

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

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ABSTRACT

Seed borne and transmission of *Bipolaris oryzae* 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 rather high level of infection at 82%, 79% respectively. There was significant relationship between incidence of infected seed and severity of infected flag leaf at 3 growth stage of rice plant; flowering ($R^2 = 0.71$, $P < 0.0001$), milky ($R^2 = 0.72$, $P < 0.0001$) and dough stage ($R^2 = 0.64$, $P < 0.0001$). Disease incidence and severity were increased with stage of plant. Transmission studies from infected seed to seedling by using test tube agar indicated that primary symptom was appeared on coleoptile and rootlets after 7 – 14 days. The first leaf of the seedling were also observed symptom after 3 – 4 weeks and some infected seedlings became browning and death in the later stage.

บทคัดย่อ

การศึกษาลักษณะการติดกับเมล็ดและการถ่ายทอดเชื้อผ่านทางเมล็ด โดยการแยกส่วนต่างๆของเมล็ดที่เชื้อเข้าทำลาย ซึ่งประกอบด้วย embryo, endosperm, palea, lemma, rachilla, และ sterile lemma พบว่า ส่วน rachilla และ sterile lemma มีการเข้าทำลายของเชื้อ 82 และ 79% ตามลำดับ การเกิดโรคของเมล็ดข้าวมีความสัมพันธ์กับความรุนแรงของโรคใบจุดสีน้ำตาลบนใบธงใน 3 ระยะการเจริญเติบโต คือ ระยะออกดอก flow ($R^2 = 0.71$, $P < 0.0001$), ะยะนํานม ($R^2 = 0.72$, $P < 0.0001$) และระยะสุกแก่ ($R^2 = 0.64$, $P < 0.0001$) โดยความรุนแรงของโรคเพิ่มขึ้นตามระยะการเจริญที่เพิ่มขึ้น การถ่ายทอดเชื้อจากเมล็ดที่ติดเชื้อสู่ต้นกล้า โดยใช้วิธี test tube agar พบว่า อาการแรกจะพบบนส่วนของ coleoptile และ รากย่อย ในระยะ 7-14 วัน ต่อมาจะพบอาการบน ใบแรก หลังจาก 3-4 สัปดาห์ ต้นกล้าที่มีเชื้อเข้าทำลายบางส่วน เริ่มเหลืองและตายในที่สุด

Key word: Brown spot, *Bipolaris oryzae*, kernel, transmission, and component.

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 the serious disease in rice production worldwide. It caused losses

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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 weight of rice grains and 11.0 to 37.3 % reduction in germination (Padmanabhan, 1977). Datnoff and Lentini (1994) reported that the disease affect the yield and milling quality of the grain and yield loss range from 16 to 40 percent in Florida. Mew and Gonzales (2002) reported that *Bipolaris oryzae* was often observed on the entire seed surface (about 32%) or on sterile lemmas (29%). 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. According Neergaard (1977) establishment and development of an infection within a seedling was linked in the process of seed transmission. Seed transmission has been established only if this completion of the infection course has been positively demonstrated to the exclusion of other means of transmission. These experiments were carried out to study the location of brown spot disease (*B. oryzae*) in/on rice kernel, transmission of *B. oryzae* from infected kernel to seedling and relationship between the severity of infected leaf and incidence of infected kernel of rice.

Materials and Methods

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

Samples of rice kernel infected with *Bipolaris oryzae* were collected from experimental field, Kasetsart University, Kamphaengsaen campus and then store at + 5°C before testing. One hundred infected kernels were selected from each lot 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, sterile lemmas. These components were collected in small plastic bag, and then surface sterilized for 1 minute with 1 % sodium hypochlorite solution (NaOCl). Each component of 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 near ultra violet (NUV) light and darkness. Each component was examined under a stereomicroscope for the growth of *B. oryzae* after 7 days of incubation with 4 replication, with 100 kernels each.

2. Disease severity at different growth stage of rice

Flag leaf of individual tiller and its panicles were collected at the flowering, milky and dough stage from experimental fields, Kasesart University, Kamphaengsaen campus in rainy season 2003. With three replications, each replication 15 tillers were collected and the leaves were dried by arranging separately on newspapers or filter paper. Infected leaf area was measured using the

method as described by Lamaban and Siddiqui (2003): Leaves of *B. oryzae* infected plants were detached and placed on a sheet of white paper. A 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 with kernels after counting number of infected kernels were also dried and stored at 4°C in the refrigerator. Additionally, the kernels and leaves with spot symptom were further investigated and examined for an infection of *B. oryzae* by blotter method before severity and incidence were calculated. Infected leaf area and incidence of infected kernel (%) were investigated.

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 growth chamber and green house using test tube agar method described by Mathur and Kongsdal (2003). The infected kernel used was obtained from the same field. The infected kernel sample was tested by blotter method to the incidence of infected kernel before preceded to transmission study. The infected seedling was monitored for appearance of the symptom and disease progress development.

* Blotter method: The infected kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and incubated on moistured blotter at 24 ° ± 1 °C in plastic tray with 100 pots (one kernel per pot was incubated) under 12 h alternating cycle of near ultraviolet (NUV) light and darkness for 7 – 14 days.

* Test tube agar: The infected kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and then grew in test tube on 20 mm of water agar. The tubes were incubated at 24 ° ± 1 °C under 12 h alternating cycle of near ultraviolet (NUV) light and darkness for 7 – 14 days. The infected kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and then grew in test tube on 20 mm of water agar. The tubes were incubated at 24 ° ± 1 °C under 12 h alternating cycle of near ultraviolet (NUV) light and darkness for 7 – 14 days.

* Germination test: The infected kernels were surface sterilized with 1 % sodium hypochlorite (NaOCl) and sown in the sterilized sand tray using 400 seeds per sample (one kernel per pot was planted). The disease was also monitored after 7 - 14 day by washing to remove sand from the tray and numbers of infected seedlings were counted and confirmed by isolation. Data were analyzed by analysis of variance (ANOVA) procedure by SAS version 6.12 (SAS Institute Inc). Statistics graphics and regression lines were used by Sigma Plot 2000 program, version 6.0 (Copyright 1986 – 2000 Inc).

Results and Discussion

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

B. oryzae infection kernels with typical brown spot symptom on pericarp were collected for studying location of inoculums. The components were separated completely and incubated for 7 – 10 day. These components were examined under stereomicroscopes. The infection of *B. oryzae* was found all of these components at different levels. The rachilla has the highest at 82% of the infection and 79% on sterile lemmas (Table 1). Embryo and endosperm infection were lower than other sites, 14 % of infected was endosperm, 9 % embryo. Infection of lemma and palea was 61% and 55 %, respectively. Suzuki (1985) reported that diseased hull are more numerous in pedicels than lemma and palea, also showed that in grains, the hilum and placenta are more severely infected than other areas. According to Fazli and Schroeder (1966) the close association of hyphae with embryonic tissues indicated that infection of the embryo might occur under favorable condition and the mycelia was reported packing in endosperm and grow along the cell walls of endosperm. In addition, Nisikado and Nakayama (1943) showed mycelia only in the pericarp and seed coat. The results showed that infection of rice kernel by *B. oryzae* has taken place through all the components of kernel and rachilla and sterile lemmas were mostly found infected.

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

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

Mean flowered by different letters in the row are significantly using Duncan's Multiple Range Test ($p = 0.05$).

Some of infected kernel with brown spot symptom on whole seed coat, embryo and endosperm also showed discoloration. Mycelium and conidia were produced only 3 to 4 days after incubation. On the infected sites, conidiophores and conidia were produced directly. Moreover, some of infection kernels with typical brown spot symptom after treated with 1 % sodium hypochlorite (NaOCl) and washed 3 times with steriled water, the conidia that were adhered on pericarp were still found. These showed that conidia could be carried on the pericarp very well.

2. Disease severity at different growth stage of rice

In the experiment fields, the difference of infected levels on flag leaf and kernel of whole panicle was observed at three stages: flowering, milky, and dough stage. The field which rice almost at mature stage was severely infected with *B.oryzae* and produced brown spot on the leaves and discoloration on the kernel of the panicle whereas at the flowering and milky stage were observed lower. The results showed the incidence and severity of brown spot were increasing according to the development stage of plant from flowering till dough stage (Figure 4). The mean of incidence of infected kernel was 26.01 % at the dough stage, and severity of infected leaf was 1. 59 %. Meanwhile, the incidence of infected kernel was 15.12 %/, 12.39 %, and severity of infected leaf was 0.55 %, 0.37 % at the milk and flower stage, respectively. The incidence of infected kernel was significantly correlated with severity of infected leaf at each stage (Figure 1, 2, 3). The percentage of infected kernel showed a significant correlation ($R^2 = 0.64$, $P < 0.0001$) of severity of infected leaves at the dough stage. The milky and flowering stage at $R^2 = 0.72$, $P < 0.0001$; and $R^2 = 0.71$, $P < 0.0001$, respectively. Padmanabhan and Ganguly, (1954) reported that *B.oryzae* was most susceptible at the flowering and mature stage. In the early stage of the development of the rice plant, only minute spots were formed. The brown spot on the leaves were larger at the later stage than early one, which was revealed during measuring infected leaf area. However, Fazli and Schroeder (1966) found that the rice kernel was more susceptible to the pathogen at the flowering and milky stages than dough stages on the resistant cultivar.

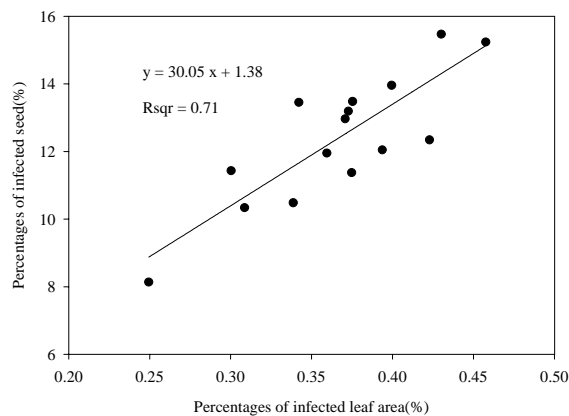


Figure 1 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the seed at the flowering stage ($R^2 = 0.71$, $P < 0.0001$)

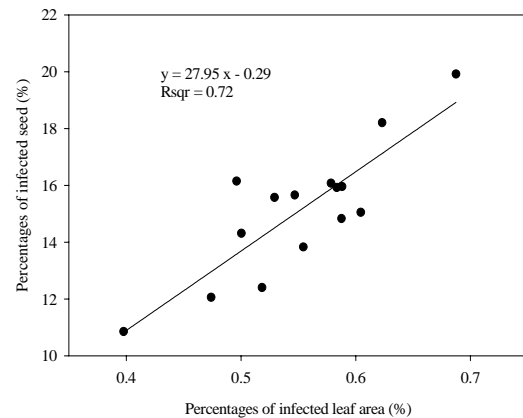


Figure 2 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the seed at the milky stage ($R^2 = 0.72$, $P < 0.0001$)

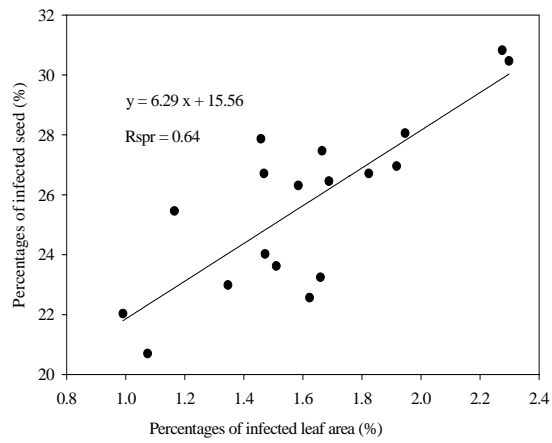


Figure 3 Relationship between severity of brown spot on flag leaf and incidence of *B. oryzae* on the seed at at the dough stage ($R^2 = 0.64$, $P < 0.0001$)

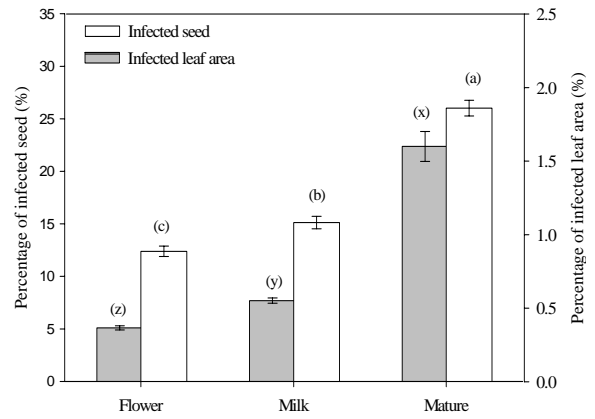


Figure 4 Incidence infected kernel (%) and severity brown spot (%) at flowering, milky, and dough stage of rice plant.

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

3.1. Comparison of testing methods

Three methods which were used this experiment were the most common method used in the health testing laboratories and the stations. These methods are easy to practice and simple. The experiment was conducted by using blotter, test tube agar and sand method to study transmission of *B. oryzae* from infected kernel to seedling was the most useful method. Infection kernel of *B. oryzae* using blotter and agar method obtained at 68 % and 76.5% of infection. Meanwhile, the sand method was the least of infection at 57% (Table 2)

* Test tube agar: Germination and seedling development was good. Moreover, the disease progress was monitored easily and more frequently. The symptoms, mycelia and conidia were observed under stereomicroscope for all of sites of seedling. However, mycelium of *B. oryzae* and other fungal could develop when seedling infected and died that prevented producing conidia to confirm the causal pathogen.

* Blotter method: This method is better for checking disease progress from infected kernel to seedling of rice but it can be used from germination to seedling stage with primary leave. The seedlings were weakness and the blotter paper easily infected by other fungi as moisture condition. With this method, the seedlings were observed directly under stereomicroscope and conidia were found on rootlets and coleoptiles of the infected seedlings.

* Sand method: The seedlings were well developed and healthy, the disease progress was monitored longer. However, one limitation for monitoring disease progress on rootlets of seedling. The seedling was taken off and cleaned to examine the symptom so the disease progress was not monitored consecutively on the same seedling. Base on these results and comparing advantages and disadvantages among 3 methods, test tube agar method was the best for studying seedling transmission of *B. oryzae*.

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

METHODS	Agar	Blotter	Sand
Infection (%)	76.5 a	68 b	57 c

Mean flowered by different letters in the row are significantly using Duncan's Multiple Range Test ($p = 0.05$).

3.2. Seedling infection from infected kernel

Infected kernel with typical symptom on pericarp was used for this experiment. Two hundred infected kernel for one replication and the experiment was replicated four times by using test tube agar method. The disease progress was examined after incubating for 7, 14, 21 and 28 days. Symptoms on the coleoptile and rootlets were observed. There was a significantly differences on the occurrence of symptoms on coleoptiles and rootlets of seedling (table 3). The symptom as brownish to black necrotic spots on coleoptile was occurred at 43.25 % and rootlets at 11 %. Meanwhile, 18 % infection was observed on both coleoptile and rootlets. The infected seedlings with browning and etiolation of coleoptiles caused seedling collapsed after 3 – 4 weeks. Some infected seedling, the browning of coleoptiles and death slowly progressed upward to primary leaves (12 %) and first leave (10 %). (Table 3)

Table 3 Transmission of *B. oryzae* from infected kernel to seedling and the appearance frequency of symptom on of seedling

Parts of seedling	Number of day of symptom appearance		
	7 day	10 – 14 day	18 – 21 day
Coleoptile	34.5 ± 1.7	43.25 ± 2.17 *	43.25 ± 2.17
Rootlets	6.25 ± 0.95	11 ± 1.47	11 ± 1.47
Coleoptile and rootlets	15 ± 1.29	18 ± 1.68	18 ± 1.68
Primary leaf	0	0	12 ± 1.08
First leaf	0	0	10 ± 1.47
Non germination	0	18.25 ± 1.47	18.25 ± 1.47
Health	0	9.5 ± 1.04	9.5 ± 1.04
Death seedling	0	0	8 ± 0.91
CV (%)	16.07	16.76	23.57

*Mean ± S. D

The roots of seedling infected by *B. oryzae* were discolored with a tinge of brown and became dark brown to black lesion during the 10 - 14 days incubation period of infected the. The lesions caused root distortion and rot. According to Rangaswami (1996), young seedling showed symptoms soon after germination on the coleoptile, and spreading to cover the other tissues of the seedling. Beside, Suzuki (1930) also found the young seedlings showed signs of infection soon after germination and the symptoms showed first on the coleoptiles. Mundkur and Chattopahyay (1967) reported that on emerged seedlings, necrotic lesions might be evident on the coleoptile and seminal roots. Addition, Tucker (1923) found that *Hemilthosporium oryzae* caused infection of the roots of seeding. Ou (1985) reported that primary infection through diseased kernel was most common and coleoptile and sometimes roots were often infected from diseased kernels. These results showed that diseased kernel were an important source of primary infection to seedling. Coleoptiles and rootlets were primary site of infection and transmission *B. oryzae* from infected kernel to seedling.

Conclusion

From these studies could be concluded that brown spot on rice is a seed borne disease and location of *B. oryzae* in/ on the infected rice kernel was observed all components including the rachilla at 82% and 79% at sterile lemma. 14 % of infected was endosperm, 9 % embryo, and at 61 and 55 % of lemma and palea. The relationship between severity of infected flag leaf and incidence of infected kernel were described as linear regression as indicated by a highly significant correlated at 3 stage of rice plant with flowering ($R^2 = 0.71$, $P < 0.0001$), milky ($R^2 = 0.72$, $P < 0.0001$) and dough stage ($R^2 = 0.64$, $P < 0.0001$). The results showed that the disease progress of *B. oryzae* on the rice plant increased with development stage of the plant. Test tube agar method was showed the best method for studying seedling transmission of *B. oryzae*. Coleoptiles and rootlets were primary infected site on the seedling obtained from infected kernel. Some of the seedlings were died of produced browning, etiolation of coleoptiles and obsoleting at the stem closed agar surface.

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