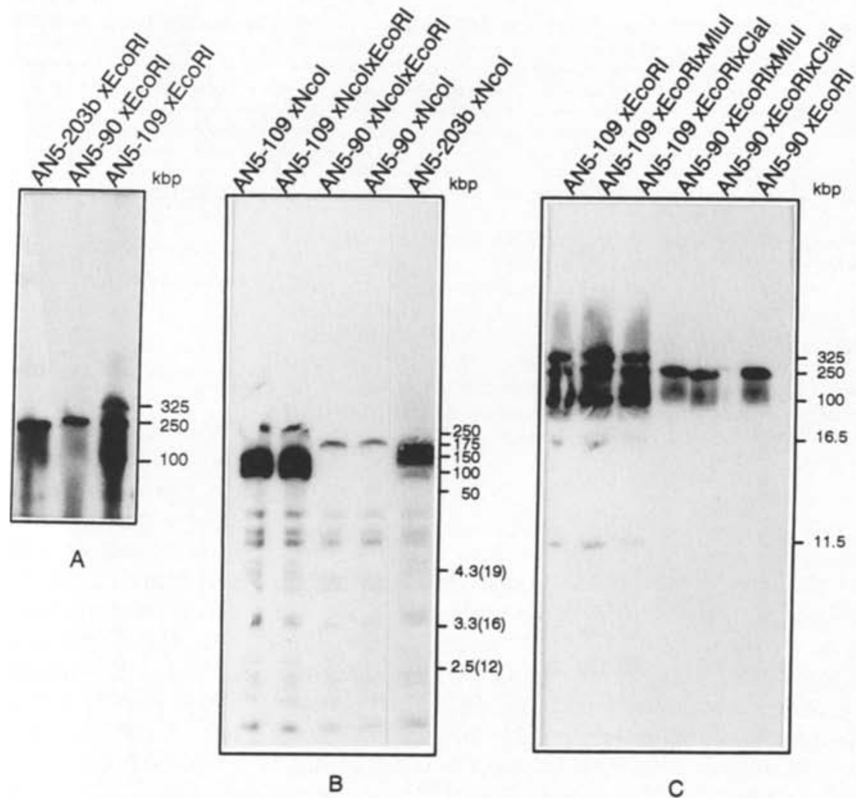

Fig. 3 A–C PFGE of high-molecular-weight DNA from the resistant fragment additions AN5-203b, AN5-109 and AN5-90 digested with the indicated restriction enzymes and hybridized with Sat-121

The long-range organization of Sat-121 near the $Hs1^{pat-1}$ locus was investigated by pulsed field gel electrophoresis (PFGE) using *Eco*RI- and *Nco*I-digested DNA. After hybridization with Sat-121 to *Eco*RI-digested DNA from the different fragment additions, three discrete hybridizing *Eco*RI fragments were evident. Fragments of 100 kbp and 325 kbp are present only in AN5-109, whereas a 250-kbp *Eco*RI fragment is present in all three fragment additions (Fig. 3A, C). Three *Nco*I fragments in the size range of 50–250 kbp hybridize to the probe. One of these (250 kbp) is only present in AN5-109; a band ranging from 50 to 150 kbp is present in AN5-109 and AN5-203b

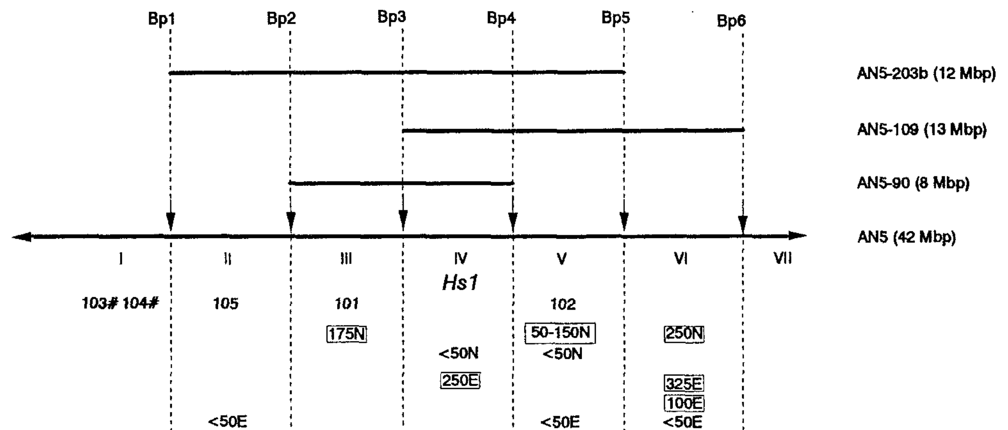

Fig. 4 Assignment of markers to different chromosomal regions (*I–VII*) of the long arm telosome of chromosome pat-1 (AN5), relative to the $Hs1^{pat\text{-}1}$ locus. Chromosomal breakpoints (*Bp1–6*) are indicated by *arrows*. 101–105, single-copy sequences CPRO101-105; E: Sat-121 clusters located on *Eco*RI fragments and *N* on *Nco*I fragments; sizes are given in kbp. <50 E and <50 N: Sat-121 sequences located on *Eco*RI- and *Nco*I-fragments <50 kbp. # = assignment to either region I or VII

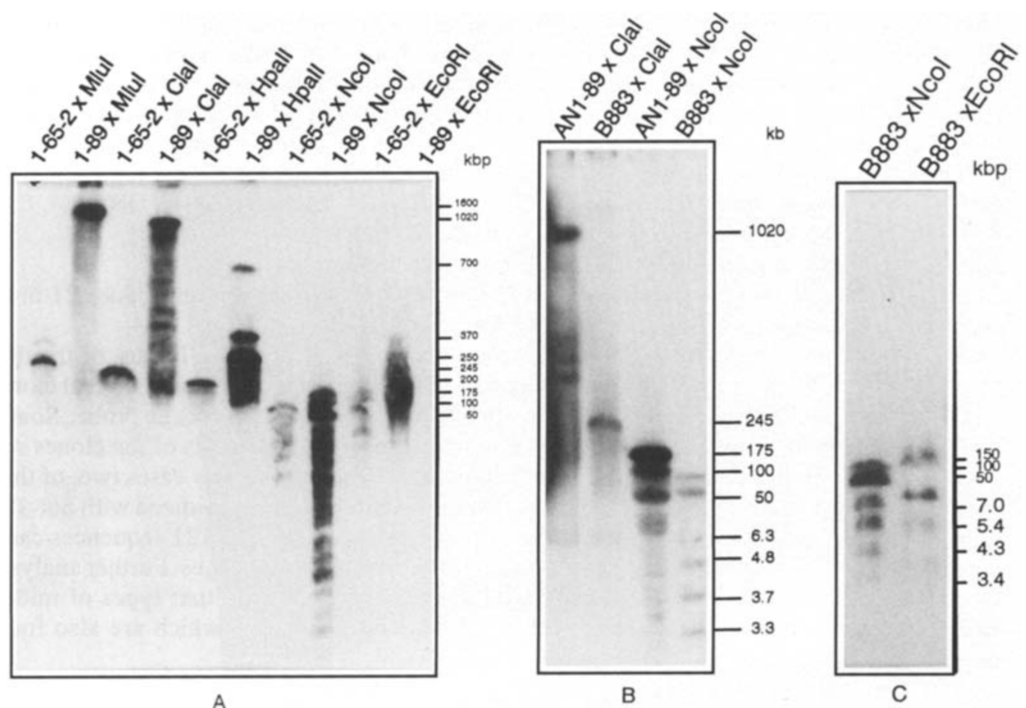

Fig. 5 A – C Pulsed-field analysis of high-molecular-weight DNA of a resistant fragment addition (AN1-89) and resistant diploid introgressions (AN1-65-2 and B883) of chromosome pro-1. DNA was digested with the indicated restriction enzymes and hybridized with Sat-121

whereas a *Nco*I fragment of 175 kbp is present in AN5-90 and AN5-203b (Fig. 3B). Double digestions with *Eco*RI and the 'rare cutters' *Mlu*I and *Cla*I, respectively, result in the *Eco*RI pattern (Fig. 3C) while double digestion with *Nco*I and *Eco*RI give rise to the *Nco*I pattern (Fig. 3B).

The results indicate a possible localization of the $Hs1^{pat\text{-}1}$ locus relative to the Sat-121 clusters. A necessary qualification for this hypothesis is that the addition fragments present in AN5-90 (8 Mbp), AN5-109 (13 Mbp) and AN5-203b (12 Mbp) have originated without rearrangements by chromosomal breakage from the large telosomic addition fragment in AN5 (42 Mbp) during backcrossing of AN5 with *B. vulgaris*. In this model (Fig. 4) Sat-121 units are assigned to regions I to VII of the long arm telosome of chromosome pat-1, based on their presence or absence in the different fragment additions. As all fragment additions are resistant against *H. schachtii* the $Hs1^{pat\text{-}1}$ locus is assigned to region IV defined by the chromosomal breakpoints 3 and 4 (Bp3 and Bp4) (Fig. 4). A cluster of Sat-121, hybridizing to a 250-kbp *Eco*RI fragment and 19 *Nco*I fragments of smaller size (< 50 kbp) is also present in all fragment additions and thus can be assigned to the same chromosomal region as the *Hs1* locus (region IV). Also the single copy markers CPRO101-CPRO105 (Salentijn et al. 1992) have been put to the model. CPRO103 and CPRO104 are only present in AN5 and must therefore be located in either region I or VII.

Organization of Sat-121 near the $Hs1^{pro\text{-}1}$ locus

The organization of Sat-121 on chromosome pro-1 was examined by Southern-blot analysis of DNA from a fragment addition (AN1-89) and from two diploid introgressions

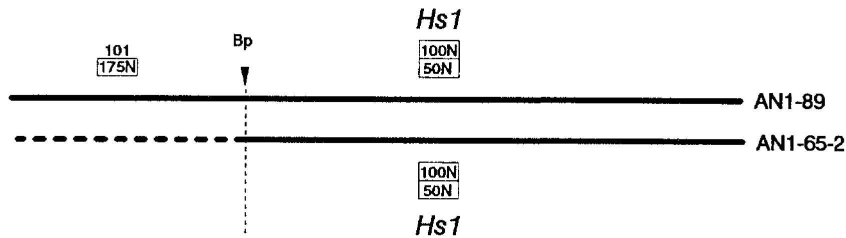

Fig. 6 Assignment of markers to the short arm telosome of chromosome pro-1 (AN1-89) relative to the *Hs1*$^{pro\text{-}1}$ locus. *101*, the single-copy marker CPRO101; *E*; Sat-121 clusters located on *Eco*RI fragments and on *N*, *Nco*I-fragments. The sizes are indicated in kbp. The *dotted horizontal line* represents flanking *B. vulgaris* DNA. *Bp* = introgression breakpoint in AN1-65-2

(AN1-65-2 and B883) of chromosome pro-1, all of which contain the *Hs1*$^{pro\text{-}1}$ locus. The *Eco*RI- (Fig. 2 C), *Nco*I- and *Sau*3A-hybridization profiles (data not shown) of AN1-89 in the low molecular weight region are similar to that of chromosome pat-1.

The long range organization of Sat-121 near the *Hs1*$^{pro\text{-}1}$ locus was investigated by PFGE analysis of AN1-89 and the diploid introgressions AN1-65-2 and B883. In AN1-89, clusters of Sat-121 are present on three *Nco*I fragments of 50 kbp, 100 kbp and 175 kbp respectively (Fig. 5A, B). *Eco*RI digestion of AN1-89 DNA generates hybridizing fragments extending from approximately 100 to 325 kbp (Fig. 5A). In addition, Sat-121 units with sizes below 50 kbp were detected on approximately 15 *Eco*RI-fragments (Fig. 2C) and 16 *Nco*I-fragments (Fig. 5A). Analysis of the resistant diploid introgressions of chromosome pro-1, AN1-65-2 and B883, indicates that the copy number of Sat-121 is reduced in these introgressions compared to AN1-89. Only clusters of Sat-121 DNAs on *Nco*I fragments of respectively 50 kbp and 100 kbp and *Eco*RI-fragments in the size range of 100 to 150 kbp, are present in AN1-65-2 and B883 (Fig. 5A, C). The reduction in copy number of Sat-121 in AN1-65-2 is also detected with the restriction enzymes *Hpa*II, *Cla*I and *Mlu*I. Sat-121 copies are present on single *Hpa*II and *Cla*I fragments of respectively 200 and 245 kbp and two *Mlu*I fragments of 250 and 370 kbp in AN1-65-2 (Fig 5 A). In addition, the difference between the introgressed chromosome pro-1 segments of AN1-89 and both diploid introgressions was confirmed by the absence of the single-copy sequence CPRO101 from the DNA of B883 and AN1-65-2 (data not shown). Although the B883 material is derived from a different monosomic addition of chromosome pro-1 (Heijbroek et al. 1988) than is AN1-65-2, the *Eco*RI-, *Nco*I- and *Cla*I- hybridization patterns with Sat-121 are identical (Fig. 5).

The pro-1 fragment addition (AN1-89) and the diploid pro-1 introgression (AN1-65-2) are both derived from crossings of the same monosomic addition of chromosome pro-1 (AN1) with *B. vulgaris*. The absence of Sat-121 on a 175-kbp *Nco*I fragment from AN1-65-2 (Fig. 5A), as well as the absence of the CPRO101 sequence, therefore reflects a chromosomal breakpoint on chromosome pro-1 (Fig. 6).

The linkage of Sat-121 to the *Hs1*$^{pro\text{-}1}$ locus was tested in backcross populations of B883 and AN1-65-2. In the B883 backcross population (187 plants) three resistant plants had lost all Sat-121 sequences, while one susceptible plant was found which contained Sat-121. In the AN1-65-2 backcross population (174 plants) eight resistant plants had lost all, or nearly all, copies of Sat-121, but no susceptible plants carrying Sat-121 sequences were obtained. The occurrence of 11 resistant plants which have lost Sat-121 sequences, and only one susceptible plant with the Sat-121 sequence, suggests that most of the resistant plants without Sat-121 do not represent crossover events, but more-likely represent deletions within the introgressed *B. procumbens* DNA.

Organization of Sat-121 in genomic B883 λ clones

A genomic library of the diploid introgression B883 was constructed and several clones were isolated using Sat-121 (clone B3-2) as probe. Southern analysis with *Eco*RI and *Nco*I digests of the clones showed that normally only one, but in a few cases two, of the restriction fragments of each clone hybridized with Sat-121. This indicates that the clustered Sat-121 sequences can be interrupted by other kinds of sequences. Further analysis showed that these sequences involve other types of middle- and highly- repetitive sequences which are also found in *Beta vulgaris* (data not shown).

Discussion

In the present study a distinct satellite DNA family (Sat-121) specific for the section *Procumbentes* of the genus *Beta* has been characterized. Since Sat-121 is present in BCN-resistant fragment additions and introgressions of chromosome-1 of both *B. patellaris* and *B. procumbens* it can be used as a marker for the presence of the BCN-resistance loci (*Hs1*$^{pat\text{-}1}$ and *Hs1*$^{pro\text{-}1}$; nomenclature according to Lange et al. 1993).

Several Sat-121 copies were isolated from fragment additions of *B. patellaris* and from an introgression of *B. procumbens*. Based on the nucleotide sequences, the different copies could be placed into two subfamilies. The repetitive sequences TS1 and TS2, described by Schmidt et al. (1990) from *B. procumbens*, belong to subfamily Sat-121.2 while the repetitive sequence RK643 isolated from *B. procumbens* material by Jung et al. (1992), belongs to the Sat-

121.1 subfamily. From this it can be concluded that the two Sat-121 subfamilies are not specific for either of the two beet species.

The genomic organization of Sat-121 was studied in more detail. The *Sau*3A monomers are approximately 159 bp in length and concatemers up to 12-mers were observed. Part of the *Nco*I-hybridization pattern exhibits the characteristics of a tandemly-arranged sequence which can be explained by the presence of a *Nco*I site generated by an A-T substitution at position 3 in the sequence 208(1). The Sat-121 sequences are typical satellite sequences with tandemly-arranged copies of a core sequence. In general, satellite DNAs are predominantly found near the centromeres and telomeres and at a few interstitial sites (Lapitan 1991; Maluszynska and Heslop-Harrison 1991; Röder et al. 1993). The Sat-121 satellite family seems to be distributed in several large clusters over the chromosome and is interrupted by other types of repetitive sequences. This organization is in good agreement with results obtained by Jung et al. (1992) who found a similar arrangement for the RK643 repeat in *B. procumbens*.

PFGE analysis of a series of related monosomic fragment additions of chromosome pat-1 revealed the presence of several Sat-121 containing *Nco*I- and *Eco*RI fragments, in the size range of 50 to 325 kbp, in different regions of the long arm telosome of pat-1. All fragment additions (AN5-203b, AN5-109 and AN5-90) have in common a cluster of Sat-121 on a 250-kbp *Eco*RI fragment and on several *Nco*I fragments of low molecular weight, indicating a tight linkage of these clusters with the $Hs1^{pat\text{-}1}$ locus. Apart from these mutual fragments the "fingerprints" of AN5-109 and AN5-90 are different showing that the chromosome fragments extend in different directions and cover different chromosomal regions [as was previously observed for the RFLP-markers CPRO101 and CPRO102 (Salentijn et al. 1992)]. A single cluster of Sat-121 on a *Nco*I fragment of 175 kbp present in AN5-90 flanks the $\mathrm{Hs1}^{\mathrm{pat\text{-}1}}$-locus on one side whereas two other clusters of 50–150 kbp and 250 kbp on *Nco*I-fragments present in AN5-109 are flanking the locus on the other side. All these clusters of Sat-121 occur on larger fragment additions (AN5 and AN5-72). The exact physical distances between the satellite clusters and the resistance gene cannot be deduced from the proposed model of the $Hs1^{pat\text{-}1}$ region, as the exact locations of the breakpoints in this model are unknown. However, the maximum distance between any cluster and the resistance gene equals the measured size of the addition fragments.

The organization of Sat-121 near the $Hs1^{pro\text{-}1}$ locus present on chromosome-1 in *B. procumbens* was studied using a resistant fragment addition (AN1-89) and resistant diploid introgressions (AN1-65-2 and B883) of chromosome pro-1. By adding the sizes of Sat-121-containing *Nco*I fragments the satellite was assigned to about 450 kbp in AN1-89 and approximately 220 kbp in the diploids. This reduction in copy number is mainly due to the deletion of a large cluster of Sat-121 located on a 175 kbp *Nco*I fragment from the diploids. Since AN1-89 and AN1-65-2 originate from the same plant line the deletion of a cluster of Sat-121 on a 175-kbp *Nco*I fragment and the CPRO101 sequence from AN1-65-2 reflects a chromosomal breakpoint. The Sat-121-containing *Mlu*I fragments (250 and 370 kbp) in AN1-65-2 most likely harbor at least one introgression breakpoint. Clusters of Sat-121 on *Nco*I fragments of 50 and 100 kbp and the $Hs1^{pro\text{-}1}$ locus, are present in both AN1-89 and the introgressions AN1-65-2 and B883. Data derived from the mapping populations of B883 and AN1-65-2 show a tight linkage between these satellite clusters and the resistance gene in *B. procumbens*.

Homology between the long arm telosome of chromosome pat-1 and the assumed short arm telosome of chromosome pro-1 was observed previously at the nucleotide level (Salentijn et al. 1992). In the present study homology between both resistance-bearing telosomes (AN1-89 and AN5) is evident from the presence of clusters of Sat-121 on *Nco*I-fragments of 50–100 kbp and 175 kbp flanking the *Hs1*-locus on chromosome pat-1. The homology, however, is not complete since a cluster of Sat-121 on a *Nco*I fragment of 250 kbp, as well as RFLP-marker CPRO102 (Salentijn et al. 1992), are present only on the long arm telosome of chromosome pat-1 (AN5). Since the only available cytogenetic mutants containing small segments of *B. procumbens* chromosome-1 are AN1-89, B883 and AN1-65-2, and the homology between the pro-1 and pat-1 chromosomes is not complete, it is not possible to localize the clusters of Sat-121 DNAs present in the diploid introgressions of chromosome pro-1 with respect to the $Hs1^{pro\text{-}1}$ locus.

The elucidation of the long-range organization of Sat-121 near the *Hs1* locus will be of great value for the isolation of the beet cyst nematode resistance gene by positional cloning (Wicking and Williamson 1990). Recently, the entire 250 kbp *Eco*RI Sat-121 cluster which is present in all *B. patellaris* fragment additions, and is thus tightly linked to the resistance locus, has been cloned in a single yeast artificial chromosome (YAC) (Klein-Lankhorst et al., in preparation). Currently, end-clones from this YAC are being used to build a YAC contig spanning the entire $Hs1^{pat\text{-}1}$ locus.

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References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

De Jong JH, Speckmann GJ, De Bock ThSM, Lange W, Van Voorst A (1986) Alien chromosome fragments conditioning resistance to beet cyst nematode in diploid descendants from monosomic additions of *Beta procumbens* to *B. vulgaris*. Can J Genet Cytol 28:439–443

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA mini-preparation: version II. Plant Mol Biol Rep 4:19–21

Flavell R (1986) Repetitive DNA and chromosome evolution in plants. Phil Trans R Soc Lond B 312:227–242.

Flavell R, Bennett MD, Smith JB, Smith OB (1974) Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem Genet 12:257–269

Hake S, Walbot V (1980) The genome of *Zea mays*, its organization and homology to related grasses. Chromosoma 79: 251–270

Heijbroek W, Roelands AJ, De Jong JH, Van Hulst CG, Schoone AHL, Munning RG (1988) Sugar beets homozygous for resistance to the beet cyst nematode (*Heterodera schachtii* Schm.), developed from monosomic additions of *B. procumbens* to *B. vulgaris*. Euphytica 38: 121–131

Hörz W, Zachau HG (1977) Characterization of distinct segments in mouse satellite DNA by restriction nucleases. Eur J Biochem 73: 383–392

Jung C, Herrmann RG (1991) A DNA probe for rapid screening of sugar beet (*Beta vulgaris* L.) carrying extra chromosomes from wild beets of the *Procumbentes* section. Plant Breed 107:275–279

Jung C, Kleine M, Fischer F, Herrmann RG (1990) Analysis of DNA from a *B. procumbens* chromosome fragment in sugar beet carrying a gene for nematode resistance. Theor Appl Genet 79: 663–672

Jung C, Koch R, Fisher F, Brandes A, Wricke G, Herrmann RG (1992) DNA markers closely linked to nematode resistance genes in sugar beet (*Beta vulgaris* L.) mapped using chromosome additions and translocations originating from wild beets of the *Procumbentes* section. Mol Gen Genet 232:271–278

Krens FA, Jamar D, Rouwendaal GJA, Hall RD (1990) Transfer of cytoplasm from new CMS sources to sugar beet by asymmetric fusion. 1. Shoot regeneration from mesophyll protoplasts and characterization of regenerated plants. Theor Appl Genet 79: 390–396

Lange W, Jung C, Heijbroek W (1990) Transfer of beet cyst nematode resistance from *Beta* species of the section *Patellares* to cultivated beet. Proc 53rd Winter Congr. International Institute for Sugar Beet Research, pp 89-102

Lange W, Müller J, De Bock ThSM (1993) Virulence in the beet cyst nematode (*Heterodera schachtii*) versus some alien genes for resistance in beet. Fundam Appl Nematol 16:447–454

Lapitan NLV (1991) Organization and evolution of higher plant nuclear genomes. Genome 35:171–180

Leutwiler LS, Hough-Evans BR, Meyerowitz EM (1984) The DNA of *Arabidopsis thaliana*. Mol Gen Genet 194:15–23

Maluszynska J, Heslop-Harrison JS (1991) Localization of tandemly repeated DNA sequences in *Saccharomyces cerevisiae*. Curr Genet 12:1–7

Röder MS, Lapitan NVL, Sorrells ME, Tanksley SD (1993) Genetic and physical mapping of barley telomeres. Mol Gen Genet 238:294-303

Salentijn EMJ, Sandal NN, Lange W, De Bock ThSM, Krens FA, Marcker KA, Stiekema WJ (1992) Isolation of DNA markers linked to a beet cyst nematode resistance locus in *Beta patellaris* and *Beta procumbens*. Mol Gen Genet 235:432–440

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Santoni S, Bervillé A (1992). Two different satellite DNAs in *B. vulgaris* L.: evolution, quantification and distribution in the genus. Theor Appl Genet 84:1009–1016

Savitsky H, Price C (1965) Resistance to sugarbeet nematode (*Heterodera schachtii*) in F1 tetraploid hybrids between *B. vulgaris* and *B. patellaris*. Am Soc Sugar Beet Technol 13:370–373

Schmidt T, Jung C, Metzlaff M (1991) Distribution and evolution of two satellite DNAs in the genus *Beta*. Theor Appl Genet 82:793–799

Schmidt T, Metzlaff M (1991) Cloning and characterization of a *Beta vulgaris* satellite DNA family. Gene 101:247–250

Schmidt T, Junghans H, Metzlaff M (1990) Construction of *B. procumbens*-specific DNA probes and their application for the screening of *B. vulgaris* x *B. procumbens* (2n = 19) addition lines. Theor Appl Genet 79:177–181

Schwartz DC, Cantor CR (1984) Separation of yeast chromosome-sized DNAs by pulsed-field-gradient gel electrophoresis. Cell 37:67–75

Speckmann GJ, De Bock ThSM, De Jong JH (1985) Monosomic additions with resistance to beet cyst nematode obtained from hybrids of *B. vulgaris* and wild *Beta* species of the section *Patellares*. Z Pflantzenzucht 95:74–83

Van Daelen RAJ, Jonkers JJ, Zabel P (1989) Preparation of megabase-sized tomato DNA and separation of large restriction fragments by field-inversion gel electrophoresis (FIGE). Plant Mol Biol 12:341–352

Wicking C, Williamson B (1991) From linked marker to gene. Trends Genet 7:288–293

Yu MH (1978) Meiotic behaviour of a disomic nematode resistant sugar beet. Crop Sci 18:615–618