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A complete physical map of a wild beet (*Beta procumbens*) translocation in sugar beet

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Abstract Two sugar beet lines carry homologous translocations of the wild beet *Beta procumbens*. Long-range restriction mapping with rare cutting enzymes revealed that both translocations are different in size, however, an overlapping region of about 350 kb could be identified. Both lines are resistant to the beet cyst nematode but only TR520 carries the previously cloned resistance gene $Hs1^{pro-1}$. Hence, a second gene for nematode resistance (*Hs1-1*) must be located within this region. A bacterial artificial chromosome (BAC) library was constructed from line TR520. The library was screened with a number of *B. procumbens* specific probes and 61 BAC clones were identified. Five BAC clones formed a minimal tiling path of 580 kb to cover the overlapping region between both translocations including the translocation breakpoint. The five BACs from the overlapping region and one additional BAC distal from that contig were sequenced. The total sequence length from the five BACs of the overlapping region amounted to 524 kb which is 74.35% of the total insert size of these BACs. The frequency of retrotransposon sequences ranged between 14.7 and 43.3%. A total of 133 ORFs were identified, none of these showed similarity to known disease resistance genes. Of these, 12 ORFs showed homology to genes involved in biotic stress resistance reactions or to transcription factors. This paper demonstrates how genome specific probes can be employed for cloning an alien gene introgression into a cultivated species.

Keywords *Beta vulgaris* · *Heterodera schachtii* · Bacterial artificial chromosome · Molecular marker · Linkage drag

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Introduction

Sugar beet (*Beta vulgaris* L.) and the wild beet *B. procumbens* both belong to the genus *Beta*. Because sugar beet is highly susceptible to the beet cyst nematode *Heterodera schachtii* Schm. major genes for resistance have been introduced into sugar beet from the resistant wild beet. Due to the lack of chromosome homology between both species (Jung 1987; Savitsky 1975), recombinations occurred by rare translocation events between non-homologous chromosomes. A number of wild beet translocations from independent translocation events have been mapped with RFLP markers to the end of sugar beet chromosome 9 (Heller et al. 1996) suggesting that a translocation hot spot is present in this region of the genome. Genetic mapping data were supported by fluorescence in situ hybridization with translocation specific probes which gave clear signals at the end of chromosome 9 (Desel et al. 2001). The translocations are inherited in a Mendelian manner, however, some meiotic instability resulting in chromosome breakage and loss of the translocation has been observed (unpublished results). Moreover, cultivars carrying the translocations show a substantial yield penalty (G. Koch, personal communication) which can be explained by complete linkage drag for all genes from the translocation.

A number of molecular markers have been selected which are located on the wild beet translocations. Some

of them are repetitive elements exclusively hybridizing with wild beet DNA (Jung et al. 1992) which makes them ideal probes for fingerprinting different translocation lines (Kleine et al. 1998) and for selecting translocation specific clones from genomic libraries (Cai et al. 1997). A number of YAC clones had been identified in this way which cover approximately 800 kb of the translocation from line A906001 (Kleine et al. 1995). The *Hs1*$^{pro-1}$ gene for nematode resistance was cloned from this line using a positional cloning approach based on the wild beet specific probes (Cai et al. 1997). There is some evidence that a second resistance gene named *Hs1-1* is located on the translocations of the beet lines A906001 and Pro4 because *Hs1*$^{pro-1}$ itself did not confer complete nematode resistance after transformation into sugar beet (unpublished results). Moreover, Pro4, which is also completely resistant, does not carry the *Hs1*$^{pro-1}$ gene (Kleine et al. 1998) although a part of its translocation clearly overlaps with the A906001 translocation from *B. procumbens* chromosome 1.

This gave us a reason to clone the overlapping region between both translocations with a purpose to identify the *Hs1-1* gene and other wild beet genes likely to cause the yield reduction. Sequence analysis of the translocation breakpoint will help to understand why wild beet chromatin is preferentially translocated into this region of the beet genome. In this paper, we describe a complete physical map of the overlapping part between both translocations, a BAC covering the translocation breakpoint and the total sequence of five BACs forming the minimal tiling path.

Materials and methods

Plant material

The biennial translocation line A906001 was backcrossed four times with the early bolting (annual), susceptible line 930190. The resulting annual BC_4 line TR520 was found to be hemizygous for the translocation and homozygous for the dominant allele for early bolting (*BB*) as determined by molecular marker analysis (Table 1).

The line TR363 is derived from the translocation line Pro4. It is hemizygous for the translocation. Both translocation lines, A906001 and Pro4, carry translocations from chromosome 1 of *B. procumbens*. They are completely resistant to the beet cyst nematode, but only A906001 carries the nematode resistance gene *Hs1*$^{pro-1}$.

Table 1 Plant material used in this study

Accession no.	Species	Short name
960051	*B. procumbens*	–
930363	*B. vulgaris*, translocation line	TR363
000520	*B. vulgaris*, translocation line	TR520
930176	*B. vulgaris*	–

The line 930176 was used as a susceptible sugar beet control (Table 1). It does not carry any wild beet chromatin. The *B. procumbens* accession 960051 (gene bank accession No. 35335) was kindly provided by Dr. L. Frese (Bundesforschungsanstalt für Landwirtschaft, Braunschweig).

DNA extraction and Southern hybridization

Genomic DNA was extracted from sugar beet leaves, as described by Rogers and Bendich (1985). BAC-DNA was isolated using the NucleoBond BAC 100 Kit (Macherey & Nagel, Düren, Germany). After restriction digest, the DNA was separated on 1% agarose gels and transferred onto hybond-N^+ membrane (Amersham Pharmacia Biotech, Freiburg, Germany) by capillary diffusion blotting overnight, using 0.25 M NaOH/1.5 M NaCl as blotting solution. Southern blots were hybridized with ^{32}P-labeled DNA-probes (Feinberg and Vogelstein 1983) at 60°C and washed twice (1 × SSC; 0.1% w/v SDS) for 20 min and exposed at -70°C.

Megabase DNA was isolated from agarose embedded nuclei according to Kleine et al. (1995). DNA was restricted with rare cutting enzymes (*Bss*HII, *Mlu*I and *Sal*I) and separated by pulse field gel electrophoresis (PFGE) on a CHEF DR II system (Biorad, München, Germany) with switching time of 5–15 s at 200 V for 16 h.

Construction of the BAC library

The BAC library from translocation line TR520 was constructed essentially as described by Hohmann et al. (2003). Beet DNA was partially digested with *Hin*dIII and cloned into the CopyControl-pCC1-BAC™-vector (Epicentre, USA). BAC clones were picked and assembled in 384-microtiter plates with LB-freezing medium. For screening the library, all plates were gridded on high-density filters with 9,216 clones on each filter in 3×3 double-spotting pattern using a Q-Pix-robot (Genetix, USA).

The average insert size was determined by *Not*I restriction analysis. Restriction fragments were separated by PFGE and insert sizes were estimated by comparison with the PFG-marker (NEB, Frankfurt/M, Germany).

PCR and DNA-sequencing

A total of 50 ng genomic DNA or 5 ng BAC-DNA were used for PCR. PCR primers were designed based on the Lasergene PrimerSelect software (GATC Biotech, Konstanz, Germany). PCR was performed in the presence of 50 pmol forward and reverse primers, 1 unit Taq polymerase (Invitrogen, Karlsruhe, Germany), 0.4 mM dNTPs, 2 mM $MgCl_2$, pH 8.3 in a 50 μl reaction vol-

ume. The optimal annealing temperature for each primer combination was determined by gradient PCR using a T-gradient thermocycler (Biometra, Göttingen, Germany). The PCR program was as follows: DNA was denatured at 95°C for 5 min, followed by 30 cycles for 1 min at 95°C, 45 s at 50–62°C (depending on primer combinations) and 1 min at 72°C. PCR products were separated on 1% agarose gels. PCR-products were sequenced with an ABI-sequencer using standard protocols, and sequence analysis was performed using the Lasergene software (GATC Biotech, Konstanz, Germany).

Plasmid clones, BAC library screening and generation of BAC-end probes

Eight translocation specific probes were employed for screening of the BAC library (Table 2). They included YAC-end probes, cDNA sequences and repetitive sequences. The YAC sequences had been cloned from YACs representing parts of the A906001 translocation (Kleine et al. 1995). The plastidal gene *pXX1.6* (probe No. 2421) and the mitochondrial gene *coxI* (probe No. 2423) were used for colony filter hybridization in order to determine the proportion of BACs carrying ct- and mt-DNA. High-density BAC filters were hybridized with P^{32}-labeled probes.

BAC termini were single pass sequenced using standard Sp6 and T7 sequencing primers. Primer sequences were designed as described above. Primer sequences are available on request. The oligomers (18–23 bp) were obtained from MWG (Ebersberg, Germany). The BAC termini were amplified by PCR and PCR-products separated on 1%-SeaKem®LE agarose-gels. PCR-products were isolated from the gel with the NucleoSpin Extract II kit (Macherey and Nagel, Düren, Germany).

Construction of BAC contigs and chromosome walking

The BAC clones were restricted with *Hin*dIII and *Eco*RI and assembled into contigs using the FPC software V7 (Soderlund et al. 2000) by adhering to the protocol described by Marra et al. (1997). The FPC contigs were verified by PCR with BAC terminal sequences and Southern hybridization on restricted BAC-DNA. Chromosome walking was performed with two BAC-end sequences of BAC100 (probe No. 3135) and BAC117 (probe No. 3136). After PCR, fragments were obtained only with genomic DNA of *B. procumbens* and translocation line TR520. The PCR products were used as probes for Southern hybridization and for colony blot hybridization.

BAC-sequencing and sequence analysis

Six BAC clones were sequenced at the Huada Sequencing Centre (Hangzhou, China) with 10 × coverage. All sequences were assembled with the Lasergene SeqMan program (GATC Biotech, Konstanz, Germany) and contig sequences were analyzed by BLAST analysis (blastn, blastx, tblastn, rpsblast) using the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). All BAC sequences have been deposited at NCBI database. An ab initio ORF-analysis with the FgeneSH program on http://www.sun1.softberry.com was performed to identify putative coding regions using the *A. thaliana* matrix. Candidate ORF-annotations are listed in Table S1.

Results

BAC library construction

A new BAC library was constructed from line TR520 carrying the *B. procumbens* translocation at the end of chromosome 9. Totally 61,056 clones were picked and assembled in 384-microtiter plates. The insert size was determined with 146 randomly chosen BAC-clones giving an average size of 97 kb. The frequency of clones containing mt- and ct-DNA was 0.87 and 1.98%, respectively, and 9.5% of the clones did not have an insert. Thus, the BAC library represents 7.5 copies of the

Table 2 Wild beet (*B. procumbens*) specific probes used for screening of the BAC library and the number of BAC-clones identified with the respective probes

Probe no.	Probe type (BAC, YAC, clone no.)	Copy number	No. of BAC clones identified	Reference
1832	cDNA (*Hs1*$^{pro-1}$)	Single copy	3	Cai et al. (1997)
1881	cDNA (23a)	Low copy	15	Unpublished
1866	cDNA (14b)	Single copy	15	Kleine et al. (1998)
3133	YAC-end (YAC58)	Single copy	2	Kleine et al. (1998)
1872	YAC-end (YAC104)	Single copy	8	Unpublished
3134	YAC-end (YAC128)	Low copy	15	Unpublished
1828	Cloned fragment (X2.1)	High copy	7	Salentijn et al. (1995)
1757	Cloned fragment (pRK643)	Low copy	0	Kleine et al. (1995)
3135	BAC-end (BAC100)	Low copy	13	This paper
3136	BAC-end (BAC117)	High copy	10	This paper
3137	YAC-end (YAC58)	Single copy	–	Unpublished
1867	YAC-end (YAC118)	Single copy	–	Unpublished

haploid sugar beet genome. Taking into account that the donor plant is hemizygous for the translocation, a given sequence from the translocation is represented ~3.7×.

Physical mapping

Translocation specific sequences provided a unique opportunity for creating a restriction map of both translocations. DNA of both translocation lines was restricted with rare cutting enzymes, *Bss*HII, *Mlu*I and *Sal*I, and separated by PFGE. Southern blots were hybridized with ten probes derived from the TR520 translocation. The probes included YAC-ends (Nos. 3133, 3134, 1877, 1867 and 3137), cDNA-sequences (Nos. 1881, 1866 and 1832) and repetitive elements 1828 and 1757 (Table 2). The *Bss*HII restriction fragments of the TR520 translocation added up to a total of 1500 kb which was estimated to be the maximum size of the translocation (Fig. 1). The proximal (centromere-near) end of the translocation was likely to be located between the proximal *Bss*HII recognition site and the 3136 locus which is represented by a BAC end (see below). Comparative analysis of restriction fragments gave rise to a physical map covering both translocations (Fig. 1).

The maximum size of the overlapping part of the translocations was estimated in the following way: comparison of both maps showed that marker loci 1866 and 3134 mapped to both translocations, however, with different hybridizing fragments (Fig. 1). The sequences 1881 and 1828 were only found on the TR520 translocation demonstrating that the distal end of the TR363 translocation is located between loci 1866 and 1881. The proximal end was determined by *Mlu*I/*Sal*I-restriction analysis. The overlapping region ends between the 3136 sequence which was cloned from a BAC end (see below) and the distal *Sal*I site from the TR520 translocation because this restriction site is lacking from the TR363 translocation. Thus, the overlapping part was estimated to be approximately 350 kb in size.

BAC library screening and contig construction

The availability of genome specific probes enabled library screening with repetitive sequences to identify clones from the translocation (Table 2). Three copies of each of the repetitive probes 1828 and 1757 were present on the wild beet translocation from line TR520 as determined by Southern hybridization (Fig. 1). These repetitive sequences are only present in the wild beet genome (Kleine et al. 1998). Three YAC-end probes (Nos. 3133, 3134,1872) derived from a YAC-contig for the translocation of line A906001 showed single- or low-copy signals after hybridization on Southern-blotted restricted nuclear DNA from *B. procumbens* and the translocation line TR520. The cDNA probes 1832 from the *Hs1*$^{pro-1}$-gene, 1881 and 1866 showed single- or low-copy hybridization signals after hybridization of Southern-blotted restricted nuclear DNA from *B. procumbens* and the translocation line TR520. However, no signals were visible after hybridization with *B. vulgaris* lacking the wild beet translocation (data not shown). Hence, these probes were regarded as *B. procumbens* specific.

The BAC library screening using the probes listed in Table 2 resulted in 46 positive BAC clones with an average of 6.7 BAC clones per probe. No BAC clone

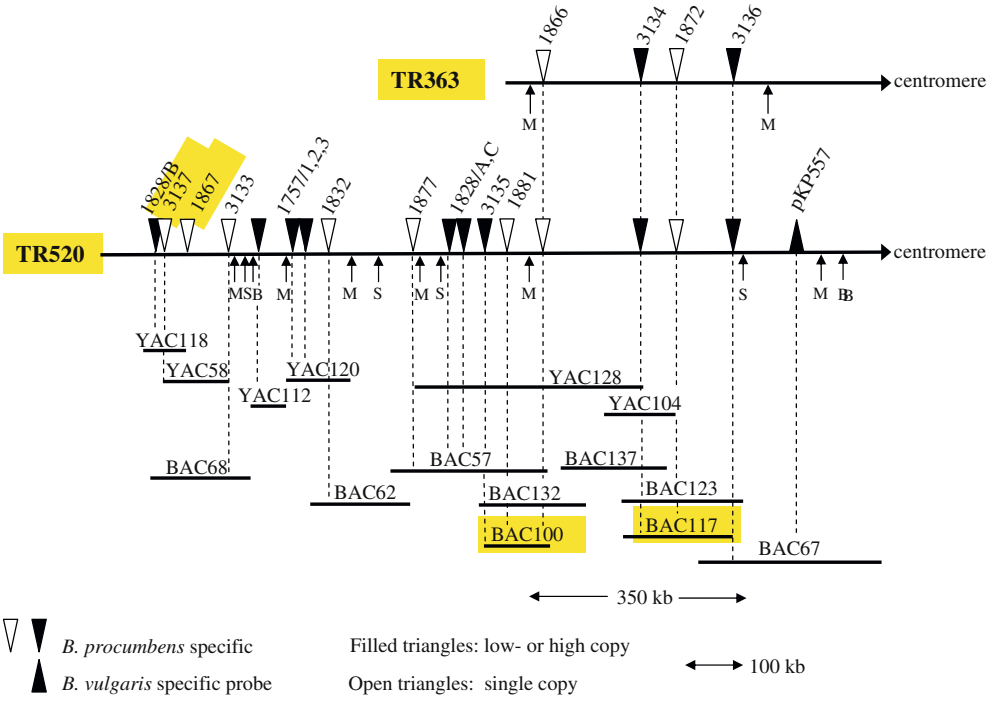

Fig. 1 Physical map of the wild beet translocations from the nematode resistant lines TR520 and TR363. *Arrows* depict restriction sites for *Bss*HII (B), *Sal*I (S) and *Mlu*I (M). A number of probes were used for anchoring the YACs originally described by Kleine et al. (1995) and BACs. Overlaps between clones were determined by restriction fragment analysis, PCR and Southern hybridization. Only BAC clones forming a minimal tiling path of overlapping clones are shown

could be identified with probe 1757. A total of 27 BAC clones hybridized with more than one probe. The insert sizes ranged from 50 to 190 kb.

The distal end of the overlapping region between both translocations was determined in the following way: a total of 15 BAC clones showed homology with the cDNAs 1866 and 1881. After Southern hybridization with probe 1881, an 8 kb *Hin*dIII fragment was visible with BAC57, *B. procumbens* and line TR520 whereas no signal was found with line TR363 (Fig. 2). On contrast, hybridization with sequence 1866 gave an 8 kb *Hin*dIII fragment with BAC57 and DNA from all resistant *Beta* accessions. Conclusively, BAC57 covers the distal end of the overlapping region.

For determination of overlaps between BACs, the BACs were analyzed by PCR and Southern hybridization. *Hin*dIII and *Eco*RI restriction patterns were used to assemble contigs with the FPC-software (Soderlund et al. 2000). Two chromosome walking steps were performed using end-probes of BAC100 (probe No. 3135) and BAC117 (probe No. 3136). In this way, the contig could be extended in the proximal direction with BAC-clones covering the translocation breakpoint. Finally, all BACs were assembled into two contigs (Fig. 1). Previously identified YACs (Kleine et al. 1995) were used for anchoring the BACs. A minimal BAC tiling path with five BACs (67, 123, 137, 132 and 57) was constructed encompassing the overlapping part between both translocations.

Identification of the translocation breakpoint

To determine which BAC extended to the sugar beet chromosome 9, the following experiments were performed. The BAC-DNA was amplified by PCR using primers derived from RFLP probes pKP942, pKP563, pKP1180 and pKP557 which had been mapped to the end of chromosome 9 in close distance to the *Hs1*$^{pro-1}$ gene (Heller et al. 1996). Only the primer combination for pKP557 yielded a PCR fragment with BACs of this region (Fig. 3a). After Southern hybridization with probe pKP557, a 2.75 kb *Hin*dIII fragment was visible with BAC67 and all *B. vulgaris* accessions but not with *B. procumbens* (Fig. 3b). Furthermore, primers were derived from the Sp6-end of BAC67 and used for amplification of DNA from the different accessions. As a result, a fragment of the expected size was visible with DNA of all sugar beet lines but not with *B. procumbens*. The PCR fragments were sequenced. The sequences derived from BAC67, TR520, TR363 and sugar beet were found to be identical (data not shown). Taken together, these data demonstrate that the right end of BAC67 is derived from the sugar beet genome.

BAC sequencing and sequence analysis

All BAC-clones of the minimal tiling path were sequenced together with BAC62. The sequence coverage for each BAC-clone ranged between 51.24% (BAC67) and 97.86% (BAC137) (Table 3). On an average, 20.1 sequence contigs per BAC-clone were generated, with contig sizes ranging between 172 and 33,216 bp. A 40 kb sequence overlap was observed between BAC57 and BAC62. Smaller overlaps of approximately 600 bp were found between BACs137/123 and BACs57/132, respectively. In total 32 anchor sequences from probes and BAC-ends were identified within the sequence-contigs. The total sequence from the five BACs from the overlapping region was 524 kb with non overlapping sequences of 482.9 kb, thus representing 74.35% of the five BACs from the minimal tiling path.

The sequences were analyzed for putative ORFs and their homology to known genes. After BLAST-analysis (tblastn, rpsblast), 133 ORF sequences were identified. The number of putative ORFs ranged between 14 and 33 per BAC-clone. Homology search with ORF-sequences resulted in a number of significant hits from Genbank (Table S1). Thirteen sequences showed homologies to sugar beet ESTs. Interestingly, no NBS-LRR-sequence

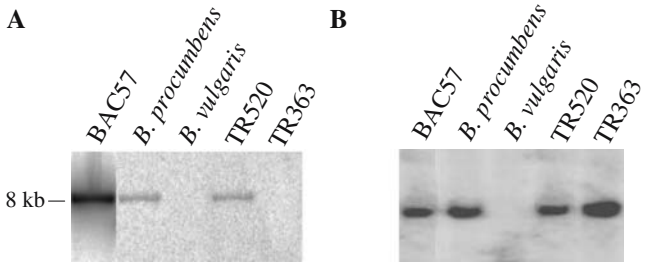

Fig. 2 Southern hybridization experiments for determining the distal end of the overlapping region between the translocations from lines TR520 and TR363. Nuclear DNA of *B. procumbens*, *B. vulgaris* and the translocation lines TR363, TR520 was restricted with *Hin*dIII and probed with **a** cDNA 1881 and **b** cDNA 1866. Exposure time: 72 h

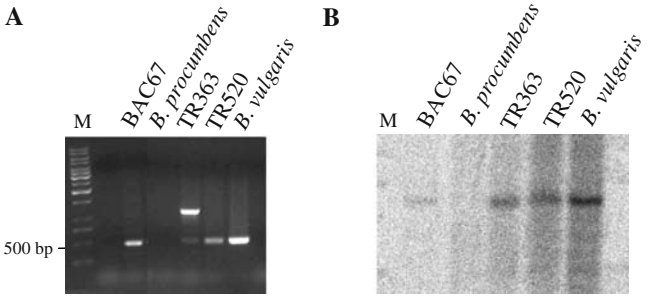

Fig. 3 Southern hybridization and PCR experiments for determining the proximal end of the overlapping region between the translocations from translocation lines TR520 and TR363. **a** Agarose gel electrophoresis of PCR fragments. For PCR, primers derived from sugar beet RFLP marker pKP557 were used. DNA from BAC67, *B. procumbens* and different sugar beet accessions was used as a template. Except for the wild beet, a 498 bp-fragment is visible in all lanes. **b** Nuclear DNA was restricted with *Hin*dIII and probed with RFLP marker pKP557. Exposure time: 24 h

Table 3 Results from sequencing six BAC clones from the translocation of sugar beet accession TR520. Except BAC62, all BACs are from the overlapping region between accessions TR520 and TR363. The percentage of transposable elements was determined by their proportion of blast-hits in relation to total blast-hits in the NCBI database. The number of ORF-sequences was determined with the FgeneSH software. Significant hits were determined after blast-analysis (tblastn) with alignment scores > 50

Clone no.	Insert-size (kb)	Total sequence contigs (kb)	Sequence coverage (%)	Transposable elements (%)	No. of ORFs (in % of total sequence)	No. of ORFs with significant hits (in % of total sequence)
BAC67	160	81.98	51.24	41.6	14 (17.2)	10 (10.4)
BAC123	140	101.66	72.62	43.3	23 (21.5)	11 (17.4)
BAC137	145	141.89	97.86	37.5	33 (22.3)	21 (19.4)
BAC132	140	99.54	71.10	14.7	18 (17.0)	11 (15.0)
BAC57	120	99.03	82.52	33.3	28 (22.9)	13 (14.5)
BAC62	115	97.06	84.40	16.1	17 (14.0)	10 (11.2)

could be found by BLAST search. Also Southern hybridization of BACs from the translocation with an NBS-LRR probe failed to give positive signals (data not shown). A high proportion of repetitive sequences were found. The frequency of transposons and retrotransposons was 14.7% for BAC132 and 43.3% for BAC123. The proportion of transposable elements was 41.6% on BAC67 which covers the translocation breakpoint. The majority were class I copia- and the gypsy-like LTR-retroelements (Table 4) which are distributed over all sequenced BAC clones. BAC57 contains a Ty3-gypsy-like retrotransposon identified from *B. procumbens* (Gindullis et al. 2001b). One non-LTR-retrotransposon (LINE) was located on BAC67. DNA transposons were identified from BAC123, BAC137 and BAC57. They included sequences for a putative Mutator-like transposase, a helitron4-element and a hAT superfamily element. Two transposons from BAC123 belong to the TC1/mariner superfamily Vulmar1 and Promar52 which were described by Jacobs et al (2004).

Discussion

A new BAC library was constructed at the beginning of the project because the libraries available at that time had been made from normal sugar beet genotypes lacking any wild beet translocation (Hohmann et al. 2003; Gindullis et al. 2001a). The translocation line TR363 was not deemed suitable because of the close distance between *Hs1-1* and the telomere in that translocation creating a danger that the most distal part cannot be cloned into a BAC vector (Kleine et al. 1998). However, there was clear evidence that the second nematode resistance gene is located within the overlapping part of the translocations from lines TR520 and TR363 because both lines are completely resistant to the beet cyst nematode.

Our study relies on the assumption that both translocations are colinear and that they do not pair with sugar beet chromosomes. To support this assumption, their pedigrees and molecular characters will be discussed in the following. Both translocation lines are derived from crosses with *B. procumbens*. After backcrossing, monosomic addition lines with one *B. procumbens* chromosome had been selected ($2n = 19$). Among these lines three different classes could be identified differing by their growth types (named 'a', 'b', 'c'), resistance characters (complete or partial) and isozyme marker pattern (Jung 1987). Conclusively, three different chromosomes of *B. procumbens* were found to house genes for cyst nematode resistance. Both lines studied here trace back to addition lines of the 'a' type carrying chromosome 1.

Chromosome pairing between wild and sugar beet chromosomes has been studied by several authors. Almost no chromosome pairing was found during metaphase I of hybrids between *B. vulgaris* and *B. procumbens* (Savitsky 1973). Correspondingly, pairing in monosomic addition lines between wild beet and sugar beet chromosomes was a rare event (Löptien 1984). Therefore, it can be expected that no pairing occurs

Table 4 Transposable elements identified from the five sequenced BAC clones

Class	Type	Element	No.	BACs
I	LTR-retroelement	Ty1/copia-like	45	BAC67, BAC123, BAC137, BAC132, BAC57, BAC62
		Ty3/gypsy-like	20	BAC137, BAC132, BAC57
	Non-LTR retroelements	LINE	1	BAC67
II	DNA transposons	MuDR/Mu superfamily	2	BAC123, BAC57
		Helitron4	1	BAC123
		TC1/mariner superfamily	2	BAC123
		hAT superfamily	1	BAC137

between the wild beet translocations and sugar beet chromosomes. This was supported by DNA fingerprinting studies with repetitive probes which resulted in identical banding pattern among all addition and translocation lines equal by descent (Kleine et al. 1998). Even after several backcross generations, no deviating fragment patterns were found although during the breeding procedure plants with smaller translocations would have been selected because they can be expected to have a better yielding potential due to their reduced linkage drag.

A special feature of this project was the availability of genome specific probes enabling library screening with repetitive sequences to identify clones from the translocation. A number of major satellites has been identified and characterized by Southern and in situ hybridization. The vast majority was specific for only the wild beet or the sugar beet genomes reflecting a high degree of genome diversity between these species (Jung et al. 1992; Dechyeva et al. 2003). Interestingly, transcribed sequences from the wild beet-like 1832 and 1881 also shared low homology with their sugar beet counterparts.

There is clear evidence that the left end of BAC57 and the right end of BAC67 are not present on the TR363 translocation. Thus, the overlapping region between both translocations is represented by BACs 57, 132, 137, 123, and 67. Correspondingly, all BACs fished with markers 1828, 3137, 3133, 1832, 1877, and 3135 could be excluded from further anlysis because they are located distal to the overlapping region.

When constructing a physical map from an alien introgression, problems may arise from syntenic regions between the donor and the recipient genome. Chromosome walking might end in a different region of the genome due to sequence conservation between both genomes. There is, however, convincing evidence that the respective BACs contain DNA from the translocation and not from a syntenic sugar beet. First, hybridization with genomic DNA of *B. procumbens*- and *B. vulgaris*-DNA on restricted BAC clones showed that more fragments are labeled with *B. procumbens*-DNA and that the signal intensity is much higher (data not shown). Second, the presence of wild beet specific repetitive sequences clearly depicted the BACs from the wild beet translocation. Third, PCR-products obtained with different primer combinations from BACs and *B. procumbens* were identical as determined by sequencing. If the BACs were of sugar beet origin, a substantial amount of sequence divergence would have been expected because both species showed very low genetic relatedness even on the level of expressed sequences (Jung et al. 1993; unpublished results).

So far, no translocation breakpoint has been sequenced from plants. Translocation breakpoints from the human genome are characterized by AT-rich regions and retrotransposon-like Alu-elements (Ballif et al. 2004; Edelmann et al. 2001; Gotter et al. 2004). A more detailed sequence analysis of BAC67 will help determine the fine structure of a plant translocation hotspot. There are several lines of evidence demonstrating that BAC67 is the candidate clone that spans the translocation breakpoint. First, the sugar beet specific RFLP-marker pKP557 is covered by this BAC. Second, the Sp6-end could only be amplified from sugar beet DNA and not from genomic DNA of *B. procumbens*. Third, the *B. procumbens* specific sequence 3136 from BAC117 is located on this BAC-clone. In the map of Schumacher et al. (1997), 28 markers extending over 20 cM had been genetically mapped distal to pKP557. Although within short regions of the genome, the marker order from the genetic map must not be fully consistent with the marker order of the physical map, it can be speculated that a substantial part from the end of chromosome 9 distal to pKP557 has been lost during the translocation event.

Sequence analysis showed the presence of various transposable elements in this region. The proportion of these elements is in the range of smaller plant genomes like rice with ca. 35% (http://www.iris.irri.org/IRGSP/) which is in accordance with an estimated genome size of 758 Mbp for sugar beet (Arumuganathan and Earle 1991). Plants with large genomes, like maize and wheat, contain up to 70% transposable elements (Li et al. 2004) which is in sharp contrast to a very low content of transposable elements in *A. thaliana* with 5% (Le et al. 2000). The majority of transposable elements from the translocation are copia- and gypsy-like retroelements (Table 4). For the *B. vulgaris* chromosomes, a dispersed distribution of these elements over all chromosomes was observed (Brandes et al. 1997; Heslop-Harrison et al. 1997), except the centromeric regions and the nucleus-organization-regions (NOR) whereas Vulmar1 and Promar52 mariner-like transposons were clustered within euchromatin and within putatively gene-rich regions at the end of the chromosomes (Jacobs et al. 2004).

The identification of candidate sequences for the *Hs1-1* gene will be a major task for the future. It will focus on sequences with homology to resistance gene analogues (RGA) and stress-related genes. Among the putative ORFs, homologies to transcription factors and to proteins that are involved in response to biotic stress were found. Whether these ORFs are involved in *H. schachtii* resistance will be analyzed by genetic complementation in transgenic hairy roots using the *Agrobacterium rhizogenes* transformation system. This strategy has been proven to be successful for the characterization of genes involved in the resistance against cyst nematodes (Cai et al. 2003; Samuelian et al. 2004). Sequence analysis will also help us understand why beet varieties carrying the wild beet translocation have a yield penalty in the absence of the pathogen which may be caused by the additional genes from a non-adapted wild species or the loss of genes from the end of sugar beet chromosome 9.

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