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Inheritance and Heritability of Resistance to Tomato Anthracnose Caused by *Colletotrichum dematium*

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Abstract. **A 6-parent diallel was used to study combining ability and type of gene action contributing to resistance in tomato (*Lycopersicon esculentum* Mill.) to anthracnose caused by *Colletotrichum dematium* (Pers. ex Fr.). The 6 parents, one set of F_1 hybrids, and 5 selected reciprocal crosses were grown at 2 locations. Ripe fruit were harvested, puncture-inoculated with the pathogen, and subsequently evaluated for resultant lesion diameter. No reciprocal effects were found at either location for the 5 crosses studied. The analysis of variance for parent and F_1 hybrid performance revealed a genotype × location interaction. Combining ability analysis based on the F_1 hybrids alone indicated a significant general combining ability (GCA) effect. The specific combining ability (SCA) and GCA × location interaction mean squares were smaller than the GCA value but were still significant. Differential performance over locations of the hybrids of one line was primarily responsible for the GCA × location interaction. Analysis of variance and covariance of parental arrays indicated partial dominance in the direction of susceptibility. Narrow sense heritability for the trait was 70% over both locations.**

Anthracnose is a fungal disease which results in a rot of ripe tomato fruits. It is an important problem for processing tomato production in the Mid-Atlantic and Mid-western United States where tomatoes are ripened on the vine, especially if environmental conditions favor growth and development of the fungal organisms (6). The organism traditionally associated with anthracnose is *Colletotrichum coccodes* (Wallr.) Hughes. However, other *Colletotrichum* and *Glomerella* spp. also have been determined to cause anthracnose lesions on tomato (4, 8, 9). Recently, *C. dematium* has been identified as a major anthracnose-causing pathogen in Indiana (18).

Because cultural techniques give only partial control of the disease (17), genetic resistance to anthracnose is desirable in tomato cultivars. Genetic studies of resistance to *C. coccodes* have revealed that inheritance is quantitative in nature with partial dominance for resistance (3, 5, 14). Although no tomato cultivar has been reported to be resistant to *C. dematium*, resistance has been found in PI 272636 (2). The objective of the present study was to determine the mode of inheritance of this resistance.

Materials and Methods

Six inbred tomato lines were crossed in a diallel design. Three resistant inbred USDA breeding lines (81B416-1, 81B1105-2, and 625-3-1) derived from PI 272636 and a 4th tolerant selection (Ark 79-90) developed by Joe McFerran, University of Arkansas, were used as resistant parents. The susceptible inbreds ('US141' and 81B9) were developed by Allan K. Stoner, USDA Beltsville Agricultural Research Center. The 6 lines were crossed in all possible combinations.

The 6 parents, one complete set of 15 F_1 hybrids, and 5 selected reciprocal crosses (Ark79-90 x 'US141', 81B1105-2 x 'US141', 625-3-1 x 81B9, 625-3-1 x Ark79-90, and 81B1105-2 x 625-3-1) were transplanted in a Norfolk sandy loam at the University of Maryland Vegetable Research Farm (VRF) at Salisbury and in a Hatboro silt loam at the USDA Beltsville Agricultural Research Center (BARC) at Beltsville, Md., during the spring of 1982. The planting was arranged in a randomized, complete-block design with 4 replications per location and 10 plants per plot. Recommended cultural and pesticide practices were used at each location. On August 24 (BARC) and August 27 (VRF), 20 mature, blemish-free, red fruit were harvested from each plot. The fruits were transported in paper bags to a shaded greenhouse in Beltsville and placed on brown paper on benches for inoculation.

Two isolates of *C. dematium* (C59 and C131) were grown on 30% filtered V-8 agar under continuous light at room temperature (1). The isolates were combined to provide an inoculum of 9.6×10^6 spores in distilled water; this concentration was determined previously to be optimum for maximum percentage of infection and mean lesion diameter on the susceptible 'US141' (12). The hypodermic inoculation technique developed by Robbins and Angell (13) was employed in screening for resistance. Lesion diameters were measured 6 days after inoculation.

Mean lesion diameter for each 20-fruit sample was computed. Orthogonal contrasts were made between selected crosses and their reciprocals. An F_{max} test indicated that a $\log_{10}$ transformation of the plot means was required due to variance heterogeneity. The transformed data were used in further analyses.

An analysis of variance was performed on the parent and F_1 data at each location and averaged over locations. A Waller-Duncan Bayesian k-ratio *t*-test (16) was performed to compare the means of the parents and hybrids across the 2 environments. General combining ability (GCA) and specific combining ability (SCA) were calculated from the set of F_1 hybrids according to

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Griffing's Model 1 Method 4 procedure (10), using the Schaffer and Usanis computer program (15). All of the effects are fixed in this model and the population about which the inferences are made is the experimental material. Individual GCA effects also were calculated for each parent.

The diallel analysis described by Hayman (11) was used to estimate the type of gene action present. Variance of parental means ($V_{\bar{p}}$), mean variance of the offspring of each parental array (V_r), means of the array (V_a), and the covariance of the offspring of the arrays with the nonrecurring parent (W_r) were calculated. The ($W_r - V_r$) values were tested by a *t*-test to determine whether certain assumptions (i.e., diploid segregation, no reciprocal differences, independent action of nonallelic genes, no multiple allelism, homozygous parents, and independent distribution of genes) were met. A 2nd *t*-test was performed on the regression coefficient of the line produced in the (V_r, W_r) graph to determine whether a nonallelic interaction existed for some arrays.

Narrow-sense heritability was calculated by using the parent–offspring regression technique (19). This estimate was used to determine the proportion of the phenotypic variance due to the additive effects of the genes.

Results and Discussion

The analysis of variance with planned comparison for the 5 F_1 hybrids (Ark79-90 x 'US141', 81B1105-2 x 'US141', 625-3-1 x 81B9, 625-3-1 x Ark79-90, and 81B1105-2 x 625-3-1) and their reciprocals is presented in Table 1. There were no significant differences to *C. dematium* at either location, which indicated the absence of reciprocal effects and confirmed the validity of using one set of F_1 hybrids without reciprocals for the diallel analysis.

The transformed lesion diameter data of the parents and one set of F_1 hybrids in response to inoculation are presented in Table 2. The Waller-Duncan Bayesian k-ratio *t*-test indicated that 7 F_1 hybrids and 2 parents exhibited significant differences across locations. The analysis of variance combined over 2 locations, including parental and F_1 genotypes, is presented in Table 3. Mean squares were highly significant for genotypes as well as for parent, hybrid, and parent vs. hybrid components. There were also genotype × location, parent × location, and hybrid × location interactions. The significant parent vs. hybrid mean squares indicated an overall difference between the 6 parents and 15 F_1 hybrids. Significant differences between means and significant interaction mean squares indicated that location influenced the phenotypic expression of reaction to *C. dematium*.

Table 1. Analysis of variance of the effect of reciprocal crosses on relative susceptibility of tomato to *Colletotrichum dematium*.

Source of variation	df	Mean square: Location BARC	Mean square: Location VRF
Replications	3	8.12 NS	8.82 NS
'US141' x Ark79-90	1	0.98 NS	22.61 NS
'US141' x 81B1105-2	1	1.28 NS	1.05 NS
81B9 x 625-3-1	1	4.96 NS	28.88 NS
Ark79-90 x 625-3-1	1	0.15 NS	25.92 NS
625-3-1 x 81B1105-2	1	2.88 NS	3.32 NS
Error	27	4.19	7.96

NSNonsignificant at 5% level.

Table 2. Fruit lesion diameters for crosses from a 6-parent diallel of tomato grown at 2 locations and inoculated with *Colletotrichum dematium*.

Crosses	Lesion diam (mm): Location BARC	Lesion diam (mm): Location VRF
81B9	1.32 ab[z,y]	1.34 a
'US141'	1.31 ab	1.35 a
'US141' x 81B9	1.26 abcd	1.29 abc
81B9 x 81B416-1	1.13 bcde	1.06 def
'US141' x 81B416-1	1.13 bcde	1.05 ef
81B9 x 81B1105-2	1.01 ef	1.08 def
81B9 x Ark79-90	0.99 efg	1.28 abc
'US141' x Ark79-90	0.90 fgh	1.25 abcd
81B1105-2	0.81 ghij	0.61 klmn
'US141' x 81B1105-2	0.76 hijk	1.02 ef
81B9 x 625-3-1	0.75 hijk	0.90 fgh
Ark 79-90 x 81B1105-2	0.75 hijk	0.81 ghij
81B416-1 x 81B1105-2	0.70 ijkl	0.46 nop
'US141' x 625-3-1	0.53 lmno	0.69 jklm
625-3-1 x 81B1105-2	0.50 mno	0.27 pq
Ark79-90	0.47 no	1.11 cde
Ark79-90 x 81B416-1	0.43 nop	0.90 fghi
Ark79-90 x 625-3-1	0.39 op	0.62 klm
81B416-1	0.16 qr	0.16 qr
81B416-1 x 625-3-1	0.14 qr	0.17 qr
625-3-1	0.00 r	0.00 r

[z]Lesion diameters were subjected to a $\log_{10}$ transformation.
[y]Mean separation in columns by the Waller-Duncan Bayesian k-ratio *t*-test, k-ratio = 100.

GCA and SCA mean squares are presented in Table 4. The GCA mean square accounted for most of the genetic variability. However, SCA also contributed a significant amount of variation as did the GCA × location interaction. Since GCA can be interpreted in terms of additive genetic variance (10), the differences noted among the F_1 hybrids were due primarily to additive genetic effects.

Estimates of the individual GCA effects for each parent at each location are presented in Table 5. Because resistance was expressed as a smaller lesion diameter in response to the pathogen, a negative GCA effect is considered desirable for this trait. The most negative GCA effect was obtained with 625-3-1. 81B414-7 and 81B1105-2 also had negative GCA effects. 81B9 had the highest positive GCA effect at both locations,

Table 3. Variance components of a 6-parent diallel analysis for resistance to *Colletotrichum dematium* in tomato.

Source of variation	df	MS
Locations (L)	1	0.37 NS[z]
Replications	6	1.07 **
Genotypes	20	1.22 **
Parents (P)	5	2.53 **
Hybrids (H)	14	0.82 **
P vs. H	1	0.26 **
Genotypes × L	20	0.10 **
P × L	5	0.16 **
H × L	14	0.08 **
P vs. H × L	1	0.01 NS
Error	120	0.02

[z]Mean lesion diameter subjected to a $\log_{10}$ transformation prior to the analysis of variance.
NS, **Nonsignificant (NS) or significant (**) at 1% level.

Table 4. Combining ability analysis of tomato for resistance to *Colletotrichum dematium* over 2 locations with and without Ark79-90 as a parent.

Source of variation	With Ark79-90 hybrids df	With Ark79-90 hybrids MS	Without Ark79-90 hybrids df	Without Ark79-90 hybrids MS
Locations (L)	1	0.29 **[z]	1	0.01 NS[z]
Replications (within L)	6	0.06 **	6	0.05 **
GCA	5	2.16 **	4	2.13 **
SCA	9	0.08 **	5	0.12 **
GCA × L	5	0.14 **	4	0.06 NS
Error	93	0.02	59	0.02

[z]Mean lesion diameter subjected to a $\log_{10}$ transformation prior to the analysis of variance.
NS, **Nonsignificant (NS) or significant (**) at 1% level.

followed by 'US141'. However, Ark79-90 exhibited a negative effect at the BARC but a positive one at the VRF.

Of the 7 crosses that displayed differences across locations (Table 2), 4 involved hybrids using Ark79-90 as a parent. The difference in GCA values from negative (BARC) to positive (VRF) for Ark79-90 and the significant GCA × location interaction indicated a differential expression of gene effects in different environments. Barksdale and Koch (7) observed that twice the amount of field infection may occur on plants grown in sandy soils compared to those grown in clay soils. The difference in the phenotypes of the hybrids of Ark79-90 between locations may be due in part to the different soil types.

Because of the differential expression with hybrids of Ark79-90, another combining ability analysis was performed on the F_1 data, excluding hybrids where Ark79-90 was a parent (Table 4). GCA and SCA mean squares remained significant; however, the GCA × location interaction was no longer significant.

The genetic analysis developed by Hayman (11) gives an indication of the degree of dominance controlling the trait. The appropriateness of Hayman's analysis was tested by a *t*-test of the ($W_r - V_r$) values across the arrays and a test of the deviation of the slope from unity of the (V_r, W_r) regression line by another *t*-test. Both *t*-tests were not significant, indicating that the assumptions of Hayman's analysis were met and that no important epistatic gene effects were present. The y-intercept of the (V_r, W_r) regression line was greater than zero, indicating partial dominance (Fig. 1). 'US141', Ark79-90, 81B1105-2, and 81B9 had the lowest V_r and W_r values, indicating that they contained

Table 5. Estimates of individual GCA effects for resistance to *Colletotrichum dematium* for each parent in the tomato diallel.

Parent	GCA effects (mm) Location BARC	VRF
81B9	0.27[z]	0.27
'US141'	0.16	0.20
81B1105-2	−0.01	−0.13
81B416-1	−0.05	−0.13
Ark79-90	−0.07	0.12
625-3-1	−0.30	−0.33
Mean	0.76	0.86

[z]Estimates derived from a $\log_{10}$ transformation of the mean lesion diameter data.

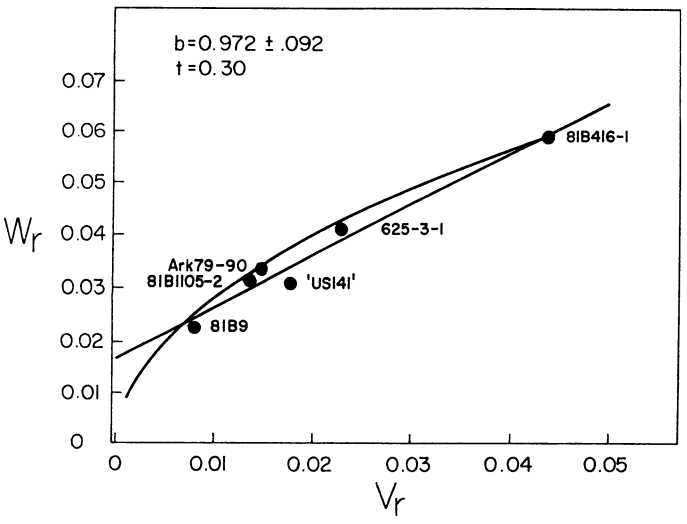

Fig. 1. Variance (V_r)—covariance (W_r) analysis for susceptibility to *Colletotrichum dematium* in a 6-parent diallel of tomato combined over 2 locations.

principally dominant alleles. These parents ranged from susceptible to tolerant. The remaining parents (625-3-1 and 81B416-1) had high V_r and W_r values and thus contained a relatively high proportion of recessive alleles. These inbreds were resistant. Thus, there appears to be partial dominance in the directions of susceptibility for this trait.

Narrow-sense heritability (h^2) was calculated by the parent–offspring regression technique. Narrow-sense h^2 was 64% at the BARC, 75% at the VRF, and 70% averaged over both locations. Again, the additive variance was relatively high, which is in agreement with the combining ability analysis.

Earlier studies by Robbins and Angell (14) and Barksdale (3, 5), using *C. coccodes* as the anthracnose organism, determined that resistance was inherited quantitatively with resistance being partially dominant. This study indicates that with *C. dematium* the variation in the F_1 hybrids was due primarily to additive effects of the resistance genes with some significant nonadditive effects. Also, partial dominance was present in the direction of susceptibility.

As additive variance was the most significant component in the experimental population, rapid genetic progress should be possible through breeding and selection. Thus, there is promise for the incorporation of resistance to *C. dematium* into susceptible genotypes with desirable horticultural characteristics. Although the differential reaction of Ark79-90 to the pathogen when grown at different locations indicates that breeding lines may need to be evaluated over a range of environments to ensure phenotypic stability for the trait, this may not be necessary if the resistance is derived from PI 272636.

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A Method for Studying the Three-dimensional Distribution of Roots Grown in an Artificial Medium

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Abstract. **A method is described for studying the 3-dimensional distribution of roots grown in a medium consisting of small pieces of glass. After growing to a desired size, the plant is sacrificed by evaporating all water from the media with flowing air. To visualize the undisturbed root system, an immersion oil with the same refractive index as the glass is added to the glass container in which the plant was grown.**

There are numerous methods to study the distribution and morphology of root systems grown in soil and soilless media. These methods have been reviewed recently by Böhm (1). Excavating root systems is tedious, time-consuming, and, unless great care is exercised, only shows the gross structure of the root system (5). Other methods, such as the cage method (3), and the needleboard method (4) were developed for ease of use or to show more structure. However, all methods require a great deal of labor to remove physically the medium from the roots and often result in damage (6). Washing media from the roots damages smaller roots by either removing them with the medium or displacing them from their original orientation, causing them to cling to larger roots (1). There are currently no methods to visualize undisturbed 3-dimensional root systems.

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Plants grown in soilless media consisting of coarse, inert material such as vermiculite, show a root distribution similar to that occurring in soil, while plants grown in other inert media such as sand or perlite, show root development similar to those grown in liquid cultures (2). Root growth and development often is studied in liquid culture to facilitate observation of the roots. However, any similarity to the 3-dimensional root distribution found in soil clearly would be coincidental.

An inert, transparent solid will seem to disappear when immersed in a liquid with the same refractive index. Flooding the root system of plants grown in a medium consisting of transparent glass, with a liquid having the same refractive index as the glass, will cause the medium to become transparent and allow the undisturbed roots to be viewed in their original 3-dimensional orientation. This paper describes such a technique for viewing roots.

Preparation of media. Glass used for the growing media must be transparent and free of inclusions and surface deposits. Safety plate glass (3.2 mm in thickness) was broken into small pieces and sieved. Pieces retained between the 3.2-mm and 1.5-mm screens yielded suitable-sized particles for growing plants. This procedure yielded about 1 liter of broken glass pieces per 22.5 cm^2 of sheet glass. Other types of glass proved unsuitable either because they contained small bubbles or surface deposits or because it was difficult to obtain enough glass pieces of the correct size.