

Seed Borne and Transmission of *Bipolaris oryzae*, the Causal Pathogen of Brown Spot of Rice

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ABSTRACT

Rice brown spot *Bipolaris oryzae*, in paddy fields was investigated to find the relationship between disease severity on flag leaf and kernel infection, at three growth stages of flowering, milky, and dough stages. Disease incidence and severity of brown spot increased with stage of plant. There was a significant relationship between incidence of infected kernel and severity of infected flag leaf at growth stage 3 of rice; flowering ($r = 0.84$, $P < 0.0001$), milky ($r = 0.84$, $P < 0.0001$) and dough stage ($r = 0.80$, $P < 0.0001$). The transmission of *Bipolaris oryzae* and location in the seed were studied. Each part of infected kernel including embryo, endosperm, palea, lemma, rachilla, and sterile lemma was found infected by *B. oryzae*. Rachilla and sterile lemma were shown high level of infection at 82%, 79% respectively. Transmission studies from the infected kernel to the seedling using test tube agar indicated that primary symptom appeared on coleoptile and roots after 7 – 14 days. The first leaf of the seedlings also had symptom after 3 – 4 weeks and some infected seedlings became brown and died at a later stage.

Key words: *Bipolaris oryzae*, Brown spot, component, kernel, transmission

INTRODUCTION

Bipolaris oryzae Breda de Haan) Shoemaker (syn. *Helminthosporium oryzae* Breda de Haan, the anamorph of *Cochliobolus miyabeanus* (Ito and Kuribayashi) Drechsler, the causal agent of brown leaf spot disease of rice, is a serious disease in rice production worldwide. It caused losses in stand due to seedling blight, in yield due to leaf and culm infection, and in quality and yield by kernel infection. Bedi and Gill (1960) estimated that *H. oryzae* caused 4.58 to 29.1 % loss in weights of rice grains and 11.0 to 37.3 % reduction in germination (Padmanabhan, 1977). Datnoff and Lentini (1994) also reported that this

disease caused yield loss ranging from 16 to 40 percent in Florida, and also affected milling quality. Nyvall *et al.* (1995) reported that *Bipolaris* spp. were isolated primarily from the awns and frequently from the palea or lemma. The incidence of seed borne *Bipolaris* spp. was related to disease severity in the field. Mew and Gonzales (2002) reported that *Bipolaris oryzae* was often observed on the entire seed surface (about 32%) or on sterile lemmas (29%). Experiments were carried out to study the relationship between the severity of infected leaf and incidence of infected kernel of rice, the location of brown spot disease (*B. oryzae*) in/on rice kernels, and transmission of *B. oryzae* from infected kernel to seedling.

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MATERIALS AND METHODS

1. Disease severity at different growth stage of rice

Flag leaf of individual tillers and its panicles were collected at the flowering, milky and dough stages from the experimental fields, at Kasetsart University, Kamphaengsaen Campus in 2003 rainy season. With three replications, each replication of 15 tillers was collected. Infected leaf area was measured using the method as described by Lamaban and Siddiqui (2003): Leaves infected by *B. oryzae* were detached and placed on a sheet of white paper then tracing paper was placed over the leaves to draw the outline of the entire leaf margin and infected portion of leaves. The total leaf area (mm²) and infected leaf area (mm²) were recorded and calculated for the percentage of infected area. Correlation between severity of the infected leaf area and incidence of infected kernel was estimated. The panicles were dried and stored at 10°C after counting the number of infected kernels. Additionally, infection of kernels and leaves showing spot symptoms was confirmed by the blotter method.

2. Location of *Bipolaris oryzae* on/ in rice kernel

Samples of rice kernels infected with *Bipolaris oryzae* were collected from the experimental fields, Kasetsart University, Kamphaengsaen campus and then kept at 10°C before studying. The infected kernels were selected from seed lots and tested using the component plating method as described by Neergaard and Mathur (1985). Individual infected kernels were dissected aseptically into six components including embryo, endosperm, palea, lemma, rachilla, and sterile lemmas. These components were collected in small plastic bag, and then surface sterilized for 1 minute with 1 % NaOCl (sodium hypochlorite solution). Each component of an individual kernel was plated on 3 layers of moistened blotters in plastic petri dishes. The dishes were incubated at 24 ± 1°C under 12h

alternating cycles of NUV (near ultra violet) light and darkness. Each component was examined under a stereomicroscope for the growth of *B. oryzae* after 7 days of incubation. There were 4 replications, (100 kernels/ replication).

3. Transmission of *Bipolaris oryzae* from infected kernel to seedling

Transmission of *Bipolaris oryzae* from infected kernel to seedling was studied under controlled environment in the growth chamber at 24 ± 1°C under 12h alternating cycles of NUV light and darkness using test tube agar and, top paper. In greenhouse experiments the seedling symptom test (sand method) described by Mathur and Kongsdal (2003) was used. The infected kernel sample was checked for *B. oryzae* infection by the blotter method before conducting an experiment.

Test tube agar: The infected kernels were surface-sterilized with 1 % sodium hypochlorite (NaOCl) and then grown on 20 mm of water agar in test tube. The tubes were incubated at 24 ± 1°C under 12 h alternating cycle of NUV light and darkness for 7 – 14 days. Kernel infection was recorded and then, the infected seedling was monitored for the appearance of the symptoms and disease development.

Top paper: The infected kernels were surface sterilized with 1 % NaOCl and incubated on moistured blotter at 24 ± 1°C in plastic tray with 100 slots (one kernel per slot was incubated) under 12 h alternating cycle of NUV light and darkness for 7 – 14 days.

Sand method: The infected kernels were surface-sterilized with 1 % NaOCl and sown the sterilized sand in plastic trays using 400 kernels per sample (one kernel per slot was planted). The disease was also monitored after 7 - 14 days by washing the Kernels to remove sand from the tray. The number of infected seedlings was counted and confirmed by isolation of *B. oryzae*.

Data was analyzed by analysis of variance (ANOVA) procedure by SAS version 6.12 (SAS Institute Inc). Statistics graphics and

correlation of coefficients were used by Sigma Plot 2000 program, version 6.0 (1986 – 2000 Inc).

RESULTS AND DISCUSSION

1. Disease severity at different growth stage of rice

In the experimental fields, the difference of infected levels on flag leaf and kernel of whole panicle of infected plant was observed at three stages: flowering, milky, and dough stage. The results showed the incidence and severity of brown spot to increase according to the developmental stage of plant from flowering till the dough stage. Field rice plants at maturity were severely infected with *B. oryzae* and produced brown spots on the leaves and discoloration on the kernel of the panicle whereas at the flowering and milky stages were lower (Figure 1). The mean incidence of infected kernels was 26 % at the dough stage, and severity of infected leaf was 1.6 %. Meanwhile, the incidences of infected kernels were 15.1 % and 12.4 %, and infection severities of the leaf were 0.6% and 0.4 % at the milky and flowering stages

respectively. The incidence of infected kernels was significantly correlated with the severity of flag leaf infection at each stage (Figure 2, 3, 4). The percentages of infected kernels showed a significant correlation ($r = 0.80$, $P < 0.0001$) of severity of infected flag leaves at the dough stage, and at $r = 0.84$, $P < 0.0001$; and $r = 0.84$, $P < 0.0001$, for the milky and flowering stages respectively. However, Padmanabhan and Ganguly (1954) reported that rice was most susceptible to *B. oryzae* at the flowering and mature stages. In the early stage of rice plant development, only minute spots were formed. The brown spots on the leaves were larger at the later stages than the earlier ones. Moreover, Imam Fazli and Schroeder (1966) found that the rice kernels were more susceptible to the pathogen at the flowering and milky stages than dough stage on a resistant cultivar.

2. Location of *Bipolaris oryzae* on/ in rice kernel

B. oryzae infected kernels with typical brown spot symptoms on pericarp were collected. The components including embryo, endosperm, palea, lemma, rachilla, and sterile lemmas were

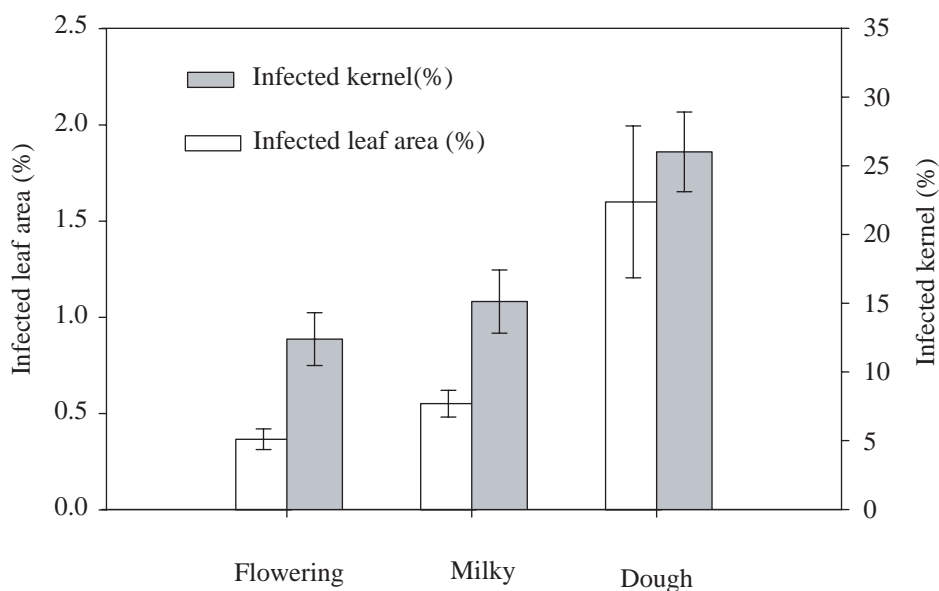


Figure 1 Incidence of infected kernels (%) and the severity brown spot (%) at flowering, milky, and dough stages of rice plant.

separated completely and incubated at the previously stated conditions for 7 – 10 days. These components were examined under a stereomicroscope. The infection of *B. oryzae* was found in all components at different levels. The rachilla had the highest at 82% and 79% for sterile lemmas

(Table 1). Embryo and endosperm infections were lower than the other sites, 14 % and 9 % respectively. Infection of lemma and palea was 61% and 55 %, respectively. Suzuki (1985) reported that diseased hull was more numerous in pedicels than lemma and palea and that in grains,

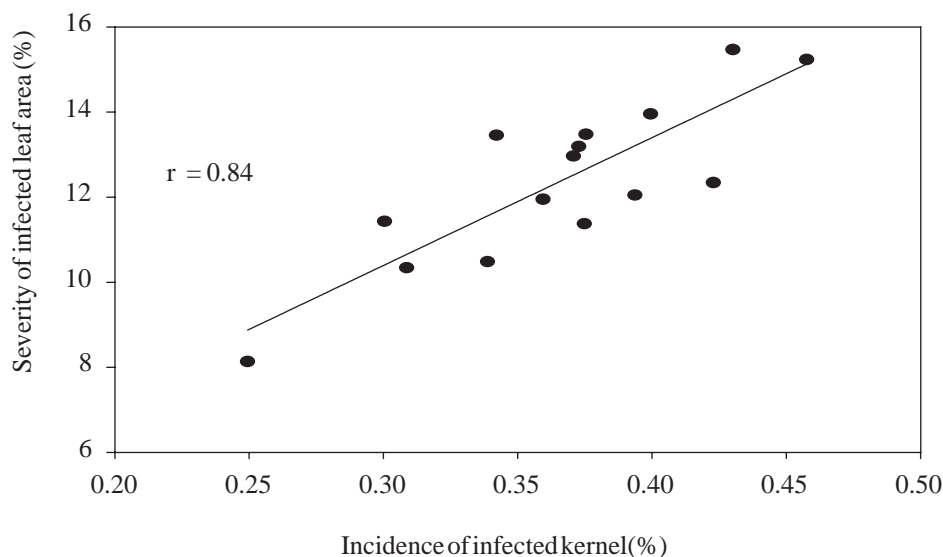


Figure 2 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the flowering stage ($r = 0.84$, $P < 0.0001$).

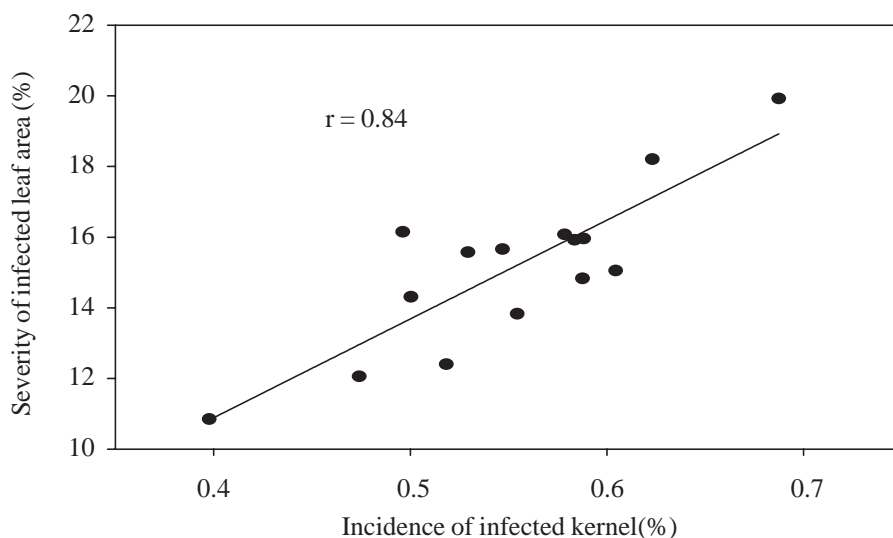


Figure 3 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the milky stage ($r = 0.84$, $P < 0.0001$).

the hilum and placenta were more severely infected than the other areas. Fazli and Schroeder (1966) stated that the close association of hyphae with embryonic tissues indicated that the infection of the embryo might occur under favorable conditions. In contrast, Nisikado and Nakayama (1943) showed mycelia only in the pericarp and seed coat. These results showed that infection of rice kernel by *B. oryzae* had taken place through all the components of kernel and the rachilla, and sterile lemmas were the most infected. Some infected kernels with brown spot symptom on the seed coat, embryo and endosperm also showed discoloration. Mycelia and conidia were produced only 3 to 4 days after incubation. On the lesion, conidiophores and conidia were produced. Moreover, some of the infected kernels with typical brown spot symptoms after being treated with 1 % NaOCl and washed 3 times with sterilized water, the conidia of *B. oryzae* still adhered to the pericarp showing that conidia could be carried on the pericarp as well.

3. Transmission of *Bipolaris oryzae* from infected seed to seedling

Comparison of testing methods: Three methods, which were used in this experiment, were the most commonly used methods in seed health testing laboratories. Tube agar, top paper, and sand method were used to study the transmission of *B. oryzae* from infected kernel to seedling. Kernal infection by *B. oryzae* using the blotter and agar

Table 1 Infection of *B. oryzae* on different components of the rice kernel with brown spot symptoms using blotter method after incubating at 24°C under 12h alternating cycles of near ultra violet (NUV) light and darkness for 12 h for 7 – 10 days.

Component	Infection (%)
Rachilla	0.82a
Sterile lemma	0.79a
Lemma	0.61b
Palea	0.55b
Endosperm	0.14c
Embryo	0.09c

CV (%) = 21

Mean followed by same letters are not significantly different by Duncan's Multiple Range Test ($p \leq 0.05$).

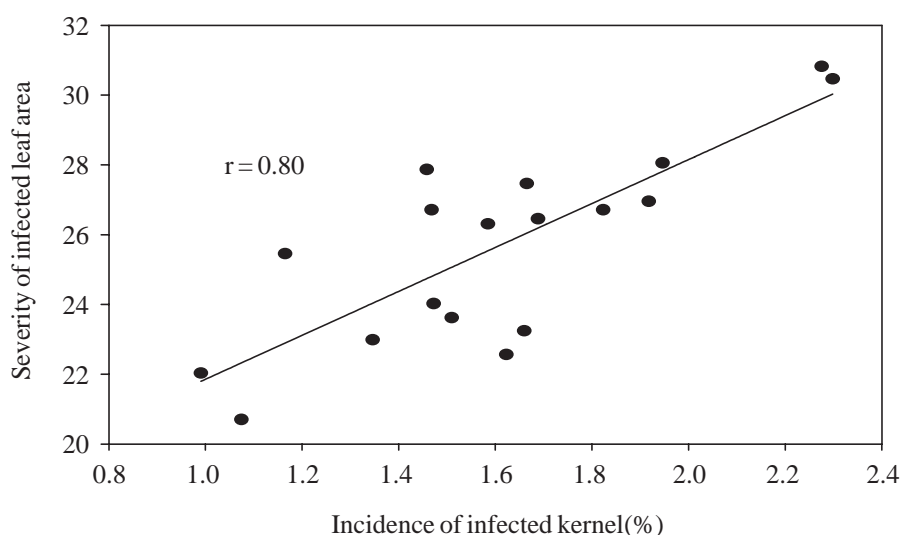


Figure 4 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the kernel at the dough stage ($r = 0.80$, $P < 0.0001$).

methods were 68 % and 76.5% while the sand method was the least of 57% (Table 2)

Test tube agar: Germination and seedling development were good. Moreover, the disease progress was easily monitored. The symptoms, mycelia and conidia were observed under stereomicroscope for all seedling sites. However, mycelium of *B. oryzae* and the other fungi could develop together and sometimes, this interfered with the production of *B. oryzae* conidia.

Top paper: This method was good to follow disease progress from infected kernel to seedling but it could not be used from germination to seedling stage with primary leaves. The seedlings were weak and the blotter paper was easily colonized by other fungi due to high moisture condition. With this method, the seedlings were observed directly under

stereomicroscope and conidia were found on rootlets and coleoptiles.

Sand method: The seedlings were well developed by this method. However, one limitation for monitoring disease progress was the symptoms on the roots of seedlings. The seedling was taken out and cleaned to examine the symptoms so the disease progress could not be monitored continuously on the same seedling.

Seedling infection from infected kernels was based on previous results and by comparing advantages and disadvantages among these methods, the test tube agar method was the best suited for studying seed transmission of *B. oryzae*. One hundred infected kernels for one replication (4 replications) used. The disease progress was examined after incubating for 7, 14, and 21 days.

Symptoms on the coleoptile and roots were observed after 7 days of incubation. There were significant differences in the occurrence of symptoms on coleoptiles and roots of seedlings (Table 3). The symptom as brownish to black necrotic spots on coleoptile occurred at 43.25 % and root at 11 %. Meanwhile, 18 % infection was observed on both coleoptile and root. The infected seedlings with browning and etiolation of coleoptiles collapsed after 3 – 4 weeks. Some infected seedlings, browning of coleoptiles and death of seedling slowly progressed upward to

Table 2 Transmission study of *B. oryzae* from infected kernels to seedlings using the blotter, test tube agar and sand methods.

Method	Infection (%)
Agar	76.5 a
Blotter	68 b
Seedling symptom test (Sand)	57 c

CV (%) = 7.9

Mean flowered by different letters are significantly using Duncan's Multiple Range Test ($p \leq 0.05$).

Table 3 Progression of *B. oryzae* infection from infected kernel to seedling and the appearance frequency of symptom on infected parts of seedlings after incubate at $24^{\circ}\text{C} \pm 1$ for 7 – 21 days.

Categories	Part of seedling	Number of day of symptom appearance		
		7 days	10 – 14 days	18 – 21 days
Total infected seedling		55.75	72.25	72.25
Infected seedlings	Coleoptile	$34.5 \pm 1.7^*$	$43.25 \pm 2.17^*$	$43.25 \pm 2.17^*$
	Roots	6.25 ± 0.85	11 ± 1.47	11 ± 1.47
	Coleoptile and roots	15 ± 1.29	18 ± 1.68	18 ± 1.68
	Primary leaf	0	0	22 ± 1.08
Health seedling		6 ± 1.22	9.5 ± 1.04	9.5 ± 1.04
Death seedling		0	0	8 ± 0.91
Ungerminated		0	18.25 ± 1.47	18.25 ± 1.47
CV (%)		16.3	16.76	22.1

*Mean \pm Standard deviation of error

primary leaves (22 %) (Table 3).

The seedling roots infected by *B. oryzae* were discolored with a tinge of brown and became a dark brown to black lesion during the 10 - 14 day incubation period, and later, caused root distortion and rot. According to Rangaswami (1996), young seedlings showed symptoms soon after germination on the coleoptile which spreaded to cover the other tissues of the seedling. Suzuki (1930) also found the young seedlings showed up signs of infection after germination and the symptoms showed first on the coleoptiles. Mundkur and Chattopahyay (1967) found that on the emerged seedlings, necrotic lesions might be evident on the coleoptile and seminal roots. In addition, Ou (1985) reported that primary infection through diseased kernels was the most common. Coleoptile and seminal roots were most often infected. These results showed that diseased kernels were an important source of primary infection to seedling. Coleoptiles and roots were primary sites of infection and transmission of *B. oryzae* from infected kernel to seedling.

CONCLUSION

From these studies, the relationship between severity of infected flag leaf and incidence of infected kernel was measured by correlation of coefficient indicated a highly significant correlated with 3 stages of rice plant: flowering ($r = 0.84$, $P < 0.0001$), milky ($r = 0.84$, $P < 0.0001$) and dough stage ($r = 0.80$, $P < 0.0001$). Disease progress of *B. oryzae* on the rice plant increased with the developmental stage of the plant. Location of *B. oryzae* in/ on the infected rice kernel was observed in all components of seed but mainly in rachilla and sterile lemma. Test tube agar method was shown to be the best method for studying the transmission of *B. oryzae*. Coleoptiles and roots were the primary infected sites on the seedling obtained from infected kernel.

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