Quantitative Relationships of Three Free Amino Acids in Fibrous Roots of Nematode Infected Sugarbeet

D. L. Doney, J. M. Fife, and E. D. Whitney[1]

Received for publication November 25, 1970

Resistance to the sugarbeet nematode *Heterodera schachtii* Schm. has been found in wild *Beta* species (6),[2] but the transfer of this resistance to the cultivated sugarbeet has been slow and difficult. No qualitative nematode resistance has been found in *Beta vulgaris;* therefore, selection in the cultivated sugarbeet has been for levels of quantitative resistance (2,8). This type of selection has been slow due to the lack of a good selection criterion and the large environmental errors involved in past selection schemes (2,10).

Chemicals or toxins affecting resistance to other diseases have been reported in other crops (7,11,13). Selection for disease resistance based on amino acids has been reported (4,5). Increases in the concentration of aspartic acid, glutamic acid, and glutamine has been observed in the fibrous roots of nematode infected sugarbeets (1).

This study was initiated to test the quantitative association of asparic acid, glutamic acid, and glutamine with nematode effects.

Methods and Materials

Nine heterogeneous populations, a uniform F_1 hybrid, and a homozygous line (doubled haploid) were selected (Table 1). Sixty plants of each entry were transplanted in 185-ml plastic vials filled with sterilized soil. Four weeks after transplanting, 4,000 surface-sterilized nematode larvae were added to 30 plants of each variety. Nematode larvae were hatched and surface-sterilized by the method developed by Whitney and Doney (12). A split-plot design of 30 replications was used with treatments (nematodes vs. healthy) as whole plots and populations as subplots.

Four weeks after inoculation the white females at the soil-vial interface were counted. Each root was washed and both tap and fibrous portions were weighed separately. A sample of fibrous root from each plant was frozen for subsequent amino acid analysis. Concentration of amino acids in fibrous root juice

[1] Research Geneticist, Chemist, and Plant Pathologist respectively, Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 5098, Salinas, California, 93901.

[2] Numbers in parentheses refer to literature cited.

was determined chromatographically from the color intensity of ninhydrin-stained amino acids (1,4,5). Juice was extracted in a hydraulic press under 5,000 lbs pressure. Data were taken on aspartic acid, glutamic acid, and glutamine.

Table 1.—Source and description of genetic material tested.

Population	Source	Description
56-408	American Crystal Sugar Co.	nematode selection
28-1	USDA	nematode selected from US 41
US 41	USDA	open pollinated variety
590-1	USDA	nematode selection from S 2
S 2	Spreckels Sugar Co.	open pollinated variety
592-3	USDA	nematode selection from US 33
US 33	USDA	open pollinated variety
594-2	USDA	nematode selection from US 22
Acc 107	G. J. Curtis (England)	mixture of nematode selections
62-9134	USDA (R. Hecker)	uniform hybrid
C5600	USDA (B. Hammond)	doubled haploid—homozygous

A small association between the mean and variance of all measured characters was present. Since nine of the entries were segregating populations, transformation of the data was not desirable. Removal of this relationship was achieved by the use of the regression of variances on means from the two non-segregating populations (uniform F_1 hybrid and doubled haploid) in order to estimate the environmental variances for the segregating populations (3). Genetic variances were estimated by subtracting the estimated environmental variance for that population from its total variance. Phenotypic and genotypic correlations were calculated from components of the variance and covariance analyses for all pairs of characters in both healthy and nematode-infected plants.

Results

The concentration of the three amino acids increased significantly under nematode conditions (nema vs. healthy); whereas, tap and fibrous root weights were unaffected (Table 2). There were significant differences among populations for all char-

Table 2.—F tests of analysis of variance for tap root weight, fibrous root weight, and concentration of aspartic acid, glutamic acid, and glutamine in the fibrous roots of 11 sugarbeet varieties.

Source of Variation	Nematode Counts	Tap Roots	Fibrous Roots	Aspartic Acid	Glutamic Acid	Glutamine
Nematodes vs. healthy		1.06	2.89	11.89**	39.66**	16.05**
Populations	<1.00	4.24**	22.91**	5.73**	2.34**	3.36**
Populations × (nema vs. healthy)		1.41	4.00**	<1.00	<1.00	<1.00

** Significant at p = .01

acters except nematode counts. The only character showing interaction between population and nematode effect (nema vs. healthy) was fibrous root weights (Table 2).

Several populations for each character except nematode counts exhibited genotypic variances significantly greater than zero (Tables 3 and 4). Negative variances are considered as random around zero. Populations Acc 107, 28-1, and 594-2 appeared to be the most heterogeneous; i.e., significant genotypic variances were obtained for these three entries for most of the measured characters (Tables 3 and 4). When the variances were pooled

Table 3.—Genotypic variances for nematode counts, tap root, and fibrous root weights in nematode infected and healthy sugarbeets.

Population	Nematode counts	Tap roots (grams)		Fibrous roots (grams)	
	White females $\times 10^3$	Nema	Healthy	Nema	Healthy
56-408	—2.65	—0.180	—0.097	—0.037	0.216
28-1	—0.09	0.545	0.175	0.867**	—0.095
US 41	—1.47	0.296	0.296	0.612*	0.392*
590-1	—2.57	0.477	0.338	0.078	0.159
S 2	—1.92	0.062	0.370	0.103	0.135
592-3	—2.00	0.217	0.431	0.109	—0.167
US 33	0.32	0.366	0.040	0.183	0.166
594-2	—3.59	1.019**	0.046	0.215	0.077
Acc 107	—2.23	1.662**	1.692**	0.097	0.263
Pooled variances					
Environmental	7.59	0.758	0.794	0.360[a]	0.222
Genotypic	—1.13	0.438**	0.507**	0.354**[a]	0.215*

* = Genotypic variance significantly greater than zero at p= .05
** = Genotypic variance significantly greater than zero at p= .01
[a] = Significant increase in total variance in nematode infected plants over healthy plants at p = .05

Table 4.—Genotypic variances for concentration (μg per gram of root juice) of aspartic acid, glutamic acid, and glutamine in nematode infected and healthy sugarbeets.

Population	Aspartic acid		Glutamic acid		Glutamine	
	Nema	Healthy	Nema	Healthy	Nema	Healthy
56-408	369**	32	—59	—33	954	—108
28-1	186	281**	1332**	134	4240**	1621**
US 41	119	—4	—131	268	761	645
590-1	—139	31	716	343	2652**	235
S 2	—1	112	504	650*	—255	—51
592-3	—55	89	—550	—11	—74	237
US 33	307*	—127	79	—186	982	—585
594-2	498**	312**	793	834*	1818*	697
Acc 107	208*	183*	1020**	1119**	2342**	1173*
Pooled variances						
Environmental	267[a]	172	1114[a]	651	2752[a]	1242
Genotypic	247**[a]	065*	386	280*	1485*[a]	382

* = Genotypic variance significantly greater than zero at p = .05
** = Genotypic variance significantly greater than zero at p = .01
[a] = Significant increase in total variance in nematode infected plants over healthy plants at p = .05

over populations, significant genotypic variances existed for all characters except nematode counts and glutamic acid in nematode infected plants (Tables 3 and 4). Pooled environmental and genotypic variances were significantly larger in nematode-infected plants than in healthy plants for all characters except tap root weights and the genotypic variance for glutamic acid (Tables 3 and 4).

The only character associated with number of white females was tap root weights (Table 5). However, this correlation was not large. Tap root weights were positively correlated with fibrous root weights (Table 5). Both tap root weights and fibrous root weights were negatively correlated with the concentration of the three amino acids (Table 5). The concentrations of the three amino acids were also correlated with each other (Table 5). Glutamic acid and glutamine were the most closely associated. In every pair of correlations (except tap root vs. aspartic acid), the correlation for nematode-infected plants was greater than that for healthy plants (Table 5).

Discussion

The lack of any measurable genotypic variance and significant F test among nematode selections and their open-pollinated parents for numbers of white females indicates the difficulty in making progress by using white female counts as a selection criterion.

In those characters affected by nematodes, there appears to be an additional environmental error introduced by the addition of nematodes. This additional error is probably the combination of the error involved in inoculation and the variation of nematode invasion between plants. The significant increase in genotypic variance in nematode-infected plants compared with healthy plants (Tables 3 and 4) implies that they are estimates of different variances, and suggests that these two variances are influenced by different groups or parts of different groups of genes. This would result in the development of the following variance equations:

Variance of healthy plants $= V_e + V_{gh}$

Where: V_e = environmental variance

V_{gh} = genotypic variance under healthy conditions

Variance of nematode-infected plants $= V_e + V_{en} + V_{gn}$

Where: V_e = environmental variance

V_{en} = additional environmental variance as a result of adding nematodes

V_{gn} = genotypic variance under nematode conditions

Table 5.—Phenotypic and genotypic correlations between nematode counts, tap root weights, fibrous root weights, and concentration of aspartic c acid, glutamic acid, and glutamine in sugarbeets.

	Tap roots		Fibrous roots		Aspartic		Glutamic		Glutamine	
	Nema	Healthy	Nema	Healthy	Nema	Healthy	Nema	Healthy	Nema	Healthy
Nema counts	.23**		.12		—.05		—.14		—.10	
Tap root			.48**[a]	.30**	—.34**	.17*	—.37**	—.02	—.31**	—.01
			.34**	.20**	—.27**	.40**	—.50**	—.01	—.52**	—.01
Fibrous root					—.47**	—.06	—.45**	—.24**	—.44**	—.17*
					—.44**	—.12	—.85**	—.32**	—.76**	—.25**
Aspartic acid							.57**	.28**	.40**	.36**
							.83**	.78**	.54**	.31**
Glutamic acid									.77**	.63**
									.86**	.74**

* = Significant correlation at p= .05
** = Significant correlation at p= .01
[a] = The upper correlation in each cell is phenotypic. The lower correlation is genotypic.

The higher correlations in nematode-infected plants compared with healthy plants (Table 5) also suggests different genetic effects.

Selection based on an alternate trait is more effective than the primary trait only if the ratio between the heritability estimates and the genetic correlation between these two traits are of the proper magnitude (9). Using tap root weight as the primary trait, the relative selection efficiency (9) of the three amino acids to tap root weight was each less than 0.50. A relative selection efficiency of greater than 1.00 is necessary for an alternate trait to be of value in a selection scheme (9). However, this test was conducted in the greenhouse with young plants. Therefore, further testing of selections needs to be conducted in the field to determine if the observed effects are related to resistance in the field.

Summary

Significant increases in concentration of aspartic acid, glutamic acid, and glutamine in the fibrous root juice of sugarbeet seedlings were found 4 weeks after inoculation with *Heterodera schachtii* larvae, compared with healthy plants of the same age. There was no measurable nematode effect in tap and fibrous root weights on sugarbeet seedlings 4 weeks atfer inoculation of nematode larvae.

Significant genotypic variances were obtained for the concentration of the three amino acids tested and for tap and fibrous root weights, but not for number of white nematode females. Populations differed in the magnitude of genotypic variance. Significantly larger environmental and genotypic variances were obtained under nematode conditions than under healthy conditions.

Tap root and fibrous root weights were positively correlated. Aspartic acid, glutamic acid, and glutamine were negatively correlated with tap and fibrous root weight and positively correlated with each other. Only tap root weight showed any association with nematode counts.

Literature Cited

(1) DONEY, D. L., J. M. FIFE, and E. D. WHITNEY. 1970. The effect of the sugarbeet nematode *Heterodera schachtii* on the free amino acids in resistant and susceptible *Beta* species. Phytopathology 60: 1727-1729.

(2) DONEY, D. L. and E. D. WHITNEY. 1969. Screening sugarbeet for resistance to *Heterodera schachtii* Schm. J. Am. Soc. Sugar Beet Technol. 15: 546-552.

(3) FEDERER, W. T., L. POWERS, and M. G. PAYNE. 1963. Studies on statistical procedures applied to chemical genetic data from sugar beets. Colo. State Univ. Agr. Exp. Sta. Bull. 77.

(4) FIFE, J. M. 1954. Chromatography as a method of attack on the problem of the chemical nature of resistance of sugar beets to curly top. J. Am. Soc. Sugar Beet Technol. 8: 207-211.
(5) FIFE, J. M. 1967. Changes in concentration of three amino acids, in mature leaves of sugar beet plants, produced by mass selection from a population infected with beet yellows. J. Am. Soc. Sugar Beet Technol. 14: 334-340.
(6) GOLDEN, A. M. 1959. Susceptibility of several *Beta* species to the sugar-beet nematode *(Heterodera schachtii)* and root-knot nematodes *(Meloidogyne spp)*. J. Am. Soc. Sugar Beet Technol. 10: 444-447.
(7) LINK, K. P. and J. C. WALKER. 1933. The isolation of Catechol from pigmented onion scales and its significance in relation to disease resistance in onions. J. Biol. Chem. 100: 379-383.
(8) PRICE, CHARLES. 1965. Breeding sugar beets for resistance to the cyst nematode *Heterodera schachtii*. J. Am. Soc. Sugar Beet Technol. 13: 397-405.
(9) SEARLE, S. R. 1965. The value of indirect selection: I. Mass Selection. Biometrics 21: 682-707.
(10) SHEPHARD, AUDRY M. 1958. Experimental methods in testing for resistance to beet eelworm, *Heterodera schachtii* Schmidt. Nematologica 3: 127-135.
(11) WALKER, J. C. and K. P. LINK. 1935. Toxicity of phenolic compounds to certain onion bulb parasites. Bot. Gaz. 96: 468-484.
(12) WHITNEY, E. D. and D. L. DONEY. 1970. Large scale hatching, disinfestation, and storage of *Heterodera schachtii* larvae. Phytopathology 60: 1191-1194.
(13) WILSKI, A. and J. GIEBEL. 1966. B-Glucosidase in *Heterodera rostochiensis* and its significance in resistance of potato to this nematode. Nematologica 12: 219-224.