

## Banana Blast Caused by *Pyricularia angulata* Hashioka

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### *Pyricularia angulata* Hashioka 에 의한 바나나 稻熱病 (假稱)

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#### ABSTRACT

Banana blast was frequently encountered in greenhouses of Jeju province during a local survey of plant diseases from 1985 to 1986. The disease incidence on banana was very severe up to 100% in a greenhouse. The causal fungus was identified as *Pyricularia angulata* Hashioka based on mycological characteristics and Pathogenicity of the fungus. The symptom of blast produced on leaves of banana was circular to oval, dark brown spots with yellow margins which measured 1-10mm in diameter, and the symptom on fruits was circular to oval reddish brown or dark brown, depressed spots which measured up to 20mm in diameter. The symptoms were scattered not only on leaves and fruits of banana but also on petioles, leaf sheaths, bunch stalks and crowns. The pathogen was characterized that conidia were hyaline, 2 septated, ovate to pyriform with a small hilum solitary at ends of denticles of conidiophores, and measured 16.0-34.0 x 7.0-12.0  $\mu\text{m}$  (average 22.5 x 9.0 $\mu\text{m}$ ) and conidiophores were mostly 2 septated, occasionally 2 or 3 branched, denticulate at the tips, and measured 75.0-100.0 x 3.0-5.0  $\mu\text{m}$  (average 90.0 x 4.0  $\mu\text{m}$ ). The optimum temperature range for mycelial growth of the fungus on PDA was 26-28°C. The result of pathogenicity tests revealed that *P. angulata* had pathogenicity only on banana. On the other hand *Pyricularia grisea* (Cke.) Sacc. isolated from *Digitaria sanguinalis* (L.) Scopio was not pathogenic to banana. It was revealed that the leaves were the most susceptible to *P. angulata* among several parts of banana in inoculation tests. Thirty-day-old fruits of banana were more susceptible to the fungus than 60-day-old fruits, and bunch stalks were more susceptible than the fruits.

**Key words:** banana blast, *Pyricularia angulata*, pathogenicity.

#### 要 約

1985年에서 1986年 地域적으로 植物病害 調査를 實施하던 중 濟州道の 바나나 栽培溫室에서 바나나稻熱病이 심하게 發生하고 있음을 發見하였다. 어떤 溫室에서는 이 病의 發生이 매우 심하여 發病率이 100%에 달하였다. 이 病을 일으킨 菌은 菌學的인 特性和 病原性에 의해 *Pyricularia angulata* Hashioka로 同定하였다. 바나나의 잎에 形成된 바나나稻熱病의 病徵은 圓形 내지 橢圓形으로 直徑 1 - 10 mm 크기의

暗褐色 斑點이며 斑點周圍는 黃色을 띠었다. 열매에 形成된 病徵은 圓形 내지 橢圓形으로 赤褐色 혹은 暗褐色의 움푹 들어간 斑點이며, 그 크기는 直徑이 20 mm에 달했다. 病徵은 바나나의 잎과 열매 뿐만 아니라 葉柄, 葉鞘, 열매의 다발줄기, 열매의 樹冠에서도 發生하였다. 病原菌의 分生子胞子는 無色, 隔膜은 2個, 하나의 작은 hilum을 가진 달걀모양 내지 서양배모양이고, 分生子梗의 작은 突起 끝에서 單生하며, 크기는 16.0 - 34.0 × 7.0 - 12.0  $\mu\text{m}$  (平均 22.5 × 9.0  $\mu\text{m}$ )였다. 分生子梗은 大部分 隔膜이 2個, 간혹 둘 혹은 셋으로 分枝하고, 頂端部位에는 작은 突起가 있으며, 크기는 75.0 - 100.0 × 3.0 - 5.0  $\mu\text{m}$  (平均 90.0 × 4.0  $\mu\text{m}$ )였다. PDA에서 이 菌의 菌絲 生育適溫 範圍는 26 - 28 °C였다. 病原性檢定 結果, *P. angulata*는 바나나에만 病原性이 있었다. 반면에 *Digitaria sanguinalis* (L.) Scop. 에서 分離한 *Pyricularia grisea* (Cke.) Sacc.는 바나나에 病原性이 없었다. 接種試驗 結果, 바나나의 여러 部位 중에서 잎이 *P. angulata*에 가장 感受性이었다. 또한 生育 30日 된 열매는 生育 60日 된 열매보다 더 感受性이었으며, 열매의 다발줄기는 열매보다 더 感受性이었다.

## INTRODUCTION

Unusual leaf spots and fruit pits of banana (*Musa sapientum* L.) have been severely occurred in greenhouses of Jeju province, Korea since 1985. The diseased samples collected from there were investigated. *Pyricularia* sp. was frequently isolated from the lesions.

A pitting disease caused by *Pyricularia grisea* (Cke.) Sacc. or *Pyricularia grisea* (Cke.) Sacc. has been recorded in banana (1, 4, 9, 10, 11, 12). Hashioka (5), however, reported *Pyricularia angulata* Hashioka as a new species of *Pyricularia* causing banana blast and *Pyricularia grisea* (Cke.) Sacc. as its synonym. On the other hand *Pyricularia musae* Hughes (6, 7, 8), *Pyriculariopsis parasitica* (Sacc. & Berl.) Ellis (2) and *Parapyricularia musae* Ellis & Peregrine (3) have been identified as fungi occurring on banana.

In this report, mycological characteristics and pathogenicity of *Pyricularia* sp. isolated from banana were investigated and inoculation tests were carried out with several parts of banana.

## MATERIALS AND METHODS

**Isolation and investigation.** Diseased samples having the symptoms of unusual spots and pits of banana were collected from greenhouses of Jeju province from 1985 to 1986. Lesions from the diseased samples were cut into 1 x 1 cm pieces, and

surface of them was disinfected in 1% sodium hypochlorite solution for two to three minutes. The pieces were plated on water agar (WA) in 9cm petri dishes after washing with sterile water and drying in filter papers. *Pyricularia* sp. was isolated from the lesions on WA after incubating at 25-28 °C under fluorescent light for 7 days.

The size of 25 conidia and 25 conidiophores of the fungus isolated from each diseased sample was measured using a micrometer under compound microscope. A total of 200 conidia and 100 conidiophores of the fungus was measured, and their characteristics were investigated.

Monoconidial isolates of *Pyricularia* sp. were transferred into slant media of potato dextrose agar (PDA) for preservation. Five isolates were tested to detect optimum temperature for mycelial growth by growing them on PDA in 9cm petri dishes in darkness. The average length of mycelial growth was measured at 24hr intervals from 3 to 10 days after transferring 5mm diam. mycelial discs to PDA. Cultural characteristics of the fungus were observed during culturing periods on PDA in darkness or under fluorescent light for 15 days. The experiments were performed with three replications.

**Inoculum.** Five monoconidial isolates of *Pyricularia* sp. isolated from banana were cultured on PDA at 27 ± 1 °C for 15 days. The aerial mycelia grown on PDA were removed by brushing with small quantity of sterile water. Then the cultures were allowed for sporulation at 27 ± 1 °C under fluorescent light for 7 days and conidial suspension was prepared by

scraping conidia with distilled water. The conidial suspension was filtered through cheese cloth, and adjusted to  $10^5$ - $10^6$  conidia per ml. Conidial suspension of *Pyricularia grisea* (Cke.) Sacc. isolated from *Digitaria sanguinalis* (L.) Scop. was prepared by the same method to compare its pathogenicity with *Pyricularia* sp. isolated from banana.

**Pathogenicity test.** Healthy plants of *Digitaria sanguinalis* (L.) Scop., *Musa sapientum* L., *Oryza sativa* L., *Setaria italica* (L.) Beauv. and *Setaria viridis* (L.) Beauv. cultivated in pots were used for the pathogenicity tests to determine host range of *Pyricularia* sp. isolated from banana and *Pyricularia grisea* (Cke.) Sacc. isolated from *D. sanguinalis*. Thirty-day-old plants of *O. sativa* namely Taebaeg, Tongil, Nagdong and Nongbaeg were tested. The growth stage of other tested plants was 30 to 40 days old. Each conidial suspension of *Pyricularia* sp. and *P. grisea* was thoroughly sprayed on the plants except banana, which was sprayed with a faint sprayer. Inoculated plants were covered with polyethylene vinyl bags and kept in a moist chamber at  $27 \pm 1^\circ\text{C}$  for 48 hours before they were placed in the greenhouse. Observations were made at 12 days after inoculation. Young leaves and 30-day-old fruits of banana were thoroughly sprayed with each conidial suspension of *Pyricularia* sp. and *P. grisea*, and incubated in moist plastic boxes at  $27 \pm 1^\circ\text{C}$  for 12 days.

Thirty-day-old fruits, 60-day-old fruits, bunch stalks and leaves of banana were inoculated with one isolate of *Pyricularia* sp. by the same method to compare the susceptibility to the fungus in several parts of banana. The age of fruits was measured by the number of days from the initiation of the finger fruits from the bunch stalk. Observations were successively made during 13 days after inoculation. Distilled water was sprayed for the control treatment in the pathogenicity tests. All the experiments of pathogenicity tests had three replications.

## RESULTS

**Disease and pathogen.** The unusual leaf spots and fruit pits of banana severely occurred in Jeju province were identified as blast caused by *Pyricularia angulata* Hashioka based on mycological characteristics and pathogenicity. The disease incidence was as severe as 100% in a greenhouse. The symptom of blast produced on leaves of banana was circular to oval, dark brown spots with yellow margins which measured 1-10mm in diameter, and the symptom on fruits was circular to oval, reddish brown or dark brown, depressed spots which measured up to 20mm in diameter (Plate 1). The centre of spots on leaves first appeared as a brown point with yellowish brown margin which measured 1-3mm in diameter, and later changed to dark brown. The centre of spots on fruits first appeared as a black point with reddish brown margin which measured 1-3mm diameter, and later changed to a dark brown or grayish black pit. The symptoms were scattered not only on leaves and fruits of banana but also on petioles, leaf sheaths, bunch stalks and crowns.

*P. angulata* isolated from banana was compared with other isolates previously reported by workers in terms of conidial size (Table 1). The average length of conidia isolated in this study was almost the same as that by Hashioka, but the average width



Plate 1. Symptoms of blast on leaf and fruit of banana caused by *Pyricularia angulata*.

Table 1. Description of *Pyricularia angulata* causing banana blast and its synonyms

Reference	Pathogen	Disease name	Size of conidia( $\mu\text{m}$ )
WARDLAW, C. W. & MCGUIRE, L. P.(1932)	<i>Piricularia grisea</i> (Cke.) Sacc.	Pitting disease	24.0-29.0 x 10.0-12.0
BERAHA, L. (1962)	<i>Piricularia grisea</i> (Cke.) Sacc.	Pitting disease	23.0-29.0 x 9.0-12.0
MEREDITH, D. S. (1962)	<i>Pyricularia grisea</i> (Cke.) Sacc.	Pitting disease	20.0-35.0 x 10.0-13.0
MEREDITH, D. S. (1963)	<i>Pyricularia grisea</i> (Cke.) Sacc.	Pitting disease	17.0-29.0 x 6.5-8.5 (Average 19.0 x 7.5)
HASHIOKA, Y. (1971)	<i>Pyricularia angulata</i> Hashioka	Banana blast	18.2-28.0 x 4.9-9.1 (Average 22.6 x 7.5)
Authors (1986)	<i>Pyricularia angulata</i> Hashioka	Banana blast	16.0-34.0 x 7.0-12.0 <sup>a</sup> (Average 22.5 x 9.0)

<sup>a</sup>Measurement of 200 conidia isolated from diseased leaves and fruits of banana.

was slightly greater than that reported by Hashioka. The range of conidial sizes investigated by authors was in accord with those of the synonyms by other workers. The shapes of conidia and conidiophores of *P. angulata* are shown in Plate 2. Conidia are hyaline, 2-septated, ovate to pyriform with a small hilum solitary at ends of denticles of conidiophores. Conidiophores are mostly 2-septated, occasionally 2- or 3-branched, denticulate at the tips, and measured 75.0-100.0 x 3.0-5.0  $\mu\text{m}$  (average 90.0 x 4.0  $\mu\text{m}$ ). The cultural characteristics of the fungus on PDA were slow mycelial growth, scanty aerial mycelium and few conidia formation. At the same time, colony appeared pale or dark brown, and the

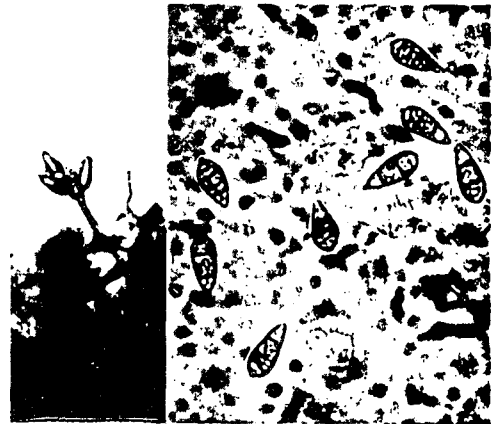


Plate 2. A conidiophore and conidia of *Pyricularia angulata* from the lesion (left, X200), and conidia (right, X 400).

Table 2. Result of pathogenicity tests of *Pyricularia angulata* isolated from *Musa sapientum* and *Pyricularia grisea* from *Digitaria sanguinalis*

Plant tested	Pathogenicity <sup>a</sup>	
	<i>P. angulata</i>	<i>P. grisea</i>
<i>Digitaria sanguinalis</i> (L.) Scop.	-(5)	+(5)
<i>Musa sapientum</i> L.	+(5)	-(5)
<i>Oryza sativa</i> L.	-(5)	-(5)
<i>Setaria italica</i> (L.) Beauv.	-(5)	-(4), $\pm$ (1)
<i>Setaria viridis</i> (L.) Beauv.	-(5)	-(4), $\pm$ (1)

<sup>a</sup>The results were recorded in a greenhouse, 12 days after spray inoculation with conidial suspension, which was followed by incubation in a moist chamber at  $27 \pm 1^\circ\text{C}$  for 2 days. Separately inoculated young leaves and 30-day-old fruits of *M. sapientum* were incubated in moist plastic boxes at  $27 \pm 1^\circ\text{C}$  for 12 days. 30-day-old seedlings of *O. sativa* namely Taebaeg, Tongil, Nagdong and Nongbaeg were tested. The symbol + signifies strongly pathogenic,  $\pm$ : weakly pathogenic, and -: not pathogenic. The number in parenthesis indicates number of isolates tested.

optimum temperature range for mycelial growth was  $26-28^\circ\text{C}$ .

**Pathogenicity.** *P. angulata* was pathogenic only to banana (*Musa sapientum* L.) among five different hosts tested (Table 2). The fungus was not patho-

Table 3. Result of inoculation tests of *Pyricularia angulata* to banana

Plant part inoculated	Symptom at days after inoculation <sup>a</sup>			
	7	9	10	13
30-day-old fruit	-	+	++	++
60-day-old fruit	-	-	+	++
Bunch stalk	+	++	++	++
Leaf	++	++	++	++

<sup>a</sup>The parts of banana inoculated with conidia suspension by spraying were incubated in a moist plastic box at  $27 \pm 1^{\circ}\text{C}$ . The symbol ++ signifies many lesions produced, +: a few lesions, and -: no lesions.

genic to *Digitaria sanguinalis* (L.) Scop., *Oryza sativa* L., *Setaria italica* (L.) Beauv. and *Setaria viridis* (L.) Beauv. On the other hand *Pyricularia grisea* (Cke.) Sacc. was not pathogenic to banana although it was pathogenic to *D. sanguinalis*. One isolate of the fungus was weakly pathogenic to *Setaria* spp.

Leaves were most susceptible to *P. angulata* among several parts of banana in the inoculation tests (Table 3). Thirty-day-old fruits were more susceptible to the fungus than 60-day-old fruits. Bunch stalks were more susceptible than the fruits.

## DISCUSSION

The disease having symptoms of unusual leaf spots and fruit pits of banana in Jeju province, Korea was banana blast caused by *Pyricularia angulata* Hashioka which was identified according to the classification criterion of Hashioka (5). *Piricularia grisea* (Cke.) Sacc. or *Pyricularia grisea* (Cke.) Sacc. has been recorded as the causal fungus of a pitting disease of banana which must be the same as the banana blast (1, 4, 9, 10, 11, 12). Hashioka (5), however, reported *P. angulata* as a new species different from *Pyricularia grisea* (Cke.) Sacc. in cultural characters, morphology and pathogenicity. He reported that a unique morphological feature of *P. angulata* was irregularly angular, sometimes stellate appressorium which was distinctly different from those of *P. grisea* and most other species of *Pyricularia* that had almost globular appressoria and

which were not pathogenic to *Digitaria sanguinalis* (L.) Scop. He used the epithet 'angulata' derive from the distinguished mark of appressoria of the fungus in nomenclature. The authors also found that *P. angulata* isolated from banana was different from *P. grisea* isolated from *D. sanguinalis* in mycological characteristics and pathogenicity. The conidiophores of *P. angulata* in this experiment were occasionally 2- or 3-branched, which was not described in that of *P. grisea* by Ellis (2). However, it was impossible to distinguish the morphological differences of conidia between the two species. In addition the result of pathogenicity tests by authors revealed that *P. angulata* was not pathogenic to *D. sanguinalis* as described by Hashioka (5), and *P. grisea* was not pathogenic to banana. Meredith (10) described the appressorial morphology of *Pyricularia* isolated from banana which was the same as that described by Hashioka (5), but he did not compare the morphology with that of *P. grisea*. The workers (1, 4, 9, 10, 11, 12) who reported *P. grisea* as a causal fungus of pitting disease on banana did not carry out pathogenicity test of the fungus against *D. sanguinalis*. Therefore there is a possibility that *P. grisea* might be misidentified as the causal fungus on banana, and also the fungus might be a synonym for *P. angulata*. *Piricularia grisea* (Cke.) Sacc. was a synonym of *Pyricularia grisea* (Cke.) Sacc. because Saccardo's original spelling for the genus was *Pyricularia* although later he changed it to *Piricularia*.

*Pyricularia musae* Hughes has been identified as a fungus occurring on banana (5, 7, 8). Ellis (2), however, recorded that the fungus was not *Pyricularia* but a synonym of *Pyriculariopsis parasitica* (Sacc. & Berl.) Ellis which was a saprophytic fungus with 3-septated conidia occurring on leaves and stems of banana. Ellis (3) also reported *Parapyricularia musae* Ellis & Peregrine occurring on leaves of banana. He indicated that it might be a saprophytic fungus occurring on banana rather than a causal fungus of banana blast.

Hashioka (5) reported that *P. angulata* easily formed conidia on PDA. The result in this experi

ment, however, revealed that few conidia of the fungus were formed on PDA, indicating the same result by Meredith (10). Accordingly it requires more investigations on sporulation using many isolates of the fungus.

There were a little differences in the width of conidia between the present result and that by Hashioka (5). The range of conidial size by authors, however, was in accord with those by other workers (1, 9, 10, 12).

The result of inoculation tests of *P. angulata* to several parts of banana revealed that 30-day-old fruits of banana were more susceptible to the fungus than 60-day-old fruits, and the leaves were the most susceptible. Meredith (10) reported that infection of the fungus mostly took place at the early stage, even though symptoms developed much later. In some cases there was no sign of pitting or spotting on immature fruits or green fruits at the time of harvesting, but the symptom was observed on the fruits in the ripening rooms during transportation (4). In addition, it was reported that rainfall and high humidity favoured the development of the disease (9, 11, 12). Accordingly much damages caused by *P. angulata* are expected in Korea from young stages to later stages of banana growth because of humid conditions in the greenhouses.

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