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BREEDING FOR TOLERANCE TO BEET-CYST EELWORM *HETERODERA SCHACHTII* SCHM. IN SUGARBEET

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SUMMARY

Successive mass selections were made in sugarbeet varieties (*Beta vulgaris* L.) and in *B. vulgaris* × *B. maritima* L. hybrids for tolerance to wilting caused by beet-cyst eelworm attack. The selected lines showed improved tolerance to wilting, but no evidence of resistance to eelworm infection was demonstrated. By repeated back-crossing of the selected lines with the commercial varieties and concurrent selection root yield could be improved without substantially decreasing the tolerance to eelworm attack. These results indicate that tolerance is partly conditioned by complete and incomplete dominant genes.

INTRODUCTION

Sugarbeet infected with beet-cyst eelworm (*Heterodera schachtii* SCHM.) shows pronounced wilting symptoms caused by a decreased uptake and transport of water in the root system. The beet-cyst eelworm larvae stimulate formation of giant cells in the lateral roots, thus decreasing the capacity for intercellular and vascular transport. Under dry conditions, therefore, evaporation can be compensated for in infected plants less easily than in healthy plants. The larvae in the root system also produce growth-promoting substances (JOHNSON & VIGLIERCHIO, 1969) that cause lateral root growth and an increase in petiole length (DONEY et al., 1971).

PRICE (1965), JORGENSON & SMITH (1966), and PAWELSKA- KOZINSKA & SZOTA (1967) reported that sugarbeet varieties or selections may differ in their response to beet-cyst eelworm infestation, but they showed no data on multiplication or cyst formation on the roots. It is not clear whether these differences in response resulted from resistance to infection, tolerance to wilting, or both. DONEY & WHITNEY (1973) suggested that some sugarbeet lines have tolerance, based on high overall plant vigor, which might be improved by further selection. The purpose of this study was to select for tolerance to wilting, caused by beet-cyst eelworm attack and determine whether this feature was inherited. In such selections the multiplication rate of the eelworms must be the same as in susceptible material, but wilting symptoms should occur less frequently.

MATERIALS AND METHODS

In a number of severely infested fields, we selected commercial sugarbeet (*B. vulgaris* L.) varieties that showed tolerance to the wilting symptoms associated with eelworm attack. The multiplication rate of the beet-cyst eelworm was the same in these selections as in susceptible plants, but wilting was less frequent.

Beta maritima L. seed was obtained from plants that grew along the Atlantic and Mediterranean coasts (English, Irish, French, and North African shores), the USDA collection, and several botanical gardens[1]. Several field selections from sugarbeet varieties and *B. vulgaris* × *B. maritima* hybrids were screened for tolerance. The commercial variety 'KWE' (later changed to Kawepoly) was used as pollinator.

The sugarbeet plants without wilting symptoms, selected from infested commercial fields, were selfed and their offspring were tested for tolerance. Wilt-tolerant plants were also selected from progeny of *B. vulgaris* × *B. maritima* hybrids backcrossed twice to *B. vulgaris* and then selected for absence of the annual character.

To increase the germplasm base, we used selections furnished by Price, Salinas, California, as parents for crosses and selections made in the Netherlands. All selections were vernalized from 4 to 6 months during the winter and then brought to flowering for self- or interpollination in isolation cabinets.

Wilting tests were made in isolated microplots (4 m^2) that contained severely infested soil. In July and August each plant was scored twice for wilting rate on a scale of 0 to 5 (0 = no wilting, 5 = severe wilting). After harvest the plants were selected on the basis of wilting rates, weight, root size, and root shape. Seed from promising lines was sown in trial plots, with or without infestation by beet-cyst eelworms, in a randomized block design with four to six replications. A soil sample was taken from each plot before drilling and sometimes after harvest, and analyzed for nematode infestation. The roots were weighed, and the concentrations of sucrose, amino-N, K, and Na were determined by standard procedures.

Because the climate in the Netherlands is unfavorable for the exact measurement of wilting rates, selections were also tested at Salinas, California, in irrigated fields. In replicated trials seeds were sown in a field that was severely infested with 20 or more viable cysts of the beet-cyst eelworm per 100 g of soil. An adjacent area was fumigated with a nematicide (either DD mixture or methyl bromide) and seed for a similar trial was sown in this non-infested area. Irrigation was withheld at least twice during the growing season and the entries were rated for wilting. Trials were also conducted at Salinas in a noninfested field following normal cultural and irrigation practices. A standard commercial variety was included as a check in each trial.

To determine the infestation level of the soil and obtain inoculum, cysts were extracted from dried soil by means of flotation according to FENWICK (1940). To determine the multiplication rate of beet-cyst eelworm on the roots, we applied a standardized inoculum of hatched larvae of the same age, derived from a culture of

[1] Dr J. Hijner, former cooperator of the Instituut voor Rationele Suikerproductie, assembled the material for tolerance screening and made the first selections.

cysts on rape (*Brassica napus* L.) and grown in silver sand with a nutrient solution (STEINER, 1968). Superficially disinfected sugarbeet seeds were germinated in germination units, and after 10 days the plantlets were transferred to pots containing 150 cc of silver sand with nutrient solution and inoculum. These trials were always performed in cooled glasshouses (18–28 °C). When necessary, additional light of Osram Fluora[2] combined with warm white was supplied. After 6 weeks the plants were immersed in water; the adhering sand was carefully removed from the roots, which were then examined for white females.

RESULTS

Progeny from the field selections had a smaller leaf area, thicker veins, and a smaller stomatal pore size than did wiltsusceptible plants of the same variety. Under high light intensity and temperature, the pores also closed earlier. With inocula of 500 and 1500 larvae per plant, beet-cyst eelworms multiplied at similar rates on the root systems of these selections as on wilt-susceptible sugarbeets (Table 1). The *B. maritima* species showed some resistance to eelworm infection but this was lost when

Table 1. Root weight and average number of cysts and eggs per cyst on the root systems of wilt-tolerant sugarbeets and of a commercial variety, 6 weeks after inoculation with 500 or 1500 beet-cyst eelworm larvae at Bergen op Zoom (50 replications).

Selection or variety	Av. number of cysts/g of roots		Av. number of eggs/cyst		Root weight (g/plant)
	500 larvae	1500 larvae	500 larvae	1500 larvae	
Tolerant selection I	18.7	29.5	119	97	0.95
Tolerant selection II	14.5	22.3	121	73	1.12
Commercial variety 'KWE'[1]	16.5	20.3	170	108	1.23

[1] This name later changed to Kawepoly.

these were crossed to *B. vulgaris*. After two backcrosses to *B. vulgaris* no differences in multiplication rates could be observed. Results of microplot experiments also indicate that the *B. vulgaris* selections were only tolerant to wilting and not resistant to eelworm. When tolerant plants were sibmated and selected for reduced susceptibility to wilting for two generations, no further improvement was detected. When plants were grown in noninfested soil, root weight was considerable lower for tolerant selections and backcrosses than for the commercial varieties both in the Netherlands and in California (Table 2 and 3).

No differences in K and Na concentration could be detected, but amino-N was higher in the tolerant lines than in the commercial varieties. Sucrose was higher in the tolerant backcrosses than in the commercial varieties at Salinas.

Without infestation, the root weight of progenies from backcrosses of tolerant selections to commercial varieties was low, (Table 3). In the Netherlands the back-

[2] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Table 2. Performance of wilt-tolerant selections and a commercial variety of sugarbeet in soil without detectable numbers of beet-cyst eelworms and in infested soil (8–12 eggs per g of dried soil) at Bergen op Zoom in 1964.

Selection or variety	Root weight (% of comm. var.)	Sucrose (%)	Amino-N (mg/100 g sugar)	K + Na (m eq/100 g sugar)	Wilt. rate (0–5)
Noninfested soil:					
Tolerant selection I	89	16.6	186	24.7	–
Tolerant selection II	86	16.9	152	22.9	–
Commercial variety 'KWE'	100	17.1	123	27.4	–
LSD 0.05	11.4	NS[1]			
Infested soil:					
Tolerant selection I	94	16.9	312	36.9	1.3
Tolerant selection II	98	17.9	295	30.6	1.5
Commercial variety 'KWE'	100	17.3	254	36.9	2.9
LSD 0.05	NS[1]	0.8			

[1] NS = not significant.

Table 3. Productivity and wilting rate for hybrids of tolerant selections and commercial varieties and for commercial varieties, in eelworm-infested and uninfested soil at Salinas, Stavenisse, and Wieringermeer in 1967 and 1968.

Hybrid or variety	Eelworm infested			No infestation		
	root weight (% of comm. var.)	sucrose (%)	wilt. rate (0–5)	root weight (% of comm. var.)	sucrose (%)	wilt. rate (0–5)
	Salinas			Salinas		
(Comm. var. × tol. sel.) F_1	145	16.8	3.0	84	16.5	1.7
(Tol. sel. × comm. var.) F_1	149	16.2	2.7	87	14.5	1.4
Commercial var. 'US H7'	100	15.8	4.7	100	15.8	1.6
LSD 0.05	24	0.8	1.1	10	0.8	0.6
	Stavenisse (init. density, 6–9 eggs/g of soil)			Wieringermeer		
(Comm. var. × tol. sel.) F_1	103	16.5	1.1	94	17.3	–
(Tol. sel. × comm. var.) F_1	102	16.0	1.2	–	–	–
Commercial variety 'KWE'	100	16.3	3.8	100	17.2	–
LSD 0.05	NS[1]	NS[1]	0.7	3.6	NS[1]	

[1] NS = Not significant.

Table 4. The performance of tolerant hybrids, three consecutive backcross generations, and a commercial variety on severely infested soils at Salinas, California in 1970.

Line or variety	Root weight (tons/ha)	Root weight (% of comm. var.)	Sucrose (%)	Wilt. rate (0 5)
A. (Tol. sel. × comm. var.) F_1	35.9	90	15.2	2.3
(Tol. sel. × comm. var.) F_2	40.8	103	15.5	2.1
Commercial variety 'US H9B'	39.7	100	13.3	3.3
LSD 0.05	NS[1]		0.9	0.7
B. First backcross	45.2	116	13.8	2.3
Second backcross	47.5	122	12.9	2.0
Third backcross	52.7	135	13.8	2.2
Third backcross (reciprocal)	45.1	116	14.1	2.4
Commercial variety 'US H9B'	38.9	100	13.1	3.3
LSD 0.05	5.9		0.6	0.4

[1] NS = Not significant.

crosses showed no improvement in yield relative to the standard even when eelworm was present. In California, the tolerant lines outyielded the commercial variety in eelworm-infested soil but yielded less than the commercial variety when eelworm was absent. By selection for shape and size of individual roots of the F_1, the yield of the F_2 was improved (Table 4). The F_2 was backcrossed several times to the same variety and selections were made concurrently for wilting tolerance. However, when no selection was applied, wilting rates increased and root weight decreased during backcrossing in plants tested in infested soil (Table 5B). In uninfested soil. root yield in the third backcross generation was not signficantly different from that in the commercial variety, when grown for 5 months with intermittent irrigation and was not significantly improved by a fourth backcross (Table 5A).

We also introduced tolerance from *B. maritima* into lines derived from field selections. Reductions in wilting rate and in crop losses caused by beet-cyst eelworm were greater from this procedure than from any other test (Table 5B). However, in a 1976 California test the F_2B_3 line that incorporated tolerance from *B. maritima* yielded significantly less than did the standard US H10B variety (76.4 vs. 88.4 tons per hectare) when each was grown for 7 months in nematodefree soil under normal irrigation procedures. No further improvement was obtained when a mixture of California selections was used as one of the parents in the original cross.

DISCUSSION AND CONCLUSIONS

Results of breeding for tolerance to wilting associated with eelworm attack is difficult to interpret because at harvest one does not know whether a portion of the yield improvement is the result of reduced invasion by eelworms. We therefore selected plants with low wilting rates, sibmated them, and determined eelworm multiplication in soil during the next generation. Most of the tolerant strains were tested at Salinas.

Table 5. Production and wilting rates of some backcross generations and the influence of different parents and procedures on final performance at Salinas, California, in 1973 and 1974.

Line or variety		Severe infestation			No infestation	
		root weight (tons/ha)	sucrose (%)	wilt. rate[1] (0–5)	root weight (tons/ha)	sucrose (%)
A. Third backcross	F_2B_3	23.0	15.2	2.1	48.3	13.9
Fourth backcross	F_2B_4	22.3	16.0	2.2	49.7	15.1
F_1 × tol. sel.		22.8	14.9	1.0	43.2	14.2
Commercial variety 'US H10B'		13.4	13.6	3.0	45.5	14.0
LSD 0.05		4.2	0.5		NS[2]	0.7
B. Third backcross	F_2B_3					
Selected for wilting tolerance during backcrossing		22.8	14.1	1.8	52.9	15.7
Wilting tolerance from *B. maritima* incorporated		26.8	13.4	0.9	49.0	15.8
Selections by Price used as original parents		22.5	13.0	1.9	48.2	14.8
Not selected for wilting tolerance during two backcross generations		17.6	14.2	2.3	52.8	16.1
Commercial variety 'US H10B'		10.1	13.0	3.0	47.4	15.1
LSD 0.05		3.8	0.6		NS[2]	0.4

[1] Wilting rate = Average of four ratings for test A and two ratings for test B.
[2] NS = Not significant.

California, where wilting could be introduced as desired by withholding irrigation. Some selections were tolerant to wilting (Fig. 1) and produced a higher root yield, even though the multiplication rate of the beet-cyst eelworm in severely infested soils was similar to that found in fields of commercial varieties. When eelworms were absent or occurred only in small numbers, the root weight of the tolerant selections was usually lower or no better than that of the standard commercial variety. Root yield was improved by successive mass selection for shape and size of individual roots and by backcrossing either to commercial varieties and/or to selections developed at Salinas (DONEY & WHITNEY, 1970). The fact that the offspring of each backcross did not show increased wilting rates (Table 4) suggests that this tolerance is dominant. However, if no selection is made during the backcross procedure a part of the tolerance is lost which indicates incomplete dominance. When biennial segregates from a hybrid between *B. vulgaris* and *B. maritima* were backcrossed to *B. vulgaris*, wilting and yield loss from beet-cyst eelworm attack were further decreased, which suggests that tolerance is also present in *B. maritima*.

We selected lines that outyielded a current commercial variety when tested in California under moderate to severe eelworm infestation. In California under eelworm-free conditions, the yields of the tolerant selections were not significantly different from those of the commercial check when grown for 5 months with restricted

Fig. 1. Wilting in a tolerant selection (on the left a six rows wide plot) as compared to an unselected check (right). Initial infestation with beet cyst eelworm 12–17 eggs per g of dried soil (Steenbergen 1974).

irrigation, but were inferior to that of the check when grown for 7 months with normal irrigation. Tolerance could also be demonstrated in the Netherlands, but in the absence of eelworm, root yields were somewhat lower in tolerant selections than in commercial varieties except when water supply was the limiting factor.

The good performance of wilt-tolerant selections under moisture stress suggests that this trait could advantageously be incorporated into varieties that are to be used in areas subject to droughty conditions. Eelworm losses could be reduced through the use of tolerant varieties, but at high infestation levels damage would continue to be severe. When these varieties are grown, the soil eelworm population will increase the same as it does with susceptible varieties. If sugarbeets are frequently used in crop rotation, exceptionally high eelworm infestations may occur and crop losses may be unacceptably high. At present, the use of tolerant varieties to reduce losses should be combined with chemical control of eelworms, but the introduction of varieties that combine resistance to eelworm invasion and tolerance to damage from eelworm attack may provide the eventual solution.

REFERENCES

DONEY, D. L. & E. D. WHITNEY, 1970. Genetic diversity in sugarbeet lines selected for nematode resistance. J. Am. Soc. Sugar Beet Technol. 16: 219–224.

DONEY, D. L., E. D. WHITNEY & A. E. STEELE, 1971. Effect of *Heterodera schachtii* infection on sugarbeet leaf growth. Phytopathology 61: 40–41.

DONEY, D. L. & E. D. WHITNEY, 1973. Individual plant selection in nematode-infested soil. J. Am. Soc. Sugar Beet Technol. 17: 375–380.

FENWICK, D. W., 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. J. Helminth. 18 (4): 155–172.

JOHNSON, R. N. & D. R. VIGLIERCHIO, 1969. A growth promoting substance occurring in an extract prepared from *Heterodera schachtii* larvae. Nematologica 15: 159–160.

JORGENSON, E. C. & C. H. SMITH, 1966. Evaluation of selected varieties of sugarbeets for response to the sugarbeet nematode *Heterodera schachtii*. Pl. Dis. Reptr 50: 650–654.

PAWELSKA-KOZINSKA, K. & Z. SZOTA, 1967. Results of experiments to estimate the degree of tolerance to *Heterodera schachtii* SCHMIDT in certain sugarbeet varieties (Polish). Biuletyn Instytutu Hodowli I Aklimatyzacji Roslin 1967 (3–4): 53–55.

PRICE, CHARLES, 1965. Breeding sugar beets for resistance to the cyst nematode *Heterodera schachtii*. J. Am. Soc. Sugar Beet Technol. 13: 397–405.

STEINER, A. A., 1968. Soilless Culture. Proc. 6th Coll. Int. Potash Inst. Florence: 324–341.