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Construction of *Beta procumbens*-specific DNA probes and their application for the screening of *B. vulgaris* × *B. procumbens* (2n = 19) addition lines

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Summary. *Beta procumbens*-specific DNA probes have been constructed by cloning digested total DNA in *E. coli* and screening the resulting recombinant plasmids in dot blot hybridizations with labelled *B. procumbens* and *B. vulgaris* DNA. Four clones (pTS1-4) have been analyzed in detail determining their degree of specificity and DNA sequence. Two clones (pTS1 and pTS2) with the highest degree of *B. procumbens* specificity were adapted for the squash dot hybridization with the aim of screening large numbers of individual hybrid plants (*B. vulgaris* × *B. procumbens*) carrying an alien *B. procumbens* chromosome (2n = 19). These addition lines carry in some cases *B. procumbens* resistance genes to the beet cyst nematode (*Heterodea schachtii Schm.*).

Key words: DNA probes – Highly repeated DNA – Squash dot hybridization – *Beta procumbens* – Nematode resistance

Introduction

Genetic engineering has induced a new quality of genome diagnosis in plant breeding research during the last decade. The new molecular methods permit faster and more sensitive screenings of large numbers of individual plants than did classical methods in the past. There are two main approaches which have contributed to this progress: (i) RFLP analysis, and (ii) the use of genome-specific DNA probes for genome identification. For the latter approach, probes with high specificity and, if possible, a high copy number in the original genome are needed. There are several examples for the use of cloned highly repeated DNA for the identification of hybrid genomes and even single chromosomes in cereals, e.g. for the rye genome (Bedbrook et al. 1980), for the wheat genome (Hutchinson and Londsdale 1982; Metzlaff et al. 1986) and for the barley genome (Ananiev et al. 1986; Junghans and Metzlaff 1988). From these three genomes, fractions of highly repeated DNA were isolated and cloned. The recombinant plasmids were screened for genome specificity and/or chromosome specificity by cross-hybridization with genomic DNA of plant species, which play a potential role in hybrid breeding programs.

In this paper we report the isolation of specific DNA sequences from the genome of *Beta procumbens*. We screened for *B. procumbens* specificity by cross-hybridization with DNA of *B. vulgaris*. By crossing *B. vulgaris* with species of the section *Procumbentes* resistance genes to the beet cyst nematode (*Heterodea schachtii Schm.*) are transmitted from wild species to the cultivated *B. vulgaris* with low frequency (Savitzky 1978). The resulting hybrids have been screened up to now by isozyme techniques (Van Geyt and Smed 1984; Jung et al. 1986; Van Geyt et al. 1988) and cytology e.g. (De Jong 1981). The aim of our work was to check whether it is possible to use *B. procumbens*-specific DNA probes for fast and sensitive identification of hybrid lines with nematode resistance.

Materials and methods

Plant material was kindly provided by Prof. R. Melzer, Institute of Beta Research of the Academy of Agriculture of the GDR, Klein Wanzleben.

DNA preparation and cloning

Total DNA from *B. patellaris, B. procumbens, B. vulgaris* and monosomic addition lines (2n = 19) carrying an alien chromosome originating from *B. procumbens* was isolated as described by Wienand and Feix (1980), except that the extraction buffer contained 2% SDS instead of 0.5% SDS. DNA was cut with Sau3A and randomly cloned into the BamHI site of the high

copy vector pUC 18. The recombinant DNA was used to transform *E. coli* JM103Y cells as described by Hanahan and Meselson (1980). Plasmid preparations were carried out using the rapid alkaline extraction method (Birnboim and Doly 1979).

Clone selection and DNA sequencing

Recombinant clones were selected on X-Gal-plates containing ampicillin. The plasmid DNA of recombinant clones was replica-blotted on two identical nitrocellulose filters (Amersham), as described by Raeder and Broda (1984). Hybridization of these filters with nick-translated total beet and wild beet DNA was carried out at 65°C overnight, according to Maniatis et al. (1982). The *B. procumbens*-specific Sau3A fragments were cut out of the linker using the enzymes EcoRI and HindIII, which cut the linker on both sides of the insert and recloned into EcoRI/HindIII-digested M13mp8 DNA. The inserts were sequenced by the dideoxynucleotid chain termination procedure (Sanger et al. 1980) using ^{32}P-dATP (Amersham) and a primer synthesized by S. Minter (UMIST, Manchester).

Dot blot and Southern blot hybridization

For preparing dot blot filters, 5 µg total DNA from *B. patellaris*, *B. procumbens*, *B. vulgaris* and monosomic addition lines was blotted on nitrocellulose, according to the procedure by Raeder and Broda (1984). Total wild beet DNA digested to completion with BglII was electrophoresed on 1.3% horizontal agarose gels (SERVA) and transferred onto nitrocellulose filters (Southern 1975). The hybridization with labelled recombinant plasmids was carried out at 65 °C overnight.

Squash dot hybridization

Leaf material from *B. procumbens*, *B. vulgaris* and monosomic addition lines was squashed onto nitrocellulose filters, as described by Hutchinson et al. (1985). The filters were hybridized with labelled *B. procumbens*-specific clones (labelling and hybridization as described above).

Results

Cloning and selection of highly repeated sequences from B. procumbens

The shotgun cloning of Sau3A-digested total DNA of *B. procumbens* resulted in a total of 700 recombinants. From 100 randomly selected recombinants (pTS1-100), the plasmids were isolated and screened for *B. procumbens* specificity by dot blot hyrbidization with labelled total DNA of *B. procumbens* and *B. vulgaris*, respectively. Nine clones showed a significantly stronger hybridization signal with *B. procumbens* DNA. Three of these nine clones did not cross-hybridize with *B. vulgaris* DNA at all, one clone cross-hybridized slightly and five strongly. For all further experimental steps, we selected the three non-cross-hybridizing clones (pTS1, pTS2, pTS4) and the slightly cross-hybridizing clone pTS3.

Southern analysis and sequencing of B. procumbens-specific sequences

Figure 1 summarizes the results of Southern hybridization experiments of labelled plasmid DNA of the clones

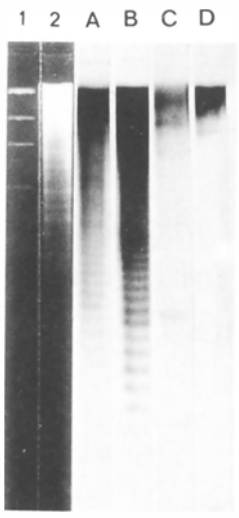

Fig. 1. Southern blot hybridization of BglII-digested *B. procumbens* DNA with labelled pTS clones. Digested *B. procumbens* DNA was electrophoresed on a 1.3% agarose gel (*lane 2*) and transferred onto nitrocellulose filters. A λ/HindIII digestion was used as size marker (*lane 1*). The filters were hybridized with labelled DNA from pTS1 (filter A), pTS2 (filter B), pTS3 (filter C) and pTS4 (filter D) overnight at 65 °C. They were washed for 20 min in 2 × SSC/0.1% SDS solution at 65°C

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pTS1
GATCTAGTAC CTATTCAAGG ACTCAAAAAT ATTTTGTAAT TATTAACAAC
AAAATTATTT GAAAGTTGTT CATACACCAC AAATCGCCTA AGAGACTATG
ACGGTTTAAC CCTTTGATTT AAATTGAGTT TGATC

pTS2
GATCAAACTT ATTTTAAAAC AAAGGGTAAA ACCGTCATAG TCTATTAGGG
GATGTGTGGG GTATGCACAC TTTTTAAGTC ATTTTTTGTG CTTAATTACC
AAATATTTTT TGTCTCCTTG AATGGGTATT AGATATATTT AAAGCATATG
AAGCCTTGGA TC

pTS3
GATCAAAAGT GAAGGATTGA GAGATGAATC TTTTGATGGA CCTTAGGTTA
GTCAATAGGT TGACTTCACC ATGAGAATAG GTGTTAGGCT TTCTAGGACC
TTAATCGAAT CGCTATGAGT TGTGGAATAC TTGATCGATT GATTTTACCA
TAGAGTAGGT AAATTGGTTG ATTAAGTCCT CCTAATTAGC TCGCGGGTAA
TTAGGAACAT ATGAGTGCTT AGTCCCTTAG TTGATC

pTS4
GATCCCCGGG AAGGCACCGG TAAAAAATAA TCACCCTACC CGAAAATGTT
TCCGATTTAC CCGTTTTCGT GGACTATTAC TAACGCCCTG GACACGTCCC
GTGGCCTGGT GTCGGATC
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Fig. 2. Sequence of four *B. procumbens*-specific DNA probes. All four clones were totally sequenced

Table 1. Length and AT-content of the cloned inserts

	Length (bp)	AT-content (%)
pTS1	135	70,4
pTS2	162	67,9
pTS3	236	61,1
pTS4	118	47,4

pTS1, pTS2, pTS3 and pTS4 with filter-fixed restriction patterns of total DNA of *B. procumbens*. Two types of hybridization patterns could be observed:

Type 1: The cloned sequence hybridizes with distinct bands within the whole lane, forming a ladder pattern.

Type 2: The cloned sequence hybridizes mainly with high-molecular-weight DNA including the relic DNA and some additional bands with lower molecular weight.

The cloned sequences of pTS1 and pTS2 show type 1 hybridization, that of pTS3 and pTS4 type 2 hybridization. All four cloned sequences have been sequenced totally. The sequences are shown in Fig. 2, their length and AT-content are listed in Table 1.

Determination of the specificity of pTS1, pTS2, pTS3 and pTS4 for B. procumbens

For the determination of the specificity of pTS1, pTS2, pTS3 and pTS4 for *B. procumbens*, 5 µg of total DNA from *B. vulgaris*, *B. procumbens* and *B. patellaris* (a second species of the section *Procumbentes*) was blotted onto nitrocellulose and hybridized at 65 °C overnight. The filters were washed at 65 °C in 2 × SSC/0.1% SDS, 1 × SSC/0.1% SDS, 0.5 × SSC/0.1% SDS and 0.1 × SSC/0.1% SDS, successively, for 20 min in each step. The slight cross-hybridization to *B. vulgaris* DNA with pTS3 can be removed by this washing procedure. The results can be summarized as follows (compare with Fig. 3): (1) The clones pTS1 and pTS2 contain specific sequences with a high copy number in the genome of *B. procumbens*. (2) The clone pTS4 also contains a *B. procumbens*-specific sequence, but with a lower copy number. (3) The clone pTS3 contains a sequence of the genome of *B. procumbens* which is also present in the genome of *B. vulgaris*. Because it is possible to remove the cross-hybridization by washing, we concluded that either the degree of homology or the copy number in the genome of *B. vulgaris* is low.

From the results of dot blot and Southern blot hybridization, the redundancy of the cloned sequences within the genome of *B. procumbens* can be expressed as follows:

$$pTS2 \geq pTS1 > pTS3 > pTS4$$

The application of the B. procumbens-specific DNA probes for squash dot hybridizations

For an extensive screening of large numbers of individual plants a fast, inexpensive but sensitive method was needed. We adapted the squash dot hybridization published by Hutchinson et al. (1985) for the screening of addition lines of *B. vulgaris* (2n = 19) which carry one additional chromosome of *B. procumbens*. The hybridization was carried out using filter-fixed leaf material and nick-translated recombinant plasmids pTS1 and pTS2. Some of the addition lines gave a clear signal indicating the addition of a *B. procumbens* chromosome (Fig. 4). From these results we concluded that the squash dot hybridization is a suitable procedure for the screening of addition lines. However, to verify the results, we isolated total DNA of addition lines including the lines identified by squash dot hybridization, and blotted 5 µg DNA of each onto nitrocellulose filters. The dot blot hybridization, using again pTS1 and pTS2 as DNA probe, verifies clearly the results of the squash dot hybridizations (shown in Fig. 5). In cytological investigations (results not shown), the chro-

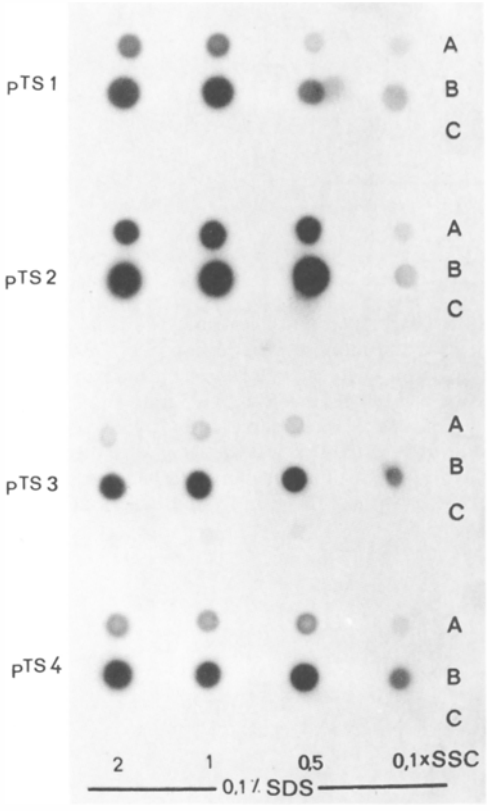

Fig. 3. Dot blot hybridization of total DNA from several species of the genus *Beta* with labelled *B. procumbens*-specific probes. Five micrograms total DNA of *B. patellaris* (A), *B. procumbens* (B) and *B. vulgaris* (C) was blotted onto nitrocellulose and hybridized with nick-translated probes pTS1, pTS2, pTS3 and pTS4. The filters were washed successively for 15 min at 65 °C under the stated salt conditions. The slight cross-hybridization of the clone pTS3 with *B. vulgaris* DNA can be removed by this extensive washing procedure. A strongh cross-hybridization of all probes to total DNA of *B. patellaris* can be observed. The *B. procumbens*-specific probes pTS1, pTS2 and pTS3 do not show any cross-hybridization with total DNA of *B. vulgaris*

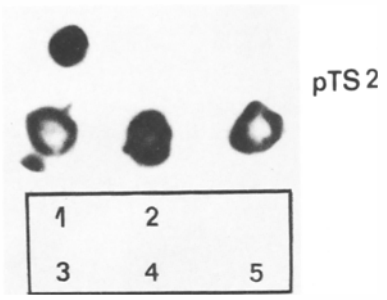

Fig. 4. Squash dot hybridization of leaf material from *B. procumbens*, *B. vulgaris* and monosomic addition lines (2n = 19) of *B. vulgaris* carrying an alien *B. procumbens* chromosome with the labelled probe pTS2. Leaf material (3 × 3 mm) from *B. procumbens* (1), *B. vulgaris* (2) and three cytologically analyzed monosomic addition lines (3, 4, 5) was squashed on a nitrocellulose filter and hybridized with labelled probe pTS2 overnight at 65 °C. The filter was washed once for 15 min in 2 × SSC/0.1% SDS solution at 65 °C

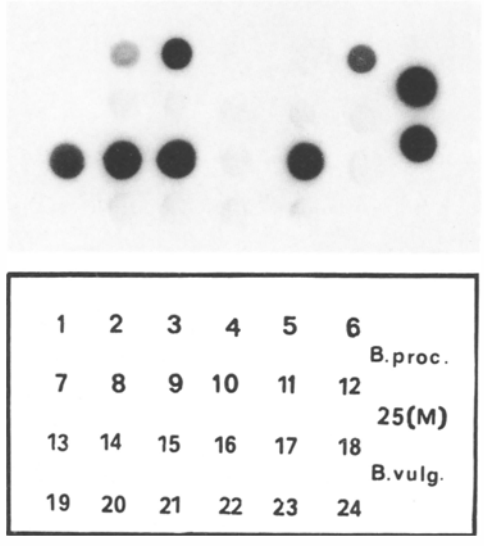

Fig. 5. Dot blot hybridization for the screening of monosomic addition lines. Five micrograms total DNA from 24 individual plants (1–24) of a cross between diploid *B. vulgaris* and a monosomic addition line (25 M) was blotted onto nitrocellulose. The filter was hybridized with the labelled probe pTS1 overnight at 65 °C and washed for 20 min with 2 × SSC/0.1% SDS solution at 65 °C

mosome numbers of the identified individual plants were determined with 2n = 19 (R. Melzer, personal communication).

Discussion

In this paper we described the cloning of highly repeated sequences of the genome of *B. procumbens*. We could show that approximately 4% of the resulting recombinant plasmids carry DNA sequences with a high degree of specificity for *B. procumbens* selected against the genome of *B. vulgaris*. We use the term specificity in the sense that the isolated sequences do not or almost do not cross-hybridize with the compared genome. It does not mean that the isolated sequences of *B. procumbens* do not exist in the genome of *B. vulgaris*. But either the degree of homology or the copy number of these sequences is so low that it is possible to distinguish between the existence and absence of *B. procumbens* genomic material. Because of the high copy number of the cloned sequences of pTS1 and pTS2 in the *B. procumbens* genome and their dispersed distribution over the whole genome, it was possible to prove the existence of a single *B. procumbens* chromosome in *B. vulgaris* × *B. procumbens* addition lines (2n = 19). The dispersed distribution of the cloned sequences is not proven in detail (e.g. by in situ hybridizations), but can be assumed from the results of the Southern blot hybridizations.

In contrast to the high amount of middle and highly repetitive sequences in cereals, the species of the genus *Beta* contain only 63% of those sequences (Flavell et al. 1974). Therefore, it was difficult to enrich the highly repeated DNA by cutting the total DNA of *B. procumbens* with a suitable restriction enzyme (e.g. BamHI, EcoRI, HindIII, BglII) and isolating the resulting relic DNA (for definition, see Bedbrook et al. 1980), as was performed for rye (Bedbrook et al. 1980), wheat (Metzlaff et al. 1986) and barley (Junghans and Metzlaff 1988). However, the results show convincingly that the shot gun cloning of Sau3A-digested total DNA is a successful and fast approach for the construction of specific DNA probes.

The sequencing of the *B. procumbens*-specific DNA fragments cloned within pTS1-4 showed a varying degree of AT-residues. The sequences of pTS1 and pTS2 have the highest degree of AT and also the highest redundancy. For these two sequences, a typical hybridization ladder was observed on BglII-digested total DNA as was described for species of *Brassicaceae* (Hallden et al. 1987), *Raphanus sativus* (Grellet et al. 1986) and *Sisymbrium irio* (Grellet et al. 1988). The computer analysis of all four cloned *B. procumbens* sequences did not show a high degree of homology between them, but revealed short directed and inverted repeats. The four cloned sequences seem to be parts of sequences of different repetition complexes within the *B. procumbens* genome. The practical approach of our work was the application of the isolated and characterized DNA probes to questions of plant breeding research. One such question consists in transferring resistance genes against beet cyst nematode (*Heterodea schachtii Schm.*) present in the genome of *B. procumbens* into the genome of *B. vulgaris*. This was possible by constructing monosomic addition lines

(2n = 19) of *B. vulgaris* × *B. procumbens* (Savitzky 1978; Jung et al. 1986). However, these monosomic addition lines are very unstable and screening for the existence of the *B. procumbens* chromosome is needed. With our *B. procumbens*-specific DNA probes in connection with the fast, simple and sensitive squash dot hybridization, we provide the opportunity to screen large numbers of hybrid plants for the existence of the *B. procumbens* chromosome. Because of the dispersed distribution of the cloned sequences over the whole genome, the identification of *B. procumbens* chromosome fragments integrated into the genome *B. vulgaris* should be also possible.

After the construction of wheat (Metzlaff et al. 1986) and barley (Junghans and Metzlaff 1988) specific probes, we demonstrated once more that the cloning of highly repeated sequences and selection for specificity by dot blot hybridization is a general procedure for obtaining a number of genome-specific DNA probes suitable for screening programs within a short time.

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