Collections of Blast Fungus from Resistant Rice Varieties and Their Frequencies of Races and Virulence Genes

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Abstract

Data on host-pathogen relationship of rice (Oryza sativa) and blast (Pyricularia oryzae) obtained in Niigata Prefecture, Japan were analyzed by virulence analysis, and analysis of distribution pattern of virulence gene numbers. The percent growing areas (frequencies) of rice varieties with various genotypes for blast resistance were obtained from statistics data, and frequencies of blast-fungus genotypes for virulence were calculated from the data from 1976 to 1986 in six areas in Niigata Prefecture. The results of virulence analysis showed that nonrandom association was found between two virulence genes, Av-a+ and Av-i+. Nonrandom associations of virulence genes were also detected by analysis of the distribution pattern of virulence gene numbers. The deviation of distribution pattern from that expected from random association of virulence genes was mostly explained by specific interaction of virulence genes, Av-a+ and Av-i+.

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Key words: virulence analysis, virulence gene distribution analysis, rice, blast, nonrandom association.

INTRODUCTION

Gene analysis has progressed for resistance of rice plants to blast disease13, and determination of genotypes for resistance and virulence of almost all rice varieties cultivated and fungal isolates collected, respectively, is possible in Japan10.

The studies on race distribution of blast fungus in Japan15) were advanced by the choice of a set of differential varieties by Goto1,2) and Kozaka11), and of new sets of differential varieties with nine and twelve single resistance genes by Yamada et al.16) and Kiyosawa7), respectively. A decrease in the number of researchers in the epidemiological field, however, brought a decrease in studies in the field. Recently, studies on the race distribution have been continued only in a few prefectures. It is very important to devise effective analytical methods to put the data in the past to practical use and to obtain new data in the future.

Three methods for analyzing the behavior of pathogen population in the field have been proposed: the virulence analysis by Wolfe et al.13,14) and two methods by Roelfs and Groth12).

Wolfe's virulence analysis13,14), in which deviation of observed frequencies of genotypes, for example, having two virulence genes from the product of frequencies of individual genes expected in random association are tested, was applied to Japanese race frequency by Kiyosawa4). Significant differences between expected and observed frequencies in various genotypes for virulence were found during the period from 1964 to 1966 in Niigata, Toyama, Yamagata and Kanagawa Prefectures.

A comparison of distribution of the numbers of virulence genes in pathogen isolates was carried out by Roelfs and Groth12), and a mean of deviations of observed virulence-genotype frequencies from
expected ones for each concerning virulence gene paired with other genes was calculated by Roelfs and Groth\textsuperscript{12}).

Kiyosawa\textsuperscript{5}) compared these three methods. One of those proposed by Roelfs and Groth\textsuperscript{12}, the comparison of distribution of the numbers of virulence genes in pathogen isolates, was the most sensitive to detect the deviation from the random association of virulence genes. Wolfe's virulence analysis\textsuperscript{13,14} is, however, evaluated to be a method useful for simply detecting an interaction between some genes in the pathogen.

In this paper, we will report yearly change of frequencies of genes and genotypes in host and pathogen, and the results of virulence analysis and analysis on distribution of the numbers of virulence genes in a propagule.

**MATERIALS AND METHODS**

Niigata Prefecture consists of six areas, *i.e.*, Joetsu, Chuetsu, Uonuma, Niigata, Kaetsu and Sado (Fig. 1).
Leaves or ears of rice plants infected by blast fungus were sampled over eleven years from 1976 to 1986 in fields on intersecting points of a lattice drawn on a map of Niigata Prefecture as shown in Fig. 1. Single spores were picked up from leaves using agar pieces of arrowhead type under a microscope. The pathogenicity tests of single-spore isolates of the fungus were performed using the Japanese differential varieties\(^{16}\). Isolates were classified into 16 genotypes shown in Table 1.

Table 1. Host and pathogen genotypes and corresponding pathogen races

<table>
<thead>
<tr>
<th>Code number</th>
<th>Host genotype</th>
<th>Pathogen genotype</th>
<th>Race(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A I K Z</td>
<td>a i k z</td>
<td>001, 101</td>
</tr>
<tr>
<td>2</td>
<td>A I K +</td>
<td>a i k +</td>
<td>041</td>
</tr>
<tr>
<td>3</td>
<td>A I + Z</td>
<td>a i + z</td>
<td>031</td>
</tr>
<tr>
<td>4</td>
<td>A + K Z</td>
<td>a + k z</td>
<td>005</td>
</tr>
<tr>
<td>5</td>
<td>+ I K Z</td>
<td>+ i k z</td>
<td>003</td>
</tr>
<tr>
<td>6</td>
<td>A I + +</td>
<td>a i + +</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>A + K +</td>
<td>a + k +</td>
<td>045</td>
</tr>
<tr>
<td>8</td>
<td>+ I K +</td>
<td>+ i k +</td>
<td>043</td>
</tr>
<tr>
<td>9</td>
<td>A + + Z</td>
<td>a + + z</td>
<td>035</td>
</tr>
<tr>
<td>10</td>
<td>+ I + Z</td>
<td>+ i + z</td>
<td>013, 033</td>
</tr>
<tr>
<td>11</td>
<td>+ + K Z</td>
<td>+ + k z</td>
<td>007</td>
</tr>
<tr>
<td>12</td>
<td>A + + +</td>
<td>a + + +</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>+ I + +</td>
<td>+ i + +</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>+ + K +</td>
<td>+ + k +</td>
<td>047</td>
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<td>15</td>
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</tr>
<tr>
<td>16</td>
<td>+ + + +</td>
<td>+ + + +</td>
<td></td>
</tr>
</tbody>
</table>

\(A + K +\) means \(P_i-a P_i-i^* P_i-k P_i-z^*\) genotype.
\(a + k +\) means \(A_i-a A_i-i^* A_i-k A_i-z^*\) genotype.

\(a\) Race code No. corresponding to the pathogen genotypes listed here.

Fig. 2. Yearly change of observed genotype frequencies for host resistance (bottom) and pathogen virulence (upper) during the eleven years from 1976 to 1986 in Niigata Prefecture. The numerals (left- and right-sides) in the figure are the code numbers of host and pathogen genotypes described in Table 1, respectively.
For virulence analysis\textsuperscript{13,14}, observed frequencies of four virulence genes (Av-a\textsuperscript{+}, Av-i\textsuperscript{+}, Av-k\textsuperscript{+} and Av-z\textsuperscript{+}) were calculated throughout the whole prefecture and individual areas. Frequencies expected from random association of two given avirulence loci (for example a and b) were calculated for four genotypes (ab, ab\textsuperscript{+}, a'b and a'\textsuperscript{+}b'). Deviation of the expected frequencies from the observed frequencies was statistically tested by chi square test to detect the presence of nonrandom association.

The number of virulence genes in each fungal isolate was calculated from the same data and analysed by the method of Roelfs and Groth\textsuperscript{12} on the deviation of distribution of virulence gene numbers expected from their random association from observed distribution of virulence gene numbers.

**RESULTS**

**Change of gene and genotype frequencies of rice plant and blast fungus**

The changes of genotype frequencies of rice plant and blast fungus in Niigata Prefecture were observed as shown in Figs. 2 (whole prefecture), 3 and 4 (six areas). The frequencies of resistance genes and virulence genes were calculated (Fig. 5). The frequency of the gene, Pi-i, decreased, and Pi-a and Pi-k frequencies tend to slightly decrease during these eleven years. The gene Pi-z began to increase in 1980.

As for the fungus, virulence genes, Av-a\textsuperscript{+}, Av-i\textsuperscript{+} and Av-k\textsuperscript{+}, continued to decrease. The gene Av-z\textsuperscript{+} continued to increase corresponding to the increase of Pi-z.

**Virulence analysis**

The significance in the deviation of the observed frequencies from the frequencies expected from
random association between two virulence genes was obtained only in years and areas in which sufficient number of isolates was collected, as shown in Table 2. When the data of the whole prefecture were examined, nonrandom associations were found between virulence genes, $Av-a^+$ and $Av-i^+$, in all the years tested from 1976 to 1986, but were not found in five other combinations of avirulence loci in most years tested. Some gene combinations could not be tested because of its unacceptably low frequency of the virulence gene(s) concerned (Table 3).

**Test of nonrandom association by a method of Roelfs and Groth**

The observed distribution of the numbers of virulence genes in isolates was compared with that expected from random association of virulence genes by the method (chi square method) of Roelfs and Groth\(^1\)\(^2\) on the data of Niigata Prefecture. The results are shown in Fig. 6. During the eleven years tested, significant differences are always observed between observed and expected distributions. Characteristics of the deviation from the expected are as follows. 1) Over the eleven years, the average of the numbers of virulence genes in isolates reduced from 2. 2) During this period, the distribution of virulence genes showed bimodal pattern.

Under the consideration of the nonrandom association detected by the virulence gene distribution test by Roelfs and Groth\(^1\)\(^2\), a distribution under the occurrence of random association between $Av-a^+$ and $Av-i^+$ was made calculating the expected number of the genotype, $Av-a Av-i^+$, by the following equation.

\[
\text{No. of } Av-a Av-i^+ = \text{no. of } Av-a Av-i \times (\text{no. of } Av-a Av-i^+ / \text{no. of } Av-a Av-i)
\]
Fig. 5. Yearly change of observed frequencies of four resistance genes (left), *Pi-a*, *Pi-i*, *Pi-k* and *Pi-z*, and corresponding virulence genes (right), *Av-a+*, *Av-i+*, *Av-k+* and *Av-z+*, during the eleven years from 1976 to 1986 in Niigata Prefecture.

Table 2. Chi square values for comparison between observed frequencies and expected frequencies for random association of virulence genes, *Av-a+* and *Av-i+*, in six areas and all of Niigata Prefecture

<table>
<thead>
<tr>
<th>Area</th>
<th>1976</th>
<th>'77</th>
<th>'78</th>
<th>'79</th>
<th>'80</th>
<th>'81</th>
<th>'82</th>
<th>'83</th>
<th>'84</th>
<th>'85</th>
<th>'86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joetsu</td>
<td>1.4</td>
<td>9.9*</td>
<td>9.6*</td>
<td>22.6**</td>
<td>16.1**</td>
<td>12.3**</td>
<td>2.0</td>
<td>10.6*</td>
<td>10.8*</td>
<td>20.3**</td>
<td>27.9**</td>
</tr>
<tr>
<td>Chuetsu</td>
<td>1.8</td>
<td>8.0*</td>
<td>9.2*</td>
<td>11.4**</td>
<td>12.5**</td>
<td>11.8**</td>
<td>7.5</td>
<td>8.8*</td>
<td>10.2*</td>
<td>6.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Uonuma</td>
<td>0.2</td>
<td>—</td>
<td>1.8</td>
<td>9.3*</td>
<td>8.6*</td>
<td>8.6*</td>
<td>15.1**</td>
<td>12.5**</td>
<td>12.9**</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Niigata</td>
<td>—</td>
<td>—</td>
<td>5.3</td>
<td>1.6</td>
<td>7.8**</td>
<td>6.8</td>
<td>7.0</td>
<td>7.1</td>
<td>0.6</td>
<td>2.9</td>
<td></td>
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<tr>
<td>Kaetsu</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
<td>—</td>
<td>7.5</td>
<td>5.0</td>
<td>4.2</td>
<td>2.3</td>
<td>5.2</td>
<td></td>
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<tr>
<td>Sado</td>
<td>—</td>
<td>0.1</td>
<td>5.6</td>
<td>0.5</td>
<td>0.2</td>
<td>0.4</td>
<td>—</td>
<td>0.7</td>
<td>0.4</td>
<td>—</td>
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<tr>
<td>Niigata Pref.</td>
<td>9.3*</td>
<td>27.8**</td>
<td>41.8**</td>
<td>52.4**</td>
<td>37.6**</td>
<td>52.8**</td>
<td>37.3**</td>
<td>43.3**</td>
<td>54.1**</td>
<td>51.9**</td>
<td>52.3**</td>
</tr>
</tbody>
</table>

a) * and ** indicate that the observed frequencies are different from the expected ones at the 5% and 1% levels, respectively.

b) — indicates that the chi square value could not be calculated, because of the small number of isolates collected.

As a result, chi square values decrease from 35.52 on average of eleven years to 4.55, indicating that the nonrandom association obtained by virulence gene distribution analysis can sufficiently be explained by the nonrandom association by virulence analysis (Fig. 6).
Table 3. Chi square values for comparison between the observed frequencies and the expected frequencies in random association of virulence genes in all six possible combinations of two avirulence loci in all of Niigata Prefecture

<table>
<thead>
<tr>
<th>Combination</th>
<th>1976</th>
<th>77</th>
<th>78</th>
<th>79</th>
<th>80</th>
<th>81</th>
<th>82</th>
<th>83</th>
<th>84</th>
<th>85</th>
<th>86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av-a and Av-i</td>
<td>9.3*</td>
<td>27.8**</td>
<td>41.8**</td>
<td>52.4**</td>
<td>37.6**</td>
<td>52.8**</td>
<td>37.3**</td>
<td>43.3**</td>
<td>54.0**</td>
<td>51.9**</td>
<td>52.3**</td>
</tr>
<tr>
<td>Av-a and Av-k</td>
<td>7.9*</td>
<td>3.1</td>
<td>1.1</td>
<td>6.1</td>
<td>0.0</td>
<td>0.3</td>
<td>0.5</td>
<td>1.0</td>
<td>1.4</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Av-i and Av-k</td>
<td>9.0*</td>
<td>3.8</td>
<td>1.3</td>
<td>3.0</td>
<td>0.0</td>
<td>1.6</td>
<td>1.7</td>
<td>0.0</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Av-a and Av-z</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>0.0</td>
<td>0.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Av-i and Av-z</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>9.8</td>
</tr>
<tr>
<td>Av-k and Av-z</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>- &amp;</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Fig. 6. Yearly difference of the number of virulence genes in isolates in Niigata Prefecture. Values on chi square tests between observed and expected (from random association) distributions are shown on upper two lines in individual figures, with year at right hand bottom: from left, chi square value, the degree of freedom and the number of isolates. In the 1st line, observed distribution was compared with the expected distribution, and in the 2nd line, observed distribution corrected under the assumption of random association between two genes, Av-a+ and Av-i+, was compared with the expected distribution (*: corrected parts by random association).

DISCUSSION

Nonrandom association: It was found that the frequency of Av-a Av-i+ genotype (2/1308) was very low in comparison with that of Av-a+ Av-i+ genotype (587/1308) through eleven years in Niigata Prefecture. From this fact, it was presumed that the nonrandom association was present between two virulence genes, Av-a+ and Av-i+. Low frequency of Av-a Av-i+ genotype was found in some past investigations11,16). The nonrandom associations between Av-a+ and Av-i+ were shown in Chiba, Niigata and Ishikawa Prefectures in 1976 and in Niigata, Ishikawa and Nagano Prefectures in 19806). This interaction of the two virulence loci was considered to be a general tendency in Japan.

The constantly low frequency of the genotype Av-a Av-i+ indicates that the selection against this genotype (specific interaction) functioned at least at or before the first year of this studies. However, it is not clear whether the function of such specific interaction was temporal or continual during the eleven years.

Kiyosawa4) considered the following as causes for nonrandom association.

a) Directional selection: growing of resistant varieties stimulates the increase of pathogen genotypes
virulent to the resistant varieties.
b) Stabilizing selection: the difference of fitness between fungus strains leads to an increase of fungus strain with higher fitness, resulting in stabilizing selection.
c) Random genetic drift: when the amount of fungus overwintering or oversummering is small, the genotype frequencies are determined by survival by chance.
d) Invasion of fungus from outside sources.
e) Difference due to varieties from which the fungus was isolated: only isolates virulent to the varieties were collected.
f) Differences due to places where the fungus was isolated: distribution of pathogen genotypes are different due to genotypes of varieties in surrounding fields, if they have specific resistance genes.
g) Differences due to the season when the fungus was isolated: seasonal variation of pathogen genotype frequency was often found.
h) Sampling error.
i) Change of genotype frequencies due to environmental conditions.
j) Error in the test for pathogenicity: the use of unsuitable differential varieties and unskilled operation lead to incorrect results.

The following cause must be added to the causes of nonrandom association from the assumptions described above.
k) Change of frequency of a genotype due to a specific interaction of two genes in the pathogen.

It is difficult to specify the cause of nonrandom associations that are found. However, a specific interaction between specific genes, the k-th cause, may also be related to nonrandom association found in this study.

The tests of nonrandom association by a method of Roelfs and Groth12) (analysis of distribution pattern of virulence gene numbers) were always significant in Niigata Prefecture, indicating that nonrandom association between virulence genes occurs. The mean numbers of virulence genes in isolates showed a decrease from 1.7 to 0.9. During this change, the pattern of distribution changed from one peak (unimodal) to two peaks (bimodal). This nonrandom association found in the virulence gene analysis of Roelfs and Groth12) was also considered to be explained at least partially by the nonrandom association of Av-a+ and Av-i+ in virulence analysis. Therefore, to test this possibility we drew virulence gene distribution under an assumption of random association of Av-a+ and Av-i+, indicating that nonrandom association of virulence analysis can sufficiently explain the nonrandom association in virulence gene distribution. Bimodal distributions of the number of virulence genes were first reported in asexual populations of rust by Roelfs and Groth12). Later, Kiyosawa8) found bimodal distributions in some prefectures in Japan.

Previously Kiyosawa9) indicated that the increase of fungal propagules without virulence genes by stabilizing selection and the increase of propagules with many virulence genes by directional selection bring a bimodal distribution of virulence genes in propagules. The interaction between virulence genes must be added as a cause of bimodal distribution of numbers of virulence genes in propagules.

We thank Dr. T. Hino, former Director of the National Institute of Agrobiological Resources, for his valuable suggestions in writing this manuscript.

Literature cited


和文摘要

清沢茂久・藤巻隆一・岩野正敬：抵抗性イネ品種からのいもち病菌の採取とそのレースと病原性遺伝子頻度

新潟県を6地区に分け、それぞれの1976年度から1986年の11年間のレース頻度からそれぞれのイネいもち病菌の病原性遺伝子(Av-a+, Av-i+, Av-k+, Av-z+)の頻度を求め、それからWolfeらの提唱した病原性分析の方法で、4つの病原性遺伝子の内の任意の2つの遺伝子組の組合せ(遺伝子型)の実際の頻度と、それぞれの組から病原性遺伝子がお互いに独立に突然変異により生じ、その後頻度の変化がなかったと仮定したときの期待遺伝子型頻度との乖離を検定した。その結果、ほとんどの年に計算が可能であった2地区で、Av-a Av-i+遺伝子型の頻度が期待値より低いこと(nonrandom association)が明らかにされた。また、同一データから、採取菌株内の病原性遺伝子が独立に生じたと考えた時の期待病原性遺伝子数の分布と、実際の分布(観察病原性遺伝子数の分布)を比較した。その結果、nonrandom associationが起こっており、病原性遺伝子数で見られたnonrandom associationは病原性分析でみられたnonrandom associationと同一原因によるものと推定された。