Genetics

Inheritance of Virulence and Uredial Color and Size in Puccinia recondita tritici

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ABSTRACT


Virulence, uredial length, width, area, and color, and chlorosis on wheat were recorded for each of 70 F2 Puccinia recondita tritici cultures from a cross between cultures 70-197 and 71-112. These variables were compared to the F1 and parents. P. recondita uredial size was normally distributed and did not fit discrete classes.  Uredial length appeared to be under genetic control. Significant correlations were found between virulence and length, and virulence and area, on wheat with genotype LrEG and between virulence and uredial length, and virulence and color on wheat with Lr23. No other significant correlations existed for the variables studied. Chi-square tests did not indicate linkage between virulence, color, uredial size, or chlorosis. Uredial color was apparently controlled by a single dominant gene that was independent of genes for virulence. Single recessive genes conditioned virulence on genotypes Lr1, Lr3, Lr11, Lr17, LrEG, and an experimental line 5534. The single recessive genes for virulence were inherited independently except for p3 and pEG and for p17 and pEG for which the chi-square tests indicated linkage. The possibility of intermediate infection types caused by cultures in the heterozygous condition is discussed.

Additional key words: virulence genes, linkage, wheat leaf rust.

Several studies on the inheritance of virulence of Puccinia recondita Rob. ex Desm. f. sp. tritici have been conducted (2,3,9,10,12,13). However, little research has been done to study factors other than virulence to develop markers to aid in more detailed genetic studies (2). Green (4) indicated that color mutants had been reported in several rust species. Some genes controlling color have been linked to virulent genes in P. recondita (2) and P. graminis (4).

Pustule size has been associated with slow rusting of wheat cultivars infected with P. recondita (7). The genetics of the fungus undoubtedly plays a role in determining pustule size, but inheritance of pustule size has not been adequately studied. Ohm and Shaner (6) reported a correlation between latent period and pustule size.

This study was initiated to study the inheritance of size and color of uredia, associated chlorosis on wheat leaves, and of virulence in P. recondita. The objective was to establish genetic markers for detailed genetic studies.

MATERIALS AND METHODS

Cultures 70-197 and 71-112 of P. recondita were purified by three sequential single pustule isolations. Purity was evaluated on isogenic lines and differentials (Table 1). The infection types are listed in Table 1.

Teliospores were produced by injecting urediospores of each culture into culms of moderately resistant plants in the boot stage. Teliospores were induced to germinate by alternate wetting and drying. After several such cycles the telia were suspended over meadow rue (Thalictrum speciosissimum Loebl.), the alternate host of P. recondita. Honeydew and pycniospores were transferred from a pycnium of culture 70-197 to a pycnium of culture 71-112. Aeciospores resulting from the cross were used to inoculate Little Club wheat, and purity of the resulting urediospore culture labeled X65 was evaluated on the lines listed in Table 1.

Teliospores of the cross X65 were produced, conditioned to germinate, and suspended over meadow rue. Sefling to produce F2 cultures was accomplished by separately transferring honeydew and pycniospores from one pycnium to another. Seventy cultures were developed from single aecial cultures resulting from selfing X65. These 70 cultures were used to inoculate isogenic lines and host cultivars listed in Table 1.

Inoculations and incubation were performed in moist chambers at 18.5 ± 2 C. Inoculated plants were held at approximately 100% relative humidity for 24 hr. After incubation, plants were returned to the greenhouse bench at 21 ± 4 C for the duration of the experiment.

The infection type expressed on each differential was classified on a standard scale (11) of 0-4 at 10-12 days after inoculation. Infection types 0 to 2 were classified as avirulent and types 3 and 4 as virulent (9). Chi-square tests were used to determine the
probabilities of the segregations fitting hypothetical ratios. Chi-
square tests for independence were used to determine if genes were
independently inherited. The recombination values were estimated
by the product method.

The length and width of each of four uredial pustules on each ofive leaves of Little Club wheat were measured with an American
Optical (Buffalo, NY 14215) binocular microscope at X60 and
expressed as millimeters. The variables analyzed were: length,
width, the width-to-length ratio, and the area. The area of the
uredia was calculated as: area = 3.14 (width/2) (length/2). A
Kolmogorov-Smirnov D statistic (1) was used to test for normality of
distribution of the above variables among the F2 cultures.

Variance of the F2 cultures was compared with that of the F1
and both parents.

Uredial color was visually compared to a Grumbacher color
wheel (Grumbacher Inc., 460 W. 34th St., New York, NY 10001)
and recorded as red-orange or orange. Color and size data for each
culture were combined with virulence data for the same culture and
and the resulting set was analyzed by correlations according to the
PROC CORR procedure of the SAS (Statistical Analysis Systems)
computer program (1).

RESULTS

Data from pustule measurement variables did not fit discrete
classes. The normality test indicated that these data were
continuous and normally distributed (P > 0.05). The frequency
distributions for length, width, and areas of uredial pustules caused
by F. recondita are in Tables 2, 3, and 4, respectively. The fact that
the F2 frequency distribution transgresses both parents by several
classes suggests that the F2 population contains a number of unique
gene recombinations not found in either parent. The variance for
pustule length of the F2 (0.0313) compared to that of the parents
(0.0118 and 0.0080) indicated that pustule length may be under
genetic control. The variance for pustule length of the F1 was
0.0240. The fact that the variance for pustule length of the F1
was larger than that of the parents does not support the data indicating
that pustule length is under genetic control. The variances of
pustule widths, width-to-length ratios, and pustule areas of the
parents, F1 cultures, and F2 cultures suggested that width may not
be under genetic control; either veins restrict width or there is little
variation in width.

Means of pustule width, length, width/length ratio, and area of
F2 cultures were analyzed for correlation with color, chlorosis, and
virulence for each single gene line. Significant correlations (P
= 0.05) were observed between virulence and pustule length, and
virulence and area on host genotype LrEG and between virulence
and pustule color and virulence on host genotype Lr23.

No significant correlations were found between virulence and the
above variables on any of the other differentials. A weak
correlation (r = 0.15) was observed between chlorosis and width
but none between chlorosis and length. However, chi-square tests
indicated that uredial size and color were independent of virulence.

TABLE 1. Segregation of F2 cultures derived from a cross (X65) of Puccinia recondita f. sp. tritici cultures 70-197 and 71-112.

<table>
<thead>
<tr>
<th>Host isogenic line or cultivar</th>
<th>Infection types of cultures</th>
<th>Number of F2 cultures with infection type or pathogenicity</th>
<th>Avirulent</th>
<th>Virulent</th>
<th>Expected ratio</th>
<th>Goodness of fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lr 1</td>
<td></td>
<td></td>
<td>0 to 1; 0; 0/2; 1 to 2; 1</td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
</tr>
<tr>
<td>Lr 2a</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 2c</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 3</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 3a</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 9</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 10</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 11</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 16</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 17</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 19</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 21</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 23</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr 24</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr T</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Lr EG</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>5534</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Transec</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Olaf</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
<tr>
<td>Thatcher</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>16 44 55 60 70 80 90 100</td>
<td>P &gt; 0.50</td>
</tr>
</tbody>
</table>

*Avirulent = 0 to 2. Virulent = 3 to 4.

*H = homozygous virulent. HA = homozygous avirulent.

TABLE 2. Frequency distributions for lengths of uredia of Puccinia recondita f. sp. tritici

<table>
<thead>
<tr>
<th>Generation</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>0.8</th>
<th>0.9</th>
<th>1.0</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>1.4</th>
<th>1.5</th>
<th>1.6</th>
<th>1.7</th>
<th>1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent 1</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent 2</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>F2</td>
<td>2</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Upper class limits in millimeters.

TABLE 3. Frequency distribution for widths of uredia of Puccinia recondita f. sp. tritici

<table>
<thead>
<tr>
<th>Generation</th>
<th>0.20</th>
<th>0.25</th>
<th>0.30</th>
<th>0.35</th>
<th>0.40</th>
<th>0.45</th>
<th>0.50</th>
<th>0.55</th>
<th>0.60</th>
<th>0.65</th>
<th>0.70</th>
<th>0.75</th>
<th>0.80</th>
<th>0.85</th>
<th>0.90</th>
<th>0.95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent 1</td>
<td>11</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parent 2</td>
<td>9</td>
<td>10</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>28</td>
<td>78</td>
<td>262</td>
<td>366</td>
<td>349</td>
<td>52</td>
<td>87</td>
<td>77</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>1</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*Upper class limits in millimeters.
Parental culture 71-112 had orange uredia. Parental culture 70-197 and the F1 culture X65 had red-orange uredia. There were 44 red-orange and 22 orange F2 cultures that fit a 3:1 model for a single gene conditioning color (P > 0.10). The observed F2 ratios and the uredia color of the F1 indicated that red-orange color was dominant. Chi-square tests indicated that uredial color (orange or red-orange) was independent of virulence in the cultures segregating for virulence.

There were 18 cultures with chlorosis and 50 with no chlorosis on Little Club wheat. This distribution fit a 1:3 ratio for a single recessive gene conditioning chlorosis (P > 0.50). Neither parent nor the F1 displayed chlorosis on Little Club.

The F1 culture (X65) and all of the F2 progenies were avirulent on lines with genotypes Lr3ka, Lr9, Lr16, Lr19, Lr21, Lr24, LrT, and cultivar Transc. The failure to observe virulent segregants among the F2 cultures indicated that culture X65 was homozygous avirulent on these cultivars. A wide range of low infection types (type 0-2) was observed in the F2 cultures, especially when the infection types on the parents were a 1 or 2.

The parental cultures, the F1 culture and all of the F2 cultures were virulent on lines with genotypes Lr2a, Lr2c, and Lr10. The failure to observe avirulent segregants on the lines with these genes indicated that the parental cultures and culture X65 are homozygous virulent on lines with genotypes Lr2a, Lr2c, and Lr10. Segregation for virulence occurred among the F2 cultures on genotypes Lr1, Lr3, Lr11, Lr17, Lr23, LrEG, and experimental line 5534. One parent was virulent, the other avirulent, and the F1 avirulent on each of these lines in every case except for genotype LrEG in which case both parents were avirulent and the F1 avirulent. Culture 70-197 and the F1 was presumably heterozygous for virulence on LrEG.

The F2 cultures segregated approximately three avirulent to one virulent on genotypes Lr1, Lr3, Lr11, Lr17, Lr23, LrEG, and experimental line 5534 when the ratings were grouped as avirulent (infection type 0 through 2) or virulent (infection type 3 or 4). Since the P values were all > 0.05, single recessive genes for virulence were indicated (Table 1). Single recessive genes for virulence p1, p11, and p12 have been previously reported (3, 9).

Most of the single recessive genes for virulence were inherited independently, but associations were found between p1 and pEG (P < 0.025) and between p11 and pEG (P < 0.005). The recombination estimate was 22 ± 11% for p1 and pEG and 37 ± 11% for p11 and p12. Linkage was not indicated between p1 and p11 (P > 0.20).

Segregation on Olaf fit a ratio of one avirulent to 15 virulent and indicated segregation for two dominant genes (P > 0.05) for virulence. Dominant genes for virulence are not common in P. recondita, but they have been reported (9, 10, 12).

When infection types 1 and 2 are classified as intermediate, segregation on genotypes Lr11, Lr17, Lr23, and 5534 provided a good fit to a ratio of one avirulent to two intermediate to one virulent (P values all > 0.05). This 1:2:1 ratio could be explained by incomplete dominance in which the heterozygous cultures produced an intermediate reaction. The F1 cultures had intermediate virulence on genotypes Lr11, Lr17, Lr23, and 5534. The F1 culture produced intermediate reactions on genotypes Lr3 and LrEG, but the F2 segregation ratios would not fit a 1:2:1 ratio because too few intermediate infection types were observed.

When the infection types 1 and 2 were classified as intermediate and 3 and 4 as highly virulent, observed F2 segregation on genotypes Lr3ka, Lr21, and LrT fit the model of three intermediate to one highly avirulent (Lr3ka, P > 0.25; Lr21, P > 0.10; and LrT P > 0.10). This observation indicated segregation of a single recessive gene conditioning avirulent infection types. Parental cultures apparently did not differ for a gene conditioning intermediate infection types. Segregation on Transc fit a three avirulent to one intermediate ratio (P > 0.50).

**DISCUSSION**

Pustule size of P. recondita is important because it has been correlated with slow rusting (7). Large pustules obviously provide more inoculum than do small ones. Latent period has also been
correlated with pustule size (6). Variance data indicated that pustule length, but not width or area, showed genetic variability.

Previous workers have reported an association between color and pathogenicity of *P. recondita* (5). However, data from this study did not demonstrate linkage or correlation between color and virulence except for a correlation between color and virulence on host genotype Lr23. Segregation ratios of red-orange to orange uredia fit a model for a single dominant gene conditioning red-orange uredia in this study. Green (4), working with *P. graminis*, also reported a single dominant gene for virulence, which was linked to the gene for normal spore color. In 1949, Johnson (5) reported that red uredospore color was dominant to orange in *P. graminis avenae*.

Chlorosis was observed on 18 of 68 cultures on Little Club. A single recessive gene may condition chlorosis. On the other hand, variation in chlorosis could be caused by variation in light, temperature, or some other environmental factor. Further studies are necessary to determine the inheritance of chlorosis of wheat infected by *P. recondita*, and to clarify the lack of variability between the parents.

Single recessive genes apparently condition virulence on genotypes Lr1, Lr3, Lr11, Lr17, Lr23, LrEG, and experimental cultivar 5534. These single recessive genes for virulence were inherited independently except for *p* and *pEG* and *p* and *pEG*.

In some cases, a wide range of infection types was observed (Table 1). Samborski (8) proposed that exceptions to the ideal situation, where only two infection types are found, may occur as a result of effects of temperature, nonallelic interaction, heterozygosity, and the possibility of alleles for virulence. He (8) further proposed that the simplest explanation is that the culture is heterozygous for virulence, and that virulence is incompletely dominant. Incomplete dominance is not common in *P. recondita*.

Incomplete dominance could be used to explain the one avirulent (ratings 0 to 0;1) to two intermediate (ratings 1 to 2) to one virulent (ratings 3 to 4) infection types for Lr11, Lr17, Lr23, and 5534. Segregation on Lr3ka, Lr21, and LrT would fit a model of three intermediate to one avirulent. This may indicate a single recessive gene conditioning avirulent infection types over intermediate infection types. A more logical explanation is that the culture was heterozygous for intermediate infection types.

The ratio of three highly avirulent to one intermediate on Transcend is difficult to explain since both parents of X65 were avirulent (infection types 0;1 and 0). The infection type of X65 was intermediate. If genes in the heterozygous condition produce the intermediate response, one parent should be intermediate. Culture 70-197 on Transcend could have been misclassified as a 0;1 instead of a 1. The results for Transcend suggest that the separation of the resistant types into classes may be arbitrary and hence not readily explained by the genetic hypotheses. The range in infection types could be attributed to gene action influenced by modifiers in the genetic backgrounds of the host and pathogen as previously theorized (10).

**LITERATURE CITED**