New pathotypes of the beet cyst nematode (*Heterodera schachtii*) differentiated on alien genes for resistance in beet (*Beta vulgaris*)

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Summary – Two populations (Schach 0 and Schach 1) of the beet cyst nematode, *Heterodera schachtii*, were used to select for new virulences to resistance genes transferred from *Beta* section *Procumbentes* into *B. vulgaris* (*Hs1*web-7 and additional resistance information on chromosome 7). Both nematode populations were able to produce a few cysts which were multiplied for six successive generations on resistant plants. The two resulting populations, selected from either Schach 0 or Schach 1, were highly virulent on four monosomic additions with chromosome web-7 or pro-7. However, the population selected from Schach 0 was unable to break the resistance of translocation lines carrying resistance gene *Hs1* from chromosome pro-1, pro-7 or web-7, whereas the population selected from Schach 1 was still virulent on these translocation lines. A resistance gene *Hs2*web-7 is postulated to exist on chromosome 7, having an epistatic effect on *Hs1*web-7. The two new virulent pathotypes are named Schach 2 and Schach 1,2. © Orstom/Elsevier, Paris

Résumé – Nouveaux pathotypes du nématode à kyste de la betterave (*Heterodera schachtii*) différenciés sur des gênes de résistance étrangers introduits dans la betterave (*Beta vulgaris*) – Deux populations (Schach 0 et Schach 1) du nématode à kyste de la betterave, *Heterodera schachtii*, sont utilisées pour sélectionner de nouvelles virulences contre des gênes de résistance transférés de *Beta* section *Procumbentes* à *B. vulgaris* (*Hs1*web-7 et d’autres caractères de résistance localisés sur le chromosome 7). Les deux populations du nématode ont produit quelques kystes sur des plantes résistantes et se sont multipliées sur ces mêmes plantes pendant six générations successives. Les deux populations en résultant, sélectionnées à partir de Schach 0 ou de Schach 1, montrent une très grande virulence à l’égard de quatre lignées d’addition monosomique avec le chromosome web-7 ou pro-7. Cependant, la population sélectionnée à partir de Schach 0, ne surmonte pas la résistance des lignées de translocation portant le gène de résistance *Hs1* du chromosome pro-1, pro-7 ou web-7, alors que la population issue de Schach 1 conserve sa virulence à l’égard de *Hs1*. Il est conclu qu’un gène de résistance *Hs2*web-7, ayant un effet épistatique sur *Hs1*web-7, existe sur le chromosome 7. Les deux nouveaux pathotypes virulents sont nommés Schach 2 et Schach 1,2. © Orstom/Elsevier, Paris

Keywords: *Beta procumbens*, *Beta vulgaris*, *Beta webbiana*, *Heterodera schachtii*, resistance, pathotype, virulence.

The beet cyst nematode (*Heterodera schachtii*) is a major pest in sugar beet production. As the use of nematicides is more and more restricted, alternative control methods are urgently needed. Therefore, attempts have been made in several countries to introduce monogenic resistance genes from three wild beet species of the *Beta* section *Procumbentes* into the sugar beet *B. vulgaris*. These resistance genes from *B. procumbens* Chr. Sm., *B. webbiana* Moq., and *B. patellaris* Moq. are inherited in a dominant way, but they are not easy to transfer into *B. vulgaris*. The research programmes have encountered crossing barriers, an extremely low frequency of introgression of alien genes into the genome of sugar beet, and a reduced sexual transmission of the introgressed genes. Savitsky (1975, 1978) was the first to produce monosomic additions (sugar beet with an added alien chromosome from wild beet, 2n = 19) and she later selected diploid plants (translocations, 2n = 18). She supposed that a single dominant gene was responsible for full nematode resistance. Morphological studies and isozyme analyses later proved the existence of six different chromosomes in the wild beet species carrying major resistance genes; chromosome 1 of all three wild beet species (pro-1, web-1, pat-1) and chromosome 7 of *B. procumbens* (pro-7) and *B. webbiana* (web-7) (Löptien, 1984; Lange et al., 1988; Reamon-Ramos & Wricke, 1992; Reamon-Büttner, 1994). Another monosomic addition with incomplete resistance had been described earlier (Jung et al., 1986; Jung & Wricke, 1987). Here, resistance was found to be located on the added chromosome 8 of *B. webbiana* (Reamon-Ramos & Wricke, 1992; Reamon-Büttner, 1994). Morphological studies and isozyme analyses do not give information on the quality of the resistance genes. Nematode-based investigations have been essential in identifying the specificity of different
genes. Using a virulent *H. schachtii* population selected by Müller (1992), it was possible to demonstrate the existence of different resistance genes on chromosomes pro-1 and pro-7, giving a specific response to the nematode populations. It was proposed to use the symbol Hs for resistance genes controlling *H. schachtii*. The different reactions to pathotypes are specified by different numbers, whereas the origin of the alien genes is indicated by an additional superscript. The two genes differentiated by the virulent nematode pathotype were named Hs1\textsuperscript{pro-1} and Hs2\textsuperscript{pro-7} (Lange et al., 1993). Further investigations proved the existence of the same genes in *B. procumbens* and in *B. weebiana* and also of a different response in resistance between monosomic additions and translocation lines. It was concluded that resistance gene Hs1 occurs on pro-1 as well as on pro-7, with chromosome 7 carrying additional resistance information (Klinke, 1995; Müller & Klinke, 1996; Klinke et al., 1996).

Molecular genetic technologies are also being used to isolate the alien genes for beet cyst nematode resistance and to transfer such genes to cultivated beet (Jung et al., 1990, 1992; Salentijn et al., 1992). After successful identification and isolation of resistance gene Hs1\textsuperscript{pro-1} of *B. procumbens*, the gene was transferred into *B. vulgaris* and proved to be effective against *H. schachtii* (Cai et al., 1997). Resistance to *H. schachtii* transferred by molecular genetic technologies is not yet available to farmers. The classical breeding techniques, however, have already resulted in marketable resistant sugar-beet hybrids and the first resistant variety was registered in France in 1996 (Mahfoud et al., 1996).

Breeders used to consider *H. schachtii* to be a constant, homogeneous factor in the host-parasite interaction. From an evolutionary point of view, however, this seems unlikely. On selected resistant monosomic additions carrying chromosome pro-1 of *B. procumbens*, a low number of cysts is usually formed. This was interpreted as an indication for the occurrence of virulence genes at low frequency in natural *H. schachtii* populations. Cysts from resistant plants were collected and tested again. After six nematode generations on plants carrying resistance of chromosome pro-1, a virulent population was selected (Müller, 1992). This population is called a pathotype if the plant-nematode interaction is based on a gene for gene relationship (Truigg, 1986, 1991). Further investigations showed that the virulent population was able to break resistance from chromosome pro-1, but not from chromosome pro-7 of monosomic additions. It was therefore considered to be a pathotype and named Schach 1 (Müller & Klinke, 1996).

The present study was undertaken to determine if more virulences exist in *H. schachtii* and if they can be used for a further differentiation of resistances derived from the *Beta* section *Procumbentes*.

**Materials and methods**

**Nematode populations**

A collection of 146 *H. schachtii* populations, obtained from different geographical origins in Germany and other European countries, is maintained at the Nematology Institute in Münster. All the populations were started from soil samples of 2-3 kg, taken from individual fields known to be infested with *H. schachtii*. Avirulent populations are maintained on rape (*Brassica napus* var. *napus*) cv. Velox. Populations virulent to resistance gene Hs1\textsuperscript{pro-1} are multiplied on a diploid homozygous resistant translocation line with gene Hs1\textsuperscript{pro-1}. This translocation line originates from B 883 (Heijbroek et al., 1988), it was called Pro 1 by Müller (1992), and was obtained from the breeding company as KWS-NR1. Nematode population N° 129 was used for the experiments reported here. The avirulent population is called Schach 0, the virulent one is called Schach 1 (Müller & Klinke, 1996). Details on maintaining the populations have been described previously (Müller, 1992).

**Plant materials**

Resistance to *H. schachtii* in the plant material listed in Table 1 came from two wild *Beta* species of the section *Procumbentes*, *B. procumbens* Chr. Sm. and *B. weebiana* Moq. (2n = 18). Eight resistant stocks originating from interspecific breeding programmes were used. Seven stocks had been selected either from resistant monosomic additions (2n = 19) or from diploid translocation lines (2n = 18), both interbred with diploid susceptible sugar beet. Because of incomplete transmission of resistance, particularly in the addition lines, the offspring segregate to less than 50% resistant plants. The transmission rates of resistance are reported to be only 10-20% in addition lines (Lange et al., 1990). The eighth stock, KWS-NR1, which contains resistance gene Hs1\textsuperscript{pro-1}, is a diploid line with homozygous resistance; its progeny is 100% resistant. One plant of the monosomic addition 14026, which contains the complete chromosome web-7, was tested twice for its resistance to Schach 0 and was then cloned in order to obtain sufficient plant material for selection of virulence in *H. schachtii*. Cloning of the monosomic addition was done by Dieckmann-Heimburg Saatzucht, Nienstädt. Seeds of monosomic additions and diploid translocation lines were obtained from Hannover University, Institut für Angewandte Genetik. Seeds of KWS-NR1 were produced by Kleinwanzlebener Saatzucht AG, Einbeck. In all experiments, the susceptible sugar beet cv. Désirée was used as control.
New pathotypes of Heterodera schachtii

Table 1. Genetic characteristics, names of plant material, and sources of resistance.

<table>
<thead>
<tr>
<th>Name of beet stock</th>
<th>Origin of added chromosome or of resistance gene</th>
<th>From wild Beta species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomic additions (2n = 19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6019</td>
<td>Chromosome 7</td>
<td>B. webbiana</td>
</tr>
<tr>
<td>6022</td>
<td>Chromosome 7</td>
<td>B. webbiana</td>
</tr>
<tr>
<td>8605</td>
<td>Chromosome 7</td>
<td>B. procumbens</td>
</tr>
<tr>
<td>14026</td>
<td>Chromosome 7</td>
<td>B. webbiana</td>
</tr>
<tr>
<td>12572</td>
<td>Chromosome 7</td>
<td>B. procumbens</td>
</tr>
<tr>
<td>Diploid translocation lines (2n = 18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Web 11</td>
<td>Chromosome 7</td>
<td>B. webbiana</td>
</tr>
<tr>
<td>Pro 3</td>
<td>Chromosome 7</td>
<td>B. procumbens</td>
</tr>
<tr>
<td>Diploid homozygous resistant translocation line (2n = 18)</td>
<td>KWS-NR 1</td>
<td>Chromosome 1</td>
</tr>
</tbody>
</table>

EXPERIMENTAL DESIGN

Tests for resistance were carried out as described by Müller (1997). Loess was used as a substrate for plant cultivation in all experiments. It was obtained from the brown coal open-cast mining near Garzweiler/Rhineland, from soil depths of 3-5 m. Steiner solution was added for better nutrient supply. The seed was treated with thiram to prevent infection with *Phoma betae*, then germinated in loess. PVC tubes (2 x 4 x 12 cm) were put into boxes containing 120 tubes each (in ten rows of twelve tubes). Single seedlings were transplanted into the PVC tubes filled with loess. The different beet stocks were planted in lines of twelve plants and these were randomised over all the boxes. The total number of plants per beet stock is indicated in Figs 2 and 3. Fourteen days later, 1000 juveniles of *H. schachtii* were inoculated per plant. The plants were cultivated in a greenhouse at ca 20°C, with 14 h/day artificial light between October and March. The tests were evaluated 6 weeks after inoculation. The loess was washed through a kitchen sieve (1 mm), then through a 100 µm sieve. The cysts were collected on the 100 µm sieve, together with some larger loess particles. Both, cysts and loess particles, were transferred onto a smaller 100 µm sieve and rinsed in 20% acetic acid for 5 min. The loess particles dissolved quickly and were removed by shaking the sieve in water. The cysts were transferred to a filter paper and counted under a stereoscopic microscope at 10 x magnification.

To gather reliable information on the resistance of a beet stock with respect to the virulence of the nematode population inoculated to this stock, it was necessary to test a high number of plants due to the often low transmission rates of resistance. The test conditions must be suitable to distinguish between resistant and susceptible plants, which means that their frequency distributions should not overlap. All conclusions concerning resistance/virulence depend on the correct classification of plants as resistant or susceptible.

The segregating addition and translocation lines could not be analysed statistically. All data are therefore presented in frequency distributions with a class width of ten cysts per plant. In all resistance tests, the susceptible cv. Désirée was used to obtain information on the test conditions. In general, it was found here that a minimum of 40 cysts per plant were needed. Lower cyst numbers indicated unfavourable test conditions and the risk of misinterpretation in the segregating breeding lines. Less than 30 cysts were found on resistant plants. The frequency distributions allowed a reliable distinction between susceptible and resistant plants.

Experiments and results

SELECTION FOR VIRULENCE

Two populations were used separately in this first screening for virulence to resistance located on chromosome 7: the avirulent 'natural' Schach 0 and the virulent Schach 1, which is able to break resistance gene Hs1<sup>web</sup>-7. In Fig. 1, the different steps for virulence selection from Schach 0 are presented. The same procedure was applied when using Schach 1 instead of Schach 0. The first step was differentiation between resistant and susceptible plants in the addition line 14026 carrying the resistance gene Hs1<sup>web</sup>-7, but also additional resistance information on chromosome 7. Both populations were able to produce a few
Fig. 1. Selection for virulence to resistance located on chromosome 7 shown at the example of Schach 0. The numbers 1-6 indicate six successive generations.

cysts on resistant plants of the monosomic addition 14026.

During the rest of the selection process, the two populations were again kept strictly separate. In the example in Fig. 1, Schach 0 produced four cysts on plant № 30. These were multiplied on rape, the juveniles hatched and they were inoculated to the same plant № 30. The resulting cysts constitute the second generation on resistant plant material. The third and the fourth generations were multiplied on addition line 12572, which carries chromosome pro-7 of *B. procumbens*. This addition line was used as no more seeds of 14026 were available. To establish nematode generations five and six, a resistant plant of 14026 was cloned in cell cultures and the clone № 395002, regenerated to normal plants, was inoculated with juveniles of the fourth generation. This clone was also used for the data presented in Table 2. After six generations, virulent populations had been selected. They were provisionally named Schach x (from Schach 0) and Schach y (from Schach 1).

The multiplication potential of these virulent populations is presented in Table 2. Resistant plants of clone 395002, grown in loess in 600 ml plastic pots, were inoculated with 3000 juveniles of Schach 0, Schach 1 and the two new virulent populations. Cysts were extracted 7 weeks after inoculation. Both populations, Schach x and Schach y, multiplied equally well and showed no difference in virulence. Only a few cysts developed from juveniles of Schach 0 or Schach 1.
**Translocation lines Web 11 and Pro 3**

Translocation lines Web 11 and Pro 3, carrying resistance genes Hs\(_1{}^{\text{web-7}}\) and Hs\(_1{}^{\text{pro-7}}\), respectively, reacted identically to the four nematode populations, as demonstrated by the frequency distributions in Fig. 2. The two beet stocks included both resistant and susceptible plants when inoculated with Schach 0. Schach 1 and Schach y break the resistance, in contrast to Schach x. The avirulence of Schach x was not expected as this nematode population was selected for virulence to chromosome 7, which carries resistance gene Hs1. More beet stocks with different sources of resistance were tested to explain this phenomenon.

**Translocation line KWS-NR1**

This diploid beet is homozygous for its resistance gene Hs1\(_{}^{\text{pro-1}}\), and consequently all plants were resistant when inoculated with Schach 0 (Fig. 3). Schach 1 overcomes the resistance, as anticipated, but Schach x is obviously avirulent. The numbers of cysts per plant produced by Schach y are not as high as expected but they do not indicate resistance in KWS-NR 1.

**Chromosome-7 Addition Lines**

Schach x and Schach y had been selected on a web-7 addition (14026) and proved to be highly virulent (Table 2). To find an explanation for the unexpected results presented in Figs 2 and 3, the virulences of the two populations were checked on different beet stocks carrying the added chromosome 7. The additions 6019, 6022, and 8605, inoculated with the four nematode populations, segregated identically and the results are therefore combined in one frequency distribution (Fig. 3). The transmission rate of these beet stocks is low and consequently the majority of plants is susceptible, independent of the inoculated population. The distributions show, however, that these addition lines include plants resistant to Schach 0 and Schach 1, whereas all plants are considered to be susceptible to Schach x and Schach y. This confirms the results presented in Table 2.

**Discussion**

Lange et al. (1993) postulated the existence of a resistance gene Hs2\(_{}^{\text{pro-7}}\) on chromosome 7 of the monosomic addition AN 101 as this beet stock proved to be resistant to the pathotype selected by Müller (1992). Later Klinke et al. (1996) demonstrated that only monosomic additions with the complete chromosome 7 give a resistant response to the pathotype but the diploid translocation lines Web 11 and Pro 3 are susceptible. They postulated the existence of more than one resistance gene on chromosome 7. As the resistance in Web 11 and Pro 3 is overcome by the pathotype - later called Schach 1 by Müller and Klinke (1996) - it was concluded that chromosomes 1 and 7 carry the same or very similar resistance genes. Moreover genes Hs1\(_{}^{\text{pro-7}}\) and Hs1\(_{}^{\text{web-7}}\) were considered to be identical. This supports the assumption of a close relationship between B. procumbens and B. veebiana (Wagner et al., 1989; Reamon-Ramos & Wricke, 1992). The results presented in Fig. 2 agree with this hypothesis.

However, the reactions of Web 11 and Pro 3 to infestation with the pathotype Schach x are inconsistent with the concept of a resistance gene Hs1\(_{}^{\text{web-7}}\) on the monosomic addition 14026. Schach x was selected on 14026 and it was able to break all resistance information of the added chromosome 7. At this point, the identity of Hs1\(_{}^{\text{web-7}}\) in Web 11 and in 14026 seemed doubtful and more information was needed. The results of further experiments (presented in Fig. 3) confirm those in Fig. 2: if chromosome 1 and chromosome 7 carry identical resistance genes and if, in addition, these genes from B. procumbens and from B. veebiana are homologous, then the translocation line KWS-NR 1 should give the same reaction as Web 11 and Pro 3 did. In fact, the population Schach x did not break resistance of KWS-NR 1 (Fig. 3).

As there was still no explanation for the unexpected results, the four nematode populations were tested on three other chromosome-7 additions. The combined data are presented in Fig. 3. The beet stocks segregated into resistant and susceptible plants when inoculated with Schach 0 and Schach 1, whereas Schach x and Schach y were able to break all resistance information. This fits with the virulence observed on addition 14026.

Another surprising fact was already evident during the process of selection for virulence. It was expected that, as the selection process started with pathotype Schach 1, an increase of virulence would occur. With Schach 0, this process was supposed to take much more time, if it occurred at all. The probability of

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**Table 2. Multiplication of four populations on cloned plants carrying chromosome 7 of Beta veebiana (monosomic addition line 14026).**

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of plants (n)</th>
<th>Mean of cysts/plant (± SD)</th>
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</thead>
<tbody>
<tr>
<td>Schach 0</td>
<td>17</td>
<td>2.4 ± 2.1</td>
</tr>
<tr>
<td>Schach 1</td>
<td>17</td>
<td>1.9 ± 2.0</td>
</tr>
<tr>
<td>Schach x</td>
<td>18</td>
<td>335 ± 143</td>
</tr>
<tr>
<td>Schach y</td>
<td>18</td>
<td>321 ± 161</td>
</tr>
</tbody>
</table>
overcoming two independent resistance genes is much higher if one virulence is already present in the nematode. However, there was no difference in selection for virulence in the two populations as multiplication rates were almost the same (Table 2).

These contradictory observations can be explained by epistasis between the resistance genes. If resistance gene Hs1\textsuperscript{web-7} is hypostatic in the presence of the other resistance gene, it will have no effect in the process of virulence selection. This would explain the rapid loss of resistance of 14026 to pathotype Schach 0 and it would also explain the lack of virulence of Schach x to Web 11, Pro 3 and KWS-NR 1. This epistatic gene is named Hs2\textsuperscript{web-7}. It is a dominantly inherited factor and it is probably identical with Hs2\textsuperscript{web-7} postulated by Lange et al. (1993). Hs2 does not seem to be located in the vicinity of Hs1 on chromosome 7. All translocation lines carrying resistance

Fig. 2. Frequency distributions of cysts per plant for the translocation lines Web 11 and Pro 3 (both carrying Hs1 of chromosome 7) inoculated with four selected populations.
New pathotypes of *Heterodera schachtii*

of chromosome 7 proved to be susceptible to Schach 1, which indicates a low probability of these two genes being translocated together. According to the two resistance genes, the two new virulent *H. schachtii* populations, named previously Schach x and Schach y, are named Schach 2 and Schach 1,2 respectively. Schach 2 is virulent only to resistance gene Hs2. Schach 1,2 is able to overcome resistance of both genes, Hs1 and Hs2, even if they occur separately. Thus, we now have to distinguish between four pathotypes.

Until now, the virulent pathotypes have been selected only in the greenhouse, but we must expect to find them in field conditions as well if resistant sugar beets are grown intensively. It is important to realize that the virulence of Schach 1, which was already present when starting the selection process on 14026, was not lost after six generations without the

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**Fig. 3.** Frequency distributions of cysts per plant for the homozygous resistant translocation line KWS-NR1 and for chromosome-7 addition lines inoculated with four selected populations.
selection pressure of an active resistance gene Hs1 (Figs 2, 3). Therefore, it is doubtful whether alternating cultivars with different resistance genes would solve the problem of resistance-breaking pathotypes in practice. Combining different and independent resistance genes in the same cultivar is more promising, but also much more difficult to achieve.

Acknowledgments

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