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Construction of a YAC library from a *Beta vulgaris* fragment addition and isolation of a major satellite DNA cluster linked to the beet cyst nematode resistance locus $Hs1^{pat-1}$

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**Abstract** A YAC library was constructed from the *Beta vulgaris* fragment addition AN5-203b. This monosomic fragment addition harbors an approximate 12-Mbp fragment of *B. patellaris* chromosome 1 accommodating the $Hs1^{pat-1}$ locus conferring resistance to the beet cyst nematode (*Heterodera schachtii*). The YAC library consists of 20,000 YAC clones having an average size of 140 kb. Screening with organelle-specific probes showed that 12% of the clones contain chloroplast DNA while only 0.2% of the clones hybridizes with a mitochondrial specific probe. On the basis of a sugar beet haploid genome size of 750 Mbp this library represents 3.3 haploid genome equivalents. The addition fragment present in AN5-203b harbors a major satellite DNA cluster that is tightly linked to the $Hs1^{pat-1}$ locus. The cluster is located on a single 250-kb EcoRI restriction fragment and consists of an estimated 700–800 copies of a 159-bp core sequence, most of which are arranged in tandem. Using this core sequence as a probe, we were able to isolate 1 YAC clone from the library that contains the entire 250-kb satellite DNA cluster.

**Key words** Nematode resistance Pulsed field electrophoresis · Satellite DNA · Sugar beet Yeast artificial chromosome library

**Abbreviations** YAC Yeast artificial chromosome BCN beet cyst nematode RAPD random amplified polymorphic DNA RFLP restriction fragment length polymorphism

**Introduction**

The beet cyst nematode *Heterodera schachtii* Schm. is a severe pest in most areas of sugar beet (*Beta vulgaris* L.) cultivation and can cause considerable losses in yield annually. Since crop rotation cannot fully overcome the nematode problem and the use of nematicides increasingly forms a threat to the environment, the introduction of resistant sugar beet varieties is highly desirable. However, no useful level of resistance is present in *B. vulgaris*. Resistance genes against *H. schachtii* were identified in wild beets (*B. procumbens* Chr. Sm. and *B. patellaris* Moq.) belonging to the section *Procumbentes* (Coens 1975; Yu 1984). In *B. procumbens* two loci conferring resistance are present, $Hs1^{pro-1}$ on chromosome 1 and $Hs2^{pro-7}$ on chromosome 7. In *B. patellaris* the $Hs1^{pat-1}$ locus on chromosome 1 confers resistance against the nematode (nomenclature according to Lange et al 1993, for further references see Lange et al 1990; Van Geyt et al 1990). Attempts have been made to transfer the nematode resistance genes to *B. vulgaris* through interspecific crossings. While direct gene transfer appeared to be impossible, resistant monosomic additions (2n = 18 + 1) containing *B. patellaris* chromosome 1, *B. procumbens* chromosome 1, or *B. procumbens* chromosome 7 were obtained. The backcrossing of resistant monosomic additions with diploid *B. vulgaris* resulted in resistant diploid cytotypes (Heijbroek et al 1988; Jung and Wricke 1987; Savitsky 1978). However, genetic studies suggest that the insertion in the recipient genome of the alien piece of chromosome carrying the gene for resistance is unstable (Lange et al 1990). The alien chromosomes in the monosomic additions also showed breakage, leading to resistant backcross individuals in which the resistance is located on a telosome (Speckmann et al 1985) or on a chromosome fragment (Brandes et al 1987; De Jong et al 1986) The chromosome fragments especially are poorly transmitted through meiosis, and they may also show some mitotic instability.

To obtain genetically stable nematode resistant sugar beet varieties, cloning of the resistance gene from one of
the wild beet species and the subsequent introduction of the gene in *B. vulgaris* forms a powerful alternative to the introduction of the resistance by classical plant breeding. Cloning of this gene, however, can not be carried out using the conventional molecular cloning strategies since no gene product from the BCN resistance gene is known. Instead, strategies such as map based cloning (Orkin 1986) can be followed in which molecular markers, like RFLP markers or RAPD markers, tightly linked to the gene of interest are identified. The markers are used to screen DNA libraries containing very large stretches of cloned DNA. These libraries mostly are constructed in YAC vectors (Burke et al 1987), which can accommodate insert DNA up to the megabase-size range (Chumakov et al 1992; McCormick et al 1989). By means of chromosome walking the isolated YAC clones are arranged into contigs spanning the region in which the gene is located. Subsequently, candidate genes are isolated from the YAC clones and the gene of interest is identified by functional analysis and/or complementation studies. The feasibility of map based cloning for the isolation of plant genes has been demonstrated by the successful isolation of a gene encoding an omega-3 desaturase (Arondel et al 1992), the ABI3 gene (Chang et al 1993) all from *Arabidopsis thaliana* and, recently, by the cloning of the *Pto* gene from tomato conferring resistance against *Pseudomonas syringae* pv. *tomato* (Martin et al 1993).

The map-based cloning of other disease-resistance genes from various important crop species can be foreseen in the near future; molecular markers linked to genes acting against plant pathogenic bacteria (Ronald et al 1992; Kunkel et al 1993), fungi (Lehners-Schippers et al 1992; Sarvatt et al 1988; Schöller et al 1992), nematodes (Barone et al 1990; Jung et al 1992; Klein-Lankhorst et al 1991; Kreike et al 1994; Salentijn et al 1992; Weiseman et al 1992) and viruses (Ritter et al 1991; Young et al 1988) have been isolated and, in addition, the construction of YAC libraries of crop species such as maize, barley, carrot, and tomato have been described (Edwards et al 1992; Guzmán and Ecker 1988; Klein et al 1993; Martin et al 1992). Also, the construction of a YAC library from sugar beet line Ar* has been reported recently (Eyers et al 1992) which, however, is a nematode-susceptible line. As a first step towards the map-based cloning of the beet cyst nematode resistance gene, we have constructed a YAC library from the resistant *B. vulgaris* fragment addition AN5-203b. This fragment addition contains a 12 Mbp chromosomal fragment derived from *B. patellaris* chromosome 1 on which the resistance gene is located. The library was screened with a wild beet-specific satellite DNA (Salentijn et al 1992), resulting in the isolation of a YAC clone containing a 250-kb satellite DNA cluster that is tightly linked to the BCN gene.

### Materials and methods

#### Chemicals

Zymolyase-100T was obtained from Seikakagu Kogyo Co, Lyticase, amino acids, adenine hydrochloride, and sorbitol were from Sigma, and the type of glucose "for microbiology" was from Merck. All growth media were obtained from Difco. High-molecular-weight DNA markers were from BioRad. Restriction and modification enzymes and β-agarase were obtained from New England Biolabs. Proteinase K was purchased from Boehringer. All agarose types used were from FMC.

#### Strains and plasmids

*Saccharomyces cerevisiae* AB1380 (Burke et al 1987), pYAC4 (Burke et al 1987), and pPHcP1 (Van Grinsven et al 1986) were obtained from R. van Daelen (LUW, Wageningen), Sat-121 (formerly referred to as 121.3) was isolated by Salentijn et al (1992), pCOXII (Fox and Leaver 1981) was obtained from C. Kick (CPR-DLO, Wageningen), and p663 was a gift of C. Jung (Christian-Albrechts Universität, Kiel). The yeast growth and selection media were prepared according to Gibson and Somerville (1991).

#### Plant DNA isolation

Total DNA was isolated (Bernatzky and Tanksley 1986) from leaf tissue sampled from at least five individual plants. High-molecular-weight (HMW) DNA was isolated basically as described by Schwartz and Cantor (1984). Mesophyll protoplasts (pps) were isolated from young leaves (20–50 mm) taken from at least five individuals of plant material 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-protopil-gallate 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 to a final concentration of 8 x 106 pps per 100 microliters by centrifugation (800 rpm), mixed (1:1) with 1.5% Incert agarose (FMC) in 0.25 M EDTA, and quickly poured into molds (V = 100 microliters). The final pps concentration in the agarose plugs of 4 x 106 pps is equivalent to 6.3 μg DNA (based on a DNA content of 1.57 picograms for the diploid *Beta* genome (Arumuganathan and Earl 1991). After solidification the plugs were immediately incubated twice for 24 h in 0.5 M EDTA supplemented with 1 mg/ml protease K and 0.02 M sodiumbisulphite (V = 15 ml per 8 plugs) at 50 °C until the green color had disappeared. For subsequent enzyme treatment the protease K activity was inhibited by incubation in TE-buffer (10 mM TRIS.HCl, 10 mM EDTA, pH = 7.5) supplemented with 1 μM PMSF (V = 15 ml per 8 plugs) for 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 over a total incubation time of 6 h.

#### Yeast DNA isolation

Total yeast DNA was isolated according to Hoffman and Winston (1987). HMW yeast DNA was isolated essentially according to Schwartz and Cantor (1984) with one modification; after incubation in protease K/EDTA/sarkosyl the agarose plugs were collected from the enzyme mixture and washed once with CPW-salts containing 9% mannitol. After isolation the pps were concentrated to a final concentration of 8 x 106 pps per 100 microliters by centrifugation (800 rpm), mixed (1:1) with 1.5% Incert agarose (FMC) in 0.25 M EDTA, and quickly poured into molds (V = 100 microliters). The final pps concentration in the agarose plugs of 4 x 106 pps is equivalent to 6.3 μg DNA (based on a DNA content of 1.57 picograms for the diploid *Beta* genome (Arumuganathan and Earl 1991). After solidification the plugs were immediately incubated twice for 24 h in 0.5 M EDTA supplemented with 1 mg/ml protease K and 0.02 M sodiumbisulphite (V = 15 ml per 8 plugs) at 50 °C until the green color had disappeared. For subsequent enzyme treatment the protease K activity was inhibited by incubation in TE-buffer (10 mM TRIS.HCl, 10 mM EDTA, pH = 7.5) supplemented with 1 μM PMSF (V = 15 ml per 8 plugs) for 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 over a total incubation time of 6 h.

#### Pulsed field gel electrophoresis

Pulsed field separations of HMW plant DNA were performed using a Rotaphor devise (type IV, Biometra). Restriction fragments in the size range of 100–1,000 kilobasepairs (kb) were separated using a linear increasing pulse time of 50–70 s at 180 V during a run of 20 h. The angle between the two fields was 120°. The agarose gels (1%, SeaKem UltraPare, FMC) were run in 0.25 x TBE buffer at 14 °C.

Size selection of HMW plant DNA for cloning purposes was carried out by CHEF electrophoresis using a BioRad CHEF DRII apparatus. Gels (1% agarose) were run in 0.5 x TBE (1 x TBE = 90 mM TRIS, 90 mM boric acid, 2 mM EDTA, pH 8.3) at 200 V, 14 °C. Pre-running of the embedded DNA was carried out for 5 h using a pulse time of 60 s. Size selection after partial EcoRI digestion was performed by a 2 h-long gel run with a pulse time of 6 s.