Components of Partial Resistance to Southern Corn Leaf Blight Caused by *Bipolaris maydis* Race O in Six Corn Inbred Lines

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Abstract

Components of partial resistance to southern corn leaf blight caused by *Bipolaris maydis* race O were analyzed using young plants of six corn inbred lines (H95rhm, R2040, Mo17Ht, H95, H93, Pa91) and compared with the progression of the disease in the field. In the greenhouse seedling tests, the infection efficiency (IE) did not vary among the inbred lines. Lesion area (LA), sporulating lesion ratio (SLR) and degree of sporulation (SP) were differed significantly among them and the resistant lines showed low values of LA, SLR, and SP. The LA values of each line corresponded to the disease progression in the field. The inbred line, H93, showed a moderate resistance in the field but the lesions expanded rapidly in young plants. However, H93 showed a low ability of spore production which might account for the moderate resistance in the field. Estimation of spore production in addition to lesion expansion as components of partial resistance should be performed for the prediction of field resistance using young plants.

(Received January 17, 1992)

Key words: partial resistance, *Bipolaris maydis* race O, corn.

INTRODUCTION

Southern corn leaf blight (SCLB) which is caused by *Bipolaris maydis* (Nisik.) Shoemaker (teleomorph: *Cochliobolus heterostrophus* Nelson) occurs all over Japan and causes severe economic losses especially in the southern part of the country. Two races have been differentiated in *B. maydis*; race T that produces T-toxin and is virulent to plants with Texas male sterile cytoplasm and race O that does not produce a host-specific toxin and is virulent to plants with any type of cytoplasm14). Although the occurrence of race T was reported in 19715) and 19738), race O has been mainly observed in Japan (unpublished data).

The control of the disease is usually achieved by the cultivation of resistant varieties. Both monogenic and polygenic resistances to the disease have been reported in corn. Monogenic resistance conferred by *rhm*, the recessive gene, effectively controls lesion expansion and sporulation12). Polygenic resistance shows additive gene effects for resistance and breeding has been carried out for improving corn populations for polygenic resistance51. Both types of resistance are "partial" resistances that restrict the growth and sporulation of the pathogen and allow a slow spread of the disease, in contrast with "complete" resistance that totally prevents the multiplication of the pathogens in the host9).

Partial resistance is related to several components like infection efficiency (the proportion of spores that produce lesions), lesion size, latent period (the time from infection to lesion appearance), spore production (the number of spores produced per unit of lesion area per unit of time), and infectious period (the period during which the lesion sporulates). The cultivar that expresses a low infection efficiency,
slow lesion expansion, long latent period, low sporulation capacity, and short infectious period is generally reported to be partially resistant and show a high level of field resistance\textsuperscript{10,17}. The components of partial resistance have been analyzed for many leaf disease pathogens, especially biotrophic fungi such as rusts and powdery mildews\textsuperscript{9,13}. However, in the case of SCLB caused by race O, there are few studies relating to the components of partial resistance.

The present study was conducted to analyze and evaluate the components of partial resistance to SCLB using corn inbred lines showing different levels of resistance in a greenhouse and in fields.

MATERIALS AND METHODS

Six corn inbred lines that were reported to differ in SCLB resistance levels in the field\textsuperscript{6} were selected as follows; three resistant lines, H95rhm, R2040 and Mo17Ht; two moderately resistant lines, H95 and H93; one susceptible line, Pa91. The resistance of H95rhm was controlled by the rhm gene and that of the other lines was controlled by polygenes. Plants of all lines were grown in plastic pots at 25°C in a greenhouse. BMZM8144, the isolate of B. maydis race O (origin: Tochigi Pref.), was used as an inoculum. The isolate was grown on V-8 juice agar for 4 days in the dark, after removing aerial hyphae, then kept in an incubation chamber under illumination with near-ultra-violet light (12 hr photoperiod) at 25°C for 24 hr to induce sporulation.

**Greenhouse seedling test.** Plants at the 4-5 leaf stage were inoculated using the single spore inoculation method described previously\textsuperscript{16} and ten spores were placed on the adaxial surface of the third leaf. After inoculation, the plants were transferred to an environment-controlled growth chamber under a day-night temperature regime of 25°C and 22°C, respectively.

The following factors of partial resistance were analyzed: infection efficiency (IE), lesion area (LA), sporulating lesion ratio (SLR) and degree of sporulation (SP). IE was determined by counting the number of lesions produced on the inoculated leaf and expressed as the ratio of the number of lesions which appeared to that of inoculated spores. LA was measured 4-19 days after inoculation at 3 day intervals. SLR was defined as the proportion of the number of sporulating lesions to that of all the lesions on the leaf. SP was expressed as the number of spores per lesion and area (mm\textsuperscript{2}) of lesion per day. Sporulation was induced by keeping the plants with lesions in an illuminated moist chamber (12 hr photoperiod) at 25°C for 24 hr. Spores from each lesion were collected by coating a water agar piece on the adaxial surface of the leaf and they were counted under a stereoscopic microscope.

**Field test.** Field tests of the disease epidemic for the six inbred lines were conducted at Nishinasuno, Tochigi in 1990. The inbred lines were sown on May 17 in a randomized complete block design with three replications of plots. Each plot consisted of four rows, with 0.8 m spacing, and each row consisted of 20 plants 20 cm apart (Fig. 1). Plots were separated by “barrier plots” consisting of four rows of the synthetic line N-2 which is highly resistant to SCLB to prevent spore dispersal among the plots. As the inoculum source, the susceptible synthetic line, BSSS, was planted in the center of each plot.

![Plant layout in a field plot.](image-url)
Table 1. Infection efficiency, lesion area, sporulating lesion ratio, and degree of sporulation of six corn inbred lines inoculated with *Bipolaris maydis* race O

<table>
<thead>
<tr>
<th>Inbred line</th>
<th>IE</th>
<th>LA (mm²)</th>
<th>SLR</th>
<th>SP (No. of spores)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cm)</td>
<td></td>
<td></td>
<td>per lesion</td>
</tr>
<tr>
<td>H95rhm</td>
<td>0.99a</td>
<td>0.4a</td>
<td>0.00a</td>
<td>0.0a</td>
</tr>
<tr>
<td>R2040</td>
<td>0.93a</td>
<td>1.6b</td>
<td>0.05a</td>
<td>1.7ab</td>
</tr>
<tr>
<td>Mo17Ht</td>
<td>0.96a</td>
<td>2.6c</td>
<td>0.02a</td>
<td>1.8ab</td>
</tr>
<tr>
<td>H95</td>
<td>0.98a</td>
<td>9.2d</td>
<td>0.39b</td>
<td>7.8b</td>
</tr>
<tr>
<td>H93</td>
<td>0.98a</td>
<td>14.0e</td>
<td>0.71c</td>
<td>7.0b</td>
</tr>
<tr>
<td>Pa91</td>
<td>0.98a</td>
<td>16.0f</td>
<td>0.80c</td>
<td>21.8c</td>
</tr>
</tbody>
</table>

a) In each column, figures followed by a common letter are not significantly different by Duncan’s multiple range test ($P<0.05$). All data refer to the results obtained 7 days after inoculation.
b) Infection efficiency.
c) Lesion area.
d) Sporulating lesion ratio.
e) Degree of sporulation.

and inoculated artificially. The inoculation was performed by depositing 10 ml of the conidial suspension ($10^6$ conidia/ml) of the isolate BMZM8144 on the whole leaf of BSSS on July 3. Disease severity was estimated as the proportion of the diseased area of the twelfth leaf using the standard diagrams proposed by Fry et al. as follows: 0: no symptoms, 1: 0.1% of the leaf area is diseased, 2: 1%, 3: 5%, 4: 10%, 5: 25%, 6: 50%, 7: 75%, 8: the whole leaf is diseased and killed. Disease severity was assessed every 7 days from July 26 to September 9. Estimations were made on the plants shown in Fig. 1. The area under the disease progression curve (ADPC) was calculated for each line.

RESULTS

Evaluation of partial resistance in greenhouse

The IE did not vary in the six inbred lines tested and there were no significant differences among them (Table 1). The values ranged from 0.93 to 0.99 and most of the inoculated spores produced lesions in all the inbred lines.

The LA differed significantly among the inbred lines. Lesions produced in Pa91 were significantly larger than those in the other lines on the 7th day after inoculation and the LA values exceeded 30 mm² after 13 days (Fig. 2). Lesions in H93 were also larger significantly than the other resistant lines. An intermediate value was obtained for H95, which was significantly higher than that for the three resistant
lines. Very small chlorotic lesions were produced in H95rhm and the lesion area was 1.7 mm² even on the 19th day. The inoculated leaf turned yellowish and was killed after 16 days in Pa91, H93, and H95, 19 days in Mo17Ht and R2040, and 21 days in H95rhm.

Significant differences among the inbred lines were also found for SLR (Fig. 3). In Pa91 and H93, 80% and 71% of the lesions sporulated on the 7th day after inoculation, respectively. The three resistant lines hardly sporulated and H95 showed an intermediate reaction. The SLR values obviously corresponded to the LA values of each line. Whereas SLR began to decrease in Pa91, H93, and H95 on the 7th or 10th day, the lesions in Mo17Ht and R2040, however, began to sporulate 10 or 13 days after inoculation.

The SP differed significantly among the inbred lines when expressed as the numbers of spores per lesion (Table 1). Pa91 showed a high SP value and the resistant lines showed very low values. H93 and H95 showed intermediate values and there were significant differences compared with Pa91. When expressed as the number of spores per mm² of lesion, the differences were not significant among these lines.

Field study

In the field, the progression of the disease varied considerably between the inbred lines (Fig. 4). A severe epidemic occurred for Pa91 and the averaged disease severity reached a value of 6.2 (the proportion of diseased leaf area >50%) 66 days after inoculation. In contrast, the disease progression was the slowest for H95rhm and the disease severity remained at a value of 1.6 (the proportion of diseased leaf area <1%). The disease occurrence in H93 was fairly severe but restricted compared with Pa91 and the ADPC value was significantly lower than that of Pa91. On the other hand, H95 was moderately resistant throughout the growth stages. Mo17Ht and R2040 were highly resistant in the previous period, but the disease severity gradually increased in the latter period.

DISCUSSION

The six inbred lines inoculated with the *B. maydis* race O differed significantly in the lesion area (LA), sporulating lesion ratio (SLR), and degree of sporulation (SP), while no differences in the infection efficiency (IE) were observed in the present study. Zhu *et al.* also reported that there were large differences in the LA, SP, and latent period among corn cultivars in the field test.

There were small differences in the IE among the inbred lines. Hill *et al.* reported that the heritability of IE was relatively high in the parasite but low in the host and IE was a highly heritable trait in the parasite, *B. maydis* race T. As indicated by the small differences in IE, the resistance did not
appeared to be expressed at the invasion stage.

The statistically significant differences which were observed in the LA among the inbred lines are in good agreement with the report that lesion expansion was mainly controlled by host genes. In particular, the effect of the \textit{rhm} gene was conspicuous and clearly restricted lesions were produced in H95\textsuperscript{rhm} compared with H95. Since the results of LA from the greenhouse seedling test corresponded to the progression of the disease in the field, \textit{La} was considered to be an important component accounting for the resistance to the disease. The resistance mechanism that restricts lesion expansion has not been determined and histopathological studies on the cell responses associated with resistance expression should be carried out in future.

The SLR values for the susceptible line increased rapidly in the early stage of infection, while those for the resistant lines showed a slow increase in the latter stage. Therefore, it was assumed that SLR was related to \textit{La} and started to increase after the production of lesions with an appropriate size.

The SP values per lesion were correlated with the \textit{La}. Even if large lesions were produced in H93, the SP value per lesion was, however, one third of that of Pa91, which indicates the low ability of spore production in H93.

Among the components of the resistance tested, \textit{La} was most closely correlated with the disease progressions in the field. Tajimi \textsuperscript{15} developed a punch inoculation method that enabled to predict the level of field resistance to SCLB based on the area of the lesion produced by punch inoculation in young plants. It was shown also in this study that if the lesions expand rapidly, spore production starts at the early stage and severe disease epidemics is likely to occur. However, the inbred line H93 expressed a moderate resistance in the field, although the lesions expanded rapidly in young plants. Since H93 showed a low level of SP compared with Pa91, it appeared that the parasite sporulated poorly on H93 and consequently the field epidemic was reduced. The estimation of lesion expansion and spore production should, therefore, be performed in young plants for predicting the field resistance level.

The inbred line, H95\textsuperscript{rhm}, was highly resistant both in the greenhouse and in the field in comparison with H95. The effect of \textit{rhm} gene was drastic but allowed slow lesion expansion and sporulation as already reported. The similar result had been obtained using the inbred line, A632\textsuperscript{rhm} (unpublished data). These results indicate that the resistance conferred by \textit{rhm} is the partial type, i.e. rate-reducing type. It is necessary to elucidate the resistance mechanism controlled by \textit{rhm} in future studies.

The resistant lines, Mo17Ht and R2040, showed some moderate resistance at the latter stage in the field epidemic. Since the data of resistance in young plants could not account for this phenomenon, it is suggested that some changes in the resistance level of these lines may occur at the adult stage.

It has been reported that it was difficult to estimate the field resistance level using young plants in SCLB. However, it was indicated in the present study that the field resistance level could be estimated on the basis of data of spore production in addition to lesion expansion using young plants. In future, a simple and effective method for the estimation of the level of lesion expansion and spore production in young plants should be developed to facilitate the breeding work for SCLB resistance.

\textbf{Literature cited}


和文摘要
月星隆雄・古賀博則・植松 勉：トウモロコシ6自殖系統のこま葉枯病に対する部分抵抗性の要因解析
トウモロコシ6自殖系統(H95rhm, R2040, M017Ht, H95, H93, Pa91)を用い、こま葉枯病(病原菌Bipolaris maydis race O)に対する部分抵抗性の要因を解析し、圃場での病勢進展との比較を行った。温室室内幼苗検定により、病斑形成効率(IE), 病斑面積(LA), 菌子形成病斑割合(SLR)および菌子形成数(SP)を測定したところ、IEでは系統間差異はなかったが、LA, SLRおよびSPでは明らかな差異が認められ、特にLA値は圃場での病勢進展と最もよく一致した。自殖系統 H93 は圃場ではやや抵抗性を示したが、幼苗上の病斑拡大は非常に速く、大型病斑を形成した。しかし、H93 は SP 値が罹病性系統より低く、このことが圃場での抵抗性に影響していると結論された。このことから、幼苗を用いた抵抗性検定で圃場での本病に対する抵抗性を予測するためには、病斑拡大要因に加え、菌子形成要因も考慮することが必要であると推察された。