Inheritance of Partial Resistance to Black Root Rot in Burley Tobacco

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ABSTRACT

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Two generation mean analyses were used to assess the inheritance of partial resistance to black root rot in burley tobacco. A cultivar moderately resistant to black root rot, KY 14, was crossed with a cultivar with low resistance, Burley 21, and with a susceptible cultivar, Judy's Pride. Five-week-old seedlings of the parental, F_1, F_2, and both backcross generations for each cross were transplanted into soil infested with 100 chlamydospores of *Thielaviopsis basicola* per gram of soil mixture and grown in the greenhouse at 21 C. Percent root necrosis was estimated 3 wk after transplanting. Dominance gene effects were significant and negative in the KY 14 × Burley 21 cross, whereas additive gene effects were significant in the KY 14 × Judy's Pride cross. Epistatic effects were significant in both crosses. Transgressive segregants were identified that may be used in a recurrent selection program to increase levels of resistance to black root rot in burley tobacco.

Additional keywords: *Nicotiana tabacum*

Black root rot, caused by *Thielaviopsis basicola* (Berk. & Broome) Ferraris, is a soilborne fungal pathogen found in all major tobacco (*Nicotiana tabacum* L.) growing regions of the world (11). Burley tobacco is grown in western Virginia and North Carolina where serious injury from black root rot can result from prolonged cool, wet weather after transplanting. Black root rot is the primary disease associated with tobacco stunting in the burley region of Virginia (18). Yield losses of 2.4% were attributed to this disease in North Carolina in 1988 (12). Yield losses of 5–7% have been reported for burley tobacco in the United States (9). Isolates of the black root rot fungus have been reported to vary widely in virulence and their ability to attack different hosts (1,11).

Clayton (1) described three types of black root rot resistance. The first type is immunity to black root rot found in *Nicotiana debneyi* Domin. This is controlled by a single dominant gene. Burley 49 was the first burley cultivar released with the *N. debneyi* resistance factor. Other burley cultivars, KY 15 (2), KY 17 (3), and TN 86 (16), that do not have the late maturity, low yields, and small leaf size characteristic of Burley 49 have been released. The second type of resistance is a high level of resistance found in the oriental tobacco introduction TI 89, which is thought to be controlled by a combination of dominant and recessive genes (1). Efforts to incorporate this resistance into a burley cultivar have failed because of tight linkage between resistance and small leaf size. Another oriental type tobacco introduction from the Near East, TI 87, has also been classified as highly resistant (10).

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The third type of resistance is a moderate level of resistance found in *N. tabacum* cultivars such as Harrow Velvet and Havana 211. The resistance in Harrow Velvet is reported to be controlled by a group of recessive genes (1). In addition, Nakamura et al (17) determined that three major genes were controlling resistance to black root rot in flue-cured tobacco cultivars such as Virginia Gold, Hicks Broadleaf, and Yellow Special.

Incorporation of disease resistance is a major objective in many tobacco breeding programs, and the mode of inheritance will affect the breeding strategy employed. Genetic control of partial resistance to black root rot in tobacco cultivars KY 14 and Burley 21 has not been previously determined. Therefore, the objective of this study was to investigate the inheritance of partial resistance to black root rot in these cultivars.

MATERIALS AND METHODS

Cultivar KY 14 was crossed with Burley 21 and Judy's Pride. The F_1, F_2, and backcross generations were produced for each cross. KY 14 (10) is a stand-up type cultivar with average yields of acceptable quality leaf. The original source of black root rot resistance in KY 14 came from a tobacco introduction, TI 87. Burley 21, also in the pedigree of KY 14, is thought to contribute to the level of black root rot resistance in KY 14 (5). Burley 21 (5) is an extreme stand-up type cultivar with average yields of acceptable quality leaf. The sources of black root rot resistance in Burley 21 include KY 16, KY 41A, and KY 56 (1). KY 14 and Burley 21 have a moderate and low level of black root rot resistance, respectively (5,10). Judy's Pride is an old cultivar highly susceptible to black root rot.

Experiments were conducted in the greenhouse during the winter of 1989–1990 at the Southern Piedmont Agricultural Experiment Station, Blackstone, VA. Parental, F_1, F_2, BC_1P_1 (parent 1 $\times$ F_1), and BC_1P_2 (parent 2 $\times$ F_1) generations for two crosses were grown in each experiment. The experimental design was a randomized complete block with three replications. The experiment was repeated four times. Each replication consisted of 10 tobacco seedlings each from the parental and F_1 generations, 50 seedlings from the F_2 generation, and 40 seedlings each from the BC_1P_1 and BC_1P_2 generations.

A virulent isolate of *T. basicola* was obtained from burley tobacco in Madison County, NC. The fungus was maintained on carrot agar (50 ml of carrot juice, 18 g of agar, and 950 ml of distilled water) in 9-cm petri dishes in the dark at room temperature. Inoculum was prepared by gently washing 3-wk-old plates with a stream of water from a squirt bottle to remove endoconidia. A rubber policeman was then used to scrape the agar surface to remove remaining spores and hyphae. The resulting spore suspension was rinsed into a Tyler Standard 500-mesh (25 μm) sieve and washed under a stream of water for approximately 5 min. This effectively removed the endoconidia, leaving only the chlamydospores and hyphae. The collected chlamydospores were washed off the sieve into a beaker, and the spore suspension was homogenized in a blender for 1 min and passed through the 500-mesh sieve again. The chlamydospores were then washed off the sieve and the spore suspension was calibrated with a hemacytometer.

Five-week-old tobacco seedlings were transplanted into 10-cm-square plastic pots containing a 1:1:1 (v/v/v) mixture of steam-sterilized soil, sand, and vermiculite. The soil mixture pH was adjusted with $Ca(OH)_2$ and ranged from 5.9 to 6.2 depending on the repetition of the experiment. Seedlings were fertilized every 6 days with 25 ml of Peters solution (W. R. Grace & Co., Fogelsville, PA) per pot. The pH of the Peters solution was 6.0. Each seedling was transplanted into the soil mixture infested with *T. basicola* at a rate of 100 chlamydospores per gram of soil mixture. Plants were rated for disease severity 3 wk after inoculation by estimating the percent of the root system with characteristic black lesions caused by *T. basicola* according to the Horsfall-Barratt rating scale (6) as follows: 0 = no symptoms, 1 = 0–3% of the root system with symptoms, 2 = 3–6, 3 = 6–12, 4 = 12–25, 5 = 25–50, 6 = 50–75, 7 = 75–88, 8 = 88–94, 9 = 94–97, 10 = 97–100, and 11 = 100% of the root system with symptoms.

A generation mean analysis procedure as outlined by Mather and Jinks (13) was used to assess the inheritance of partial resistance to black root rot. The variances of the six generation means in each cross were unequal; therefore, each generation mean was weighted by taking the reciprocal of the squared standard error of that mean. A three-parameter model (*m*, *a*, and *d*) was fitted and tested for goodness of fit by a chi-square test with three degrees of freedom. The three genetic parameters were defined as follows: *m* = the midparent value, *a* = the amount of variation among the means resulting from the additive effect of the genes, and *d* = the amount of variation among the means resulting from the dominance effect of the genes (4).

If a significant chi-square value was obtained (indicating a lack of fit to the three-parameter model), a six-parameter model (*m*, *a*, *d*, *aa*, *ad*, and *dd*) was fitted. The definition of the six genetic parameters are as follows: *m* = the mean of the inbred population, *a* and *d* as defined for the three parameter model, *aa* = the amount of variation among the means attributable to additive $\times$ additive epistasis, *ad* = the amount of variation among the means resulting from additive $\times$ dominance epistasis, and *dd* = the amount of variation among the means resulting from dominance $\times$ dominance epistasis (4). Standard errors of genetic estimates were obtained from variances of individual plant data after removal of replicate effects. Significance of the genetic estimates was determined by comparing estimated values with their standard errors. If the absolute value of an estimate exceeded twice its standard error, the estimate was considered significantly different from zero (4).

RESULTS

Each cross and related generations were analyzed by an analysis of variance for each run and combined analyses were conducted over runs. Significant differences were found among generation means for each cross in each run. When a combined analysis was made for each cross over runs, a significant difference between runs and a highly significant run by generation mean interaction were observed. A graphical presentation of the data showed this interaction to be the result of changes in magnitude between the generation means and not changes in rank; therefore, a combined analysis could be justified.

Generation means for each cross are presented in Table 1. Although KY 14 is generally considered to have a higher level of partial resistance than Burley 21, there was no significant difference (P = 0.05) in the disease severity rating for KY 14 and Burley 21 in three of the four runs of the current experiment. The F_1 of KY 14 $\times$ Burley 21 had a higher disease severity rating than Burley 21 in three of the four runs, although the differences were not significant. When the four runs were averaged, the F_1 was significantly more susceptible than either parent. The F_2 generation was generally more resistant than the F_1, but again the difference was not significant.

KY 14 always had a significantly lower disease severity rating than the susceptible cultivar Judy's Pride (Table 1). The F_1 of KY 14 $\times$ Judy's Pride was significantly different from either parent in three of the four runs. The F_2 generation was significantly more resistant than the F_1 in three runs. The backcross of the F_1 to the partially resistant parent (BC_1P_1) had a significantly lower disease severity rating than the backcross to the susceptible parent (BC_1P_2) in three of the four runs (Table 1).

The values of the genetic effects fitted to an additive-dominance (three-parameter) model for both crosses are presented in Table 2. The chi-square value was high enough to reject the three-parameter model in each run for each cross with the exception of run 3 in the KY 14 $\times$ Judy's Pride cross. Therefore, epistasis

(nonallelic interactions) appears to be important in the inheritance of partial resistance to black root rot in these cultivars.

Chi-square estimates for the six-parameter model for both crosses (Table 3) were all very low and nonsignificant, indicating a fit to the model. Only run 1 in the KY 14 × Burley 21 cross had significant additive genetic effects. Dominance genetic effects were significant for all runs in the KY 14 × Burley 21 cross. Most of the estimates for dominance genetic effects were negative. In runs 1 and 3 for the KY 14 × Burley 21 cross, all three interaction components were either significant or highly significant. Only the additive × additive effect was significant in run 2.

Additive genetic effects were significant for all runs in the KY 14 × Judy's Pride cross (Table 3). Runs 1 and 4 had significant dominance effects in the KY 14 × Judy's Pride cross. All three interaction components were either significant or highly significant in run 4 and when the runs were averaged for the KY 14 × Judy's Pride cross. In runs 1 and 2 for KY 14 × Judy's Pride cross, only the additive × additive effect was significant.

DISCUSSION

Development of resistant cultivars is imperative to efficient crop production. Incorporation of resistance from different sources is essential for developing stable cultivars that are not easily overcome by pathogens. Resistant cultivars are the primary method for control of black root rot in burley tobacco. When *T. basicola* is present in the soil, black root rot symptoms are likely to occur in nearly all burley cultivars grown today (15). Meyer et al (15) observed no black root rot symptoms on TN 86 in fields with high inoculum densities. Reported yield losses attributable to black root rot in the burley region may decrease slightly as the percent acreage planted to TN 86 increases. Nevertheless, resistance to black root rot in TN 86 is controlled by a single dominant gene that may or may not be durable. Development of cultivars with high levels of partial resistance is worthwhile in the event that new races of *T. basicola* appear capable of overcoming the dominant resistance gene currently being employed.

Additive gene effects were more important in the inheritance of partial resistance to black root rot in the KY 14 × Judy's Pride cross. Several studies have observed a predominance of additive gene action in burley tobacco for most agronomic characters (7,8,14). Nonadditive gene action and significant estimates for heterosis have been observed on occasion (8,14). In a more recent study using burley cultivars of diverse origin (19), nonadditive gene action and heterosis were indicated to play a greater role in the inheritance of agronomic characters than previously indicated.

Dominance gene effects for black root rot resistance were observed in the KY 14 × Burley 21 cross. Most of the estimates of dominance gene effects in this study were negative, indicating that

Table 1. Generation means for disease severity of black root rot on two burley tobacco crosses

Generation	Mean disease severity index[y]				
	Run 1	Run 2	Run 3	Run 4	Average
KY 14 × Burley 21					
KY 14	4.58 ab[z]	3.71 ab	7.37 ab	4.54 c	5.12 bc
BU 21	3.96 c	3.41 b	7.67 ab	5.11 a–c	5.10 bc
F_1	4.54 a–c	3.42 b	7.80 a	5.56 a	5.44 a
F_2	4.20 bc	4.04 a	7.60 ab	5.29 ab	5.32 ab
KY 14 × F_1	4.65 ab	3.59 ab	6.30 c	4.83 bc	4.90 c
Bu 21 × F_1	4.83 a	3.53 ab	7.01 b	4.66 bc	5.09 bc
KY 14 × Judy's Pride					
KY 14	4.65 c	4.73 c	6.23 d	3.11 d	4.72 d
Judy's Pride	7.52 a	8.31 a	8.34 a	7.41 a	7.90 a
F_1	5.48 b	6.32 b	8.20 a	4.62 b	6.17 b
F_2	4.07 d	5.31 c	7.51 bc	4.29 b	5.29 c
KY 14 × F_1	4.32 cd	4.78 c	7.34 c	3.56 cd	5.07 c
JP × F_1	5.57 b	6.54 b	7.98 ab	4.09 bc	6.07 b

[y]Disease severity was an estimate of the percent of the root system with characteristic black lesions caused by *Thielaviopsis basicola* according to the following rating scale: 0 = no symptoms, 1 = 0–3% of the root system with symptoms, 2 = 3–6, 3 = 6–12, 4 = 12–25, 5 = 25–50, 6 = 50–75, 7 = 75–88, 8 = 88–94, 9 = 94–97, 10 = 97–100, and 11 = 100% of the root system with symptoms.

[z]Any two means within a column, within a cross, having a letter in common are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Estimates of genetic effects for disease severity of black root rot on two burley tobacco crosses fitted to a three-parameter model

Cross and run	Parameter[x]			χ^2 value[y]
	m	*a*	*d*	
KY 14 × Burley 21				
Run 1	4.20**[z]	0.12	0.57*	23.30
Run 2	3.67**	0.09	−0.07	22.11
Run 3	7.35**	−0.37**	−0.46	47.37
Run 4	4.80**	−0.19	0.58	15.33
Average	5.06**	−0.07	0.19	25.59
KY 14 × Judy's Pride				
Run 1	4.63**	−1.34**	0.05	152.52
Run 2	5.70**	−1.63**	0.07	50.74
Run 3	7.24**	−0.88**	0.79**	5.23
Run 4	4.28**	−1.00**	−0.43	70.75
Average	5.64**	−1.18**	−0.12	108.38

[x]Mean, additive, and dominance genetic effects for the model $y = m + a + d$, where y equals the generation mean.

[y]For values larger than 7.81, the probability of a fit is less than 0.05.

[z]* And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

Table 3. Estimates of genetic effects for disease severity of black root rot on two burley tobacco crosses fitted to a six-parameter model

Cross and run	Parameter[x]						χ^2 value[y]
	m	*a*	*d*	*aa*	*ad*	*dd*	
KY 14 × Burley 21							
Run 1	2.10**[z]	0.31*	5.96**	2.17**	−0.97*	−3.51**	4.36
Run 2	5.46**	0.15	−3.65*	−1.89**	−0.18	−0.18	7.54
Run 3	11.31**	−0.15	−11.30**	−3.79**	−1.11*	7.79**	8.40
Run 4	7.02**	−0.29	−5.44**	−2.19**	0.93	3.98**	0.21
Average	6.41**	0.01	−3.38**	−1.30**	−0.40	2.40**	2.72
KY 14 × Judy's Pride							
Run 1	2.57**	−1.43**	3.08*	3.51**	0.37	−0.16	0.74
Run 2	5.10**	−1.79**	−0.40	1.42*	0.07	1.61	0.27
Run 3	6.67**	−1.06*	1.81	0.62	0.82	−0.28	0.21
Run 4	7.13**	−2.15**	−8.83**	−1.87**	3.25**	6.32**	3.25
Average	5.20**	−1.59**	−0.59	1.12**	1.17**	1.57**	0.93

[x]Mean, additive, dominance, additive × additive, additive × dominance, and dominance × dominance genetic effects for the model $y = m + a + d + aa + ad + dd$, where y equals the generation mean.

[y]Values are $\times 10^{-24}$.

[z]* And ** = estimate is larger than its standard error by a factor of 2 and 3, respectively.

alleles for susceptibility are dominant to alleles for resistance. Because the estimates of dominance gene effects (*d*) and dominance × dominance gene effects (*dd*) are of opposite sign, this type of interaction can be classified as the duplicate, dominant epistatic, or recessive suppressor kind (13). It is not possible to distinguish between these alternatives, and it refers only to the predominant type of interaction present in cases where different pairs of genes are showing different types of interactions.

Burley 21 and KY 14 may have some black root rot resistance gene(s) in common since Burley 21 is in the pedigree of KY 14. Three of the parents in Burley 21 (KY 16, KY 41A, and KY 56) each contributed to the level of black root rot resistance, and these were derived from various producer selections. Therefore, it is possible that more than one source of black root rot resistance is present in Burley 21. TI 87, which is not present in Burley 21, is reported to have contributed to the black root rot resistance found in KY 14. It is also possible that KY 14 possesses different resistance factors than those found in Burley 21. The presence of alternative factors could have contributed to the occurrence of epistatic effects and transgressive segregation observed in both crosses.

In conclusion, choice of a breeding strategy would depend on the type and magnitude of gene effects. If additive gene effects were more important in the inheritance of partial resistance to black root rot, such as in the KY 14 × Judy's Pride cross, a breeding program that leads to the development of desirable homozygous cultivars would be suggested. If dominance gene effects were more important in the inheritance of partial resistance to black root rot, such as in the KY 14 × Burley 21 cross, development of hybrids would be more appropriate. The presence of epistatic gene effects, combined with dominance for susceptibility, indicates the need for screening large numbers of progeny to identify transgressive segregants followed by a recurrent selection program. The combination of different sources of resistance could lead to enhanced levels of black root rot resistance that may be more stable to changes in the pathogen.

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