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Inheritance of resistance in cucumber to race 2 of *Colletotrichum lagenarium*

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Summary. The resistant breeding line, AR79-95, and the susceptible cultivar, Model, were crossed to develop F_1, F_2, F_3, and backcross populations for genetic analysis of resistance in cucumbers to race 2 of *Colletotrichum lagenarium* (Pass.) Ellis & Halsted., the causal agent of cucurbit anthracnose. There was no maternal effect on resistance and a small amount of F_1 heterosis toward the susceptible parent. Generation means analysis showed that there was additive and dominance but no epistatic gene action detected on the scale used. Additive and dominance genetic variances were estimated, and narrow-sense heritability was low to moderate. Based on effective factor formulae, at least five effective factors contrtolled the resistance. Some of these factors were dominant and others recessive. Implications for breeding procedures are discussed.

Key words: *Cucumis sativus* – Anthracnose – Disease resistance – Quantitative inheritance – Vegetable breeding

Introduction

Colletotrichum lagenarium is a major foliar pathogen causing anthracnose of cucumber. Although seven races have been identified (Jenkins et al. 1964), Sitterly (1973) stated that races 1 and 2 appear to be most common. Selection for resistance is important during the development of cultivars adapted for the southern United States. Knowledge of the genetic basis and heritability of resistance is essential for efficient development of resistant cultivars.

Although Abul-Hayja et al. (1978) have reported that a single recessive gene conditions resistance to race 1, the inheritance of resistance to race 2 has not been fully characterized. Plant Introduction (PI) 197087 has commonly been used as the source of resistance to race 2 (Sitterly 1973). Barnes and Epps (1952) observed that F_1 progeny resulting from matings between PI 197087 and three susceptible varieties were intermediate in resistance, and that F_2 individuals could not be classified into discrete classes. However, no F_2 or F_3 plants were as resistant as PI 197087. Therefore, these authors concluded that resistance in PI 197087 is controlled by several "major" genes and that modifying genes are present. Since Barnes and Epps published their results before Goode (1956) first reported the occurrence of races in *C. lagenarium*, it is not clear which race(s) they used.

Using PI 197087, Goode and Bowers (1973) developed AR79-95, which possesses a level of resistance to race 2 equal to or better than the resistance in PI 197087. Goode (personal communication, 1984) concluded that resistance of AR79-95 is controlled by several genes.

Our objective was to perform a detailed study on the inheritance of resistance to race 2 of *C. lagenarium* in AR79-95. A study was designed to: (1) study maternal effects; (2) determine whether heterosis for resistance exists; and (3) determine gene action and estimate genetic variances, minimum number of effective factors, and heritability of resistance.

Materials and methods

Genetic material and field experiments

Model has been described as being extremely susceptible to anthracnose (Sitterly 1973). Therefore, inbred sources of AR79-95 (P_1) and Model (P_2) were crossed to develop reciprocal F_1 and F_2 progenies, two backcross populations (B_1 and B_2), and 16 F_3 families. The F_3 families were developed by selfing eight random F_2 plants in each F_2 population.

Two field experiments were conducted at the Edisto Research and Education Center. In the first (summer and fall

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Table 1. Means and variance of anthracnose severity ratings for the cucumber genotypes used in experiments 1 and 2

Genotype	Experiment 1[a]			Experiment 2[c]		
	n	Mean	Variance	*n*[b]	Lsmean[d]	Variance[e]
AR79-95 (P_1)	9	0.59	0.041	26	1.09	0.0288
Model (P_2)	5	1.34	0.088	24	2.02	0.0367
AR79-95 × Model	19	1.06	0.061	21	1.67	0.0113
Model × AR79-95	14	1.08	0.048	22	1.63	0.0159
Pooled F_1	33	1.07	0.054	43	1.65	0.0147
(AR79-95 × Model) F_2	9	0.93	0.360	20	1.75	0.0551
(Model × AR79-95) F_2				20	1.62	0.0432
Pooled F_2				40	1.69	0.0506
$P_1 \times (P_1 \times P_2)$ (B_1)				21	1.36	0.0589
$P_2 \times (P_2 \times P_1)$ (B_2)				23	1.83	0.0239
F_3-1				10	1.67	0.0623
F_3-2				18	1.61	0.0442
F_3-3				18	1.57	0.0106
F_3-4				18	1.77	0.0320
F_3-5				21	1.78	0.0139
F_3-6				16	1.55	0.0442
F_3-7				22	1.63	0.0206
F_3-8				22	1.62	0.0238
F_3-9				22	1.76	0.0506
F_3-10				18	1.81	0.0128
F_3-11				23	1.50	0.0186
F_3-12				17	1.48	0.0430
F_3-13				18	1.75	0.0305
F_3-14				19	1.50	0.0552
F_3-15				16	1.60	0.0328
F_3-16				19	1.92	0.0089

[a] Disease severity ratings in experiment 1 were transformed using logarithms
[b] *n* = number of plants summed across replications
[c] Disease severity ratings in experiment 2 were transformed using fifth roots
[d] Lsmean = least squares mean
[e] Variances in experiment 2 were adjusted for location

1984), an unbalanced completely randomized experimental design was used. Ten plants of each parent, 20 plants of each reciprocal F_1, and 30 plants of one F_2 population were transplanted at the first leaf stage from the greenhouse into the experimental site. Approximately one-third of the plants died because of transplant shock and insect damage. The number of plants surviving for each generation is listed in Table 1.

A single-conidial culture of *C. lagenarium*, designated AR2, was obtained from Love (1984), who had identified it as race 2 by the method of Jenkins et al. (1964). AR2 was cultured and increased by standard procedures (Littrell and Epps 1965), with the modification that the isolate was cultured on the susceptible parent every 3–6 months to maintain virulence. Two weeks after transplanting, the plants were inoculated at dusk with 20,000 spores/ml using a tractor-drawn sprayer. The rig, travelling at 30 m/s, delivered 193 l/ha through six nozzles (no. 803) at 4.2 kg/cm^2.

Plants were rated for disease 21, 23, and 29 days after inoculation, by estimating the percentage of leaf area exhibiting typical anthracnose lesions. The scale used was 1%, 2%, 5%, 10%, 15% and higher, increasing by 5% intervals. The highest disease rating assigned to any plant was 80%. The ratings from the three dates were averaged prior to analysis.

The second field experiment (spring and summer 1985) utilized an unbalanced completely randomized experimental design, which was replicated in four adjacent locations. Each location contained 170 single plant hills. The reciprocal F_1s, reciprocal F_2s, B_1, B_2, and each F_3 were represented by seven hills per location. The two parents had eight hills per location. Each hill was direct seeded and thinned to one plant per hill. Hills which lacked plants were replanted 11 days later with seed or with transplants (first leaf stage) started in the greenhouse. The final number of plants surviving for each genotype is listed in Table 1. Because of possible age effects on resistance, the number of leaves on each plant was counted on the day preceding inoculation for covariance analysis.

Forty days after planting, the plots were inoculated at dusk with 60,000 conidia/ml of isolate AR2 using a backpack sprayer (Solo, Model 485, fan-type nozzle) until run-off occurred. On the 10th day after inoculation, plants were visually rated for percentage leaf area infected. The scale used was 0%, 1%, 5%, 10%, 20%, 30%, and higher, increasing by 10% intervals. The maximum disease rating given was 60%.

Data analyses

In both experiments, the original disease severity ratings were not found to meet the criteria of scaling (Mather 1949) necessary for genetic analysis of the resistance. In experiment 1, a logarithmic conversion was made to equalize parental and F_1 variances. In experiment 2, a fifth root transformation was most suitable for equalizing the parental variances without excessively depressing the reciprocal F_1 variances.

In both experiments, the presence or absence of maternal effects and F_1 heterosis were tested via analysis of variance

(ANOVA) of the parental and reciprocal F_1 data and by comparing the appropriate means with linear contrasts. (A11 ANOVAs described herein were checked and found to meet the assumptions of constant variance and normal distribution of errors.)

The parental, F_1, backcross (experiment 2 only), and F_2 means were analyzed for dominance and additive genetic effects with the weighted generation means analysis of Mather and Jinks (1971). As a complementary analysis for experiment 2, the additive and dominance genetic variances (V_A and V_D, respectively) were estimated with Mather's (1949) unweighted variance method. The equations for $\bar{V}_{F2}$, V_{F3}, V_{B1} and V_{B2}, E_1, and E_2 were used to obtain least squares estimates of V_A and V_D. Broad [$(V_{F2}-V_{F1})/V_{F2}$; Falconer 1981] and narrow [$1/2V_A/(1/2V_A+1/4V_D+E_1+E_2)$; Mather and Jinks 1971] sense heritabilities were estimated.

In experiment 2, the minimum number of effective factors controlling resistance was calculated by five methods. Methods one through four were described by Lande (1981) and are based on various combinations of the observed parental, F_1, backcross, and F_2 variances. Method five (Mather 1949) uses the estimate of V_A obtained above. Each method also uses the difference observed between the parental means. The specific formulae used in each method are described in Table 2.

Results

Maternal and heterotic effects

Means and variances of the transformed data for both experiments are presented in Table 1. In experiment 1, a linear contrast comparing reciprocal F_1s showed no difference ($F=0.07$, $P=0.80$) in the disease ratings, indicating lack of maternal effects. Furthermore, the contrast comparing the pooled reciprocal F_1 mean (1.07) with the parental midpoint (0.96) showed no difference ($F=1.82$, $P=0.18$).

No location or genotype by location interactions were detected in experiment 2. Number of leaves at inoculation or age of plants did not covary with important traits. A linear contrast comparing the reciprocal F_1 populations showed no difference ($F=0.58$, $P=0.44$), indicating no maternal effect. A linear contrast comparing the pooled F_1 mean (1.65) with the parental midpoint (1.56) showed a difference ($F=9.77$, $P<0.01$), indicating heterosis toward the susceptible parent.

Genetic effects

In experiment 1, the F_2 population, although limited in size, did not segregate into discrete classes, thus confirming observations made in the early summer of 1984 with 118 F_2 plants inoculated with the same isolate. Likewise, in experiment 2, the F_2 did not segregate into discrete classes. Resistance was concluded to be inherited as a quantitative trait.

In experiment 1, the weighted generation means analysis yielded estimates of the mid-parent (u), the sum of the additive genetic effects (a), and the sum of the dominance genetic effects (d) (Table 3). Fit to the additive-dominance model was adequate ($\chi^2_{(1)}=0.19$, $P=0.90-0.75$). Additive genetic effects were larger and accounted for a greater percentage of the ANOVA genotype sums of squares than dominance genetic effects.

Table 2. Minimum number of effective factors formulae and estimates provided by them in experiment 2 for resistance to race 2 of *C. lagenarium* in the cucumber line, AR79-95

Method	Formula	Estimate
1	$(P_1-P_2)^2/8\ (V_{F2}-V_{F1})$	3.0
2	$(P_1-P_2)^2/8\ (V_{F2}-(1/2V_{F1}+1/4V_{P1}+1/4V_{P2}))$	4.0
3	$(P_1-P_2)^2/8\ (2V_{F2}-V_{B1}-V_{B2})$	5.9
4	$(P_1-P_2)^2/8\ (V_{B1}+V_{B2}-(V_{F1}+1/2V_{P1}+1/2V_{P2}))$	3.1
5	$(1/2(P_1-P_2))^2/V_A$	8.6

Table 3. Estimates of additive and dominance genetic effects as calculated by weighted generation means analysis in experiment 1 and 2 and the percent genotype sums of squares they accounted for in the analysis of variance

Parameter[a]	Experiment 1		Experiment 2	
	Estimate	% Genotype SS	Estimate	% Genotype SS
u	0.96 ± 0.07**[c]	–	1.56 ± 0.02**	–
a	0.37 ± 0.07**	95.5	0.46 ± 0.02**	94.8
d	0.11 ± 0.08^{ns}	3.4	0.10 ± 0.03*	4.0
residual (epistasis)		1.1		1.2

[a] u=midpoint, a=sum of the additive genetic effects, d=sum of the dominance genetic effects

[b] The estimates of parameters in experiment 1 and 2 are not expected to be the same because different transformations were used

[c] ** =significant at 5%, *=significant at 1%, ns=not significant

In experiment 2, similar results were obtained from the weighted generation means analysis. Fit to the additive-dominance model was adequate ($\chi^2_{(3)}=2.75$, $P=0.50-0.25$). Additive genetic effects were larger in magnitude and accounted for a larger percentage of the genotype sums of squares than dominance genetic effects (Table 3).

Mather's unweighted variance method gave the following estimates for the diffeent variance components in experiment 2: $V_A=0.251\pm0.0087$ ($t_{(6)}=2.90$, $P=0.05-0.02$), $V_D=0.0527\pm0.0177$ ($t_{(6)}=2.97$, $P=0.05-0.02$), $E_1=0.0222\pm0.0030$ ($t_{(6)}=7.48$, $P<0.001$), and $E_2=0.0013\pm0.0030$ ($t_{(6)}=0.40$, $P>0.50$).

Broad- and narrow-sense heritability were calculated to be 70.9% and 26.0%, respectively, in experiment 2. The 95% confidence interval for narrow-sense heritability was 3.8%–47.1%.

The estimates of the minimum number of effective factors controlling the resistance ranged from 3.0 to 8.6 (Table 2).

Discussion

Although data from both experiments suggest that there was no maternal effect on resistance, disparity among experiments regarding the existence of heterosis in the F_1 was evident. With regard to midparent values, the F_1 mean in both experiments was closer to the susceptible parent. Since the parental midpoint was probably more accurately estimated in experiment 2, it appears that there was a small, but measurable heterotic effect. These data suggest that both inbred components used for hybrid development must have high levels of race 2 resistance.

Generation means analysis suggested that additive genetic effects are much larger than dominance genetic effects. In experiment 2, V_A and V_D were both significant, with V_D being estimated as the larger of the two. It is not surprising that there is little correspondence between estimates provided by the generation means analysis and Mather's variance method. As discussed in Hallauer and Miranda (1981), generation means analysis estimates the sum of the unsigned genetic effects, whereas variance components are the sums of squares of individual genetic effects. Since in both experiments the F_1 mean is much closer to the midparent than to the mean of susceptible parent, it can be concluded that V_D is caused predominantly by the balance of dominance in two directions. Therefore, some of the resistance genes in AR79-95 are at least partially dominant and others are at least partially recessive.

Narrow-sense heritability is low to moderate, suggesting that selection be delayed until advanced generations. Moreover, low heritability estimates should discourage the use of backcrossing for transfer of quantitatively inherited traits such as resistance to race 2 of *C. lagenarium* (Knott and Talukdar 1971). Barnes' recombination cross technique as described by Sitterly (1973) can be used to combine anthracnose resistance with resistance to other diseases.

Results support Goode's (1956) claim that the resistance in AR79-95 is quantitatively inherited and controlled by several genes. Methods one through four for estimating minimum numbers of effective factors are dependent upon the assumption that there is no dominance genetic variation. When, as is the case here, this assumption has not been met, the formulae underestimate the number of effective factors. However, the estimate given by method five is not affected by the presence of dominance, and the value of 8.6 obtained with this method is consistent with the other estimates by being a larger number. Whereas method five, unlike other methods, is not affected by the presence of dominance and uses all of the data (indirectly in the estimation of V_A) in experiment 2, we feel that this method provides the most reliable estimate of the minimum number of effective factors. Conservative estimates using method five (adding two standard errors to V_A) would reduce the number of effective factors to 5.1.

The resistance to race 2 derived from PI 197087 has been used widely in commercial varieties in the United States for about 30 years and can be accurately described as durable (Johnson 1981). The basis of this durability is not known. However, it is attractive to speculate that this resistance may be durable because the pathogen reproduces asexually and the resistance derived from PI 197087 is too complex for the pathogen to easily adapt to through mutation, somatic recombiantion, or heterokaryosis, We suggest that all varieties developed for the southern United States, even those used primarily in the spring when anthracnose is usually not a problem, should possess a high level of resistance to race 2 *C. lagenarium*, in order to prevent erosion of the effectiveness of this resistance.

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