The Syringe Inoculation Method for Selecting Rice Plants Resistant to Sheath Blight, _Rhizoctonia solani_ Kühn*

Kikuo WASANO, Satoru ORO and Yasuhiro KIDO
Faculty of Agriculture, Saga University, Saga, 840, Japan

Abstract

The syringe method was newly adopted as an easy and reliable technique for selecting the rice plants resistant to sheath blight, _Rhizoctonia solani_. The method was as follows: 1) the fungus was cultured on PSA medium at 28 °C for 2 days, 2) about 0.25 ml aliquot of crushed agar culture was injected into the 3rd leaf sheath interstice at heading stage, and 3) in order to evaluate the resistant level of plants to sheath blight, the ratio of areas of disease lesion to that of total leaf sheath (disease scores) was used on 2 and 4 weeks after inoculation; the most effective evaluation of the resistance level to the disease was made by reading the disease scores on the 2nd leaf sheath on 4 weeks after inoculation. Some of the F7 lines selected for the resistant to bacterial leaf blight were also found to be resistant to sheath blight.

Introduction

Sheath blight has become one of the most serious diseases on rice plants in Southeast Asia and Japan as well as blast and bacterial leaf blight disease. For evaluating the resistant levels of rice varieties in fields many inoculation methods of the fungus have been done by using such inocula as follows; the fungus cultured either on rice straw, a mixture of unhulled rice/rice hull, rice bran or PSA medium. When inoculating many individuals in fields some of the above methods are easy but unreliable, the other reliable but troublesome. Therefore, it is critically important to develop an easy and reliable inoculation method of the causal fungus, _Rhizoctonia solani_. The present investigation was conducted to develop a new easy and reliable inoculation method which could be adopted in fields for selecting resistant rice varieties to sheath blight.

Materials and Methods

The rice varieties and lines used in this experiment were presented in Table 2. Following after Marshall and Rush some varieties were used for discriminating the levels of resistance to sheath blight; Taducan and Tetep as resistant varieties, Saturn and Zenith as moderately susceptible, and Bluebelle as susceptible. Table 1 showed the cross combinations of the F7 lines selected for polygenic resistance to bacterial leaf blight. The experiments were conducted by using plants grown in field and those cultured in polyethylene pots. For field study the rice seedlings were transplanted individually by hand on June 25 in a randomized block design with two replications in the paddy field of Saga University. Each experimental plot was consisted of three rows, 36 plants a row, and spacing among hills were 18 × 18 cm within a variety and 36 cm between rows of different varieties. Fertilizer N, P2O5 and K2O were applied at the rate of 80 kg/ha, respectively, as basal dressing. The fertilizer used was the slow acting compound chemical fertilizer which contains same rate of

* Received 31 May 1982
three elements. For pot culture study, two each seedlings were transplanted on July 6, in every polyethlene pot (8 litre in volume), two pots per variety, and randomly arranged. The potted plants were amended with an equivalent amount of the chemical fertilizers used in the field study. Four inoculation methods used in this experiment were as follows. Method 1: Pieces of rice straw (about 3 cm in length) were washed with tap water and submerged in water for about 12 hours and then autoclaved at 120°C for 30 minutes. A piece of fungal mats of *R. solani* was transferred onto the sterilized rice straw and cultured at 28°C for 40 days. About five pieces of rice straws thus prepared were inoculated to each plants in fields by laying them among tillers of a rice hill. Method 2: The mixture of unhulled rice and rice hulls was washed with tap water and submerged in water for about 12 hours and autoclaved at 120°C for 30 minutes. A piece of fungal mat of *R. solani* was transferred onto the sterilized mixture mentioned above and cultured at 28°C for 20 days. About 12–15 ml of inoculum was given to each hill. Method 3: Disks (about 5 mm in diameter and 3 mm in thickness) of the fungal mat were punched out by using a cork borer and inserted into the interstices between the 3rd leaf sheath from flag leaf and a culm. Method 4: The fungus was cultured at 28°C for 2 days on PSA medium containing 1.6% agar as an ingredient. The fungal mats of *R. solani* were completely crushed in a mortar with a pestle and about 0.25 ml of the fungal suspension was injected by using a syringe into the interstices between the 3rd leaf sheath and a culm of each five tiller per plant as shown in Fig. 1. Numbers of the inoculated plants per variety in a block were 8 for the method 1 and 2, and 13 for the method 4. For each variety the plants in the center of three rows were selected for being inoculated. As shown in

![Fig. 1](image1.png)

**Fig. 1** The fungal suspension of *R. solani* is injected by using a syringe into the interstice between the 3rd (from flag leaf) leaf sheath and a culm.

![Caloro and Norin 22](image2.png)

**Caloro**

**Norin 22**

**Fig. 2** Disease symptoms on the leaf sheaths of rice plants inoculated by the syringe method. Photo was taken on three weeks (Caloro and Norin 22) after inoculation. ▶ indicates the site of inoculation. Each figure on the photo indicates the disease on the 2nd leaf sheath.
on each plant were inoculated at heading stage by four needle prick method. Evaluations of resistance to bacterial leaf blight were made by reading the disease scores (0-7) on 3 weeks after inoculation. Inoculations were made to 10 plants in a block of every variety.

**Results**

Table 2 showed the mean values of disease scores, the percentages of plants infected by sheath blight, and the heading dates of rice varieties or lines. Table 3 showed the correlations among the evaluated resistance levels to sheath blight in the varieties and lines inoculated by four methods. The four different resistance levels due to line means were significantly positively correlated each other except for the relations between the disease scores on the 3rd leaf sheaths in the method 4 and the percent infections in the methods 1 and 2. As shown in Fig. 4, the disease score on the 2nd leaf sheath inoculated by the syringe method was significantly negatively correlated with the
Table 2  Percent infections in the plants inoculated by the methods 1 and 2, disease scores on the 3rd, 2nd and flag leaf sheaths of the plants inoculated by the methods 3 and 4, and heading dates in the all varieties and lines used in this experiment. Percent infections were recorded on two weeks after inoculation, and disease scores were on two and four weeks after inoculation.

<table>
<thead>
<tr>
<th>Varieties and Lines</th>
<th>Dates of Heading</th>
<th>% Infections Meth. 1 Meth. 2</th>
<th>Disease Scores Meth. 3 Meth. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>2 weeks</td>
<td>3rd 2nd</td>
</tr>
</tbody>
</table>

**Discrim. Var.**
- Taducan: 9.60 0.0 5.9 4.9 0.1 5.7 0.3 0.0
- Tetep: 9.10 0.0 3.2 4.4 0.2 6.2 1.0 0.0
- Saturn: 8.26 0.0 6.3 5.0 0.2 6.1 0.4 8.7 2.1 0.0
- Zenith: 8.31 0.0 6.3 10.0 0.0 5.0 0.2 6.8 1.3 0.1
- Blue Belle: 9.80 0.0 6.3 10.0 4.4 9.7 2.9 10.0 7.5 0.5

**Parent. Var.**
- Belle Patna: 9.10 6.3 6.3 10.0 0.2 7.9 1.2 9.6 6.2 0.6
- Norin 22: 8.26 6.3 31.3 10.0 0.0 8.8 0.5 9.8 4.8 0.2
- Norin 18: 9.15 6.3 12.5 7.3 0.0 8.1 0.8 8.8 2.1 0.1
- Nipponbare: 8.25 6.3 43.8 10.0 1.2 9.3 4.5 10.0 7.6 0.8
- Caloro: 8.31 0.0 6.3 8.4 0.2 8.0 0.6 9.0 3.0 0.0
- Reioh: 9.1 6.3 6.3 8.3 1.9 8.2 1.6 9.0 4.3 0.2

**Suscept. Line**
- A18: 8.22 56.3 100.0 9.4 0.5 7.5 2.7 9.1 6.7 0.4
- K1 233: 8.21 62.5 56.3 9.6 0.2 8.0 3.8 9.3 8.3 2.4
- D1 52: 8.25 18.8 37.5 9.9 0.9 9.9 4.4 10.0 8.2 0.5
- E1 67: 8.20 75.0 87.5 9.7 2.4 8.0 7.3 9.8 9.1 6.3
- F1 77: 9.10 0.0 12.5 7.7 0.5 7.3 2.7 8.7 4.4 0.2

**Resist. Line**
- A1 3: 9.23 0.0 18.8 6.0 1.4 6.4 3.5 7.2 6.4 0.3
- A11 (II) 3, 9: 9.21 0.0 12.5 8.2 0.4 7.2 1.5 8.5 5.6 0.1
- K1 223: 9.12 0.0 0.0 7.4 0.0 7.7 0.3 9.0 0.4 0.0
- K1 (I) 223: 9.90 6.3 6.3 8.7 0.2 7.5 0.0 8.7 0.3 0.0
- D1 48: 8.29 0.0 12.5 9.5 0.5 9.2 1.7 9.7 5.0 0.1
- D1(III) 48: 8.20 56.3 87.5 8.9 1.7 8.2 4.8 9.9 8.9 2.6
- E1 57: 8.23 81.3 100.0 8.8 0.3 7.1 3.4 8.7 7.0 1.2
- E1(III) 57: 8.23 68.8 87.5 6.5 0.9 7.4 2.6 8.8 6.7 0.1
- F1 72: 9.10 6.3 12.5 8.0 0.0 7.7 0.3 8.8 1.5 0.1
- F1 (II) 72d: 9.11 6.3 50.0 8.8 0.3 8.3 2.8 9.2 5.4 0.5

Method 1.: Inoculation with the fungus cultured on rice straw
Method 2.: Inoculation with the fungus cultured on the mixture of rice seed and hull
Method 3.: Agar disk inoculation in pot culture
Method 4.: Syringe inoculation
Abbreviations (A-F) for susceptible and resistant lines were the same to that shown in Table 1.
Table 3  Relations between the disease scores or the percent infections obtained on two weeks after inoculation by the different methods

<table>
<thead>
<tr>
<th>Inoculation method†</th>
<th>Leaf sheath positions observed</th>
<th>Coefficients of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 1 × Method 2</td>
<td>—</td>
<td>0.99**</td>
</tr>
<tr>
<td>Method 4 × Method 1</td>
<td>3rd</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.63**</td>
</tr>
<tr>
<td>Method 4 × Method 2</td>
<td>3rd</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.70**</td>
</tr>
<tr>
<td>Method 4 × Method 3</td>
<td>3rd</td>
<td>0.83**</td>
</tr>
<tr>
<td></td>
<td>2nd</td>
<td>0.71**</td>
</tr>
</tbody>
</table>

†: For details of each method, see Table 2.
**: significant at 1 % level

The date of inoculation. This correlation was mainly attributed to the high susceptibility of the F7 selected lines which headed as early as in mid or late August in the west Japan. There were large differences in the resistance levels among lines or varieties in inoculated on September 4th and 12th. Two F7 polygenic resistance lines for bacterial leaf blight showed the highest resistance to sheath blight as Tadukan did. As shown in Fig. 5, relations between the disease scores on two and four weeks after inoculation by the syringe method were positively correlated in both 3rd and 2nd leaf sheaths. These correlations were fitted to the quadratic equations. Figs. 6 and 7 showed the relations between the disease scores on the 3rd and 2nd leaf sheaths and also between those on the 2nd and flag leaf sheaths, respectively. These data showed that disease infections spreaded from the lower leaf sheath to the upper one, from the 3rd to the 2nd leaf sheath and then...
to the flag leaf sheath, when the developed disease lesion covered over the half area of lower leaf sheath. Disease scores on the 2nd leaf sheath linearly increased with increasing those on the 3rd leaf sheath. But, the relations between the disease scores on the 2nd leaf sheath and those on the flag leaf sheath fitted to the quadratic equation as shown in Fig. 7. The Table 4 showed the genetic and some statistical parameters estimated from the analyses of variances for disease scores. The variance components due to variety, i.e. genetic variances, and also the heritabilities were found to be the largest values in the 2nd leaf sheath on 4 weeks after inoculation. And the coefficients of variation calculated from the values of error variances and general means were much smaller in the 2nd leaf sheaths than in the flag leaf sheaths. These results showed that individual selections for the resistance to sheath blight can be most effectively carried out by evaluating the disease scores on the 2nd leaf sheath on 4 weeks after inoculation.

Table 4 Heritabilities and some other statistics for the disease scores (Aresin/) of sheath blight on the 3rd, 2nd and flag leaf sheaths, on 2 and 4 weeks after inoculation. These estimates were obtained from the analyses of variance of the disease scores in the six parental varieties or in 15 F7 lines selected for the polygenic resistance to bacterial leaf blight as shown in Table 2.

<table>
<thead>
<tr>
<th>Materials Week</th>
<th>Posit.</th>
<th>v2</th>
<th>e2</th>
<th>h2 (%)</th>
<th>Mean</th>
<th>C.V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental varieties</td>
<td>2 3</td>
<td>17.06</td>
<td>24.06</td>
<td>41.5</td>
<td>68.0</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>2 4</td>
<td>46.75</td>
<td>12.69</td>
<td>78.7</td>
<td>80.1</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>2 1</td>
<td>18.11</td>
<td>4.49</td>
<td>80.1</td>
<td>5.3</td>
<td>40.0</td>
</tr>
<tr>
<td>F7 Iloe</td>
<td>2 3</td>
<td>59.78</td>
<td>10.80</td>
<td>84.7</td>
<td>63.7</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>2 2</td>
<td>233.98</td>
<td>19.98</td>
<td>92.1</td>
<td>28.3</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>2 1</td>
<td>464.84</td>
<td>15.03</td>
<td>96.9</td>
<td>47.8</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>1 1</td>
<td>207.37</td>
<td>10.07</td>
<td>95.4</td>
<td>10.6</td>
<td>29.9</td>
</tr>
</tbody>
</table>

Posit.: Leaf sheath positions from leaf
v2: Variance component due to variety
e2: Error variance or environmental variance
h2: Broad sense heritability calculated from, h2 = v2/(v2 + e2)
C.V(%) = √e2/mean × 100 mean = general mean

Fig. 8 showed the relation between the resistance level to bacterial leaf blight and that to sheath blight. It was not significantly correlated but some F7 lines selected for the polygenic resistance to bacterial leaf blight also showed the resistance to sheath blight. But the F7 lines selected for being susceptible to bacterial leaf blight showed very low resistance to sheath blight.

**Discussion**

The varietal different levels of resistance to sheath blight have been recorded among rice varieties. Hashioka showed that most of Japanese lowland rice varieties were susceptible and some of native Formosan and tropical ones were resistant to the disease. The genes for resistance to the disease were shown to be dominant by the cross experiments between the resistant and susceptible varieties in which the ratios of resistant and susceptible plants were 3:1 or 15:1. But Hashioka also reported the difficulties in classifying F2 plants into the resistant and susceptible due to the continuous variations in the resistant levels among F2 segregants. Excepting the report by Hashioka, no further genetical studies have been done on the relations of disease scores for bacterial leaf blight on 3 weeks after inoculation and for the latter on 4 weeks after inoculation.
the breeding of varieties resistant to sheath blight because of the following reasons; 1) a difficult classification of *Rhizoctonia*, 2) no availability of resistant varieties to be crossed, 3) no simple and reliable inoculation method for individual selections from hybrid populations, and 4) a complicated procedure for evaluating the resistance level of a plant. For selecting lines resistant to sheath blight, the breeding programs have been done in some local paddy fields where the disease was prevailing. But, natural infections under field conditions are not efficient for selecting resistant lines because of a limited number of lines to be tested at once and an incomplete occurrence of the disease. All research workers concluded that the varietal differences in the levels of resistance to sheath blight were attributed to the differences in some ecological and morphological characters such as heading date, plant height, number of tillers per hill in early developmental stage and number of leaves per plant. They reported such characters as earliness, shortness, large number of tillers and leafy plant type were considered to be suitable for an attachment of sclerotia onto a rice hill, and also for the growth and development of hyphae on an upper leaf sheath of rice plant. On the other hand, late-maturing varieties could escape the attack by fungus because of a lower temperature at heading stage which is the most susceptible stage to the disease. The yields and qualities of rice were severely affected when the infection of sheath blight established at or after the heading stage. The syringe inoculation method adopted in this experiment would cause little pathological differences in the conditions such as suitability in the sclerotia attachments to a rice hill and the fungus development on a leaf sheath. And this inoculation method could correctly infect the disease to rice plants at the time when rice plants were, in its developmental stages, most severely infected with the disease. The varieties and F₇ lines showed wide variations in the levels of resistance to sheath blight, even when they were inoculated at a same time, on August 4th or September 12th. But, all early-maturing F₇ lines when they were inoculated on August 24th were susceptible to the disease. Based on the above experimental results, we concluded that the syringe inoculation method can be used as an useful technique to find the real difference of the genotypic values in the resistance to sheath blight of plants in a segregating hybrid population.

**Acknowledgment**

We are very grateful to Dr. F. Nonaka of Saga University for technical instructions in culturing the fungus of *Rhizoctonia solani* and his presentation of the fungus No. 395. Thanks are also due to Mr. M. Yoshida of Kyushu Tokai University for his useful suggestions to the syringe inoculation method.

**Literature Cited**


*: Theme of the original papers were translated from Japanese to English by K. Wasano
摘 要

注射器接種法による稲穂枯病抵抗性の選抜*

和佐野喜久生・尾路 悟・城戸 康博

佐賀大学農学部 840 佐賀市本庄町

稲穂枯病は、いもち病、白葉枯病ならんで、日本お
よび東南アジア諸国において重大な病害となっている。
しかし、稲品種の育成に際して稲穂枯病抵抗性の選抜
は、今日まではほとんど行われていない、その原因の一
つに、本病原菌の接種法が考えられる。すなわち、慣行の
接種法（稲わら培養法、もみ・もみがら混合培養法及び
PSA 培養コルクボーラ打抜法など）は、接種方法は容
易であるが接種株の発病が不確実である、あるいは発病
は確実であるが圃場で接種するには煩雑すぎるなどの欠
点がみられた、そのために、多くの個体を対象とする雑
種群から個体選抜に適用できなかった。本研究は、
接種方法が容易で、しかも発病が圃場の環境条件あるい
は穂個体の生態的・形態的特性に影響されない接種法の
確立と、抵抗性系統の育成に目的として行った。本実験
では、新に注射器による接種法を採用し、従来の慣用法
との比較を行なった。注射器接種法は、寒天量を 20％少
なくした PSA 培地で病原菌を 28℃ で 2 日間培養し、
よく攪拌後 100 ml 容の注射器に入れ、出穂期に未熟から
3 番目の穂位の葉鞘内に約 0.25 ml を注入する方法であ
る。抵抗性的判断は、接種後 2 および 4 週目に接種穂位
およびその上位 2 枚の葉鞘での病斑面積率を指数（0～
10）で判断して行なった。慣用の稲わら、もみ・もみが
ら混合接種法（いずれも出穂期に接種）は、接種個体の
発病が不確実であったので、発病株率で抵抗性の大小を
判別した。慣用の 3 接種法による品種抵抗性は、注射器
接種法の第 2 穂位の 4 週目病斑指数と有意な正の相関関
係がみられ、品種あるいは系統の抵抗性を比較する場合
はいずれの接種法によっても差がないことがわかった。
注射器接種法は、出穂期にすべての接種個体を確実に発
病させることができたが、同じ時日に接種された品種の
間でも病斑指数に大きな差がみられた。なお、8月24日
に接種した極早生系統には抵抗性を示すものは見られな
かった。病斑指数は、接種後 2 および 4 週目の間、第 3 お
よび第 2 穂位の間、および第 2 穂位と出穂の間でいずれ
の場合にも正の相関関係がみられ、一次あるいは二次の
回帰式が適合した。注射器接種法による選抜は、遺伝力
及び誤差の変動係数の大小から判断して、第 2 穂位の 4
週目の病斑指数によって行うのが最も効率的であると結
論された。穂枯病抵抗性と白葉枯病ボリージ án 3）
との間には有意な相関関係はみられなかったが、穂枯病
抵抗性強系統は白葉枯病ボリージ án 3）抵抗性強系統の中の
みにみられた。

* 1982年 5 月31日受理