Inheritance of Resistance to Erwinia Root Rot in Sugarbeet

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ABSTRACT

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Increased susceptibility to Erwinia soft rot of sugarbeet (*Beta vulgaris* L.) was introduced inadvertently into commercial hybrid sugarbeet cultivars grown in California and Arizona. Two noninbred sugarbeet lines with different gene frequencies for resistance and susceptibility to infection by a variety of *Erwinia carotovora* were used as parents to study the inheritance of resistance. Individual roots from the parental lines and their F_1, F_2, B_1P_1, and B_1P_2 generations were grown and inoculated in 2 yr of field testing at two locations. The difficulty of establishing maximum rates of infection in susceptible genotypes caused some problems in the interpretation of the data. On the basis of frequency distributions for resistant and susceptible roots in the segregating generations and in the progeny from resistant and susceptible selections, we concluded that resistance is simply inherited and controlled by dominant gene action. A single dominant allele may be responsible for a high level of resistance in the root. The presence of a second, quantitative genetic mechanism that partially controls the rate of rot development within susceptible roots also was suggested.

Additional key words: disease resistance, genetic vulnerability, *Erwinia carotovora*, bacterial vascular necrosis and rot.

A root rot of sugarbeet (*Beta vulgaris* L.) was observed initially in the San Joaquin Valley of California in 1968 on the first semicommercial plantings of a new hybrid cultivar, US H9. Increased incidence of the disease subsequently was observed in cultivar trials of hybrids in which the pollinators of US H9 (3) and US H10 (4) were involved (J. D. Schulke, *personal communication*). As US H9 and US H10 were adopted statewide, the rot-causing organism was found to be indigenous to most areas of California. The disease incidence was so intense in certain areas of the western San Joaquin Valley that production was limited. Besides causing direct losses to the growers, infected roots prevent efficient extraction of sugar by the refiners (9). The causal agent of this root rot was identified as a variety of *Erwinia carotovora* (6, 7, 8).

The purposes of this study were to develop procedures to determine the genetic nature of this increased susceptibility and, if possible, to determine the mode of inheritance of resistance.

MATERIALS AND METHODS

Preliminary screening tests failed to identify breeding lines of sugarbeet that were uniformly resistant or susceptible to *Erwinia*. The noninbred parental lines, C17 (5) and C64, then were chosen for testing because of commercial use and wide differences in reaction to *Erwinia* (9). Line C17, the pollinator of US H10, was used as the susceptible parent, and C64, the pollinator of US H7, was used as the resistant parent. Vernalized stecklings of these self-incompatible lines were chosen at random and subsequently pair-crossed under paper bags in the greenhouse. The following season, randomly chosen self-incompatible F_1 stecklings were similarly backcrossed with stecklings from C17 and C64 to produce the B_1P_1 and B_1P_2 generations, respectively. The F_1 was increased by sib mating in isolation to produce the F_2. Sufficient F_1 seed for field testing was obtained by again randomly pair-crossing stecklings of C17 and C64. Within each generation, approximately equal amounts of seed from each cross were bulked. No distinction was made between seed from reciprocal crosses. The number of individual crosses to produce the initial F_1, the second F_1, B_1P_1, and B_1P_2 generations was 20, 32, 26, and 22, respectively. The F_2 was produced from 300 open-pollinated F_1 plants. In all cases, self-incompatibility was relied upon to prevent unwanted selfing.

Reaction of the individual plants in the parental lines and in the F_1, F_2, B_1P_1, and B_1P_2 generations to *Erwinia* was determined in field tests. In 1975, tests were at two locations in the Salinas Valley and, in 1976, at a single location. The tests were in randomized complete block designs with four to 10 replications. The F_2 was repeated three times within each replication to give a larger number of plants. Plants in the test plots were thinned and singled to a spacing of 20 to 25 cm. At 2-3 mo of age the leaf blades, petioles, and crowns of each plant were injured mechanically to provide avenues of infection through wounds (8, 9). Immediately following injury, the plants were sprayed with a suspension of *Erwinia* strains (9, 10).

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Five strains of *Erwinia,* UR-7, SB-4, SB-6, SB-7, and SB-13, were grown on medium B of King et al. (1) for 40 hr at 26 C. Suspensions of each strain in tap water were standardized to a concentration of cells equivalent to an absorbancy of 200 with a Klett-Summerson colorimeter and equal proportions of each were mixed. The composite stock solution was diluted 1:7 (to approximately 10^7 cells/ml) with tap water and was applied to the injured plants with a pressurized back-pack sprayer. Approximately 6 ml of inoculum was applied to each plant.

At harvest (about 3 mo after inoculation), the test plants were lifted and each beet was sliced progressively with a knife through the crown and root and scored for amount of rot. The roots were scored on a pretransformed scale with ratings from 0 to 6 (2). These ratings approximated 0, 7, 25, 50, 75, 93, and 100% soft rot of the root. In addition, a 1% rating was used to distinguish roots with vascular necrosis (VN) in some vascular bundles of otherwise apparently healthy roots. We considered VN in a root, with no accompanying soft or dry rot, as evidence of a resistant reaction. A disease index (DI) or average percent rot per root was calculated for each plot [DI = (Σ% rot)/(no. of roots)]. Roots with 0% rot or a VN reaction were considered resistant.

Analyses of variance were made on individual tests to determine if differences in DI and percent resistant roots between generations were significant. The combined test data also were analyzed to determine if generation × year and generation × location interactions were significant. Homogeneity χ^2 values for percent resistant roots over test environments were calculated for each generation.

Because data obtained in these tests suggested that reaction to *Erwinia* may in part be inherited as a single, dominant gene, the data were analyzed by use of χ^2 analyses to test for goodness of fit between the observed and expected frequencies of resistant and susceptible plants in the F_1, F_2, B_1P_1, and B_1P_2 generations. The frequencies of the resistant and susceptible alleles in the parental lines were estimated from their phenotypic frequencies on the basis of the Hardy-Weinberg principle of gene equilibrium in a population. These allelic frequencies then were used to calculate the expected number of resistant and susceptible plants in each of the segregating generations.

When the frequency distributions and the means of the parents and their segregating generations were examined only for the disease classes considered susceptible (i.e., 7 to 100% rot) there was prima facie evidence that other factors condition the differential amount of soft rot exhibited by the two parents. Comparisons were made within this susceptible range of the disease classes to determine if additional factors could be identified that modified the degree of susceptibility or rate of rotting.

In another test in 1976, selections made from line C13 (5) for resistance or susceptibility to *Erwinia* (10) were evaluated for disease reaction. This evaluation test was planted, injury-inoculated, and scored for rot on May 6, July 29, and October 10, 1976, respectively. Approximately 60 roots of each sugarbeet line were scored.

RESULTS

The means for the DI and percent resistant roots between generations usually were significantly different (Table 1). As expected, the reactions of the parental lines to *Erwinia* differed markedly. The rank of the generation means from most resistant to most susceptible was $P_2 > B_1P_2 > F_1 > F_2 > B_1P_1 > P_1$. Comparisons showed that the F_1 and F_2 were significantly different except for percent resistant roots at the Spence location in 1975. The variation in the generation means from test to test and the relatively high CV values within tests for percent resistant roots showed that considerable variability was associated with scoring sugarbeet roots for infection by *Erwinia.*

TABLE 1. Disease indices for *Erwinia carotovora* in sugarbeet and percent resistant roots at two locations and for 2 yr and combined over test environments

Generation[t]	DI[u]				Resistant roots[v]			
	1975		1976	Combined	1975		1976	Combined
	Salinas[w]	Spence[x]	Salinas[y]		Salinas[w] (%)	Spence[x] (%)	Salinas[y] (%)	(%)
P_1	73.0 f[z]	72.3 e	70.3 d	72.0 f	14.6 d	7.1 e	11.8 d	9.4 f
P_2	15.7 a	21.1 a	24.4 a	20.8 a	66.0 a	50.6 a	48.7 a	53.1 a
F_1	27.9 c	30.7 b	33.6 b	30.7 c	46.9 b	32.9 c	35.3 b	36.1 c
F_2	41.6 d	39.0 c	43.3 c	40.4 d	34.0 c	27.9 c	23.9 c	28.2 d
B_1P_1	47.7 e	47.2 d	49.4 c	47.7 e	31.0 c	17.0 d	16.8 cd	19.8 e
B_1P_2	20.1 b	22.6 a	24.7 a	22.5 ab	55.7 b	43.7 b	45.0 ab	46.3 b
C.V. (%)	11.4	10.1	11.4	10.6	23.1	26.4	21.6	23.9

[t]Breeding line identities; P_1 = C17, P_2 = C64, $B_1P_1 = F_1 \times P_1$, $B_1P_2 = F_1 \times P_2$. The abbreviation, C.V., stands for coefficient of variability.

[u]Disease index: DI = (Σ% rot)/(no. of roots). Roots scored on a scale of 0, 1 [vascular necrosis only (VN)], 7, 25, 50, 75, 93, and 100% rotted.

[v]Percent resistant roots = (No. with 0% rot with VN × 100)/total.

[w]Eight replications, single-row plots, 6.1 m long, 71 cm wide.

[x]Ten replications, single-row plots, 16.2 m long, 71 cm wide.

[y]Four replications, single-row plots, 13.1 m long, 71 cm wide.

[z]Means followed by different letters within columns differ significantly ($P = 0.05$) according to Duncan's multiple range test.

Because of the variability usually encountered with root rot data, these CV values are not inordinately high.

Homogeneity χ^2 values were significant for each generation. The deviation from homogeneity was caused by an excess of resistant plants in the 1975 Salinas test (Table 1). Year and location effects also were significant for the DI and percent resistant plants. However, because of the lack of significant generation × test interactions and the presence of homogeneous variances, the data for the three tests were combined for further analyses (Table 2).

The data for percent resistant plants (Table 2) were tested for fit to a single gene model in which resistance to soft rot (0%, VN) is conditioned by a dominant allele. By use of the combined data from Table 2, the frequencies of the dominant allele in the P_1 and P_2 lines were calculated to be 4.83% and 31.49%, respectively. By use of these estimated gene frequencies, the observed frequency of resistant and susceptible plants in the F_1, F_2, B_1P_1, and B_1P_2 were tested against their expected frequency. A good fit was obtained for the F_1 and B_1P_2 generations. The B_1P_1 showed a relatively poor fit, and the F_2 showed no evidence of fit.

Results of breeding and selection for resistance or susceptibility starting with line C13 showed that reaction to *Erwinia* is highly heritable (Table 3). Whereas one cycle of selection for susceptibility from C13 essentially eliminated resistant roots, one cycle of selection for resistance dramatically increased the number of resistant roots. A second selection cycle for resistance again substantially improved the level of resistance. Although the first cycle of selection for resistance did not produce the frequency of resistant plants expected, these results are in general agreement with the expectations of selecting a trait governed by a single dominant factor from a heterogeneous line.

When only the susceptible classes (7 to 100%) of the parents and their segregating generations were considered, a second genetic mechanism influencing the rate and extent of root rot was suggested. Regression analyses between percent resistant roots and the percent rot per root (in the susceptible classes) gave regression coefficients that were not significantly different from zero, which suggested that the amount of rot in susceptible plants was independent of the major gene for resistance. Within the 7 to 100% rot range, C17 was again

TABLE 2. Frequency distributions of root rot ratings and number of observed and expected sugarbeet roots resistant to *Erwinia carotovora* from experiments combined over test environments

Generation[a]	Roots with a rot rating of: 0 or VN (%)	7 (%)	25 (%)	50 (%)	75 (%)	93 (%)	100 (%)	Roots observed (no.)	Resistant roots[b] Observed (no.)	Expected (no.)	χ^2 Value	P[c]
P_1	9.4	3.7	7.3	9.1	16.0	15.2	39.3	1,115	105	...	...	...
P_2	53.1	10.4	11.3	11.2	9.5	2.7	1.7	1,095	581	...	...	...
F_1	36.1	11.3	16.0	15.8	10.6	5.1	5.2	1,153	416	401.2	0.84	0.25-0.50
F_2	28.2	8.2	15.4	16.4	14.1	8.0	9.6	3,269	923	1,137.6	62.09	< 0.001
B_1P_1	19.8	6.5	17.9	16.5	16.6	9.7	13.0	1,063	210	246.1	6.89	0.005-0.01
B_1P_2	46.3	12.4	14.3	13.6	8.1	3.8	1.6	1,087	503	485.7	1.11	0.25-0.50

[a]P_1 = C17, P_2 = C64, $B_1P_1 = F_1 \times P_1$, $B_1P_2 = F_1 \times P_2$.

[b]Resistant roots are those with 0% rot and vascular necrosis only (VN) ratings.

[c]Probability of obtaining a χ^2 value as large or larger when the expected gene frequencies were calculated from the observed P_1 and P_2 frequencies (4.83 and 31.49%, respectively) for the dominant allele that conditions resistance.

TABLE 3. Disease indices (DI) and percent resistant sugarbeet roots in lines derived from root-rot resistant and susceptible selections from line C13 inoculated with *Erwinia carotovora*

Line	Description[a]	DI[b]	Resistant roots (%)
C13	Pollinator of US H9	56.4	15.8
E540	One mass sel. for ES	72.4	1.7
E538	One mass sel. for ER	29.9	58.9
E534	Two mass sel. for ER	7.6	80.4
E502	Two mass sel. + progeny test for ER[c]	5.8	84.9
E506	Two mass sel. + progeny test for ER[c]	3.7	90.0
E536	Two mass sel. + progeny test for ER[c]	0.2	100.0

[a]Erwinia-resistant selections were made from field plantings. Most of these lines were derived through different lines of descent. Erwinia-susceptible selection was made from greenhouse plants. The abbreviations, ES and ER = *E. carotovora* susceptibility and resistance, respectively, and "sel." = selection.

[b]Disease Index: DI = (Σ% rot)/(no. of roots). Root rot was scored on a scale of 0, 1 [vascular necrosis only (VN)], 7, 25, 50, 75, 93, and 100% rotted.

[c]After the second mass selection, half-sib seed from each plant was evaluated in the field for resistance to *Erwinia*, root yield, and percent sucrose. On the basis of the best combination of these traits, remnant half-sib seed from the selected lines was bulked and increased.

more susceptible than C64. The average rot per root in these parents was 81.1 and 44.2%, respectively, a highly significant difference. The segregating generations had mean rot values close to their midparent values or regressed toward the more resistant parent. Although the design of these tests did not permit an accurate analysis of this genetic system, the amount of rot appeared to be due primarily to additive gene action, with some dominance or heterotic effects.

DISCUSSION

The data obtained from the segregating populations and the selection experiments show that resistance to *Erwinia* in sugarbeet is simply inherited and has a large dominance component. With the exception of the F_2, adequate fits for the F_1 and backcrosses were obtained to substantiate the single-gene hypothesis. However, because of the lack of fit for the F_2 and the observed variation for percent resistant roots, we cannot exclude completely the possibility that more than a single gene is responsible for the resistance to rotting in the sugarbeet root. We believe that the poor fit of the F_2 may have been caused in part by unequal sampling of the parental gene frequencies.

The lack of homogeneity in the data in Table 1 show that the proportion of resistant and susceptible roots was somewhat different from test to test. Thomson et al. (8) have shown that injury is necessary for infection and that other environmental factors influence disease expression. We believe that most of the variability and deviation from homogeneity in our field tests was caused by our inability to establish maximum rates of infection in susceptible genotypes. We visualize that despite uniform injury and inoculation procedures, varied numbers of escapes occurred that could not be differentiated from resistant plants. These escapes would increase the error for both the inheritance study and the selection for resistance.

A second and probably quantitative genetic system appears to govern the rate of development of the soft rot within the root. This system appears to reinforce the proposed major gene when present, but may condition fairly high levels of resistance even in the absence of the major gene. Thus, a plant that is classified as resistant genotypically may be susceptible for the major gene. The phenotypes conditioned by the two systems probably overlap causing the expression of the major gene resistance to be less discrete.

Our experience in field and greenhouse testing has suggested that, although the shoot (leaves and crown) tissues of all injury-inoculated beets show visible symptoms of vascular infection, the root tissue is generally susceptible to infection only in the genotypes without the major gene for resistance. For the 0% rot reaction class, no visible evidence of infection in the root was observed. For the VN reaction class, a few vascular bundles extending from the crown into the root showed some necrosis. This necrosis usually extended only for 1 or 2 cm into the root. However, the root tissue appeared to resist further damage or tissue breakdown (rotting) caused by the bacterium. It is probable that most of the roots scored as 0% rot also would have shown vascular necrosis in the root and/or transition zone between the root and the shoot if they had been examined in closer detail.

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