Peanut Science (1979) 6, 51-54

Heritability of Cylindrocladium Black Rot Resistance in Peanut[1]

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ABSTRACT

In order to estimate heritability of resistance to Cylindrocladium black rot (CBR), caused by *Cylindrocladium crotalariae*, in peanuts (*Arachis hypogaea*), the F_1 F_2 and parental generations from a four-parent diallel cross were rated for resistance under optimum greenhouse conditions. General combining ability was significant for both generations suggesting that resistance to Cylindrocladium black rot in these lines was primarily due to additive genetic effects.

The four parents produced progeny having different levels of resistance. NC3033, Argentine and NC2 produced progeny from which resistant selections could be made, while Florigiant produced susceptible progenies in all crosses.

Estimates of heritability ranged from 0.48 to 0.65 depending on the method of calculation. Based on these estimates, early generation selection for CBR-resistance in the greenhouse should be effective.

Key Words: disease resistance, diallel cross, general combining ability, *Arachis hypogaea*.

Cylindrocladium black rot (CBR), caused by *Cylindrocladium crotalariae* (Loos) Bell and Sobers, was first reported in Georgia in 1966 as a peg, pod, and root rot of peanut, *Arachis hypogaea* L. (1). Microsclerotia (ms) are thought to be the primary source of inoculum and are responsible for spread of the fungus, however, other reproductive propagules also are capable of inciting the disease (7, 13). Since its first appearance in 1970, CBR has been a perennial problem in North Carolina (2). By 1973, 13 of the 17 peanut producing counties had reported incidences of CBR and intensive disease control efforts were begun (14).

Field and greenhouse evaluations of peanut germplasm have identified several cultivars with varying degrees of resistance (17). Of these, NC 3033 and Argentine were found to possess moderate to high levels of resistance, as characterized by Phipps and Beute (9) and Phipps, et al. (11). Approximately 50 ms of the fungus per gram of soil, or 100 times the amount for the susceptible cultivar Florigiant, are required before severe necrosis of the tap root and ultimate death of the plant occurs in either resistant line. Root infections of the resistant cultivars occur at lower inoculum densities, however, disease symptoms are not evident.

[1]Paper No. 5784 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, N. C.

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[3]Use of trade names in this publication does not imply endorsement by the N. C. Agricultural Research Service of the products named, nor criticism of similar ones not mentioned.

Knowledge of the inheritance of CBR resistance should allow the development of more effective breeding procedures. The purpose of this study is to determine the heritability of CBR resistance in the two most highly resistant and two widely grown but susceptible peanut cultivars.

Materials and Methods

The heritability of CBR resistance was studied by analyzing the progeny derived from a four-parent complete diallel cross. Parents were chosen such that two represented the maximum degree of CBR resistance available, and the remaining two were susceptible cultivars widely grown in North Carolina. In order to reduce the possibility of heterogeneity for CBR resistance within parent entries, each of the two resistant parent populations was screened for disease reaction. Ten seed lots of NC 3033 and three seed lots of Argentine, representing both single plant and bulk harvested progeny, were evaluated at an inoculum density of 65 ms per gram of soil according to the methods described by Phipps et al. (11). Single genotypes were selected, based in disease severity indices described by Rowe and Beute (12), for clonal propagation as diallel parents. Likewise, the two susceptible parent populations, Florigiant and NC 2, were evaluated for general plant quality and single genotypes were selected.

A diallel cross procedure, including reciprocals, was followed to produce F_1 families (3). Approximately 30 F_1 seed were produced for each of the 12 crosses. Ten seed from each F_1 family were then planted to produce F_2 families of between 50 and 100 progeny. All seed was produced in a greenhouse by growing parents and F_1's in 55 x 37 x 15 - cm wooden boxes filled with a 1:1 mixture of Norfolk sandy loam and peat moss. Land plaster ($CaSO_4$) was applied at an equivalent rate of 1344 kg/ha at flowering to ensure adequate pod development. Seed was harvested 90 days after flower initiation.

The 20 remaining F_1 and the 50 to 100 F_2 progeny from each of the 12 crosses, plus 20 selfed seed from each of the four parents, were evaluated for CBR resistance in the greenhouse. Infested soil was prepared by thoroughly mixing equal quanities of ms from five representative pathogenic isolates of *C. crotalariae* with Norfolk sandy loam soil from a peanut field having no known history of CBR. Microsclerotia were produced on potato dextrose agar (PDA) and were extracted by blending and washing through nested sieves (11). Sufficient ms were added to provide an inoculum density of 30 ms per gram in the infested soil. Replicate samples of the infested soil were quantitatively assayed for microsclerotial density and varified by the elutriation technique (10).

Prior to germination, seeds were treated with a 2:2:1 mixture of Botran 30% (2,6-dichloro-4-nitroaniline), Captan 30% (N[(trichloromethyl) thio] -4-cyclohexene-1,2-dicarboximide), and ethephon [(2-chloroethyl) phosphonic acid], respectively, to break dormancy and prevent damping-off of seedlings. Seed were placed in plastic trays filled with moistened vermiculite for three days, at which time 1-2 cm radicles had emerged. Seedlings were transplanted into 10 cm plastic pots containing the *C. crotalariae*-infested soil, and were arranged in a randomized complete block design. Soil temperatures were continuously monitored in all three replications by means of an electronic multipoint temperature recorder (Rustrac Model 166, Science Associate Inc., Princeton, N. J.), and were maintained at 25 ± 2°C. Soil moisture was ketp at or near field capacity by frequent watering.

Plants were grown for eight weeks, at which time the root systems were washed free of soil and rated for disease. A subjective root disease index was used which ranged from 0 (healthy root system

with no lesions) to 5 (completely rotted root system and dead plant) (12).

Data consisting of replicate full-sib family means of disease indices for both F_1 and F_2 generations were analyzed by a computer program (DIALL) developed by Shaeffer and Usanis (15). Variances were calculated by the general least squares method (5, 6) and the analysis is suitable for any diallel data set, balanced or unbalanced, or with missing observations.

Estimates of heritability were obtained by parent-offspring regression analysis and by variance comparisons involving segregating and non-segregating generations.

Results

Parents were screened for CBR resistance and individual plants selected to ensure homozygosity for resistance within genotypes. Seed derived from a number of single plants and bulk harvested lots were rated for disease development. Variances were calculated and compared (Table 1).

For both resistant parents, the variances among seed lots were not significantly different, indicating that loci conditioning resistance to CBR are probably homozygous and selfed progeny will not segregate for disease reaction. Also, the resistant parent populations appear to be homogeneous for CBR resistance, since all seed lots tested had nearly identical disease ratings. The single genotypes selected as resistant parents were highly resistant, and the resistances were maintained through succeeding generations of selfing.

Table 1. Variances associated with single plant and bulk harvested seed lots of resistant parents for reaction to *C. crotalariae*.

	Resistant Parent	
Seed Lot	NC 3033	Argentine
Single plant	0.34	0.17
Bulk harvested	0.37	0.06

Table 2. Mean disease ratings of parental lines for resistance to *C. crotalariae*.

Parent	Mean[a]
Argentine	2.21
NC 3033	2.13
NC 2	3.50
Florigiant	4.28
LSD (.05)	0.28

[a]Means are based on a subjective root disease index (o = healthy to 5 = completely rotted) and are weighted by the numbers of observations within each replicate.

The two resistant cultivars (Argentine and NC 3033) were significantly different from NC 2 and Florigiant (Table 2). NC 2 was intermediate, being significantly different from both the resistant parents and Florigiant. The overall full-sib family means, weighted by the number of observations within each replicate, were compared in diallel tables (Table 3). In F_1 combinations, the resistant parental cultivars Argentine and NC 3033 consistently produced more resistant progeny except when in combination with the susceptible parent Florigiant. This same trend occurred among F_2 progeny.

When data from F_1 and F_2 generations were analyzed by Griffing's Model I (3), only the variation due to general combining ability was significant (Table 4). All other sources of variation were not significant.

General combining ability effects were calculated for each of the four parents from the overall disease rating of full-sib families, according to the methods described by Griffing (3). The value or importance of a parent in hybrid combination for the measured character can be assessed by the relative magnitude

Table 3. Diallel table of F_1 and F_2 full-sib family means for disease reaction to *C. crotalariae*.

Female Parent	F_1				F_2			
	Argentine[1/]	NC3033	NC2	Florigiant	Argentine[a/]	NC3033	NC2	Florigiant
Argentine	----	2.53	2.57	3.39	----	2.78	2.34	3.01
NC 3033	3.09	----	2.73	3.27	2.62	----	2.46	2.88
NC 2	3.13	3.00	----	3.32	2.45	2.28	----	2.84
Florigiant	3.77	3.45	3.70	----	3.09	3.23	3.28	----

[1/] LSD (.05) = .0.69

[2/] LSD (.05) = 0.56

Table 4. Analysis of variance of F_1 and F_2 full-sib family means for disease reaction to *C. crotalariae*.

Source of Variation	d.f.	F_1 Mean Square	F_2 Mean Square
Mean	1	359.73	278.39
Replicates	2	0.31	0.53
General Combining Ability	3	1.28 **	0.95 **
Specific Combining Ability	2	0.04	0.08
Maternal	3	0.38	0.14
Reciprocal	3	0.09	0.04
Error	22	0.17	0.11

** Indicates significance at P=.01

of the estimated general combining ability effect (3, 16). The more negative the estimate, the greater the value of the parent for CBR resistance in breeding systems. In the F_1 analysis (Table 5), NC 3033, Argentine, and NC 2 all contributed to CBR resistance. The estimate of the general combining ability effect for Florigiant was positive, indicating that this parent contributed to CBR susceptibility, and was the least suitable for use in breeding for CBR resistance. The same trend occurred in estimates based on the F_2 data in which case NC 3033, Argentine, and NC 2 were ranked favorably as parents. Again, Florigiant was ranked the least resistant parent.

Heritability estimates were calculated from mid-parent values and overall full-sib family means for the analysis of F_1's on the midparents and for F_2's on the F_1's. Heritability, based on the regression of F_1 on mid-parent values, was 0.51, a moderate level. A slightly larger estimate, 0.65, was obtained when F_2 data were regressed on F_1 means.

Table 5. Estimates of general combining ability (GCA) effects for parents based on F_1 full-sib family means of disease reaction to *C. crotalariae*.

Parent	F_1 GCA[1/]	F_2 GCA[2/]
Argentine	-.1238	-.0850
NC 3033	-.2263	-.0950
NC 2	-.1313	-.2450
Florigiant	+.4813	+.4250

1/ Standard deviation = 0.29

2/ Standard deviation = 0.23

From the DIALL analyses of variance of both F_1 and F_2 generations, only the variation associated with general combining ability was found to be significant. Thus it can be assumed that additive genetic variance is probably most important for CBR resistance. Estimating heritability by assuming that the general combining ability variance is due to additive genetic variance gives a heritability estimate of 0.48.

Discussion

Analyses of combining ability for both the F_1 and the F_2 generations were very similar. Only variation due to general combining ability was significant, indicating that CBR resistance is primarily controlled by additive genetic effects. Since maternal and reciprocal effects were not significant, resistance to *C. crotalariae* in the peanut cultivars studied is governed solely by nuclear genes.

Estimates of general combining ability effects rank the parental cultivars for their effectiveness in contributing CBR resistance to hybrid progeny. Based on F_1 data, NC 3033 was the most resistant parent. Argentine and NC 2 also contributed CBR resistance to their progeny although to a lesser degree than NC 3033. Similar estimates of general combining ability effects were found for the F_2 generation. Argentine, NC 3033, and NC 2 should give progeny with higher levels of CBR resistance when intercrossed. Florigiant did not combine favorably with any of the parents, and it can not be used as a source of CBR resistance.

Changes in the environment have been shown to drastically influence CBR disease development in peanut (8). In the present study, parents and progeny were evaluated for CBR resistance in a greenhouse environment in which soil temperature and moisture were optimum for disease development and symptom expression. By comparison, heritability estimates derived from field evaluations will be deflated due to the great environmental variation. Consequently, estimates of heritability and their implications concerning breeding and selection techniques must be prefaces with the appropriate environmental circumstances.

In the present study, estimates of heritability ranged from 0.48 to 0.65, depending upon the method used for calculation. These values represent a moderate level of heritability and suggest that resistant progeny can be selected under greenhouse conditions.

A distinction must be made as to the selection environment. If progeny are evaluated in a greenhouse where soil temperature and moisture, and inoculum density can be controlled, selection of superior individuals could begin in the F_2 generation since the CBR resistance of progency can be measured. Because heritability declines as environmental variance increases and the probability of disease escape is larger, selection of F_2 plants in naturally infested field sites would be less effective.

Since it was determined that resistance to CBR was due to additive gene effects, genetically distinct sources of resistance could be combined producing genotypes having superior CBR resistance and the ability to buffer potential pathogen adaptation to that resistance (4). Evaluation of segregating generations prior to flowering would permit intercrossing of resistant individuals, thus allowing the use of recurrent selection

methods with only two generations per cycle. As resistant germplasm are located, they can be incorporated into the recurrent selection programs.

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Accepted January 24, 1979