Resistance of Trisomic and Diploid Hybrids of Beta vulgaris and B. procumbens to the Sugarbeet Nematode, Heterodera schachtii

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Abstract: Trisomic and diploid hybrids of sugarbeet (*Beta vulgaris* L.) × wild beet (*B. procumbens* Chr. Sm.) inherited the gene for resistance to *Heterodera schachtii* Schm. from *B. procumbens*. The hybrids showed partial resistance to *H. schachtii*, manifested in failure of larvae to reach maturity. Although significantly greater numbers of female nematodes developed on plants inoculated with populations from the Netherlands or Italy than on plants inoculated with a population from the Salinas Valley, California, the totals for all populations on resistant plants were small. Greater numbers of males than females developed on root-slice cultures of resistant hybrids when compared to a susceptible cultivar. *Key words:* interspecific hybrids, *Beta* species, beets, nematode, resistance, geographic populations.

The sugarbeet nematode, *Heterodera schachtii* Sch., is the most important pest limiting production of sugarbeet, *Beta vulgaris* L., in the United States. In many areas of production, chemical control is not economically feasible, and in regions where crop variation is limited, control by rotation is not practical. Consequently, there is considerable interest in developing commercial cultivars of sugarbeet resistant to this nematode.

Early attempts to detect resistance or tolerance in *B. vulgaris* were unsuccessful (9,10,13), and resistance (2,3,4,5) or tolerance (12,14,24) in wild *Beta* species has not been successfully transferred to sugarbeet cultivars. Of 13 wild *Beta* species investigated, only *B. procumbens, B. patellaris,* and *B. webbiana* were found to have genes for resistance to *H. schachtii* (6,7,8, 11,19,23,27,28), and these species have similar morphologic characters and are members of the taxonomic section *Patellares Tr.* (25).

Savitsky (15,16,17,18) crossed *B. vulgaris* with the three *Beta* spp., and investigations have continued on the cytogenetics and

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nematode resistance in hybrids *B. vulgaris* × *B. procumbens* only. Savitsky (15) crossed *B. vulgaris* with *B. procumbens* and obtained viable resistant trisomic progeny having 19 chromosomes (18 from *B. vulgaris* and the single chromosome from *B. procumbens* which contained the gene for resistance). Resistant trisomics backcrossed to diploid sugarbeet to the eighth generation gave about 12 percent transmission of resistance. All diploid (2n = 18) progeny from the trisomic backcross were susceptible to *H. schachtii*. A gene for resistance, however, was incorporated into a chromosome of sugarbeet by crossover transfer of a segment of a chromosome from *B. procumbens* (17). In the transfer, dominant genes linked to the gene for resistance and controlling undesired features such as bolting, narrow leaves, and tumor formation were deleted.

Studies were undertaken to determine whether resistance of trisomic and diploid hybrids is due to inability of larvae of *Heterodera schachtii* to penetrate fibrous roots or to failure of larvae to develop after penetration. In addition, tests were conducted to determine if diploid hybrids resistant to local populations of *H. schachtii* would exhibit susceptibility to one or more geographically isolated populations of the nematode.

MATERIALS AND METHODS

Penetration and development: To determine whether resistance is due to failure of larvae to enter roots or the failure of larvae to develop to maturity after penetration, we investigated these parameters in resistant trisomic (2n+1 = 19), resistant diploids (2n = 18), and susceptible diploid 'US 75' (2n = 18) sugarbeets inoculated with a local isolate of *H. schachtii*. Chromosome numbers of host plants were verified by cytological examination of root tips; the disease reactions of the hosts were known from earlier studies (29). Two tests were conducted to compare resistant trisomics with susceptible diploids (Test 1) and resistant diploids with susceptible diploids (Test 2). Fifteen plants each of resistant trisomic, resistant diploid, and susceptible sugarbeet were individually transplanted into individual 15-cm clay pots containing a steam-sterilized sand-clay soil mixture. Nematode cysts for inoculum were collected from 'US 75' sugarbeet grown in infested soil in a greenhouse. The cysts were broken, and the contents of 30 cysts (about 7,000 larvae and eggs) were added to each pot at the time of transplanting. Plants were randomized on a bench in a greenhouse. Air temperature was maintained at 68–75° C. Roots were harvested 15, 30 and 45 d after transplanting, stained in a hot acid fuchsin-lactophenol solution, cleared in lactophenol, fragmented in a Waring Blender, and the various developmental stages of *H. schachtii* determined. Soil from the pots of harvested plants was extracted by the flotation method (1) and the stages of *H. schachtii* determined.

Comparison of H. schachtii isolates: Three tests were designed to compare the development of geographic and host isolates of *H. schachtii* on resistant trisomic hybrids and resistant and susceptible diploid plants. One test studied development of populations of *H. schachtii* from Salinas, California, Bergen op Zoom, the Netherlands, and a virulent Salinas population (designated as Tri-1) which had developed on a resistant trisomic hybrid and susceptible 'US 75' sugarbeet. Each treatment was replicated 10 times. Each pot contained single plants inoculated with the contents of 30 cysts. Forty days after the inoculation, roots and soil were examined for adult female nematodes.

In the second test the susceptibility of the diploid hybrids to *H. schachtii* populations from Bari, Italy, the Netherlands, and the common local population were compared. Each of ten seedlings for each nematode population was transplanted 10 d after germination and inoculated with 35 cysts.

The third test evaluated 20 resistant and 20 susceptible diploid hybrids for susceptibility to an Italian population of *H. schachtii*. Each plant was inoculated with 50 cysts. Sixty days after inoculation, the plants were harvested and the numbers of adult female nematodes recovered from roots were analyzed by the t-test for nonpaired variates.

Development on root slices: The development of male and female *H. schachtii* (local population) on storage roots of susceptible 'US 75' sugarbeet and resistant diploid hybrids (*B. vulgaris* × *B. procum-*

bens) were compared. The nematode was cultured on storage root slices from six resistant diploid plants and six susceptible sugarbeet. After surface sterilization, two transverse segments about 1.5 cm thick and 4 cm in diameter were cut from the storage roots. The contents of 30 cysts of *H. schachtii* were placed on the cut surfaces of the root slices. The root slices were then placed in loosely capped, sterile crystallizing dishes and maintained at 100% relative humidity and 24 C. After 33 d the root slices were removed and examined for adult male and female nematodes. Data were analyzed for statistical significance.

RESULTS AND CONCLUSIONS

Penetration and development: The numbers of second-stage larvae recovered from roots of resistant trisomic, resistant diploid hybrids, and susceptible 'US 75' sugarbeet at 15 and 30 d after inoculation indicated that resistance could not be attributed to failure of larvae to enter roots (Table 1). This agrees with the conclusions reached by Golden (6) from his study on the hatching activity of root diffusates of wild *Beta* spp.

After 45 d the number of second-stage larvae recovered from susceptible diploid roots were three times greater than numbers within roots of trisomic plants (Table 1, Test 1). This is apparently due to penetration by progeny of nematodes which had successfully reproduced on these plants. Roots of resistant trisomic and resistant diploid hybrids (Tests 1 and 2, respectively, Table 1) examined 30 and 45 d after inoculation had fewer third- and fourth-stage larvae and adult females than did susceptible diploid sugarbeet. This would indicate that larvae died at each stage of development within the roots. Histological examination of sectioned and stained roots has confirmed this assumption (29). Also, second-stage larvae may have migrated from the roots. Forty-five days after inoculation, roots of resistant trisomics and resistant diploid hybrids contained equal numbers of larvae and adult females. The low numbers of adult females indicate a high degree of resistance. Total numbers of nematodes on susceptible diploids were similar for

Table 1. Mean numbers of the various stages of *Heterodera schachtii* recovered from *Beta vulgaris* and *B. vulgaris* × *B. procumbens* trisomic and diploid hybrids.

Plant genotype	Number of days after inoculation*	2nd stage larvae	3rd stage larvae	4th stage larvae	Adult female	Total nematodes
			Test #1			
Resistant trisomic†	15	12	1	0	0	13
	30	39	23	35	9	106
	45	14	16	18	7	55‡
Susceptible diploid§	15	18	4	0	0	22
	30	38	67	121	176	402
	45	42	13	17	641	713
			Test #2			
Resistant diploid‖	15	39	46	9	0	94
	30	2	1	0	5	8
	45	21	18	2	4	45
Susceptible diploid§	15	37	33	9	0	79‡
	30	1	1	2	299	303
	45	2	2	5	730	739

*Each plant was inoculated with 30 broken cysts.
†*B. vulgaris* × *B. procumbens* (2n+1 = 19 chromosomes).
‡Mean of four plants. All other figures are means of five plants.
§*B. vulgaris* 'US 75'.
‖Resistant diploid plants (2n = 18) derived from the above trisomics.

each test. Methods employed in these tests did not permit recovery of adult male *H. schachtii.*

Comparison of H. schachtii isolates: Trisomic hybrids were highly resistant to the *H. schachtii* populations tested (Table 2). The total numbers of adult females of individual populations recovered from trisomics amounted to about 1% of the total from susceptible sugarbeet. Twice as many female nematodes were found on susceptible diploid sugarbeet inoculated with the nematode populations isolated from resistant trisomics (Tri-1) than were recovered from plants that were inoculated with an unselected population from Salinas. This suggests that population differences were due to invasion of unequal numbers of nematodes. The number of females of the Netherlands population that matured on trisomics amounted to nearly four times the number for Salinas populations. Although the different susceptibility of trisomics to these populations is evident in the significant entry-population interaction, population differences were not of sufficient magnitude to suggest the occurrence of a "resistance-breaking biotype."

Significantly more nematodes from the Italian than from the Netherlands population matured to females on roots of diploid resistant plants. The number of plants on which female *H. schachtii* matured and the mean numbers of females per plant, respectively, for the tested nematode populations were Italy—10, 20; the Netherlands—9, 1; and Salinas—5, 7. However, prolonged periods of greenhouse temperatures in the 55–65 C range caused by a faulty heating system resulted in fewer nematodes per plant in the population from the Netherlands than were obtained in the previous test (Table 2). Nevertheless, inoculations of plants with populations from Italy or Salinas resulted in greater numbers of infected plants and higher numbers of nematodes per plant than were previously observed for any of the three consecutive screening tests conducted before this study. A subsequent test comparing the development of a nematode population from Italy on resistant diploid hybrids and a susceptible diploid sugarbeet revealed that, as in the previous test, mature females developed on all susceptible and resistant diploid plants. The mean numbers of adult females on resistant diploid hybrids (57) amounted to only 3.2% of the mean numbers on susceptible diploid sugarbeet (1,765). Population differences of this size permitted easy distinction between resistant and susceptible plants and supported the theory that resistance was probably conferred by a single dominant gene (15,16,17).

Development on root slices: Greater numbers of adult males than adult females were observed on root-slice cultures of both resistant hybrids and susceptible diploid sugarbeet (Table 3). The male:female ratios were 10.9:1 for hybrids and 3.9:1 for the susceptible diploid sugarbeet. That various forms of environmental stress can alter sex ratios in nematodes is well known. Triantaphyllou (26) noted that unbalanced sex ratios in the genus *Heterodera* were due to failures of female larvae to reach ma-

Table 2. Mean numbers of females of three populations of *Heterodera schachtii* on roots of sugarbeets (*Beta vulgaris*) and resistant trisomic (*B. vulgaris* × *B. procumbens*) plants 40 d after inoculation.

Plant†	Nematode populations*			Entry mean
	Netherlands	Salinas	Tri-1 isolate‡	
Trisomic	41.3	11.3	21.2	24.6a
Susceptible diploid	2,742.7	1,582.2	2,866.5	2,397.1b
Pop. mean§	1,392.0z	796.8y	1,443.9z	

*Individual plants inoculated with 30 cysts. Figures listed are means of 10 replications.

†Resistant trisomics (2n+1 = 19) and susceptible *Beta vulgaris* cultivar 'US 75' (2n = 18).

‡Population from Salinas, California, which reproduced on resistant trisomics and was increased for inoculum on 'US 75' sugarbeet.

§Values in the same column with unlike lower-case letters were significantly different according to Duncan's multiple-range test.

Table 3. Numbers of adult *Heterodera schachtii* on storage root slices of nematode-resistant sugarbeet hybrids and susceptible diploids.*

Test plant	Adult males	Adult females	Total nematodes
IH-1	89	1	90
IH-2	148	2	150
IH-3	17	5	22
IH-4	124	8	132
IH-5	255	25	280
IH-6	415	57	472
Mean†	175	16	191
Susceptible diploid	555‡	141‡	696

*Mean counts of nematodes/root slice 33 d after inoculation of each of two root slices/plant with contents of 30 broken cysts.

†Mean counts of nematodes on 12 root cultures.

‡Mean counts of nematodes on 10 root cultures (two slices from each of five susceptible sugarbeet storage roots).

turity under adverse conditions. Steele (21) reported that slight overcrowding of *H. schachtii* on sugarbeet and tomato resulted in shifts in the sex ratio favoring development of males. Steele (22) also found high male:female ratios (about 10–12 males/female) of *H. schachtii* cultures on nontreated and aldicarb treated root sections. He suggested these ratios might be typical for root-slice cultures. However, Golden (6) found greater numbers of males than females developed on resistant *Beta patellaris, B. procumbens,* and *B. webbiana.*

The numbers of females found on root-slice cultures were greater than those found on roots of individual whole plants in three consecutive screening tests conducted before this study. These inconsistent results were probably due to the root-slice culture which allowed closer control of temperature and moisture than did the whole plant method and made more root surface area available for invasion by the nematode larvae. Nevertheless, where plants can be sacrificed, the root-slice method (20) may be a useful alternative in evaluating resistance, particularly when data on both male and female nematodes is required.

Although not immune, trisomic and diploid hybrids were highly resistant to *H. schachtii.* This resistance was expressed in failure of the majority of larvae to develop and not in decreased invasion of fibrous roots. Trisomic and diploid hybrids were highly resistant to nematode populations obtained from the Salinas Valley of California, the Netherlands, and Italy. However, the hybrids appeared to be more susceptible to populations from Italy and the Netherlands than to local populations used for selecting resistant lines. This apparent susceptibility was not, however, of sufficient magnitude to indicate that the Netherlands and Italian populations are "resistance-breaking biotypes" of *H. schachtii.* The males:female ratio of nematodes developed on root-slice cultures of resistant hybrids was nearly three times the ratio developed on similar cultures of susceptible sugarbeet. This difference in sex ratios was probably due to higher mortality of females.

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