Pre-breeding for Nematode Resistance in Beet

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ABSTRACT

Pre-breeding for partial resistance to the beet cyst nematode (BCN), *Heterodera schachtii*, included repeated mass selection in descendants of *Beta vulgaris* subsp. *maritima* accession BMH and preliminary studies on inheritance and mechanism of this resistance. The selection in accession BMH and in progenies of a cross with fodder beet resulted in rather high levels of partial resistance. The mean numbers of cysts per plant for the BMH selections and for progenies of a cross with fodder beet were 20-40% and 15-30%, respectively, of those of the susceptible control varieties. The progenies of crosses between accession BMH and sugar beet were less advanced, and additional selection for bienniality may have decreased the progress in selection for BCN resistance. The resistant materials showed a wide variation in cyst size, and many of the cysts were considerably smaller than those on the susceptible control varieties. This phenomenon will reduce the multiplication rate of the nematodes. The numbers of cysts in progenies of pair crosses between accession BMH and cultivated materials generally were slightly below the mid-parent values. The results are discussed in the light of pre-breeding strategies and the possible application of marker-facilitated selection procedures.

Additional Key Words: *Heterodera schachtii, Beta vulgaris* subsp. *maritima,* repeated mass selection, partial resistance.

Beet cyst nematodes (BCN) are a serious threat to the cultivation of beet. In the major regions of sugar beet production, 10-25% of the area is infested with this pest. Cultivated beet *(Beta vulgaris* L. subsp. *vulgaris)* is a host plant for two cyst nematode species, *Heterodera schachtii* Schm. and *H. trifolii* Goff. f.sp. *beta,* of which the first-mentioned species is by far the most important. The use of resistant cultivars would be desirable, but high levels of BCN resistance have not been found in cultivated materials (Doney and Whitney, 1969; Heijbroek, 1977).

At present, two sources of resistance are known. Major gene resistance occurs in the three *Beta* species of section *Procumbentes,* and much attention has been paid to the transfer of such genes into sugar beet (for references see reviews by Lange et al., 1990; Van Geyt et al., 1990; Nakamura et al., 1991; Roberts, 1992). Reduced sexual transmission of the introgressed genes stimulated studies to isolate the genes and to transfer them into sugar beet by means of molecular technologies (Jung et al., 1990; 1992; Salentijn et al., 1992). However, the approach through monogenic resistance may have its limitations, as is indicated by the fact that virulence to at least one of the resistance genes of section *Procumbentes* occurs in some populations of *H. schachtii* (Müller, 1992; Lange et al., 1993).

The second source for BCN resistance was identified in the wild sea beet, *B. vulgaris* subsp. *maritima* (L.) Arcang., in material collected in France (Hijner, 1951). Studies by Heijbroek (1977) revealed that the resistance was partial, that selection resulted in raising the level of resistance to a limited extent only, and that the inheritance of this type of resistance was polyfactorial and largely recessive. With tolerance to wilting as a selection criterion for BCN resistance in hybrids between sugar beet and the partial resistant material, no evidence for nematode resistance was observed (Heijbroek et al., 1977).

From 1977 onwards, the partially resistant stocks have been handled at the Foundation for Agricultural Plant Breeding (SVP) at Wageningen, the Netherlands, a research institute which at present is part of CPRO-DLO, Wageningen. Repeated selection was carried out, both in the original material and in hybrids with fodder and sugar beets, and rather high levels of resistance were reached (Mesken and Lekkerkerker, 1988; Mesken, pers. comm.). In 1990 several of the selected stocks were released to the breeding companies.

In the present paper an overview is given of the latest results of the (pre-)breeding activities with the BMH material, with special emphasis on the results of successive cycles of selection, and with a discussion on pre-breeding strategies. In this context pre-breeding activities are considered to be at least two-fold: (1) the acquisition of knowledge on the target characteristic, especially on its genetical behaviour, and (2) the development of strategies to be used in selecting for the target characteristic, in addition to selection for other desirable traits or against undesirable traits from the donor material.

MATERIALS AND METHODS

Resistant plant materials originated from the annual accession PI 198758 of *B. vulgaris* subsp. *maritima*, which had been collected in Le Pouliguen, Brittany, France. In 1977, G. Cleij and coworkers (SVP, Wageningen, the Netherlands) selected four resistant plants, which were multiplied and named accession BMH. Progenies of this accession were used in a breeding programme (Mesken and Lekkerkerker, 1988). Raising the level of resistance was attempted by repeated mass selection, i.e. plants without, or sometimes with only a few cysts, were selected and used to produce the next generation. Through crosses with fodder beet or sugar beet, followed by repeated mass selection, the transfer of the resistance into cultivated materials was attempted. Mesken and co-workers also used accession BMH in pair crosses with fodder beet or sugar beet, which were made in isolation cages.

The plant material of the present study consists of a selection out of the more advanced stocks that were produced in the above-mentioned breeding programme, as well as some pair crosses. Series of progenies were chosen in such a way that the families within a series were directly related to each other, nearly always as parent and offspring.

Tests for BCN resistance were done as described by Toxopeus and Lubberts (1979). Young seedlings were transplanted into 36 ml PVC tubes filled with quartz sand, which was moistened with a nutrient solution. After 10 days each plant was inoculated with a suspension containing about 250 pre-hatched juveniles of *H. schachtii*, by means of a veterinary syringe. The experiments were carried out in an air-conditioned greenhouse at about 22°C. After four weeks, the root systems were carefully washed free of sand and the white females (further on called cysts) were counted using a magnifying glass. The tests were carried out using the nematode population normally used at CPRO-DLO. In Lange et al. (1993) this population was called BCN-WA.

Four experiments were carried out, using randomised complete block designs with twelve replicates. The number of plants per plot per replicate was eight. All experiments included, as control, the oldest available material of accession BMH. This material is called BMH 0, and it went through three cycles of selection for BCN resistance. The susceptible sugar beet cultivars 'Regina' (four experiments) and

'Monohil' (three experiments) were included as controls. Other controls were related to the target material of the respective experiments. The data were square root transformed before statistical analysis. Data in the tables are non-transformed numbers.

RESULTS

Repeated selection

The first experiment (Table 1) included three series of four progenies each of accession BMH, viz. BMH 1, BMH 2 and BMH 3. Within each series the second number relates to the generation, e.g. BMH 1-2 is a direct descendant of BMH 1-1, etc. Between the generations mass selection for BCN resistance was applied, and the number of selection cycles has been indicated.

Table 1. Results of testing for resistance to the beet cyst nematode (BCN), *Heterodera schachtii,* in three series of progenies of accession BMH of *Beta vulgaris* subsp. *maritima,* which progenies originated through repeated selection in consecutive generations.

Plant materials	Number of BCN selection cycles	Number of cysts/plant mean	range	Significance (P<.05)[†]
BMH 1-1	4	13.2	2-46	abc
1-2	5	18.6	5-60	efg
1-3	6	15.0	2-62	abcd
1-4	7	14.5	3-48	abcd
BMH 2-1	4	12.8	1-40	ab
2-2	5	14.7	2-43	abcd
2-3	6	11.9	2-26	a
2-4	7	12.7	2-33	a
BMH 3-1	4	19.0	2-44	fg
3-2	5	21.3	1-45	g
3-3	6	16.1	2-44	cdef
3-4	7	15.4	2-32	bcde
Controls:				
BMH 0	3	16.9	2-37	def
'Monohil'	-	54.9	21-88	h
'Regina'	-	53.5	20-93	h

[†] different letters indicate significant differences

The results of this experiment show three phenomena: (1) there is a large and significant difference between the two susceptible sugar beet varieties and the progenies of accession BMH, (2) both the low mean numbers of cysts per plant and the absence of plants without cysts confirm the partial resistance of the BMH stocks, and (3) the range of numbers of cysts per plant indicate a large variability, which is not unusual for experiments with cyst nematodes. The mean numbers of cysts per plant in the BMH stocks were between about 20% and 40% of those of the susceptible controls. However, the pattern observed for the three BMH series is not the same. In series BMH 1, repeated selection has had no additive effect. The deviating results with BMH 1-2 could neither be understood nor explained. In series BMH 2, a high and relatively stable level of resistance appeared to be reached, whereas in series BMH 3 the level of resistance showed some improvement to repeated selection.

In addition to the reduced numbers of cysts per plant, the cysts on the roots of accession BMH often were considerably smaller than in susceptible materials (Fig. 1). This effect has not been quantified for the BCN, but for the potato cyst nematode strong positive correlations were found between the volume of the cysts and the number of eggs per cyst (Stelter and Gaur, 1969). Such decrease of numbers of eggs, through the development of smaller cysts, may add to the effect of the resistance, i.e. the nematode multiplication rate may be reduced further.

The results of BCN resistance tests of three series of progenies of a cross between fodder beet and accession BMH are presented in Table 2. As in Table 1, the second number under 'plant materials' relates to the generation. The number of BCN selection cycles is indicated in the second column. All materials based on accession BMH showed more or less the same level of resistance. The mean numbers of cysts per plant were about 15-30% of those of the susceptible controls, and the plants were significantly more resistant than the susceptible varieties. In general, the mass selection in the advanced generations had little or no effect on the already attained level of resistance. Generation 3-2 showed an unexplainable drop in resistance. As in the original BMH progenies (Table 1), the resistant materials showed a wide variation in cyst size, i.e. a number of the cysts was considerably smaller than those on the susceptible controls.

The results shown in Table 3 describe the tests carried out with progenies of five crosses between sugar beet and accession BMH. This material generally is less advanced than the progenies of fodder beet x accession BMH (Table 2). In many cases, additional selection for bienniality was carried out. However, both selections were not carried out on the same plants. In each generation either the one or the other type of selection was performed. The numbers of the two types of selection cycles are indicated. The second number of the plant materials relates to the generation, e.g. both 1-2a and 1-2b are direct descendants of 1-1. In Cross 5 the parentage is slightly more complicated: 5-3a is a direct descendant of 5-2a, but 5-(3b) and 5-(3c) are descendants of 5-1 along another route than that of 5-2a and 5-3a.

The highest level of resistance was attained by BMH 0, and even in Cross 4, having five cycles of selection for BCN resistance, the level of BMH 0 was not reached. The comparison of results in routes, with or without selection for bienniality in the various series of progenies, revealed a tendency towards a decrease in BCN resistance after the

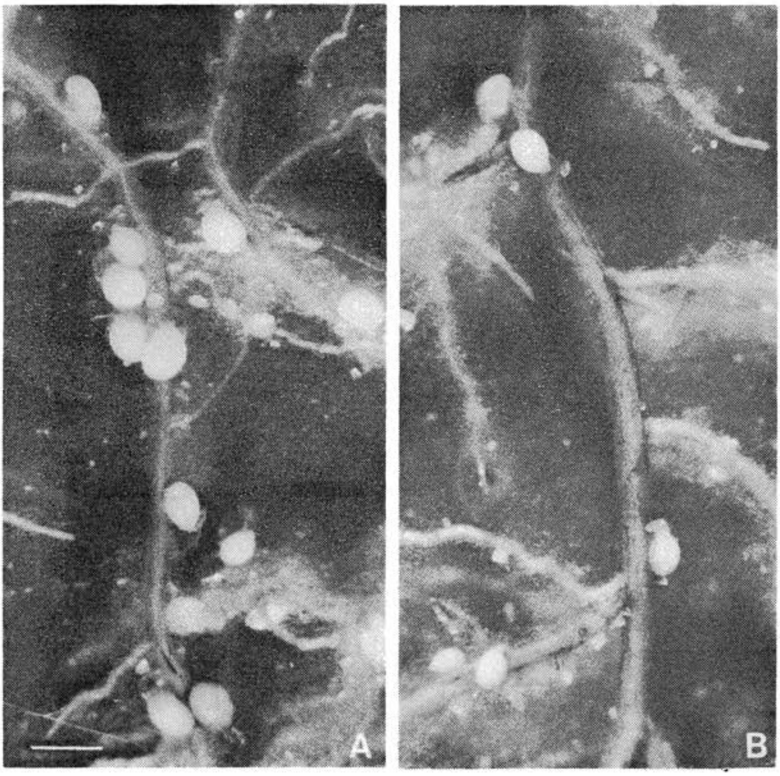

Fig. 1. Young white females (cysts) of the beet cyst nematode, *Heterodera schachtii;* A. on susceptible sugar beet, and B. on the partial resistant accession BMH, *Beta vulgaris* subsp. *maritima.* Bar = 1 mm.

selection for bienniality. The clearest example is presented by the difference between 2-2b and 2-1 or 2-2a.

F_1s of pair crosses

Crosses were carried out between individual plants of accession BMH and fodder beet or sugar beet (Table 4). In cases where the plants of BMH were used as female parent, selfing was not expected to occur, because of self-incompatibility. This was tested in the crosses with a stock having dark red hypocotyls (NK 150). All F_1 plants had dark red hypocotyls. When BMH was the male parent, male sterile (ms) plants of cultivated beet were used as females.

Table 2. Results of testing for resistance to the beet cyst nematode (BCN), *Heterodera schachtii,* in three series of progenies of crosses between fodder beet {('Eureka' x 'Neo-K') x 'Buffalo'}ms and accession BMH of *Beta vulgaris* subsp. *maritima,* which progenies originated through repeated selection in consecutive generations.

Plant materials	Number of BCN selection cycles	Number of cysts/plant mean	range	Significance ($P<.05$)[†]
Series 1-1	4	18.2	0-56	e
1-2	5	17.8	2-46	de
1-3	6	17.1	1-56	de
1-4	7	14.7	0-63	bcd
Series 2-1	4	12.2	2-40	abc
2-2	5	16.7	1-46	cde
2-3	6	12.6	1-40	abc
2-4	7	10.1	1-37	a
Series 3-1	4	12.3	1-59	ab
3-2	5	18.1	1-54	e
3-3	6	11.8	1-42	ab
Controls:				
BMH 0	3	19.4	2-43	e
'Neo' (fam.)	-	62.0	13-108	f
'Monohil'	-	64.7	23-104	f
'Regina'	-	63.3	15-101	f

[†] Different letters indicate significant differences.

Table 3. Results of testing for resistance to the beet cyst nematode (BCN), *Heterodera schachtii,* in series of progenies of five crosses between sugar beet and accession BMH of *Beta vulgaris* subsp. *maritima;* the progenies were selected for BCN resistance and bienniality.

Plant materials†	Number of BCN selection cycles		Number of cysts/plant		Significance ($P<.05$)‡
	BCN	Biennial	mean	range	
Cross 1-1	4	0	23.5	7-58	b
1-2a	3	0	17.8	6-91	bc
1-2b	2	1	28.2	5-69	bcd
Cross 2-1	2	0	26.8	7-59	bc
2-2a	3	0	25.7	8-61	bc
2-2b	2	1	33.8	11-61	ef
Cross 3-1	2	0	33.1	9-67	def
3-3	2	2	39.3	12-88	fg
Cross 4	5	1	26.4	9-54	bc
Cross 5-2a	3	0	30.1	5-56	cde
5-3a	4	0	35.2	6-72	ef
5-(3b)	2	2	43.7	18-104	gh
5-(3c)	1	3	39.0	7-70	fg
Controls:					
BMH 0	3	0	18.1	1-43	a
'Nemee' (fam.)	-	-	47.6	11-86	hj
'KWE' (fam.)	-	-	56.8	17-158	k
'Monohil'	-	-	50.9	13-97	hjk
'Regina'	-	-	52.7	17-98	jk

† Cross 1 = Acc. BMH x 'Nemee' (fam.); Cross 2 = Acc. BMH x 'Nemos' (fam.); Cross 3 = Acc. BMH x 'KWE' (fam.); Cross 4 = {('Dippe-N' x 'Nemee-B') x 'KWE'}ms x Acc. BMH; Cross 5 = 'Nemee' (fam.)ms x Acc. BMH.

‡ Different letters indicate significant differences.

The results of testing for BCN resistance are presented in Table 4. The mean number of cysts per plant in the F_1s was in most cases slightly lower than the mid-parent value of the cross. All F_1s had a significantly lower number of cysts than the susceptible parent, and with BMH 1 and BMH 4 the F_1s had a significantly higher number of cysts than the resistant parent. BMH 5 was less resistant than the other BMH

Table 4. Results of testing for resistance to the beet cyst nematode (BCN), *Heterodera schachtii,* in progenies of pair crosses of accession BMH of *Beta vulgaris* subsp. *maritima* and fodder beet (FB) or sugar beet (SB).

Plant materials (crosses, parents, controls)	Number of cysts/plant mean	range	Significance ($P<.05$)[†]
BMH 1 x 'Buffalo'	30.3	1-62	bcd
"	30.7	4-8	bcd
BMH 4 x 'Buffalo'	31.8	6-58	cde
"	31.9	6-57	cde
BMH 1 x NK 150	27.3	3-55	bc
"	28.2	10-55	bcd
BMH 4 x NK 150	26.8	0-52	b
'Neo'ms (fam.) x BMH 5	31.7	3-60	
"	36.7	7-83	efg
'Nemee'ms (fam.) x BMH 5	32.9	13-65	def
"	38.2	9-100	fg
BMH 1 (5x BCN sel.)	16.6	2-27	a
BMH 4 (5x BCN sel.)	16.1	5-34	a
BMH 5 (3x BCN sel.)	29.2	6-70	bcd
'Buffalo' (FB)	57.5	12-107	j
MS (SB) x NK 150 (SB)	41.3	0-86	g
'Neo'ms (fam.) (FB)	55.3	11-106	j
'Nemee'ms (fam.) (SB)	46.8	15-91	h
Controls:			
BMH 0 (3x BCN sel.)	15.8	3-31	a
'Regina' (SB)	50.1	11-94	hj

[†] Different letters indicate significant differences.

parents. The F_1s based on BMH 5 were more variable and showed on average a higher number of cysts per plant than the average number of the F_1s based on BMH 1 and BMH 4.

DISCUSSION

Resistance of accession BMH

The stock BMH 0 and the sugar beet variety 'Regina' were included in all four experiments, and other cultivated materials in two or three of the experiments. From the results of BCN testing in these control materials it can be concluded that, although the level of infection varied between the four experiments, the ratio between BMH 0 and cv. 'Regina' was very stable, i.e. mean value 0.32, range 0.31-0.34. This stability indicates that these controls are adequate for comparison of the experiments. The ratio 0.32 indicates that the resistance of accession BMH reduces the number of cysts by about two-thirds. In addition, it was shown that the BMH resistance results in many of the cysts being much smaller than those on the susceptible control varieties. These small cysts certainly will contain fewer eggs, and thus will reduce the multiplication rate of the nematodes even further. This suggests that this source of BCN resistance may be very valuable for beet breeding. There is still a need for more detailed studies concerning the multiplication rate of the nematodes on accession BMH.

The type of resistance in accession BMH appears to be reduced susceptibility (Trudgill, 1991). According to Trudgill (1991), such resistance is likely to be sensitive to environmental factors (especially those that influence pathogen development), it will be nonspecific, possibly recessively inherited, and may influence the sex ratio of the nematodes. The resistance also might be more durable than the monofactorial, major gene resistance from the species of section Procumbentes.

The supposed sensitivity to environmental factors might explain the observed differences between the present experiments. Until now, there has been no evidence for specificity, although only a few nematode populations have been tested on the BMH material (Lange et al., 1993). Preliminary studies have indicated that accession BMH is also partially resistant to *H. trifolii* f.sp. *beta* (Mesken and Lekkerkerker, 1988), and Heijbroek (1977) suggested a possible influence of this type of resistance on the sex ratio of the nematodes. The BMH resistance does not appear to show a recessive inheritance, although this has been suggested earlier (Heijbroek, 1977). The observation

(Table 4) that most F_1 families have mean numbers of cysts that are slightly below the mid-parent value suggests either incomplete dominance, or oligogenic inheritance, in which part of the genes for resistance are dominant and the others recessive. More detailed genetic analyses are needed.

Pre-breeding strategies

Comparing the results of the F_1s (Table 4) with those of the advanced generations (Tables 1 and 2) it can be concluded that repeated mass selection has been effective. However, the level of attack, as measured through the number of cysts per plant, appears not to reach values lower than about 15-20% of those of the susceptible controls. The results concerning crosses with fodder beet (Table 2) would suggest that four cycles of selection is sufficient to reach such levels, although in Series 1 a higher number of selection cycles still led to a slight improvement. The crosses between sugar beet and accession BMH (Table 3) gave slightly different results. The resistance level of BMH 0 was not reached, and the number of cysts in the repeatedly selected progenies was about 50% of that of the susceptible controls. The observed difference between the progenies of crosses with fodder beet and with sugar beet is difficult to explain. There might either have been a difference in the level of resistance between the BMH stocks used in the crosses, or the genetic background in sugar beet is less favourable for the expression of the resistance. A difference between crosses with fodder beet and sugar beet was not observed for the F_1s of the pair crosses (Table 4). A possible influence of the BMH parent was supported through the general difference between results of pair crosses with BMH 1 or BMH 4 and BMH 5 (Table 4). In addition, the selection for bienniality tended to decrease the progress in the selection for BCN resistance (Table 3).

The above described results may help to formulate a pre-breeding strategy for the transfer of the partial BCN resistance from accession BMH to cultivated beets. Because of the testing system, it seems unrealistic to carry out mass selection for BCN resistance and selection for other agricultural traits on the same plants and in large numbers. Thus both types of selection should be executed in alternation, either after each generation or after a series of selections for the one trait or the others. A possible strategy would be: (1) repeated BCN selection within accession BMH to acquire the threshold level of resistance, followed by (2) crossing with the best breeding stocks, (3) repeated BCN selection to acquire a good level of resistance again (4) selecting for other characters by using the selected resistant stocks only, and if needed, (5) repeating the cycles (2) to (4). This sequence of activities is elaborate, is restrictive to the repeated use of unselected germplasm in the breeding programme, and can only be used if the genetic variability for the agricultural traits is highly heritable. Therefore, a breeder should also consider the second possible strategy, i.e. the alternation per generation of selection for BCN resistance and selection for other traits.

Molecular technologies might present attractive possibilities to speed up the selection process, but only if the genetics of the BCN resistance in BMH is not too complicated, e.g. not more than three or four genes. Two RFLP (Restriction Fragment Length Polymorphism) maps have been published for beet (Pillen et al., 1992; Barzen et al., 1992), and Uphoff and Wricke (1992) described RAPD (Random Amplified Polymorphic DNA) markers for this crop species. Both types of marker could be used in marker-facilitated selection programmes (Melchinger, 1990; Michelmore et al. 1991). In that way the selection for the partial BCN resistance from accession BMH could be done with the help of the molecular markers and on the same plants that are used for the general breeding activities.

If eventually a major gene for BCN resistance from one of the species of section *Procumbentes* can be isolated (Jung et al., 1990; 1992; Salentijn et al., 1992) and incorporated in beet using transformation techniques, the desired pyramiding of both systems of BCN resistance can be done. However, if a major gene is present, the selection for the maintenance and improvement of the partial resistance from accession BMH can not be done without markers. The pyramiding of both systems would likely lead to a higher level of durability of BCN resistance in beet.

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