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Physical mapping and cloning of a translocation in sugar beet (*Beta vulgaris* L.) carrying a gene for nematode (*Heterodera schachtii*) resistance from *B. procumbens*

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Abstract Two diploid (2n = 18) sugar beet (*Beta vulgaris* L.) lines which carry monogenic traits for nematode (*Heterodera schachtii* Schm.) resistance located on translocations from the wild beet species *Beta procumbens* were investigated. Short interspersed repetitive DNA elements exclusively hybridizing with wild beet DNA were found to be dispersed around the translocations. The banding pattern as revealed by genomic Southern hybridization was highly conserved among translocation lines of different origins indicating that the translocations are not affected by recombination events with sugar beet chromosomes. Physical mapping revealed that the entire translocation is represented by a single *Sal*I fragment 300 kb in size. A representative YAC (yeast artifical chromosome) library consisting of approximately 13,000 recombinant clones (2.2 genome equivalents) with insert sizes ranging between 50 and 450 kb and an average of 130 kb has been constructed from the resistant line A906001. Three recombinant YACs were isolated from this library using the wild beet-specific repetitive elements as probes for screening. Colinearity between YAC inserts and donor DNA was confirmed by DNA fingerprinting utilizing these repetitive probes. The YACs were arranged into two contigs with a total size of 215 kb; these represent a minimum of 72% of the translocation.

Key words Sugar beet · Nematode resistance · Yeast artificial chromosomes (YACs) · Contig analysis

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Introduction

The beet cyst nematode (*Heterodera schachtii* Schm.) causes severe damage in sugar beet (*Beta vulgaris* L.). Monogenic resistance has therefore been introduced from three wild species of the *Beta* section IV (*B. procumbens*, *B. patellaris*, *B. webbiana*). The resistant material available can be classified into two groups: (1) monosomic addition lines (2n = 19), which contain the original wild beet chromosome (Savitsky 1975; Speckmann and De Bock 1982; Löptien 1984; Speckmann et al. 1985) or a chromosome fragment conferring resistance (de Jong et al. 1986; Jung and Wricke 1987; Brandes et al. 1987), and (2) diploid sugar beets (2n = 18), which carry a translocation from the wild beet chromosome. The latter are intensively used as introduction lines for sugar beet breeding (Yu 1981, 1983; Heijbroek et al. 1983; Yu 1984; Jung and Wricke 1987; Heijbroek et al. 1988). However, since the agronomic performance of these lines is poor and the transmission rates for nematode resistance genes are low due to meiotic disturbances (Brandes et al. 1987), attempts have been made to isolate the gene for transfer into susceptible sugar beet lines of high breeding vlaue. The chosen strategy can be separated into four steps: (1) the selection of single-copy and repetitive probes closely linked to the resistance genes in *B. procumbens* and *B. patellaris* fragment addition lines or translocation lines (Jung et al. 1990; Jung and Herrmann 1991; Jung et al. 1992; Salentijn et al. 1992), (2) the construction of representative libraries, preferably with yeast artificial chromosomes (YACs) from appropriate resistant plant material, (3) the cloning of long coherent stretches of DNA (contigs) using YAC vectors, and (4) the identification of the resistance gene via genetic complementation of susceptible sugar beet using the "hairy root" system generated by *Agrobacterium rhizogenes* transformation (Paul et al. 1990).

During the past few years the YAC technology has gained significant relevance for the analysis of complex

eukaryotic genomes since it provides a means to isolate relatively large DNA fragments (size range between 100 and > 1,000 kb). If sequence tagged sites (STS) from a high-density linkage map are available as landmarks for screening such libraries and for arranging individual YACs into contigs of overlapping fragments, long stretches of DNA can be cloned and genes selected. In this way a single array of overlapping YACs with an average insert size of 600 kb has been established using an STS map for the human chromosome 21q (Chumakov et al. 1992). This contig spans 40–50 Mbp. Recently, a first attempt to generate a physical map of the entire human genome by overlapping YAC clones has been made (Cohen et al. 1993). The map-based cloning of plant genes has been demonstrated with the isolation of a fatty acid desaturase gene (Arondel et al. 1992), a gene of the abscisic acid response pathway (Griaudat et al. 1992), and a gene from tomato conferring resistance against *Pseudomonas syringae* (Martin et al. 1993).

Sugar beet offers various advantages for studying the map-based cloning of the nematode resistance gene. It possesses a relatively small genome of 750 Mbp (Arumuganathan and Earle 1991), a restriction fragment length polymorphism (RFLP) linkage map has been established (Pillen et al. 1993), and it is readily transformable with *A. tumefaciens* or *A. rhizogenes* vectors (Krens et al. 1988; Lindsey and Gallois 1990). Transformation with the latter results in hairy roots, which can be monitored for nematode resistance (Paul et al. 1990). In the article we present a physical map of a translocation carrying the beet cyst nematode resistance gene from *B. procumbens* chromosome 1 (*Hs1 pro-1*, Lange et al. 1993), the construction of a representative YAC library from the resistant sugar beet translocation line, and the isolation of YAC clones covering more than 70% of the translocation.

Materials and methods

Plant material and DNA preparation

Two diploid (2n = 18) introduction lines carrying translocations from the *B. procumbbens* chromosome 1 (type 'a', Löptien 1984) were used in this study. L86 was kindly provided by Dr. M. H. Yu, USDA, Salinas (Yu 1984). Line A906001 was selected from a diploid resistant sugar beet produced at the Institute of Applied Genetics in Hannover, Germany (Jung and Wricke 1987). It was kindly supplied by the breeding company A. Diekmann-Heimburg Nienstädt, Germany (c.f. Jung et al. 1992). A backcross population segregating for nematode resistance was produced by pollinating a susceptible breeding line with an A906001 population heterozygous for nematode resistance. Susceptible plants from the segregating population together with the monosomic fragment addition line PRO1 carrying the *B. procumbens* chromosome 1 (Jung and Wricke 1987) served as a control. Genomic DNA was isolated from A906001 as described by Jung et al. (1990). The resistance test was performed according to Toxopeus and Lubberts (1979).

High-molecular-weight (HMW) DNA for pulsed field gel electrophoresis (PFGE) analysis and YAC library construction was prepared in the following way: 5g of fresh leaves from 8- to 10-week-old greenhouse-grown sugar beet plants kept in the darkness for 2 days before DNA extraction were ground in liquid nitrogen. The fine powder was suspended in a buffer containing 20 m*M* Tris-HCl, pH 7.8, 250 m*M* sucrose, 5 m*M* $MgCl_2$, 5 m*M* KCl, 0.25% (v/v) Triton X-100, and 0.1% (v/v) β-mercaptoethanol (Steinmüller and Apel 1986) by gentle stirring for 5–10 min on ice. Cell debris was retained by filtering the suspension through two layers of nylon mesh (pore size 50 µm), and the chromatin was collected by centrifugation (1,200 *g* for 5 min at 4°C). The pellet was washed once with isolation buffer and resuspended in 300 µl sterile water. An equal volume of 1.5% (w/v in H_2O) liquefied "InCert"-Agarose (Biozym, Hameln) was added to the chromatin fraction and gently mixed by pipetting up and down with a blunted pipet tip. The agarose solution was carefully poured into 200-µl plug molds (Biorad, Richmond) and solidified for 30 min at 4 °C. The plugs were then incubated with three volumes of 0.5 *M* EDTA, pH 9.5, 1% (w/v) lauroyl sarcosinate and 1 mg/ml Proteinase K (Merck, Darmstadt) for 24 h at 50 °C. After this step the HMW DNA was ready for use either for PFGE or YAC cloning.

λ-DASHII library construction and clone analysis

Genomic DNA of the nematode-resistant sugar beet line A906001 was partially restricted with *Sau*3AI and separated in a 0.75% agarose slab gel. DNA in the size range between 15 and 20 kb was electroeluted onto NA45 membrane (Schleicher und Schüll, Dassel) as described (Jung et al. 1990), ligated with *Bam*HI-restricted λ-DASHII, and amplified according to the manufacturer's (Stratagene, Heidelberg) protocol. Approximately 2×10^6 pfu were transferred to Biodyne A membrane (Pall, Dreieich) and hybridized in combination with the probes pRK643 and pCJ1794 (Benton and Davis 1977). DNA was purified from 27 positive clones using Qiagen tip 100 columns (Diagen GmbH, Düsseldorf), restricted with *Eco*RI or *Xba*I, and separated in 0.75% agarose gels. Southern transfer and filter hybridization were performed as described below. For further analysis, *Eco*RI-restricted λ fragments were isolated and cloned into the plasmid vector pT7T318U (Pharmacia, Freiburg).

YAC library construction

The vector pYAC4 (Burke et al. 1987) and the *Saccharomyces cerevisiae* strain AB1380 (*MAT*a, ψ^+, *ura3, ade2-1, can1-100, lys2-1, his5*) were kindly provided by Dr. M. Olson (St. Louis, USA). Vector DNA was prepared as described by Kleine et al. (1993). HMW DNA was treated with TE/PMSF as described in Kleine et al. (1993). Agarose plugs were equilibrated with 50 m*M* NaCl, 0.75 m*M* spermidine, and 0.30 m*M* spermine 3 times for 30 min each at room temperature, melted at 68 °C for 10 min, and cooled down to 42 °C for 10 min. The β-agarase (USB, Cleveland, 1 unit/100 µl) was then added to remove agarose for partial digestion with *Eco*RI (Boehringer, Mannheim). Digestion of agarose was complete after 1 h at 42 °C. The liquefied sample containing HMW DNA was handled cautiously to avoid shearing. Aliquots of 50 µl were incubated with various *Eco*RI concentrations ranging from 0.1 to 10 units in *Eco*RI restriction buffer (Boehringer, Mannheim) containing 3 m*M* spermidine and 0.5 mg/ml bovine serum albumine for 1 h at 37 °C. The restriction enzyme was inactivated by incubation for 10 min at 65 °C, and the extent of partial digestion was checked by PFGE using the CHEF DR II system (Biorad, Richmond; ramped switching time from 60 to 90s at 200 V for 18 h). Between 0.5 and 4 units *Eco*RI per microgram DNA were used for large-scale digestions. Prior to ligation the partially digested DNA was dialyzed twice for 2 h at room temperature against TE (10 m*M* Tris, 1 m*M* EDTA, pH 8.0).

For ligation, the restricted and dephosphorylated vector pYAC4 was added to the digested and dialyzed DNA in a molecular weight ratio of 1:1 to ensure a 50- to 100-fold molar excess of vector DNA. Ligation buffer and T4 DNA ligase (Boehringer, Mannheim; 1 unit per microgram DNA) were then added and mixed by gentle stirring with a pipet tip; the ligation assay was then incubated at 14 °C overnight. Ten microliters of the ligation assay were used to transform 150 µl ($3–5 \times 10^8$ cells/ml) of yeast spheroplasts. The preparation of yeast spheroplasts, the transformation procedure,

and the selection and storage of recombinant YAC clones in microtiterplates were performed essentially as described in Kleine et al. (1993).

YAC library screening

Yeast clonies from four microtiterplates were grown for 2–3 days on solidified YPD medium (Rose et al. 1990), pooled in batches of 384 individual recombinant YACs, and the DNA was isolated from each pool as described in Green and Olson (1990) with the following modifications. Yeast clonies were scraped off the YPD plate, transferred into 50 ml of liquid YPD, and grown for an additional 1.5 h at 30 °C. The cells were then collected by centrifugation (2,100 *g*) and washed twice with 10 ml 50 m*M* EDTA, pH 7.5, and 40 m*M* β-mercaptoethanol. Also, Zymolyase was replaced by Lyticase (Sigma, St Louis; 2,000 units per 384 colonies) for spheroplasting the yeast cells. The DNA was finally recovered by ethanol precipitation. For dot-blot screening, 20 µg DNA per pool was denatured in 0.5 *M* and 1.5 *M* NaCl for 10 min at room temperature. The DNA solutions were individually transferred under vacuum onto Biodyne B membrane (Pall, Dreieich) using the dot-blot device of Schleicher and Schüll (Dassel). After 20 min at 80 °C, the membrane was neutralized in 2 × SSC for 5 min at room temperature. Radiolabelled (Feinberg and Vogelstein 1983) probes were hybridized at 60 °C (Jung et al. 1990), and the filters were washed twice in 0.5 × SSC and 0.2% SDS at 60 °C for 30 min. The membranes were exposed to an X-ray film (Amersham, Braunschweig) with a Quanta III intensifying screen (Du Pont, Bad Homburg) for 7 days at −70 °C.

Individual clones were finally identified by colony hybridization. For colony lifts, autoclaved Biodyne A membranes (Pall, Dreieich) were placed onto solidfied YPD plates, and the yeast cells were transferred from the microtiterplates onto the membrane using a 96-pin device. After incubation at 30 °C overnight the membranes were removed and placed, colony side up, on 3MM Whatman soaked with 1 *M* sorbitol, 100 m*M* Na_3citrate, pH 5.8, 60 m*M* EDTA, 40 m*M* β-mercaptoethanol, and 200 units Lyticase/ml in a total volume of 20 ml for 384 colonies. Cells were spheroplasted at 37 °C overnight in a wet chamber to avoid evaporation and lysed by treatment with denaturing solution for 20 min followed by subsequent washes in 1 *M* TRIS-HCl, pH 7.6, 1.5 *M* NaCl, and 0.1 *M* NaCl for 5 min each. Cell debris was thoroughly removed from the membranes with a soft tissue. The membranes were ready for hybridization as described above after treatment at 80 °C for 20 min and incubation in 2 × SSC for 5 min. HMW DNA of individual YACs was isolated, separated on plused field gels, and analyzed by Southern hybridization as described in Kleine et al. (1993).

Physical mapping

HMW DNA was digested in agarose to completion with the rare-cutting restriction endonucleases *Sal*I or *Mul*I, or double digested with *Sal*I/*Mlu*I (Pharmacia Biotech, Freiburg). Aliquots of 4–7 µg DNA were incubated overnight with 100 units of the enzymes in restriction buffer containing 3 m*M* spermidine at 37 °C. The DNA was separated by PFGE on a CHEF DRII system with ramped switching time of 60–120 s at 200 V for 24 h. The DNA was transferred onto nylon membranes and subsequently hybridized as described in Kleine et al. (1993).

Analysis of isolated YAC clones

Prior to digestion of the YAC DNA with restriction endonucleases (Boehringer, Mannheim), the agarose was liquefied by agarase treatment. Ten nanograms of the YAC DNA and 10 µg of the genomic DNA of the translocation line A906001 were restricted with *Eco*RI or *Xba*I, or double digested with *Eco*RI/*Xba*I (20 units each) for 2 h at 37 °C. The DNA was separated on a 0.75% agarose slab gel, transferred to nylon membrane, and hybridized with the wild beet-specific probes.

Plasmid probes

The recombinant plasmid pRK663 carrying a 189-bp tandemly repetitive satellite DNA element of the sugar beet genome was selected from a shotgun library of genomic DNA of the addition line PRO1 (Kleine, unpublished). Probes pRK643 (Jung et al. 1992), pCJ1794 (identical with PCR1012; Jung et al. 1992), and pCE1757 share homology to different dispersed repetitive DNA elements present only in the wild beet *B. procumbens*. The latter probe is an *Eco*RI subclone of the λ-clone L1710 (Jung et al. 1995). All these probes display homology to resistant sugar beet lines carrying translocations from *B. procumbens*.

The DNA fragments were labelled by random priming (Feinberg and Vogelstein 1983) in the presence of α-[^{32}P] dCTP.

Results

Construction and characterization of a YAC library from sugar beet

Initial attempts to isolate large amounts of HMW DNA from the translocation line A906001 with the customary protoplast embedding technique (Jung et al. 1990) failed since the amounts of DNA obtained were too low for the YAC cloning procedure. The protocol outlined above proved to be suitable for the construction of a representative YAC library. The procedure yielded between 20 and 40 µg HMW DNA (> 1,000 kb) per gram plant material which, after liquefication of the agarose by agarase, was digestable to completion with the restriction endonuclease *Eco*RI after 1 h of incubation at a concentration of 10 units enzyme per microgram DNA. The transformation efficiency was monitored by transforming the centromeric yeast plasmid pRS316 (Sikorski and Hieter 1989), which gave 5 × 10^5 cfu of the Ura$^+$ phenotype per microgram plasmid DNA. Recombinant YACs transformed with a frequency of 200–300 cfu of the Ura$^+$ phenotype per microgram DNA.

The DNA of 107 individual Ura$^+$/Trp$^+$ yeast colonies was analyzed by PFGE to estimate the frequency, size distribution, and origin of the inserts. All of the yeast clones checked contained an additional DNA fragment that hybridized with vector-specific sequences as well as with genomic sugar beet DNA, though at a different signal intensity, probably because of varying portions of repetitive DNA. For further characterization of the library, a subset of 768 colonies was screened by colony hybridization with the sugar beet satellite probe pRK663. Nine colonies (1.2%) gave signals of varying intensity (data not shown). YAC clones carrying plastid DNA were not detected in the library because isolated chromatin had been used for DNA extraction (data not shown).

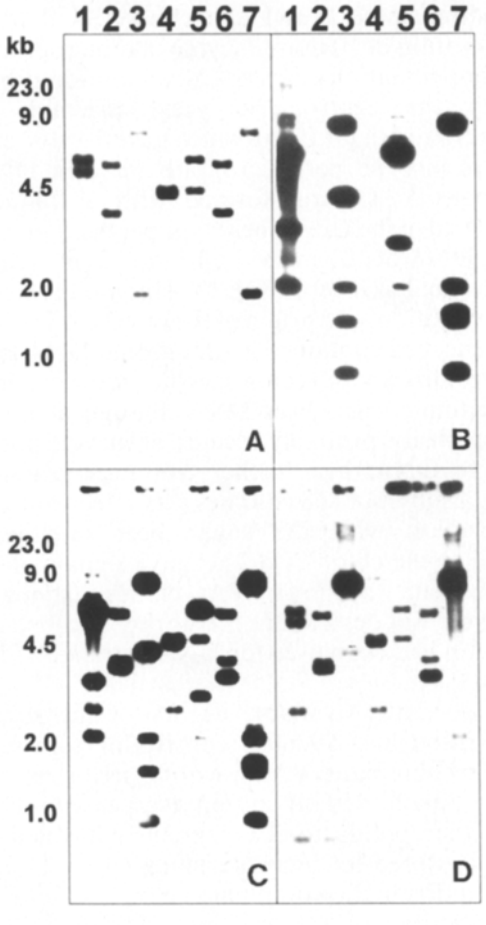
The currently available library of the sugar beet translocation line A906001 consists of approximately 13,000 recombinant YACs with insert sizes ranging between 50 and 450 kb and an average size of 130 kb, which corresponds to 2.2 genome equivalents. This library is stored in microtiterplates at −70 °C in the form of individual recombinant cells.

λ clones

Screening of the A906001 λ library with probes pRK643 and pCJ1794 resulted in the identification of 27 clones with an average insert size of 14.5 kb. As expected, all inserts hybridized with the repetitive probes pRK643 and/or pCJ1794, 11 clones displayed homology to only 1 of these probes. The individual inserts were characterized by restriction site mapping (data not shown). Of the 132 *Eco*RI/*Xba*I restriction fragments that hybridized with labelled DNA of *B. vulgaris* or *B. procumbens*, 45.4% of the fragments displayed differential homology, 8.3% hybridized exclusively with *B. procumbens* DNA, and 37.1% showed a strong signal with *B. procumbens* and only a weak signal with *B. vulgaris* DNA (Fig. 1). Differential homology was also apparent after hybridization with the wild beet-specific probes: 37.9% of the fragments hybridized exclusively with 1 probe; 53.0% did not contain any of the 643/1794 repetitive elements.

To further verify the origin of the λ inserts, 33 *Eco*RI/*Xba*I fragments were used as probes for RFLP analysis of 10 resistant and 10 susceptible plants from the segregating population. Of these, 12 probes (36.4%) hybridized exclusively with DNA from resistant plants, and 16 gave polymorphic banding patterns. Four low-copy probes gave identical results with DNA from resistant and susceptible plants, while the remainig probes gave complex banding patterns that could not be evaluated. One 0.5-kb fragment (pCE1757, Jung et al. 1995) that exclusively hybridized with DNA from resistant plants was further used for screening the YAC library.

Fig. 1A–D Insert analysis of seven λ clones from the A906001 library. λ DNA was double-disgested with *Eco*RI and *Xba*I and subsequently hybridized with radiolabelled DNA: **A** pCJ1794, **B** pRK643, **C** *B. procumbens*, and **D** *B. vulgaris*. A number of fragments hybridized exclusively with DNA from *B. procumbens*



Dot-blot screening of the YAC library and isolation of individual YACs

A novel approach that had been developed to allow systematic screening of complex libraries in two steps resulted in the isolation of the different YAC clones. Thirty YAC-DNA pools consisting of 11,520 individual YAC clones in total were checked by dot-blot hybridization with the repetitive *B. procumbens*-specific probes pCJ1794 and pCE1757 (Fig. 2A). After colony hybridization with the corresponding filters of three pools 1 YAC clone per pool could be isolated (Fig. 2B). Size and origin of the YACs were determined by PFGE analysis resulting in three YACs of 150 kb (YAC120E7), 60 kb (YAC112G9), and 50 kb (YAC42D12) (Fig. 2C), respectively.

To verify the colinearity between the YAC inserts and the genomic DNA of the translocation line A906001, Southern blots of the digested DNA were hybridized subsequently with probes pCE1757 and pCJ1794 (Fig. 3). YAC-DNA restriction fragments generated by single *Eco*RI digests and *Eco*RI/*Xba*I double digests perfectly matched those fragments visible with the donor DNA. All of the genomic fragments hybridizing with the repetitive probes pCE1757 and pCJ1794 are located within the YAC clones. Only a single genomic *Xba*I fragment (Fig. 3B, asterisk) was not detectable with YAC DNA. Instead, a larger YAC restriction fragment (Fig. 3B, two asterisks) appeared that was not visible with genomic DNA, indicating that this fragment is homologous to the single *Xba*I genomic fragment and located next to the cloning site of YAC120E7.

Comparative RFLP analysis of different translocation lines

To study the inheritance of the repetitive elements linked to the resistance gene, translocation lines of various origins were tested with the probe pCJ1794. The experiment included two introduction lines (A906001, L86) housing translocations from the *B. procumbens* chromosome 1 (type 'a', Löptien 1984) and a population resulting from a backcross between a heterozygous A906001 and a susceptible sugar beet plant. Southern analysis revealed that both introduction lines displayed equal banding patterns (Fig. 4). Moreover, the distribution of

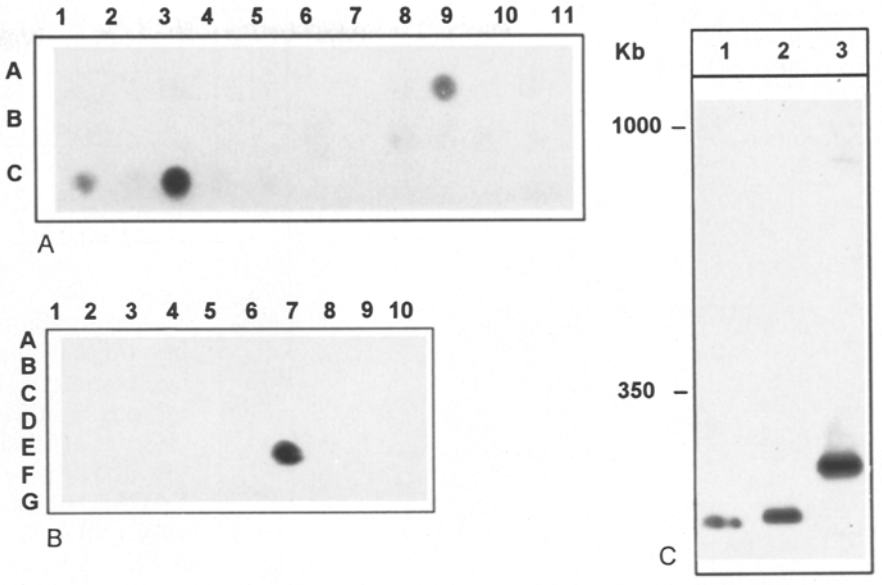


Fig. 2A–C Isolation of three recombinant YAC clones from the resistance-carrying translocation using the probe pCE1757. **A** Dot-blot screening of the YAC library, **B** colony hybridization, **C** PFGE analysis

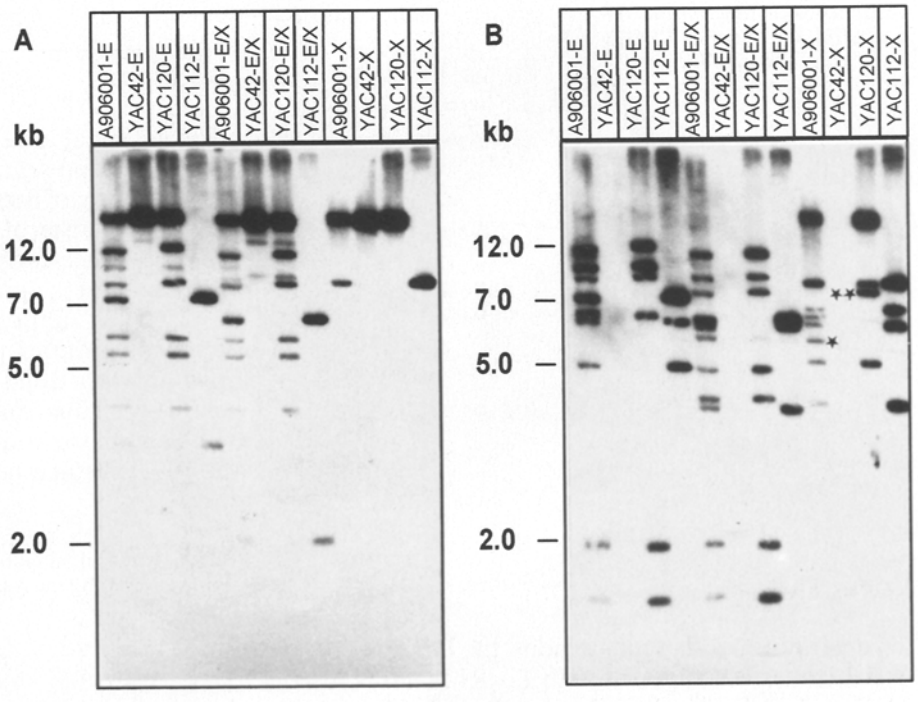


Fig. 3A, B Analysis of colinearity between genomic DNA of the translocation line A906001 and the recombinant YAC clones, each digested with *Eco*RI (*E*), *Eco*RI/*Xba*I (*E*/*X*), and *Xba*I (*X*). **A** Hybridization with probe pCE1757, **B** hybridization with probe pCJ1794. The *asterisk* indicates a single genomic *Xba*I fragment, *two asterisks* indicate a single *Xba*I fragment of YAC120E7

the repetitive elements was conserved among all resistant individuals from the segregating population. In contrast, all of the susceptible plants analyzed lacked the wild beet DNA elements.

Physical mapping

The size of the translocation was determined by PFGE mapping using different rare-cutting restriction endonucleases. After hybridization with either pCE1757 or pCJ1794, 1 *Sal*I (300 kb), 2 *Mlu*I (250 and 140 kb) and 2 *Sal*I/*Mlu*I (160 and 140 kb) fragments were visible (Fig. 5). Comparative analysis with DNA of the original wild beet chromosome (line PRO1) uncovered that neither the 4 *Sal*I fragments (1,300, 1,030, 940 and 440 kb, c.f. Jung et al. 1992) nor the *Mlu*I fragment (1,500 kb, data not shown) present on the original chromosome were present in the translocation line (Fig. 6). It is therefore conclusive that both *Sal*I sites flanking the repetitive elements in the translocation line A906001 must originate from the sugar beet chromosome while the internal *Mlu*I site is derived from the translocation itself.

Fig. 4 Genomic Southern analysis of *Hin*dIII-restricted DNA from two nematode-resistant introduction lines A906001 and L86 hybridized with probe pCJ1794

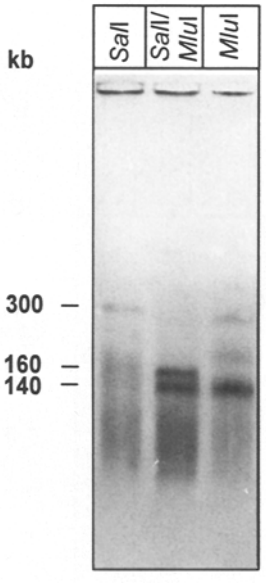


Fig. 5 Physical mapping of the resistance-carrying genome region. HMW DNA was restricted with *Sal*I, *Sal*I/*Mlu*I and *Mlu*I, separated by PFGE, and hybridized with the probe pCE1757. Exposure: 4 days

Contig analysis

To determine overlapping regions, the DNA of all YAC and λ clones was digested with *Eco*RI, *Xba*I, and *Hin*dIII and hybridized with the 2 repetitive probes pCJ1794 and pRK643. Overlapping regions were established with at least 2 common restriction fragments. In this way, 21 λ and three YAC inserts could be arranged into two contigs (A, B/C; Table 1), each resulting in a defined restriction map (data not shown). The repetitive elements were more or less evenly distributed within the contigs. The total length of both contigs was approximately 216 kb. It should be noted that none of the inserts contained a *Sal*I restriction site. However, a single *Mlu*I restriction site was present in YAC120E7 and YAC42D12 in the proximity of the right vector arm (small arm, YAC120E7) and left vector arm (YAC42D12).

Table 1 Contig analysis on the basis of *Eco*RI/*Xba*I restriction sites and DNA fingerprinting with the probes pRK643 and pCJ1794

	Number of λ clones	Number of YAC clones	Total length (kb)
Contig A	9	2	150
Contig B/C	12	1	66
Total	27	3	216

The combining of all the mapping data resulted in a first physical map of the translocation carrying the beet cyst nematode (BCN) resistance locus (Fig. 6). Contig A extends continuously 15 kb in one direction from the internal *Mlu*I site and stretches approximately 135 kb towards the right translocation breakpoint. Both YAC42D12 and λ cluster A overlap completely with YAC120E7. Contig B/C resides within the segment bordered by the *Mlu*I site and the left translocation breakpoint. λ cluster B/C extends the end of YAC112G9 for 5.6 kb, as judged from restriction mapping (data not shown).

Discussion

The principal aims of this study were: (1) the construction of a representative YAC library of the nematode-resistant sugar beet line A906001, (2) the isolation and characterization of recombinant YAC and λ clones from the resistance-carrying translocation from *B. procumbens*, and (3) the physical mapping of the translocation.

The YAC library presented here corresponds in size to plant libraries previously reported for *Arabidopsis thaliana* (Ward and Jen 1990; Grill and Somerville 1991), carrot (Guzmán and Ecker 1988), maize (Edwards et al. 1992), tomato (Martin et al. 1992), and barley (Kleine et al. 1993). A sugar beet library has been constructed from

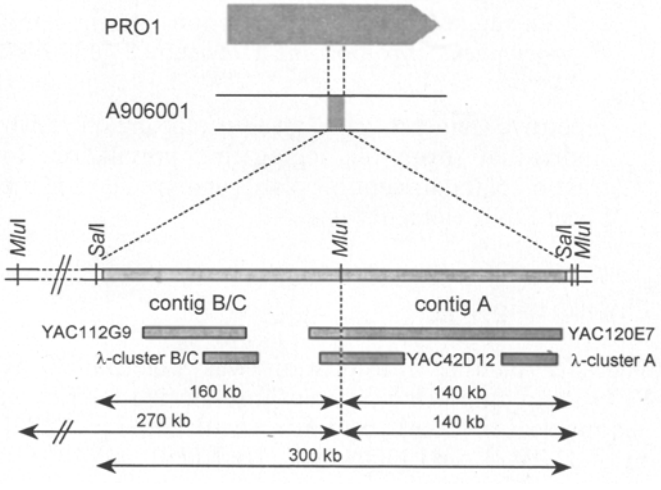


Fig. 6 Physical map of the *B. procumbens* translocation (*shaded regions*) carrying the resistance gene present in the diploid sugar beet line A906001

a non-resistant breeding line consisting of approximately 15,000 clones with an average insert size of 140 kb (Eyers et al. 1992). In contrast to the YAC libraries mentioned above, our library does not contain organellar DNA because isolated chromatin was used as the source for megabase DNA instead of protoplasts. The protocol outlined above yielded HMW DNA highly concentrated and completely digestable in less than 24 h. It may also be useful for establishing YAC libraries from a broad variety of plant species.

We have identified three YACs with the aid of previously isolated λ clones originating from the resistance-carrying translocation. The YAC inserts hybridized with wild beet-specific repetitive DNA elements, thus proving that they originate from the resistance-carrying *B. procumbens* translocation. In addition, subclones used in the RFLP analysis clearly distinguished between resistant and susceptible plants.

The YAC inserts were characterized by DNA fingerprinting employing SINE (short interspersed repetitive DNA) elements spread around the wild beet genome. Basically, the same technique has been successfully used for establishing large YAC contigs of the human genome (Bellanné-Chantelot et al. 1992). In our study, YAC fingerprinting yielded three main results. (1) All of the repetitive elements present on the translocation are represented by the three YACs, indicating that there is no evidence for rearrangements within the YACs. (2) Two YACs overlap, as judged from common restriction fragments. (3) The repetitive elements are evenly distributed around the cloned part of the translocation.

Previous data have suggested a close linkage between wild beet-specific elements and the resistance gene (Jung et al. 1992, Salentijn et al. 1992). To corroborate this hypothesis and to compare the nature of the translocations, two translocation lines of different origins have been analyzed with these probes. Although both lines resulted from independent translocation events they displayed equal banding patterns, indicating the possibility of well-defined translocation breakpoints in the *Beta* genomes. In addition, strict cosegregation between the repetitive pRK643 elements and the resistance gene was verified by the analysis of a segregating population. These data suggest that all introduction lines housing the *B. procumbens* chromosome 1 resistance gene *Hs1 pro-1* (Löptien 1984; Lange et al. 1993) carry wild beet translocations of equal size.

To estimate the size of the wild beet translocation, we combined physical mapping data from the translocation line and the addition line. The large-scale mapping around the resistance gene of the fragment addition line PRO1 resulted in 4 *Sal*I fragments of 1,300, 1030, 940, and 440 kb that hybridized with probe pRK643 (Jung et al. 1992). None of these fragments around the resistance gene was present in the translocation line, indicating that at least one translocation breakpoint is located within any of these *Sal*I fragments. Conclusively, the left *Sal*I site in the translocation line resides in the sugar beet chromosome (Fig. 6). The right site was localized by means of double digestion with *Mlu*I and *Sal*I: since no information was available about the linking fragments located between the 4 *Sal*I fragments, the translocation might extend far more to the right. In that case, 1 of the *Mlu*I fragments from A906001 could be expected to be present also on the original chromosome. However, comparative restriction analysis of the translocation and addition lines showed that none of the *Mlu*I fragments in the size range of the A906001 fragment was present on the original chromosome. In contrast, common bands were found in *Eco*RI- or *Hin*dIII-restricted DNA (data not shown), which provides compelling evidence that both *Sal*I sites are located on the sugar beet chromosome and that the entire translocation from the wild beet is present in the 300-kb *Sal*I fragment.

Finally, this inference allows estimation of the fraction of the translocation represented by the contigs isolated to date. Using the *Mlu*I site for anchoring contig A, we could place the contigs on the translocation as shown in Fig. 6. At least 72% of the 300-kb *Sal*I fragment are represented by the YAC and λ clones, which contain all of the wild beet repetitive elements of that segment. Experiments are in progress to identify overlapping YACs filling the gap between contigs A and B/C and to screen a root cDNA library with the YACs in order to transfer coding regions into susceptible sugar beet using the hairy root transformation assay (Paul et al. 1990).

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