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DIFFERENTIAL GRAYWALL DEVELOPMENT IN TOMATO STOCKS INFILTRATED WITH BACTERIA

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Abstract

A comparison of several varieties and breeding lines which differed in field resistance to graywall was made using an infiltration technique. The varieties and breeding lines having field resistance to graywall tended to have less graywall than the field susceptible types after inoculation. Considerable variability occurred in the tests which might be the result of variation in individual fruit susceptibility. Plant to plant variation in susceptibility within certain lines was found to be very high. This plant to plant variation is characteristic of graywall occurring naturally in the field.

Intrduction

A bacterium isolated from graywall-affected tomato fruit caused graywall symptom development to the same degree in both susceptible and resistant cultivars when injected into the pericarp of green tomato fruits (1, 3, 4). Graywall symptoms were also produced after placing a suspension of the bacterium about the stem scar with stem attached and subjecting the fruit to a vacuum. Using this vacuum infiltration technique, graywall resistant cultivars developed less graywall than susceptible cultivars. Thus it appeared that the graywall resistance might be due to some structural characteristic of the stem scar area. If the stem were removed before infiltration, as with injections no cultivar difference was found, since the bacteria entered each type equally well through the exposed vascular bundles. Floradel and Manapal cultivars, which showed resistance with the infiltration technique, had narrower corky rings and smoother shoulders with a general absence of deep creases. Samish et al. (2) found that bacteria placed on the sepals (which are in close proximity to the corky scar tissue) entered tomato fruit under natural conditions.

Since preliminary results showed the infiltration technique might be useful in distinguishing between resistant and susceptible cultivars, the possibility of using this technique for screening lines for graywall resistance was investigated. A number of cultivars and lines with differences in field resistance to graywall were evaluated. A study was also made of plant variation in resistance within single lines.

Materials and Methods

The tomato cultivars and lines were grown at the Gulf Coast Experiment Station (GCES) (staked culture) and Sub-Tropical Experiment Station (STES) (ground culture) during the

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1967-1968 season. Four harvests were made at the former and three at the latter station. The fruits were harvested with stems attached about a week prior to the mature-green stage. Random samples were taken for inoculation. In the studies on plant variation in susceptibility, the fruit from each plant were treated separately. The samples were transported to Gainesville for treatment.

Injection inoculations with *Erwinia ananas* Serrano were as detailed elsewhere (4). Infiltration inoculations were made by spraying fruit (stems attached) with a bacterial suspension of about 10^6 cells/ml until the stem scar area was filled. Immediately afterward a vacuum of 25 inches of Hg was applied for one minute. In most of the studies injections and infiltrations were made on separate groups of fruit. With the GCES harvests, sample size was 10-12 fruit for each inoculation method. With the STES harvests, sample size was 15 fruit for the infiltration inoculations. At the first harvest 10 separate fruit samples were inoculated by injection, while at the third harvest the same 15 fruit samples inoculated by infiltration were also inoculated by injection. No injection inoculations were made at the second harvest.

All fruit were held at 45°F for 7 days following inocluation to increase their susceptibility (1). The injection inoculations were made as a check to determine if the tissue were susceptible. The fruits were placed at 70°F following the chilling treatment for symptom development.

Infiltrated fruit were checked for browning by peeling around the stem scar and shoulder area. Each injection site was examined by removing a thin slice from the surface.

Three randomly selected replications were used at the first two harvests and two for the last two harvests from the GCES. Four replications were used for the first two harvests and three for the third from the STES.

RESULTS

Comparison of cultivars and lines: Homestead 24, when inoculated by infiltration, developed a greater amount of browning than any of the other cultivars and lines following all four harvests at the GCES (Table 1). However, the difference was significant statistically only for the first two harvests. None of the other lines, all of which exhibited greater field resistance than Homestead 24, differed statistically although

Table 1. The incidence of browning (graywall) of fruits from several cultivars and lines inoculated with bacteria by vacuum infiltration or by injection into the wall following 4 harvests at the Gulf Coast Station.[1]

		Inoculation Method							
		Infiltration				Injection			
		Harvest date				Harvest date			
Cultivar or line	Relative field resistance[2]	11/2	11/9	11/21	12/6	11/2	11/9	11/21	12/6
		Percent of fruit				Percent of sites			
Homestead 24	S	70a	37a	55a	25a	65cd	12c	15c	46bc
Floradel	R	23b	7b	20a	5a	92ab	44a	82a	71a
Indian River	R	28b	10b	10a	15a	79abc	34ab	92a	58ab
294 BK-1-D1-DBK	R	20b	10b	10a	0a	23e	12c	8c	6d
56-7-DBK	R	14b	0b	0a	0a	48cd	7c	13c	7d
STEP 407	R	30b	7b	30a	5a	82abc	24abc	26c	33c
37-5-1-6-BK	R	22b	0b	20a	10a	94a	22bc	59b	48abc
STEP 461-5-BK	R	34b	3b	35a	15a	68bcd	21bc	14c	40bc
Coefficient of variation		47.1	97.9	68.1	360.6	19.2	50.8	34.1	32.6

[1]Cultivar or line means within each harvest date with the same letter are not different at the 0.05 level of significance.

[2]R = resistant; S = susceptible.

the range was rather large. The lack of significance was probably due to the great variation in results as shown by the high coefficients of variation.

The injection method gave much different results when compared to the infiltration method. Floradel and Indian River, which were low to intermediate in browning by infiltration, had much more browning than Homestead 24. Lines 56-7-DBK and 294 BK-1-D1-DBK were low in browning with both methods of inoculation.

Results from infiltration of fruit from the harvests at the STES show Homestead 24 had more browning than the other stocks except for the January 18 harvest, when Indian River and line 56-7-DBK did not differ significantly from Homestead 24 (Table 2). The graywall resistant varieties Floradel, Tropi-Gro and Tropi-Red had less browning than Homestead 24 at all harvests. The line 294 BK-1-D1-DBK had much less browning with either inoculation method. Line 56-7-DBK had low browning when injected but not when infiltrated. The latter result differed from that obtained with fruit grown at the

Table 2. The incidence of browning (graywall) of fruits from several cultivars and lines inoculated with bacteria by vacuum filtration or by injection into the wall following 3 harvests at the Sub-Tropical Station.[1]

		Inoculation Method				
		Infiltration			Injection	
		Harvest Date			Harvest Date	
Cultivar or line	Relative field resistance[2]	1/11	1/18	2/23	1/11	2/23
		Percent of fruit			Percent of sites	
Homestead 24	S	85a	67a	47a	55a	75a
Floradel	R	44bcd	46bcd	13b	71a	68a
Indian River	R	42cd	60ab	-	76a	-
294-BK-1-D1-DBK	R	3e	8f	-	4c	-
56-7-DBK	R	55bc	53abc	-	28b	-
STEP 407	R	34d	25ef	-	59a	-
37-5-1-6-BK	R	47bcd	33de	-	59a	-
Tropi-Red	R	45bcd	45bcde	16b	72a	79a
Tropi-Gro	R	58b	39cde	22b	76a	75a
1175	R	44bcd	40cde	-	19bc	-
Coefficient of variation		20.6	31.4	53.9	23.3	19.7

[1] Cultivar or line means within each harvest date with the same letter are not different at the 0.05 level of significance.

[2] R = resistant; S = susceptible.

Table 3. The variation between 20 plants in the percent of fruits which developed browning following infiltration with bacteria. The breeding line used was a susceptible selection of Sub-Tropical line 183. The plants were observed about 2 weeks later for presence of graywall fruits.

Plants with graywall		Plants without graywall	
Percent brown	No. of fruits	Percent brown	No. of fruits
66	6	20	10
50	10	50	10
50	8	40	10
100	8	30	10
86	7	20	10
90	10	100	7
50	10	30	10
90	10	20	10
100	5	20	10
40	10	70	10
Mean 72		40	

GCES. Considerable variation occurred in browning between harvest dates with both inoculation methods.

Plant variation in susceptibility: The percentage of fruit which developed browning following infiltration with bacteria varied greatly between plants of the same line. In one test using 20 plants of the STES line 183, the percentage of inoculated fruit showing browning from each plant ranged from 20 to 100 (Table 3). When these results were compared with the appearance of graywall on remaining fruits on the same plants a few weeks later the plants which developed graywall in the field had a much higher average percent of fruit showing browning following inoculation (72%) than those which did not develop graywall (40%).

In another test using Tropi-Red plants, which have considerable graywall resistance, a range of 0 to 88% of the fruits showed browning (Table 4). The mean value was 43 percent, which closely compared with the 40% of the previous test with graywall-free plants.

Discussion

The infiltration method can be used to distinguish between very susceptible and very resistant stocks but apparently cannot be used to determine differences between lines having moderate resistance to graywall. Larger samples and more replications might reduce the variation and so give more precise results. However, this amount of fruit and time would not be available in a screening program. The great plant to plant variation found in these tests precludes the use of the method for screening small plant populations. The plant to plant variation in susceptibility to browning of the fruit is characteristic of graywall development in the field.

Table 4. The variation between 20 Tropi-Gro plants in the percent of fruits which developed browning following infiltration with bacteria.

Percent brown	No. of fruits	Percent brown	No. of fruits
69	13	36	14
69	16	31	16
38	16	33	15
80	10	80	15
19	16	69	13
66	12	33	15
36	14	22	18
25	16	36	14
40	15	58	19
0	14	25	16

A comparison of the results from both methods of inoculation indicates that there may be two types of resistance. One would be resistance to entrance of the bacteria, which is exemplified by the differences between the susceptible Homestead 24 and the resistant Floradel in the infiltration tests. The other type of resistance would be some factor in the tissue which prevents bacterial development after invasion of the tissue. When the bacteria are injected into the tissue, and the fruits subjected to chilling, Floradel is very susceptible whereas Homestead 24 is moderately susceptible. The low values obtained with line 294 BK-1-D1-DBK with both inoculation methods may be due to an extremely high tissue resistance, which was not decreased by chilling to the level which allowed bacteria to multiply.

Resistance to entrance, or a combination of it and tissue resistance, would be preferred to tissue resistance only. Since the latter type can be altered by chilling, it is presumed that other factors such as change in environment, change in nutrition, or virus infection could also reduce the tissue resistance.

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