Improvement of Levels of Bacterial Wilt Resistance in Eggplant Through Breeding

R. W. GOTH, Research Plant Pathologist, K. G. HAYNES, Research Plant Geneticist, and T. H. BARKSDALE, Research Plant Pathologist, Vegetable Laboratory, Plant Sciences Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD 20705

ABSTRACT

Goth, R. W., Haynes, K. G., and Barksdale, T. H. 1991. Improvement of levels of bacterial wilt resistance in eggplant through breeding. Plant Dis. 75:398-401.

Significant differences in Kaplan-Meier survival curves were calculated by the log-rank test for root-dip inoculated parental lines and their corresponding S_3 families in five of seven eggplant (*Solanum melongena*) accessions. The accessions from the U.S. Department of Agriculture's Plant Introduction (PI) Collection had been selected based on resistance to bacterial wilt. Resistance in one of these seven S_3 families was significantly greater than that of the parents. The survival curves were plotted for 21 days after inoculation of 3-wk-old seedlings with a broth culture of *Pseudomonas solanacearum*, based on time from inoculation to initial wilt symptoms for plants grown in a greenhouse at temperatures of 33-37 C in daytime and 24-27 C at night. Significant differences were found in the survival curves of parental PI lines and their corresponding F_3 families of all four crosses in the hybridization program. Resistance in three of the four families was significantly improved over levels expressed by the parents. Levels of resistance that equalled the most resistant parent were found in the fourth family. Both crossing and selfing of resistant eggplants offer promise in developing lines with improved bacterial wilt resistance.

Bacterial wilt of eggplant (*Solanum melongena* L.), caused by *Pseudomonas solanacearum* (Smith) Smith, is an important disease worldwide in warm, moist climates where soils do not freeze in winter (7,15). This disease can be a limiting factor for crop production. Field losses of 50% have been observed by midsummer in North Carolina (18), and losses between 75 and 81% have been recorded in India (15).

In 1985, all 524 eggplant accessions in the U.S. Department of Agriculture's Plant Introduction (PI) Collection were evaluated for resistance to *P. solanacearum*; although none of the accessions was found immune, some had considerable tolerance (4). After the 1985 evaluation, we took two approaches toward improving and stabilizing a useful level of bacterial wilt resistance in eggplant. First, selections were made in the lines that showed the most resistance, and these, in turn, underwent two cycles of selfing and selection in greenhouse inoculation tests. Second, crosses were made between several of the most resistant lines resulting in an advance in the level of resistance in the F_1 and F_2 progenies of some of these crosses (8,11).

Various methods have been used to evaluate the performance of a plant breeding population in disease resistance studies (17). Quantitative measurements, such as stand percentage, days to 50% wilt, yield reduction relative to a standard, percentage of diseased tissue, or calculated disease indices, have all been analyzed by standard statistical techniques, and significant differences have been detected by various mean separation techniques. The assumptions underlying the analysis of variance are rarely examined by nonstatisticians. Most researchers assume that the variances among the different classes are homogeneous. However, certain types of data should be examined for abnormality, e.g., data composed of small whole numbers, data recorded as percentages, and data in which the classes produce multiplicative effects (5,16).

Most models of disease development used in the evaluation of host resistance are based on the assumption that the variance of disease initiation, disease severity, or time to death is normally distributed. In an initial evaluation, it is unlikely that the researchers will have adequate estimations of the population parameters or populations to allow them to determine distributions. Moreover, insufficient knowledge of the disease progress may not allow models to be fit to progress curves.

A statistical method widely used in medical research but unknown in plant host resistance studies is Kaplan-Meier survival analysis (1,2,7,12,13). This nonparametric method can be used when the distribution of the population is unknown or obviously abnormal and population parameters are of little interest. The nature of the distribution of a certain response, such as wilt, over a range of time at which the event of interest occurs rather than the value of a particular parameter, such as the mean or variance, is investigated. The observations are event times. At the termination of the experiment, all individuals that have not wilted have a censored observation. Censored observations also occur if plants wilt or die for some reason

Accepted for publication 17 October 1990 (submitted for electronic processing).

(disease or other), other than the one under investigation. The values assigned to censored observations are the length of time the plant survives.

If T denotes the disease-free time of a plant selected at random from a test population and t denotes the event time, then the Kaplan-Meier survival curve provides an estimate of the probability of surviving past t: Pr $(T > t)$. The number of plants that are at risk at each each t_l time ($l = 1, 2, 3,...$) equal $r_1, r_2,...r_l$. Similarly, the number of plants that are diseased at each t_l time equal $d_1, d_2,..., d_l$. Then, the probability of having no disease symptoms from the beginning of the experiment ($t_l = 0$) to time t_1 can be estimated as: $S(t_1) = 1 - (d_1/r_1)$, because d_1/r_1 is the estimated proportion of diseased plants occurring in that time interval. The probability of no disease from t_1 to t_2 is: $1 - (d_2/r_2)$. Because non-overlapping time intervals are independent events, the probability of having no disease symptoms from the beginning of the experiment ($t_l = 0$) to time $t_2 > t_1$ is: $\hat{S}(t_2) = [1 - (d_1/r_1)][1 - (d_2/r_2)]$. The estimated survival (no disease) function for any arbitrary time t assuming non-overlapping time intervals is: $\hat{S}(t) = \pi_{l|t_l<t} [1 - (d_l/r_l)]$. Greenwood's formula (13) can be used to estimate the variance of the survival estimate at any arbitrary time t as: var $[\hat{S}(t)] = [\hat{S}(t)]^2 \Sigma_{l|t_l<t}\, d_l/[r_l(r_l - d_l)]$. An approximate 95% confidence interval at time t would be: $\hat{S}(t) \pm 1.96[\hat{S}(t)]^{1/2}$.

The purpose of the study described here was to determine if improvements in resistance to bacterial wilt in genetically diverse eggplant germ plasm can be made by selfing or through hybridization.

MATERIALS AND METHODS

Strain G-4 of *P. solanacearum* used for inoculation was originally isolated from potato tissue collected from a field test at Tifton, GA. Seven strains (two from Georgia, three from Florida, and two from North Carolina) had been assayed for aggressiveness in eggplant and G-4 was the most aggressive. Bacteria were suspended in 5 ml of sterile distilled water in screw-capped vials and stored at room temperature to reduce development of avirulent mutants. For preparation of inoculum, samples of the suspension were transferred to nutrient broth, (3,8–10). After 3 days, the broth cultures were diluted to about 10 colony-forming units (cfu) per milliliter. The number of colony-forming units was determined with a Klett-Summerson Photo-Electric Colorimeter (Klett Manufacturing, Inc., New York, NY) coupled with a 10-fold dilution series.

Inoculation. Plants were sown in 2.5-cm polystyrene Speedling flats (Speedling, Inc., Sun City, FL) containing Jiffy Mix Plus (Jiffy Products of America, West Chicago, IL) and were grown in a greenhouse. Daytime temperatures fluctuated between 33 and 37 C and night

Table 1. Comparison of the amount of bacterial wilt in eggplant lines from the U. S. Plant Introduction (PI) Collection and their S_3 progeny as measured by the number of days to 50% of the plants wilted and the percentage not wilted (symptomless) 21 days after root-dip inoculation of young seedlings where significant differences were indicated by the log-rank test

PI line	Generation	Days to 50% wilt	Not wilted (%)
381236	S_0	11.0	36.7 a[y]
	S_{3-1}	7.0	40.0 a
	S_{3-2}	7.0	33.3 a
	S_{3-3}	5.0	13.3 a
381237	S_0	NR[z]	53.3 ab
	S_{3-1}	NR	83.3 a
	S_{3-2}	NR	76.7 a
	S_{3-3}	NR	76.7 a
	S_{3-4}	NR	70.0 a
	S_{3-5}	NR	70.0 a
	S_{3-6}	NR	60.0 a
	S_{3-7}	6.5	26.7 b
386254	S_0	5.0	10.0 c
	S_{3-1}	NR	60.0 a
	S_{3-2}	6.0	43.3 ab
	S_{3-3}	6.0	20.0 bc
	S_{3-4}	5.0	6.7 c
386263	S_0	NR	83.3 ab
	S_{3-1}	NR	96.7 a
	S_{3-2}	NR	96.7 a
	S_{3-3}	NR	96.7 a
	S_{3-4}	NR	90.0 ab
	S_{3-5}	NR	83.3 ab
	S_{3-6}	NR	83.3 ab
	S_{3-7}	NR	66.7 b
419020	S_0	5.0	26.7 ab
	S_{3-1}	NR	56.7 a
	S_{3-2}	6.0	13.3 b

[y] Within each family, means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.
[z] NR = not reached.

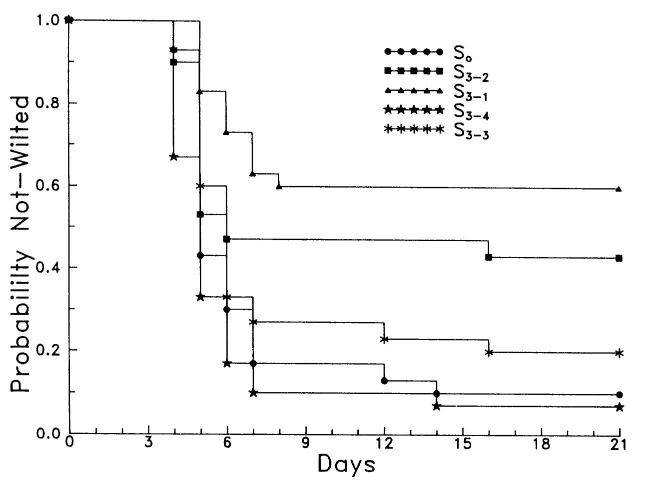

Fig. 1. Survival distribution of bacterial wilt susceptible eggplant PI 386254 and its S_3 families after inoculation with *Pseudomonas solanacearum.*

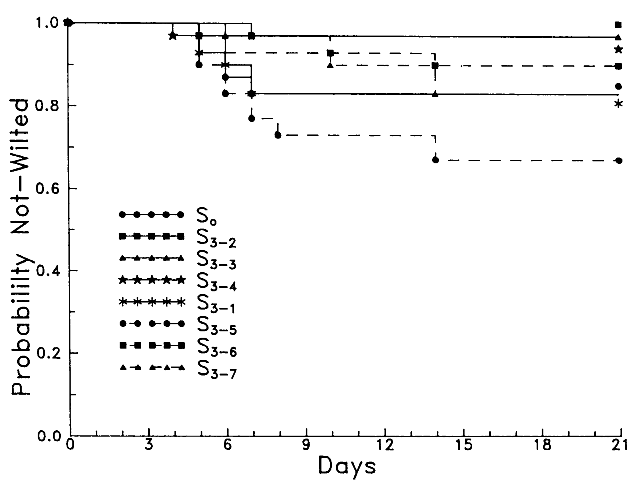

Fig. 2. Survival distribution of bacterial wilt resistant eggplant PI 386263 and its S_3 families after inoculation with *Pseudomonas solanacearum.*

Table 2. Comparison of the amount of bacterial wilt in parental eggplant lines from the U.S. Plant Introduction (PI) Collection and their F_3 progeny as measured by the number of days to 50% of the plants wilted and the percentage not wilted (symptomless) 21 days after root dip inoculation of young seedlings where significant differences were indicated by the log-rank test

Hybridization	Generation	Days to 50% wilt	Not wilted (%)
PI 176761 × PI 169663	P_0	5.0	10.0 g[y]
	P_1	6.5	16.7 efg
	$F_{3\text{-}1}$	NR[z]	70.0 a
	$F_{3\text{-}2}$	NR	56.7 ab
	$F_{3\text{-}3}$	NR	53.3 abc
	$F_{3\text{-}4}$	6.0	46.7 abc
	$F_{3\text{-}5}$	7.0	43.3 bcd
	$F_{3\text{-}6}$	7.5	40.0 b–e
	$F_{3\text{-}7}$	8.0	36.7 b–f
	$F_{3\text{-}8}$	7.0	26.7 c–g
	$F_{3\text{-}9}$	7.0	26.7 c–g
	$F_{3\text{-}10}$	7.0	26.7 c–g
	$F_{3\text{-}11}$	5.0	23.3 c–g
	$F_{3\text{-}12}$	5.0	20.0 d–g
	$F_{3\text{-}13}$	4.5	20.0 d–g
	$F_{3\text{-}14}$	6.5	20.0 d–g
	$F_{3\text{-}15}$	5.0	16.7 efg
	$F_{3\text{-}16}$	6.0	13.3 fg
PI 220120 × PI 173106	P_0	4.0	0.0 e
	P_1	5.0	0.0 e
	$F_{3\text{-}1}$	NR	50.0 a
	$F_{3\text{-}2}$	7.0	30.0 b
	$F_{3\text{-}3}$	7.0	26.7 bc
	$F_{3\text{-}4}$	7.5	26.7 bc
	$F_{3\text{-}5}$	7.0	26.7 bc
	$F_{3\text{-}6}$	6.0	26.7 bc
	$F_{3\text{-}7}$	6.0	20.0 bcd
	$F_{3\text{-}8}$	5.0	20.0 bcd
	$F_{3\text{-}9}$	7.5	20.0 bcd
	$F_{3\text{-}10}$	6.0	10.0 cde
	$F_{3\text{-}11}$	6.0	10.0 cde
	$F_{3\text{-}12}$	5.0	6.7 de
	$F_{3\text{-}13}$	5.0	6.7 de
	$F_{3\text{-}14}$	5.0	6.7 de
	$F_{3\text{-}15}$	5.0	3.3 de
	$F_{3\text{-}16}$	5.0	3.3 de
	$F_{3\text{-}17}$	5.0	0.0 e
	$F_{3\text{-}18}$	5.0	0.0 e
PI 230334 × PI 320502	P_0	7.0	36.7 b–h
	P_1	6.0	6.7 gh
	$F_{3\text{-}1}$	NR	83.3 a
	$F_{3\text{-}2}$	NR	70.0 ab
	$F_{3\text{-}3}$	NR	66.7 ab
	$F_{3\text{-}4}$	NR	63.3 abc
	$F_{3\text{-}5}$	NR	63.3 abc
	$F_{3\text{-}6}$	NR	60.0 abc
	$F_{3\text{-}7}$	NR	53.3 a–d
	$F_{3\text{-}8}$	NR	50.0 b–e
	$F_{3\text{-}9}$	10.0	43.3 b–f
	$F_{3\text{-}10}$	7.0	40.0 b–g
	$F_{3\text{-}11}$	77.0	40.0 b–g
	$F_{3\text{-}12}$	6.5	36.7 b–h
	$F_{3\text{-}13}$	7.0	30.0 c–h
	$F_{3\text{-}14}$	7.0	26.7 d–h
	$F_{3\text{-}15}$	5.0	20.0 e–h
	$F_{3\text{-}16}$	5.5	13.3 fgh
	$F_{3\text{-}17}$	5.5	13.3 fgh
	$F_{3\text{-}18}$	7.0	13.3 fgh
	$F_{3\text{-}19}$	6.0	6.7 gh
	$F_{3\text{-}20}$	5.0	3.3 gh
PI 349612 × PI 173106	P_0	NR	66.7 a
	P_1	5.0	6.7 c
	$F_{3\text{-}1}$	NR	73.3 a
	$F_{3\text{-}2}$	NR	56.7 ab
	$F_{3\text{-}3}$	NR	50.0 ab
	$F_{3\text{-}4}$	12.0	46.7 ab
	$F_{3\text{-}5}$	11.0	43.3 ab
	$F_{3\text{-}6}$	7.0	43.3 ab
	$F_{3\text{-}7}$	8.0	43.3 ab
	$F_{3\text{-}8}$	7.0	26.7 bc
	$F_{3\text{-}9}$	6.0	26.7 bc
	$F_{3\text{-}10}$	6.0	23.3 bc

[y] Within each family, means followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.
[z] NR = not reached.

temperatures between 24 and 27 C. Seedlings were carefully lifted from these flats when they were about 21 days old and had one true leaf. Roots were washed in tap water and then dipped into the cell suspension. The cvs. Black Beauty and Classic served as checks, and their roots were dipped in the inoculum suspension or into nutrient broth as a negative check. The inoculated seedlings were immediately transplanted into a pasteurized soil mix in 10-cm-diameter pots and kept in a greenhouse. There were three replications of 10 plants for each entry from the two different breeding programs.

Breeding programs. In the selfing program, remnant seed from seven PI lines previously found to possess resistance to *P. solanacearum* comprised the S_0 population. Two or more S_1 and S_2 lines with resistance to *P. solanacearum* from each of the original PIs were selfed to give rise to S_3 families. Thirty seed representing each PI line, S_3 family, and check were sown on 2 June 1988.

Four crosses were made between plant introductions with varying levels of resistance in the hybridization program. Resistant F_2 plants were selfed to give rise to F_3 families. Remnant seed from the original PIs comprised the parental populations. Thirty seeds of each PI, F_3 family, and check were sown on 5 July 1988.

After inoculation, data were taken daily for 21 days and records kept of the day when plants first wilted. Plants that had not wilted after 21 days had a censored observation. Kaplan-Meier survival curves (1,2,7,12–14) were plotted for these data, where the event time was days to wilt rather than days to death. The log-rank test (12–14) was used to compare the survival curves of the parental population(s) and their corresponding S_3 and F_3 families.

RESULTS

In the selfing program, five of the seven families derived from PI lines showed significant differences in their survival distributions as measured by the log-rank test, $\chi^2 = 12.2$–40.0 ($P < 0.01$). In PI 381236, plants of remnant seed from the original parental population were at least as resistant as two of the S_3 families as measured by the percentage of plants that were not wilted after 21 days (Table 1). Significant differences among the S_3 and parental lines were probably attributable to excessive disease development in one particular S_3 family. No significant progress was made in selecting for bacterial wilt resistance by selfing PI 381236.

For PI 386254 and 419020, less than half of the progeny in one S_3 family wilted after 21 days, whereas, in 6 days or less, at least 50% of the progeny wilted in the parental populations and the remaining S_3 families (Table 1). Twentyone days after inoculation, the percentage of plants not wilted in one S_3 family from PI 386254 was significantly higher than that of its parental population (Fig. 1).

In PI 381237, 27% of the progeny in one S_3 family had not wilted 21 days after inoculation (Table 1). In the remaining six S_3 families, the percentage of plants that remained symptomless was greater than that of the original parental population. This indicates progress in stabilizing bacterial wilt resistance by selfing in this family.

In PI 386263, extremely high levels of bacterial wilt resistance were found in all S_3 families and in the original parental population (Table 1). In three of the seven S_3 families, 97% of the plants had not wilted after 21 days (Fig. 2). Bacterial wilt resistance obtained by selfing was excellent.

In the hybridization program, all four of the PI families showed significant differences in the survival distribution, $\chi^2 = 67.6\text{–}180.9$ ($P < 0.01$). The cross between a highly resistant line, PI 349612, and a line with little resistance, PI 173106, resulted in seven of the 10 F_3 families with resistance that was not significantly different from the resistant parent line as measured by the percentage of plants not wilted (Table 2). The resistance of these seven F_3 families was greater than the resistance exhibited by the calculated midparental value (37% not wilted), indicating that progress in selecting for resistance is being obtained.

The cross between two PI lines with little or no resistance, PI 220120 × PI 173106, produced nine F_3 families that were significantly more resistant to bacterial wilt than either parent (Table 2). However, the overall levels of resistance, with the exception of one F_3 family, were not outstanding. In nine of the 18 F_3 families, 10% or less of the plants were wilt-free after 21 days.

In the two remaining hybridizations involving parents with low to moderate levels of bacterial wilt resistance, significant progress was made in improving bacterial wilt resistance in some of the F_3 families (Table 2).

DISCUSSION

The eggplant PI collection is maintained by periodic increase of seed in the field. Although eggplant is self-pollinated, a degree of outcrossing can occur, and the rate of outcrossing may vary among PI lines. This may result in PI lines expressing varying degrees of heterozygosity despite similar treatment.

The level of homozygosity in the PIs used in this study is not known nor is the rate of outcrossing. However, PI 386263 apparently has considerable homozygosity for bacterial wilt resistance and a very low or nonexistent level of outcrossing because there was so little variation in the reaction of the S_3 families to infection by *P. solanacearum*. In contrast, it appears that PI 386254 either has considerable heterozygosity for bacterial wilt resistance or a high level of outcrossing because the reaction of its S_3 families to infection by *P. solanacearum* was so varied.

Both selfing and hybridization methods can be used to improve bacterial wilt resistance in eggplant. In cases where several recessive genes are involved, selfing and selection can be one way to enhance a character of interest, and this may have occurred during a breeding program for resistance to bacterial wilt in tomatoes (6). Another way is through hybridization, and our data is evidence for the improvement of resistance in some F_3 lines derived from four crosses. The cross of PI 220120 × PI 173106 may be an example of transgressive segregation, that is, the appearance in the F_2 or later generations of a cross of individuals showing more resistance than either parent.

ACKNOWLEDGMENTS

We thank Karen Bergling and David Maas for technical assistance.

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