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Genetic localization of four genes for nematode (Heterodera schachtii Schm.) resistance in sugar beet (Beta vulgaris L.)

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Abstract Sugar beet (Beta vulgaris L.) is highly susceptible to the beet cyst nematode (Heterodera schachtii Schm.). Three resistance genes originating from the wild beets B. procumbens (Hsl proc-1) and B. webbiana (Hsl web-1, Hs2 web-2) have been transferred to sugar beet via species hybridization. We describe the genetic localization of the nematode resistance genes in four different sugar beet lines using segregating F2 populations and RFLP markers from our current sugar beet linkage map. The mapping studies yielded a surprising result. Although the four parental lines carrying the wild beet translocations were not related to each other, the four genes mapped to the same locus in sugar beet independent of the original translocation event. Close linkage (0.46 cM) was found with marker loci at one end of linkage group IV. In two populations, RFLP loci showed segregation distortion due to gametic selection. For the first time, the non-randomness of the translocation process promoting gene transfer from the wild beet to the sugar beet is demonstrated. The data suggest that the resistance genes were incorporated into the sugar beet chromosomes by non-allelic homologous recombination. The finding that the different resistance genes are allelic will have major implications on future attempts to breed sugar beet combining the different resistance genes.

Key words Sugar beet · Beta vulgaris · Nematode resistance · RFLP · Genetic maps · Bulk segregant analysis

Introduction

The beet cyst nematode (Heterodera schachtii Schm.) is one of the major pests in sugar beet (Beta vulgaris L.) cultivation. Sugar beet and closely related cultivated beet species (fodder beet, red table beet, swiss chard) are susceptible to this pathogen, and within B. vulgaris there is no genetic variability for resistance. Only a partial resistance has ever been found in one accession of the wild species B. vulgaris ssp. maritima (Lange et al. 1993), and due to its polygenic and recessive mode of inheritance this resistance is difficult to handle in backcross breeding programs.

Attempts have therefore been made worldwide to introduce monogenic resistance genes from three wild species of the Beta section IV (B. procumbens, B. patellaris, B. webbiana). These resistances are inherited in a dominant way, thus facilitating the breeding process. Using monosomic analysis Löptien (1984a) located three resistance genes in each of the species B. procumbens and B. webbiana. These genes are located on chromosome 1 (Hsl proc-1, Hsl web-1), chromosome 7 (Hs2 proc-7, Hs2 web-7, Löptien 1984a; Lange et al. 1993) and chromosome 8 (Hs3 proc-8, Hs3 web-8 (Löptien 1984a; Reammon-Ramos and Wricke 1992). Only one chromosome in B. patellaris (Hs1 proc-1, Löptien 1984a) was found to carry a resistance gene. Results from isozyme and molecular marker studies indicate that the resistance-carrying chromosomes from B. webbiana and B. procumbens are homoeologous (Wagner et al. 1989; Jung et al. 1993).

Gene transfer from wild to cultivated beet has been achieved in the following way. Species' hybrids were backcrossed to sugar beet giving rise to a series of alien monosomic addition lines (2n = 19) that contain the original wild beetle chromosome (Savitsky 1975; Löptien 1984a; Speckmann et al. 1985). An almost complete lack of homology between sugar beet and wild beetle chromosomes was found when metaphase I pollen mother cells were analyzed. The added chromosome almost exclusively formed a univalent. Trivalent formation ranged...
between 2% (de Jong et al. 1985) and 5% (Löptien 1984b). Therefore, the transfer of resistance to sugar beet can only be accomplished by rare crossover or translocation events. Among the offspring of monosomic addition lines diploids (2n = 18) have been selected for with a more or less stable inheritance of the resistance trait (Yu 1981; Jung and Wricke 1987; Heijbroek et al. 1988). These lines are intensively used as introduction lines for sugar beet breeding. However, transmission rates have been considerably low, ranging from 70.6% to 100% (Jung and Wricke 1987). With only one exception has resistance been proven to be durable. Lange et al. (1993) presented data on a pathotype which is able to complete its life cycle on beets carrying the resistance gene Hs1^pro-1 from chromosome 1. However, beets carrying the gene from chromosome 7 were still found to be resistant against this pathotype.

Attempts have been made to isolate the resistance gene following a positional cloning strategy. A set of probes has been identified of which all the probes are closely linked to the resistance genes Hs1^pro-1 and Hs1^pro-2 (Jung et al. 1992; Salentijn et al. 1992). Also, two RFLP linkage maps of sugar beet have been published (Pillen et al. 1993; Barzen et al. 1995). Some genes of agronomical value have been included like annual behavior (Boudry et al. 1994), restorer (Pillen et al. 1993; Barzen et al. 1995). Rizomania resistance and monogermy (Barzen et al. 1995).

The purpose of the work presented here was to localize the wild beet translocations in sugar beet conferring nematode resistance using existing restriction fragment length polymorphism (RFLP) libraries and to select markers differentiating between individuals that are homozygous or heterozygous at the resistance locus. The resistance genes Hs1^pro-1, Hs1^pro-2, and Hs2^web-7 were mapped in four breeding lines of different origin. The mapping data are discussed together with the subsequent implications for future breeding of nematode resistant sugar beet.

Materials and methods

Plant material, resistance test and DNA preparation

Four different F₂ sugar beet populations segregating for nematode resistance were established by selling individual F₁ plants hemizygous (R*) for nematode resistance. The selection of the donor lines has been described previously (Table 1). Each line originated from an individual translocation event in the offspring of monosomic addition lines. Except for PRO4, translocations were not induced by irradiation. The lines investigated here are not related by descent, they carry genes for nematode resistance from the wild species B. procumbens and B. webbiana as described in Table 1. A number of plants from each F₂ population were tested for nematode resistance (Table 1) as described by Toxopeus and Lubberts (1979). Genomic DNA was isolated from 5 g of developing green leaves following the protocol of Saghai-Maroof et al. (1984).

RFLP analysis

DNA restriction, Southern blotting and radioactive hybridization were performed as described by Pillen et al. (1993). For bulk segregant analysis (BSA) and most of the mapping experiments, a non-radioactive hybridization system based on enhanced chemiluminescence was used (ECL-Direct, Amersham). Blotting, labelling and hybridization were carried out according to the manufacturer's protocol (Amersham). Non-radioactive autoradiograms were scored after exposure for periods of several hours up to 1 day.

Bulk segregant analyses, linkage analyses

The probe PCR1012 was isolated from B. procumbens chromosome 1 and hybridizes with DNA from Procumbentes species only (Jung et al. 1992). In this study it was used for distinguishing between different translocation lines carrying the resistance gene Hs1^pro-1. For BSA (Michaelmore et al. 1991) previously mapped RFLP probes were used (Pillen et al. 1993). DNA from resistant and susceptible plants was restricted with the enzymes EcoRI, EcoRV, HindIII and XbaI, subsequently, bulked in two independent pools and separated on 0.75% agarose gels. The DNA was transferred to a Biodyne B membrane (PALL, Dreieich) using 20 x SSC as transfer buffer. Each bulk consisted of 12 different plants and the total DNA concentration in each lane was about 5 µg.

To shorten the BSA procedure, up to four single-copy RFLP probes were hybridized in parallel to the filters. In this way a representative set of marker loci from each linkage group was analyzed. Probes linked to the resistance genes showed RFLPs between the pools of resistant and susceptible plants. In the next step each of the probes was tested on a survey filter with 24 individuals, and linkage to the resistance gene was estimated by calculating the distance in recombination units. If the RFLP locus was more closely linked to the nematode resistance gene than 25 recombination units, more RFLP loci in the vicinity were mapped.

Linkage was calculated with the computer program MAP-MAKER/EXP Ver. 3.0 (Lander et al. 1987) processed on a SUN Sparc 10 workstation (Unix). Linkage groups were formed using the Haldane transformation (Haldane 1919) with thresholds of LOD = 3.0 and 0.3 recombination units.

Statistical analysis of distorted segregation of linked marker loci

Segregation ratios for codominant RFLP markers and for the dominant nematode resistance gene were expected to be 1:2:1 and 3:1, respectively. Segregation ratios were tested by χ² analysis for the RFLP loci in proximity to the nematode resistance genes and for the nematode resistance locus itself. Also, a test for gametic and zygotic selection (unequal frequencies of the three observed classes) was conducted for the closest linked codominant RFLP loci using the formulas from Wagner et al. (1992) with an α = 0.01.

### Table 1: Plant material and origin of the resistance genes

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Resistance gene</th>
<th>Origin of resistance gene</th>
<th>Reference</th>
<th>Number of F₂ individuals investigated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A906001</td>
<td>Hs1^pro-1</td>
<td>B. procumbens chromosome 1</td>
<td>Jung et al. 1992</td>
<td>48</td>
</tr>
<tr>
<td>PRO4</td>
<td>Hs1^pro-1</td>
<td>B. procumbens chromosome 1</td>
<td>Jung and Wricke 1987</td>
<td>101</td>
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<tr>
<td>WEB6</td>
<td>Hs1^web-1</td>
<td>B. webbiana chromosome 1</td>
<td>Jung and Wricke 1987</td>
<td>96</td>
</tr>
<tr>
<td>WEB11</td>
<td>Hs2^web-7</td>
<td>B. webbiana chromosome 7</td>
<td>Jung and Wricke 1987</td>
<td>61</td>
</tr>
</tbody>
</table>