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Planning Workshop on Red Stripe

T.W. Mew, Editor

IRRI
INTERNATIONAL RICE RESEARCH INSTITUTE

IAS
Institute of Agricultural Sciences of South Vietnam

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Contents

Welcome address

Dear Dr. T.W. Mew, Head of the Entomology and Plant Pathology Division, IRRI; dear Prof. Pham Van Bien, Director of the Institute of Agricultural Sciences of South Vietnam; dear scientists, ladies, and gentlemen,

I am very pleased to be here today at the opening of the workshop on red stripe organized in Ho Chi Minh City by the Institute of Agricultural Sciences of South Vietnam in collaboration with the International Rice Research Institute.

I believe that the results of this workshop will be a significant contribution to the agriculture of Vietnam in general and to rice production in particular, including rice production in Ho Chi Minh City.

In Vietnam's 4,000-year history of development, agriculture has always played the most important role.

In appraising the achievements obtained during the renovation of Vietnam's economy, many economists in the world have agreed that the biggest gains were made in agriculture.

Agriculture in Vietnam has seen changes in management systems since 1988. Before the renovation, Vietnam's agriculture had been characterized by self-reliance and self-sufficiency, and food shortages had continuously occurred. Before 1975, South Vietnam had to import rice. In the early years after the reunification of the country, difficulties in maintaining the food supply remained.

In recent years, Vietnam's agriculture has produced enough food to not only feed 73 million people, and thereby attain national food security, but also to export. Today, it is one of the leading rice exporters in the world. In 1998, rice exports reached 3.7 million t, earning more than US$1 billion for the country. After only 15 years (1983-98), total annual food production, in rice equivalent, of Vietnam has doubled, from 15 million t to 30 million t.

Vietnam has developed a commodity agriculture with many agricultural products for export—rice, coffee, rubber, cashew nut, tea, pork, and vegetables.

We are very proud of these achievements. These successes resulted from sound and appropriate decisions and policies instituted by the Vietnamese government; effective research and technology transfer between research institutes and universities; dynamic collaboration with international institutions, such as IRRI, on crop breeding, fertilization, pest and disease management, and irrigation; and, especially, the Vietnamese farmers' creativeness and willingness to adopt new technologies.

Rice production is threatened by many pests and diseases, such as brown planthopper, rice blast, sheath blight, etc. From 1989 to 1990, a new disease, red stripe, which farmers call "early maturation," appeared in the Mekong Delta. The infected area reached 250,000 ha in some years. Though this disease reduced rice yield to a limited extent and only in small areas, researchers must pay closer attention to this.

Red stripe has recently occurred in areas near Ho Chi Minh City, the most populated city and the biggest industrial center of the country. Agriculture is an important sector, with more than 75,000 ha devoted to rice and crops such as beans and other vegetables. The city is also a big producer of meat earmarked for both domestic consumption and export.

In recent years, the Institute of Agricultural Sciences of South Vietnam has contributed considerably to the development of agriculture in the city through the transfer of technologies such as new rice varieties, safe vegetable production, and the production of dairy cows and backyard chickens.

I believe that, through this workshop, we can reach sound conclusions, which directly or indirectly will contribute effectively to the management of red stripe.

On behalf of the People's Council of the city, I warmly congratulate all of you and express my sincere welcome to all scientists participating in this workshop. I wish you all the best of health. Congratulations for making this workshop a success.

Mr. Nguyen Minh Nhat
Vice Chairman of the People's Council of Ho Chi Minh City

Opening remarks

Dear Mr. Le Minh Nhat, Vice Chairman, People's Council of Ho Chi Minh City; dear Dr. T.W. Mew, Head, Entomology and Plant Pathology Division, IRRI; ladies and gentlemen,

In 1987, when red stripe disease (RS) was first reported by Dr. Mogi and others in Indonesia, only a few people knew about this disease. Several years later, RS was found in other Southeast Asian countries such as Malaysia, the Philippines, Thailand, and Vietnam.

In Vietnam, RS first occurred in 1988-89 in Tien Giang Province, an intensive rice-growing area in the Mekong Delta. Later, the disease spread to all areas in the Delta and some surrounding coastal provinces, covering about 40,000–60,000 ha.

The RS lesions occur on the leaf, then develop rapidly on the flag leaves, when severe damage at heading may be noted, resulting in empty seeds. Vietnamese farmers gave it the name "early matured rice disease."

Different institutions in Vietnam have conducted studies on RS in the last 10 years. The focus of research is the causal agent. Several scientists have suggested that some bacteria or fungi are the culprit. Many fungi have been isolated from infected tissues, but no fungus was found to be associated with this disease.

Some of the fungal isolates used for inoculation were *Curvularia*, *Helminthosporium*, *Sclerotium*, and *Trichoconis,* but results suggested that these fungi did not cause RS. Research results also suggested that RS was not transmitted through dry leaf tissues, debris, soil, or seed collected from diseased plants.

The disease problem attracted the attention of some foreign scientists. RS-infected samples were collected and identified in different laboratories around the world. Unfortunately, the etiology of this disease is still not clear. The area infected by RS is high (about 150,000–250,000 ha a year) with an increasing tendency to spread in the last few years.

Some pathologists who have carried out chemical trials advised using fungicides such as benlate, Tilt, and zinc-copper to control RS in the Mekong Delta.

In view of the frequent occurrence of RS in Southeast Asia, this workshop is indeed timely and the following objectives are very relevant:

1. To plan joint efforts to test different hypotheses on RS etiology.
2. To review progress of research on RS and to evaluate methods in establishing its etiology.
3. To solicit ideas for use in developing a concept note for potential collaboration and donor support.

I am sure the workshop outputs will be useful not only to researchers but also to farmers who are most affected by this unknown disease. Thank you very much for your attention.

Prof. Pham Van Bien
Director, Institute of Agricultural Sciences of South Vietnam
Ho Chi Minh City

Introduction

Red stripe, a rice malady, was first reported in Indonesia in 1987. Initially, the symptom appears as a pinpoint spot of yellow to orange-red color at the base of the leaf blades. The spot then expands to a stripe toward the leaf tip.

Red stripe is observed most commonly at the heading stage, although some reports suggest that it occurs at any stage of the rice crop. The causal agent was thought to be a bacterial pathogen of the genus *Pseudomonas*. However, this was questioned later and, until now, the etiology of red stripe remains obscure.

In the past 10 years, in addition to Indonesia, the "disease" has also been reported in Vietnam, Thailand, and Malaysia. In the Philippines, it was found occasionally in Mindanao; but occurs erratically. We have difficulty securing enough samples for use in isolation procedures.

Some reports associate its occurrence with intensive rice production systems. Lately, it has occurred in upland rice in Thailand. Red stripe has also been observed on wild rice grown in pots in Thailand.

Many trials that attempted to establish the etiology of red stripe failed. We have attempted to isolate the "causal agents" from different stages of red stripe lesions. No uncommon organisms were isolated by conventional laboratory methods. Inoculation tests also failed to establish any causal relationship of these isolated microorganisms, which may range from bacteria to fungi. Growing seeds harvested from "infected" rice plants in soil collected from the field where red stripe was intensive also failed to reproduce the symptoms by inoculating the plants with a different bacterium or fungus.

Although some reports showed that transmission of red stripe was possible through leaf contact or by placing healthy plants together with "infected" ones in moist chambers, these experiments, when repeated, did not confirm previous results.

In view of the frequent occurrence of red stripe in Southeast Asia and the failure to establish its etiology, a workshop was held with the following objectives:

1. To plan joint efforts to test different hypotheses on red stripe etiology;
2. To review progress of research on red stripe and to evaluate methods in establishing its etiology; and
3. To solicit ideas for use in developing a concept note for potential collaboration and donor support.

The outputs expected from the workshop are (1) information about red stripe and methods used in current research to establish its etiology, (2) new strategies identified to continue the study of etiology of red stripe, and (3) a collaborative research proposal for possible funding.

The workshop was attended by 17 participants and observers from IRRI and five countries: Indonesia, Japan, Malaysia, Thailand, and Vietnam (Appendix).

Etiological studies on the yellow leaf syndrome of rice (*Oryza sativa* L.)

Mai Thi Vinh, T.W. Mew, and Pham Van Bien

Yellow leaf syndrome (YLS) was produced and developed in healthy rice plants placed in tandem with infected plants in plastic frames of the screenhouse. High relative humidity (RH) was maintained continuously during the experiment. The disease was not transmitted to other plants by crude extract from infected tissues. Fresh leaf tissues with YLS lesions were scattered onto the canopy of healthy plants and YLS lesions were produced 2 wk later at 28–32 °C and 90–100% RH. Typical YLS lesions occurred on leaves of healthy plants 2 wk after placing YLS-infected plants next to healthy ones, and letting leaves overlap or leaving them a short distance apart in plastic frames. No evidence proved that YLS was transmitted through infected soil or roots, and leaves of ratoon rice derived from plants with YLS. Leaf contact between healthy leaf and YLS leaf produced new, typical YLS spots on healthy leaves within 3–8 d. A plastic frame under conditions of high temperature and humidity appears to establish a system of YLS transmission. New and fresh YLS leaves were detached and placed in test tubes with water. The YLS lesions increased in size; sometimes, new streaks were formed toward the leaf tips within 3–10 d. When 0.1% Viben C 50WP (benzimi-dazole 25% + copper oxychloride 25%) was applied to healthy plants or YLS source plants before leaf overlapping, this chemical failed to control YLS lesion development and transmission in screenhouse conditions.

Yellow leaf syndrome as reported in Vietnam or bacterial red stripe (RS) as reported in Indonesia and the Philippines is one of the new rice diseases found to occur in countries such as Indonesia (Mogi et al 1987), Malaysia, Philippines (Barroga and Mew 1994, pers. commun.), and Vietnam (Bien et al 1988, unpubl.).

More than 10 years ago, YLS first occurred in Tien Giang Province, Vietnam, an intensive rice-growing area with three crops per year (Kim et al 1992, unpubl.). Later the disease appeared in almost all rice-growing areas in the Mekong Delta. Bien et al (1990) and Du et al (1992) showed that YLS occurred and developed year-round, but most severely during the winter-spring crop season (December-March). Since YLS began occurring in Vietnam, it has been observed yearly in rice fields of the Mekong Delta. Total infected area was 64,200 ha in 1990 and 199,000 ha in 1991. In winter-spring of 1998-99, total diseased area was about 4,000 ha in the provinces of Long An, Tien Giang, Tra Vinh, and Ben Tre. IR64 appeared to be a very susceptible rice variety (Mogi et al 1988). All common rice varieties grown under different cropping seasons in many provinces were attacked by this disease (Bien et al 1998).

Symptoms appearing as lesions occurred on the leaf, sometimes on the upper part of sheaths (Mogi et al 1988). At the heading stage, RS develops rapidly on the upper leaves of rice plants, then the flag leaf and the second and third leaves wither completely when severe damage occurs. Bien et al (1992, unpubl.) noticed that transparent, hyaline to greenish tiny dots are scattered on the leaf surface at the beginning of disease development. Ultimately, they form long stripes elongating toward the leaf tips. Infected leaves become partly or completely wilted, depending on disease severity. These authors observed that, under field conditions, YLS often starts at the lowest part of the leaves where the microclimate is favorable for development (high air humidity, shady places, high plant density, fertile, and healthy plants). Barroga and Mew (1994, pers. commun.) observed that the disorder starts as pinpoint yellow specks on the leaves. As the lesions grow older, they darken in color (orange) with the center of the lesion becoming more distinct.

Some authors (Bien et al 1992, unpubl.) reported that, under high air humidity, primary lesions may be observed on any leaves at different positions of the plant. Also, in this condition, the YLS pathogen easily infects rice seedlings. In the field, the syndrome is often observed at the heading stage or

sometimes earlier, during the tillering stage, but rarely at the seedling stage and on ratoons left after harvesting (Mogi and Baskoro 1990, unpubl.).

Extensive research has been carried out simultaneously in different countries since 1988, concentrating on the causal agent of this syndrome. But no definite results have been obtained.

Pseudomonas sp. was suggested as the causal agent of RS (Mogi et al 1988). The bacterium was described as rod-shaped, 1–2 polar flagella, Gram-negative. Colonies on potato agar are yellowish milky white. However, Du et al (1992) and Bien et al (1992, unpubl.) agreed that no ooze streams from the margin of YLS lesions, but there is a clear appearance of a transparent margin around the leaf spot, especially when observing new fresh lesions early in the morning with high relative air humidity. Du et al (1991) suggested that *Curvularia lunata* is the causal agent of this disease. Bien et al (1991) used an isolate of nonfluorescent *Pseudomonas* sp. for artificial inoculation. Seven to 10 d after inoculation, YLS lesions occurred on the leaf surface in all treatments, and even later on the leaf surface of control plants sprayed with sterile distilled water. Thus, it was not clear that YLS is caused by the *Pseudomonas* isolate used. Tuat (1991, unpubl.) proposed that *Pseudomonas setariae* is the causal agent of this syndrome. Many fungi have been isolated from the infected tissues but there is no consistent fungus associated with this disease. Vinh (1991, unpubl.) used some of the common fungal isolates (*Curvularia* sp., *Helminthosporium* sp., *Trichoconis* sp., *Sclerotium oryzae*) for inoculation, but results suggested that the fungi were not the primary cause of YLS (RS).

In the Philippines, bacteria isolated from the lesions were inoculated on rice plants (IRRI 1994). No symptoms were produced. Conidia of *Alternaria* sp. were also observed on the lesions of the disease. They were considered as saprophytes or surface contaminants (IRRI 1994). Extensive isolations have been done from leaf lesions at different stages of disease development and from seeds. The tissue plating technique to isolate fungi and bacterial ooze and "growing on" technique to detect bacterial pathogens have been used. Fungal isolation from seeds by blotter technique indicates that 13% of young lesions were due to *Nigrospora* sp., and 86% of old lesions were due to *Nigrospora* sp. Results of preliminary research have not yet shown any distinct microorganisms (bacteria or fungi) associated with the disease syndrome. Pathogenicity tests of some microorganisms have been investigated on rice plants grown in soil collected from diseased fields. Vinh and Mew (1997, unpubl.) observed that not one of the fungi and bacteria isolates from new, fresh YLS lesions could reproduce the YLS. Their results also showed that YLS was not transmitted through dry leaf tissues, debris, soil, and seeds collected from diseased plants.

Mark Holderness (CABI Bioscience, England, pers. commun. 1992) mentioned that four isolates are obtained from the YLS spots—*Xanthomonas oryzae* pv. *oryzicola*, *Erwinia herbicola*, *Bacillus* sp., and a fluorescent *Pseudomonas*.

H. Kaku (pers. commun.) has done extensive sectioning through the lesions and examined them under both light and electron microscopes. No virus particles were observed. Some other researchers proposed that RS and YLS may be the result of a physiological disorder such as extra nitrogen or deficient potassium. Barroga and Mew (1994) proposed that the disease occurred in areas of intensive cultivation, where there is high nitrogen fertilization combined with nutrient deficiency, particularly potassium. This may be a complex disease, resulting from an interaction between soil fertility and a weak pathogen. Some studies on this aspect did not give positive results. Besides, the diagnosis based on symptoms of nutrient deficiency and toxicity of rice has been studied carefully over the years. No reported symptoms are identical to those of YLS.

The application of chemicals such as benlate, fundazole (benzimidazole), derosal (carbendazim), and copper B (copper and benlate) has retarded and prevented disease development (Bien et al 1990, Du et al 1992, Kim et al 1992).

The objectives of this study are to determine whether YLS is caused by a biotic agent including bacteria, fungi, virus, mycoplasma-like organisms, or RLO and to establish the transmission of the disease.

Materials and methods

Sample collection

Diseased leaves showing YLS were collected from rice fields in Nhi Quy and Cai Lay villages, Tien Giang Province; Moc Hoa, Long An Province; and Cu Chi and Hoc Mon districts, Ho Chi Minh City.

Soil samples were also collected in these infected rice fields for nutrition analysis and other observations in the laboratory and screenhouse. Collected rice hills were grown in pots placed in plastic cages and used for inoculation work and other observations.

Inoculation experiments

YLS transmission under favorable conditions
YLS-infected rice plants were collected in Cai Lay, Tien Giang Province, during the rainy season. Six pots were put in plastic cages (28–32 °C; >90% RH) and six others inside an inoculation chamber. The severity and incidence of YLS were observed regularly at 3-d intervals.

Effect of freshly infected tissue scattering on formation and development of YLS symptoms
In each treatment, 3 g of freshly infected tissues were cut into 4–5-cm segments and scattered onto the canopy of leaves at different stages: heading, soft dough, and hard dough. The plants were kept in plastic cages under high RH after 14-d inoculation before estimating YLS severity and incidence. Different kinds of soil were used for growing IR64 rice plants: infected soil collected in diseased rice fields, sterilized soil, and screenhouse soil. Experiments with two factors (different soils; with or without tissue scattering) were laid out in a randomized complete block design with three replications.

Mechanical transmission
Young lesions and lesions with streaks were used in the mechanical transmission experiment. Two grams of young infected tissue were cut into small tissues with sterile scissors and placed into a precooled mortar. One mL of 0.01 M phosphate buffer, pH 7.2, was added. The mixture was ground thoroughly and quickly with a pestle. The extract was placed on ice. Two fully expanded healthy green leaves of each IR64 rice plant at the tillering stage were stroked gently in one direction onto marked leaves. Inoculum was washed off from the test plants. Inoculated plants were incubated in the shade in the screenhouse for 20 d. Each type of lesion represented one treatment. Control was treated with phosphate buffer only. The experiment was laid out in a randomized complete block design with four replications.

Transmission by association
Leaf association
There is a significant difference among treatments in the degree of transmitted YLS infection.

Leaf separation or leaf overlapping of canopy of diseased leaves to healthy one. The following treatments were prepared: pricked healthy leaves and unpricked healthy leaves of IR64 rice plants. Inoculated plants were grown in healthy soil and diseased plants in infected soil. Both source plants (diseased plants) and inoculated plants were placed with leaves overlapping or separated from each other inside the high RH of plastic cages. The experiment was laid out in a randomized complete block design with three replications per treatment. Disease severity and incidence were monitored after 14 d.

Leaf overlapping and leaf apart. Diseased and healthy plants were placed a little apart or such that they overlapped the canopy of leaves inside the plastic cage. Leaves of healthy plants were unwounded or wounded by rubbing with glass cotton before inoculation. Disease appearance and development were observed 2 wk after treatment. Three replications (12 plastic cages of $0.5 \times 0.5 \times 1.2$ m) of the two-factor experiments were laid out in a randomized complete block design. This observation was repeated twice in November and December 1997.

Leaf overlapping and different degrees of transmitted YLS infection. IR64 rice plants were grown in the screenhouse from healthy seeds. Before treatment, all healthy test plants were fertilized with urea. A 7-d-old culture of fungus *Sclerotium oryzae* was inoculated 24 h before treatment. Two-square-centimeter block agars with this fungus were kept between two tillers, four in each pot. Inoculated tillers were punctured with sterile needles before fungus inoculation. The source of inoculum was YLS rice plants collected in Binh My, Cu Chi District, Ho Chi Minh City. One source plant and two inoculated plants were put together with leaves overlapping in a plastic cage. In total, there were 16 source plants, 16 plastic cages, and 64 inoculated plants laid out in a complete random design with four replications. There were four treatments: YLS-healthy plants, YLS-fungus inoculation, 0.1% Viben C 50WP (benzimidazole 25% + copper oxychloride 25%), sprayed YLS-healthy, and healthy-healthy. Viben C 50WP was sprayed only once at the beginning of this experiment. The severity and incidence were observed at 14-d intervals. This observation was repeated twice in 1998. (The first was from 12 Aug to 23 Sep; the other was from 16 Oct to 27 Nov 1998.)

Leaf and root association
Diseased plants collected in rice fields of Hoc Mon Village, Ho Chi Minh City, were grown in big pots (25 cm diam) of infected soil. Healthy plants of IR64 were grown in small pots (11 cm diam) of healthy soil. The small pot (with or without a 2-cm hole at the bottom) contained a healthy plant. The root of the healthy plant was dipped into the water layer of the infected plant pot (root association). Simultaneously, the canopy of the healthy plant was overlapped or

separated from leaves of YLS plants (leaf association). All treatments were maintained under high RH in plastic cages for 2 wk. The experiment was laid out in a randomized complete block design with three replications per treatment. This observation was repeated three times (in September and October 1997) in the screenhouse. The plants were monitored for disease appearance and development.

Transmission by leaf contact
IR64 healthy plants were grown in two different types of soil: infected and sterilized. They were inoculated at different stages: tillering, heading, soft dough, and hard dough. Two types of YLS lesions were chosen for leaf contact inoculation—(1) pinpoint: young lesions 1–2 mm × 0.5–1.5 mm and (2) young lesions: dark yellow to orange 4–5 mm × 2 mm. YLS lesions of diseased leaves were placed onto the punctured healthy leaves. Then these inoculated leaves were fixed by wrapping both ends with pieces of small sterile cotton and plastic tape. The two-factor experiment (different soil and different contact time) was kept in a plastic cage (30–32 °C; >90% RH). This was laid out in a randomized complete block design with three replications. The percentage of positive infected positions was estimated for various times of leaf contact: 3, 6, and 8 d.

Transmission through ratoons
Hypothesis: Newly grown leaves from infected ratoons left after harvest may dispose of YLS lesions.

YLS-infected rice plants were cut and moved away; only 15-cm ratoons were left for observation. One treatment was placed in a plastic cage. Another was put in open air at the screenhouse. Four replications of each treatment were observed after 15, 45, and 60 d for YLS appearance and development.

YLS lesion development and streak formation
YLS leaves with lesions at the heading stage were collected in infected rice fields of Tien Giang Province. One by one, leaves were put into a test tube of tap water or distilled water. Only the petiole part of each leaf was dipped into the water layer.

Observations were made after 1, 3, and 5 d.

Transmission system
YLS rice plants—VND-10 collected in Moc Hoa, Long An Province—were put in pots in the screenhouse. IR64 healthy rice plants in separated pots were placed next to the diseased ones. Each YLS plant was named first inoculum and two healthy plants (one got direct sunlight in the morning, the other did not) were put together in a plastic cage. There were six replications (16 plastic cages, 32 healthy plants, 16 YLS plants) for this observation. Then, healthy rice plants that became infected with YLS in the previous experiment were used as second inoculum in the following experiment. The same procedure was designed to get new YLS-infected rice plants (third inoculum). Finally, the third inoculum was placed next to the other healthy plants for transmission. Each observation was repeated twice in the screenhouse of IAS in 1998.

Transmission control
Spraying Viben C 50WP (benzimidazole 25% + copper oxychloride 25%) onto YLS plants
YLS rice plants collected in the fields were sprayed with 0.1% Viben C 50WP (781 g ai ha^{-1}) or tap water (control) before being placed next to healthy plants for inoculation. Viben C or tap water was applied at 7-d intervals to canopies of source plants. Inoculated plants were arranged such that there were leaves separating from or overlapping infected plants. One source plant and two healthy ones were used in one treatment. This two-factor experiment was laid out in a randomized complete block design with three replications and repeated twice in the screenhouse. Severity and incidence were observed at 14-d intervals.

Spraying Viben C 50WP (benzimidazole 25% + copper oxychloride 25%) onto healthy plants
Chemical or tap water was applied to the leaf surface of healthy rice plants before they were put next to the infected plants. Sprayed plants were placed with leaves overlapping or separating from source plants. Application was done at 7-d intervals. Observations of YLS severity and incidence were carried out at 14-d intervals. One treatment included one diseased and one healthy plant in the same plastic cage. The total number of pots was 24; 12 source plants and 12 inoculated plants. This experiment was laid out in a randomized complete block design with three replications per treatment.

Results and discussion

Inoculation experiments

YLS transmission under favorable conditions
By maintaining YLS rice plants in two different conditions of the inoculation chamber and plastic frames (Fig. 1), favorable conditions for YLS transmission were examined. Lower disease severity and incidence were obtained in the inoculation chamber. The internal conditions of the plastic frames (higher RH) encouraged disease development. On the

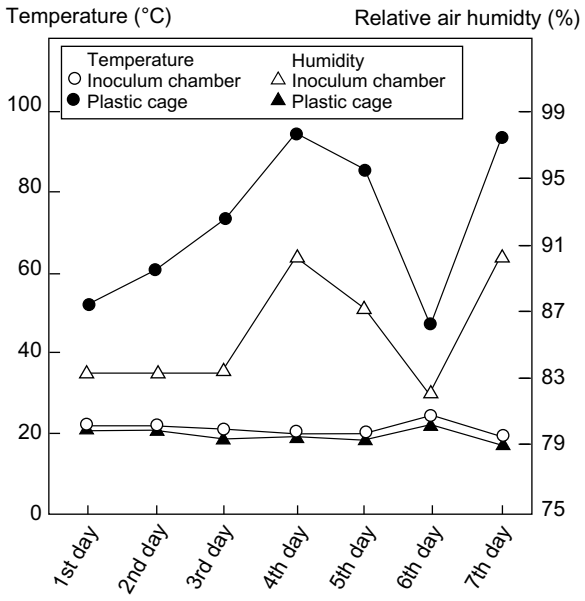

Fig. 1. Temperature and relative air humidity in plastic cage and inoculation chamber during observation time, 1997.

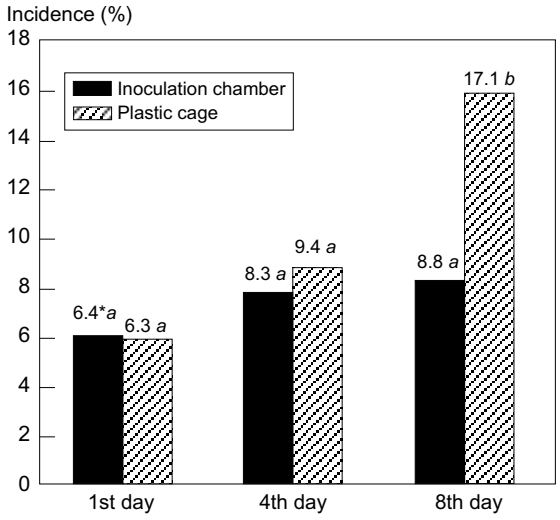

Fig. 2. YLS incidence of infected plants in the plastic frame and inoculation chamber after 8 observation days. *Av of 3 replications. Means followed by different letters are significantly different at p=0.05 level.

first day of observation, incidences in both the inoculation chamber and plastic cage were equal. After 8 d, there was a significant difference at P = 0.05 level of incidence of infected plants in plastic frames (17.1%, compared with the ones inside the inoculation chamber, 8.8%). There was also a significant interaction between time and different place for severity and incidence at P = 0.05 (Fig. 2).

YLS transmission by crude extract

YLS freshly infected tissues were macerated in a sterilized mortar and pestle. The crude extract was rubbed on punctured healthy leaf tissues. The inoculated plants were incubated under high RH for 20 d. No local lesions and systemic symptoms were induced on rice plants during incubation time. This observation had the same result as our 1997 experiment with air-dried YLS specimens. This again indicated that YLS was not caused by crude extract as mechanically transmitted by a virus. Our observations in the rice fields from 1992 to 1998 also showed that no persistent insects were involved with the YLS syndrome.

Effect of freshly infected tissue scattering on formation and development of YLS symptoms

Healthy rice plants grown in various types of soil (sterilized, infected, and screenhouse) were scattered with fresh YLS tissues onto the canopies at different stages. The scattered rice plants had a more severe disease than nonscattered ones at both stages: hard dough (15.9% and 1.4% in sterilized soil, 10.7% and 0.0% in infected soil, 3.4% and 0.0% in screenhouse soil, respectively) and at maturity (12.5% and 6.6% in sterile soil, 8.1% and 5.3% in infected soil, 10.0% and 4.4% in screenhouse soil, respectively). This showed that fresh YLS tissues are a source of inocula for disease transmission and the causal agent of YLS may be an airborne pathogen because only 33.3% of the nonfresh tissue-scattered plants are free from YLS after 14 d (Table 1).

YLS transmission by leaf association

YLS severity and incidence of inoculated plants differed when different inoculation methods were used (Table 2). The wounded healthy leaves that overlapped YLS leaves (first treatment) always obtained the highest infection, followed by unwounded, overlapped leaves (second treatment). Only 75% of the unwounded healthy leaves separated from YLS leaves had the lowest incidence compared with the two preceding treatments. Soil factor again did not affect YLS infection level.

In another observation, wounded overlapped healthy leaves also had the highest severity and incidence. However, even when YLS plants were placed adjacent to or far from healthy rice plants, they became an important source of YLS transmission (Table 3).

At this point, YLS may be attributed to an airborne pathogen because YLS can be transmitted through the canopy, overlapping leaves, or separated leaves between infected or healthy rice tillers. The

Table 1. Frequency of appearance (%) of YLS on IR64 rice plants grown in different types of soil and exposed to either infected tissue or noninfected tissue scattering, 1997.

Treatment	First experiment (hard dough)		Second experiment (maturity)	
	Severity (%)	Incidence[a,b,c] (%)	Severity (%)	Incidence[a,b,c] (%)
Tissue scattering				
Sterilized soil[d]	29.2	15.9	22.8	12.5
Infected soil[e]	23.3	10.7	15.9	8.1
Screenhouse soil[f]	6.8	3.4	22.2	10.0
Nontissue scattering				
Sterilized soil	2.5	1.4	14.9	6.6
Infected soil	0.0	0.0	10.7	5.3
Screenhouse soil	0.0	0.0	10.1	4.4

[a]Significant difference in severity and incidence between tissue scattering treatments (P<0.05). [b]No significant difference in severity and incidence among soil treatments (p<0.05). [c]No significant interactions between tissue scattering and soil type (P<0.05). [d]Sterilized soil: soil collected in YLS rice field sterilized for 2 consecutive days at 121 ˚C for 1 h. [e]Infected soil: soil collected in YLS fields in Long Thanh, Cai Lay, and Tien Giang provinces. [f]Screenhouse soil: soil collected in the IAS.

Table 2. YLS frequency (%) of IR64 rice plants under leaf separation or leaf overlapping treatment, 1997.

Treatment	First experiment (maximum tillering)		Second experiment (hard dough)	
	Severity (%)	Incidence[a,b] (%)	Severity (%)	Incidence[a, b] (%)
Sterilized soil				
Pricked leaf—overlapping	49.8	17.8	26.7	13.4
Nonpricked leaf—overlapping	33.3	14.2	26.2	13.7
Nonpricked leaf—separating	8.7	3.2	8.5	3.7
Infected soil				
Pricked leaf—overlapping	39.4	21.0	27.9	13.0
Nonpricked leaf—overlapping	14.3	6.8	22.3	10.4
Nonpricked leaf—separating	14.6	6.5	0.0	0.0

[a]Significant difference among treatments at P = 0.05 level. [b]No significant difference between soil treatments (P<0.05).

Table 3. YLS frequency (%) of IR64 rice plants with leaves overlapping or leaves set apart from canopy of the diseased ones, 1997.

Treatment	First experiment (tillering)		Second experiment (heading)		Third experiment (hard dough)	
	Severity[a,b,c,d] (%)	Incidence (%)	Severity[a,b,c,d] (%)	Incidence (%)	Severity[a,c,d] (%)	Incidence (%)
Leaves overlapping						
Pricked leaf	17.8	8.2	3.7	1.7	28.0	12.03
Unpricked leaf	13.9	6.9	3.6	1.6	26.9	10.8
Leaves apart						
Pricked leaf	15.5	6.8	7.9	5.0	37.2	15.6
Unpricked leaf	19.9	8.9	3.5	1.5	23.6	10.3

[a]No significant difference between leaf contact treatments at P = 0.05 level. [b]No significant difference between pricked and unpricked leaf treatments at P = 0.05 level. [c]No significant difference among treatments at P = 0.05 level. [d]No significant interaction between leaf contact method and pricked or unpricked inoculation at P = 0.05 level.

biotic agent can actively penetrate intact leaf tissues without previous entry (unwounded leaves).

Different degrees of transmitted YLS infection by leaf association

Different degrees of transmitted YLS infection were presented in Figure 3A,B. The lowest incidences were seen in the control treatment, when healthy plants were used as the inoculum source. YLS plants or YLS ones sprayed with 0.1% Viben C 50 ai had almost equal amounts of severity and incidence on inoculated plants. Healthy plants inoculated with *S. oryzae* before contact with YLS-infected plants had the highest incidence—23.7% and 14.0% in both observations, respectively. The fungus *S. oryzae* resulted in inoculated rice tillers being infected with YLS more severely. Thus, there was a significant difference ($P<0.05$) of YLS infection when various YLS inocula were used.

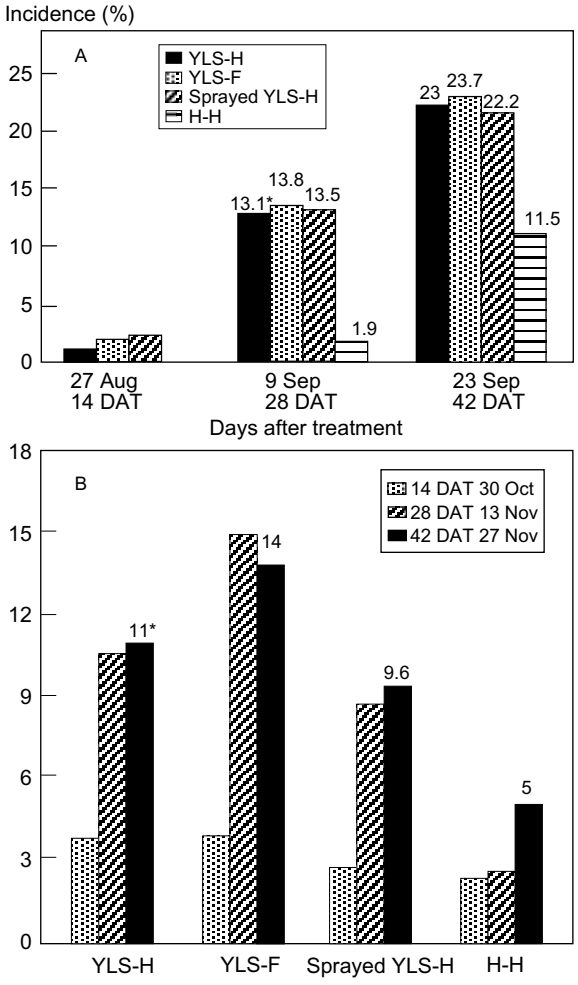

Fig. 3. (A) Different degrees of transmitted YLS to IR64 healthy plants by leaf overlapping, 1998. *Av of three replications. Significant differences in incidence among treatments and time ($P<0.05$). (B) Different degrees of transmitted YLS infection to IR64 healthy plants by leaf overlapping, 1998. *Av of three replications. Significant differences in incidence among treatments and time ($P<0.05$).

YLS transmission with leaf and root association

YLS severity and incidence on rice leaves of root association and check treatments are equal, and not significantly different (1.6% and 3.8%, respectively, at the tillering stage, or 4.9% and 2.03%, respectively, at the soft dough stage). In leaf association or leaf and root association treatments, YLS infection reached the highest percentage, 15.1% and 17.4%, respectively, at the tillering stage, or 14.5% and 11.2%, respectively, at the soft dough stage (Fig. 4). The results therefore indicated that YLS was transmitted from infected plants to healthy ones by the inocula of the canopies and the YLS causal agent may be an airborne pathogen.

Transmission by leaf contact

The ability of YLS transmission by leaf contact between infected and healthy leaves was examined. At the heading stage, YLS severity and incidence were always higher than those at the tillering stage (22.6% and 11.9%, respectively, in sterilized soil, or 16.6% and 8.6%, respectively, in infected soil). The longer the leaf contact time, the higher the severity and incidence (Table 4). There were significant differences in incidence at $P = 0.05$ level between different inoculated stages and different leaf contact durations. Sterilized soil and infected soil did not

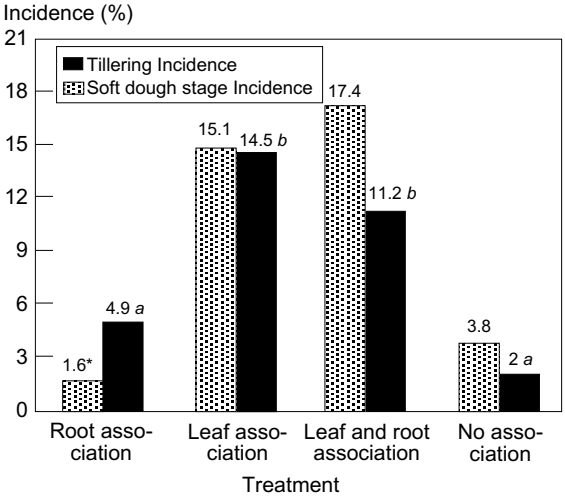

Fig. 4. YLS frequency (%) of IR64 plants inoculated by leaf and root association. *Av of three replications. Significant differences in incidence among treatments ($P<0.05$).

Table 4. YLS frequency (%) of IR64 rice plants inoculated by leaf contact * in plastic cage.

Contact time	First experiment (tillering)		Second experiment (heading)	
	Severity (%)	Incidence[a,b,c,d] (%)	Severity (%)	Incidence[a,b,c,d] (%)
Three	10.0	4.3	0.0	0.0
Six	25.0	10.4	30.0	10.3
Eight	30.0	11.9	55.0	22.6
Three	0.0	0.0	15.0	7.6
Six	20.0	8.6	25.0	11.9
Eight	20.0	8.6	40.0	16.6

*Two types of YLS lesions used for leaf contact inoculation: pinpoint 1–2 mm × 0.5–1.5 mm and young lesion: dark yellow to orange 4-5 mm × 2 mm. [a]Significant difference between stage treatments at $P = 0.05$. [b]No significant difference between soil treatments at $P = 0.05$. [c]Significant difference between day treatments at $P = 0.05$. [d]No significant interaction among factors at $P = 0.05$.

Table 5. YLS frequency (%) of IR64 rice plants growing from infected ratoons under two different conditions.

Day	First experiment		Second experiment	
	Severity (%)	Incidence[a,b,c] (%)	Severity (%)	Incidence[a,b,d] (%)
In plastic cage				
15 d	0.0	0.0	0.0	0.0
30 d	0.0	0.0	0.0	0.0
60 d	15.5	9.9	7.8	3.4
In screenhouse				
15 d	0.0	0.0	0.0	0.0
30 d	0.0	0.0	0.0	0.0
60 d	0.0	0.0	9.1	5.2

[a]Significant difference among day treatments at $P = 0.05$. [b]No significant difference between place treatments at $P = 0.05$. [c]Significant interaction between day and place at $P = 0.05$. [d]No significant interaction between day and place at $P = 0.05$.

affect YLS infection of observed rice tillers. After some leaf contact time, YLS typical spots occurred on healthy leaves, which were placed in contact with infected ones under favorable environmental conditions.

Transmission through ratoons of YLS rice plants

Severity and incidence were observed on new rice leaves growing from ratoons left in two different conditions (plastic frames and screenhouse). After 60 d, 75% of the observed plants showed YLS lesions. However, the result was much lower than other observations. This indicates that YLS may not be transmitted through ratoons of YLS plants because, under high RH in plastic frames, typical spots appear on the leaf surface only after 14 d. The infections in both conditions (plastic frames and screenhouse) were caused by air transmission and not by the inocula from ratoons (Table 5).

YLS lesion development and streak formation

Young lesions were selected for this observation. After 3 d, 10% of the new streaks were formed on detached leaves (dipped into water to maintain freshness). This percentage increased to 36.7% after 10 d. From 10% to 46.7% of YLS lesions increased in size. No new spots occurred on these leaves (Table 6).

This observation method is too difficult to repeat because of early wilting of detached leaves. We also failed to wrap black material around the test tubes of water (containing detached leaves) for observation of fungi sporulation. The reason is that all the leaves became yellow and wilted after only 2 d.

Table 6. Formation percentage (%) of new streaks, enlarged lesions, and newly formed YLS lesions observed on detached leaves in test tubes.

Days after treatment	New streaks formed[a,b,c] (%)	Enlarged lesions[a,b,c] (%)	New lesions formed[a,b,c] (%)
		Tap water	
0	0.0	0.0	0.0
3	10.0	0.0	0.0
6	20.0	36.7	0.0
10	36.7	46.7	0.0
		Sterile distilled water	
0	0.0	0.0	0.0
3	10.0	10.0	0.0
6	10.0	10.0	0.0
10	10.0	20.0	0.0

[a]Significant difference among day treatments at $P = 0.05$ level. [b]No significant difference among water treatments. [c]No significant interaction between day and water.

Transmission system

When YLS inocula were used for leaf overlapping inoculation, IR64 plants always got infected. The frequency of infected leaves inoculated through three cycles was observed (Fig. 5). With the first inoculum, incidence was 24.0%under direct sunlight treatment and 24.6% under no-morning sunlight treatment. When the second and third inocula were considered as the inocula source, the same results were obtained. The incidences were 12.6% (sunlight treatment) and 14.6% (no-sunlight treatment) in the second passage and 3.2% and 4.1%, respectively, in the third passage. However, there were no significant differences at $P = 0.05$ in the third cycle was lowest (Fig. 6). The infection efficiency of the YLS source decreased

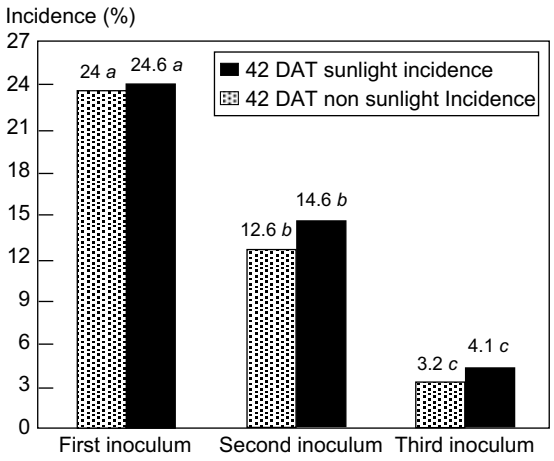

Fig. 5. Schematic representation of YLS incidence (%) on IR64 rice plants with different inocula at 42 DAT, 1998. *Av of three replications. Means followed by a common letter are significantly different at *P* = 0.05 level.

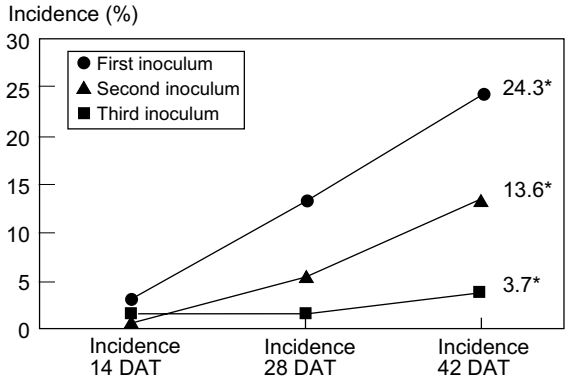

Fig. 6. YLS frequency of appearance (%) on IR64 rice plants inoculated with different inocula. *Av of three replications.

gradually after each cycle. This may be explained by the long maintenance of rice plants in the plastic cage during the experiment.

Transmission control

Spraying 0.1% Viben C 50WP onto canopies of YLS rice plants

In both observations, most leaf overlapping treatments had higher YLS severity and incidence than the leaf-separating treatments. Source plants sprayed with 0.1% Viben C 50WP had less infection frequency than water-sprayed rice plants. However, there were no significant differences in YLS incidence between the two treatments at P = 0.05 (Table 7). Viben C 50WP (781 g ai ha^{-1}, 25% benzimidazole + 25% copper oxychloride) did not prove efficient in controlling YLS at 7-d application intervals under strong YLS pressure in plastic cages in our experiments.

Spraying 0.1% Viben C 50WP onto canopies of IR64 healthy rice plants before inoculation

In this experiment, leaf overlapping and leaf separating treatments resulted in the same levels of severity and incidence. Application of 0.1% Viben C 50WP at 7-d intervals could not control YLS appearance and development effectively. Although in most cases the severity of this treatment was lower than that of the tap water spraying, no significant differences between the chemical treatment and tap water treatment were observed (Table 8).

Table 7. YLS frequency (%) of IR64 plants either leaf overlapping or leaf apart to the canopies of diseased ones sprayed with 0.1% Viben C 50WP or tap water, 1998.

Treatment	First experiment (soft dough, 42 DAT)		Second experiment (tillering, 28 DAT)		(heading, 59 DAT)	
	Severity** (%)	Incidence[a,b] (%)	Severity** (%)	Incidence[a,c] (%)	Severity** (%)	Incidence[a,c] (%)
Leaves overlapping						
Viben C 50WP***	48.8	27.3	11.1	5.6	12.2	6.8
Tap water	45.5	28.8	23.9	12.5	17.8	7.8
Leaves apart						
Viben C 50WP	42.0	25.2	0.0	0.0	4.8	2.2
Tap water	56.4	30.3	4.5	1.3	11.4	6.4

*DAT = days after treatment. **Av of three replications. ***0.1% Viben C 50WP (25% benzimidazole + 25% copper oxychloride) 781 g ai ha^{-1} applied at 7-d intervals. [a]No significant difference between sprayed treatments (*P*<0.05). [b]No significant difference between leaf contact treatments (*P*<0.05). [c]Significant difference between leaf contact treatments (*P*<0.05).

Table 8. YLS frequency (%) of IR64 rice plants with either 0.1% Viben C 50WP or tap water sprayed onto leaves of healthy plants, 1998.

Treatment	First experiment (tillering)				Second experiment (tillering, 28 DAT)	
	14 DAT		28 DAT			
	Severity** (%)	Incidence[a] (%)	Severity (%)	Incidence[b,c] (%)	Severity (%)	Incidence[b,c] (%)
Leaves overlapping						
Viben C 50WP***	0.0	0.0	11.2	5.2	7.5	3.4
Tap water	13.7	6.5	17.1	8.1	10.8	5.8
Leaves apart						
Viben C 50WP	0.0	0.0	5.5	2.7	9.9	7.7
Tap water	8.8	4.5	18.7	9.4	15.2	7.0

*DAT = days after treatment. **Av of 3 replications. ***0.1% Viben C 50WP (25% benzimidazole + 25% copper oxychloride) 781g ai ha^{-1} sprayed at 7-d intervals. [a]Significant difference between sprayed treatments (P<0.05). [b]No significant difference between sprayed treatments (P<0.05). [c]No significant difference between leaf contact treatments (P<0.05).

Conclusions

Until now, the causal agent of YLS or RS is still not known.

YLS may develop and be transmitted under a high RH condition in plastic frames.

YLS may not be transmitted by crude extract, unlike other diseases spread by mechanical transmission of virus.

From infected leaves, YLS lesions may infect healthy leaves of YLS-free tillers, not considering overlapping or separation of YLS leaves.

Once healthy leaves got into contact with fresh YLS leaves (undetached from rice plants), they are easily infected with YLS after 3–8 d at high RH condition of plastic frames. The causal agent can actively penetrate leaf tissues without previous wounds.

Diseased rice tillers and fresh YLS-infected leaf tissues may be considered as YLS inocula of disease transmission under favorable conditions.

YLS is not transmitted through soil collected from infected fields and through ratoons of diseased tillers and root contact.

YLS lesions may continue increasing in size, forming new streaks toward the leaf tips when detached leaves are maintained fresh in test tubes of water.

The methods used in the present study did not suggest that YLS could be controlled or prevented effectively by spray application of Viben C 50WP (benzimidazole 25% and copper oxychloride 25%) (781 g ai ha^{-1}) before leaf overlapping in plastic frame condition.

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Notes

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Appendix. Soil sample analysis.

Site	N (%)	Available Olsen P (mg kg^{-1})	Exchangeable K (ppm)	pH (H_2O)
Long Thanh, Cai lay, Tien Giang	0.284	104	0.332	5.2
Nhi Quy, Cai lay, Tien Giang	0.249	76	0.397	5.5
Diem Hy Cai lay, Tien Giang	0.127	19	0.288	6.2
Diem Hy Cai lay, Tien Giang	0.157	49	0.382	5.7
Tan Hiep, Hoc Mon, HCM City	0.822	22.3	7.205	5.4
Screenhouse IAS	0.239	149	0.32	6.3

Studies on some aspects of red stripe disease of rice in the Mekong Delta

Pham Van Du, T. Noda, and Lai Van E

"Red stripe," "bacterial red stripe," or "rice leaf yellowing" are different names for the same syndrome, a new disease of rice recorded in some Asian countries. It was reported for the first time in 1986 (Mogi et al 1988) in Indonesia. In Vietnam, it was seen for the first time in the Mekong Delta during the 1987-88 dry season. Subsequently, it was also observed in Mindanao, southern Philippines, Thailand, Cambodia, and Lao PDR. The disease was found in all areas with intensive rice cultivation under the humid tropical conditions of Asia. Until now, this syndrome has not been reported in temperate rice-growing countries such as mainland China, Korea, Taiwan (China), and Japan.

In the Mekong Delta, a severe attack can cause yield losses up to 50% where no-till was commonly used with high-density planting, direct seeding, and high N application. Disease appearance at the early stage leads to early maturity but grains do not completely mature. At present, red stripe disease develops endemically in every season at the hard dough stage of the rice crop.

Symptoms

A typical symptom is a lesion with a grayish center at the late development stage. An orange stripe extends from the lesion. The initial type of lesion may either be a development type (acute) or oval type (chronic). The development-type lesion is water-soaked, which is clearly seen in early morning, and it develops upward rapidly from one end of the spot within a few days (3–4 d). The entire leaf may be rolled and dried. The stripe extends downward in some cases. Numerous stripes may occur on the same leaf. Lesions are also found in the leaf sheath. The chronic lesion type maybe seen as yellowing spots but this type will not develop into a large stripe as does the acute type.

In the field, when leaves are dirty with "pollen grains" at the heading stage, the plant becomes sensitive to the disease. In the milky stage, a few lesions can be found in the 1st and 2nd leaves at the base of the plant. Disease is severe at the hard dough stage. Lesions are concentrated more in the leaf angle than in other parts of the flag leaf.

Resistance to red stripe syndrome

Resistance of rice cultivars to this disease has been observed since 1989-90. Of more than 1,000 lines and cultivars of short duration, none were resistant to the disease, based on disease assessment using leaf area covered by the disease (%). Disease was recorded during the milky and maturity stages; some cultivars were infected earlier while others showed less severity (tolerance) at the maturity stage. OM1490 and OM1721-50-2 were the two cultivars that showed early infection to red stripe (Tables 1 and 2). TH54-5 and TH24 showed less severity than did others that were nearly 50% infected. A survey conducted in the 1998 dry season found several varieties with severity of <20% (Table 3, Fig. 1).

Red stripe disease progress curve

When red stripe lesion develops from the milky to dough stage, severity can go up to 80–100% at the time of harvest. Disease severity in short-duration varieties is normally observed starting at 60 d after transplanting (DAT); by 70–80 DAT, red stripe on the rice leaf is fast spreading (Fig. 2).

Testing of pesticides against disease development

Since the 1990 dry season, many kinds of fungicides and bactericides have been tested to control the disease. Only a few fungicides—benomyl (benlate), fundazol, other products from benomyl mixture (copper-B, benlate-C), carbendazim (Derosal), and propiconazole (Tilt)—could suppress disease

Table 1. Reaction of cultivars to red stripe syndrome observed at the milky stage (60 d after transplanting). CLRRI, 1996-97 dry season.

Cultivar	Disease severity (%)
OM1490	2.54
OM1721-50-2	1.48
IR59673	0.00
IR64	0.00
IR62030	0.00
IR62032	0.00
KSB288-24	0.00
KSB140-5	0.00
MTL148	0.00
MTL145	0.00
MTL149	0.00
MTL153	0.00
MTL48	0.00
MTL152	0.00
OMCS 94	0.00
OM1314	0.00
OM1248-27	0.00
OM1706	0.00
OM1704	0.00
OM1270-49	0.00
OM1666	0.00
OM1633	0.00
OM1630	0.00
OM269	0.00
OM997	0.00
OM1271-50-1	0.00
OM1570	0.00
OM1726	0.00
TH17	0.00
TH24	0.00
TH54-5	0.00
TH50-2	0.00
TH66	0.00
TNDB100	0.00
VND95-19	0.00
VN95-19S2	0.00
VN97-1	0.00

Table 2. Reaction of cultivars to red stripe syndrome observed at the maturity stage (78 d after transplanting). CLRRI, 1996-97 dry season.

Cultivar	Disease severity[a] (%)
OMCS 94	59.20 a
OM1248-27	55.42 ab
IR59673	55.01 ab
OM1490	53.83 abc
MTL145	53.34 abcd
OM1706	51.61 abcde
MTL149	51.15 abcde
MTL153	50.30 abcde
TH50-2	49.61 abcdef
OM1270-49	49.18 abcdef
VND95-19	48.78 abcdefg
OM1630	48.61 abcdefg
IR62030	47.62 abcdefg
OM269	45.90 abcdefgh
VN95-19S2	45.29 abcdefgh
OM997	45.26 abcdefgh
IR62032	44.46 abcdefgh
OM1570	44.30 abcdefgh
OM1726	44.25 bcdefgh
OM1314	44.11 bcdefgh
OM1721-50-2	44.04 bcdefgh
MTL148	43.92 bcdefgh
TNDB100	43.32 bcdefgh
IR64	43.30 bcdefgh
TH66	42.69 bcdefgh
KSB288-24	41.23 bcdefgh
OM1704	39.68 cdefghi
TH17	39.65 cdefghi
OM1666	38.52 cdefghi
OM1633	38.04 cdefghi
KSB140-5	37.31 cdefghi
MTL 48	34.86 fghij
MTL152	33.97 ghij
VN97-1	32.23 hij
OM1271-50-1	31.38 hij
TH54-5	26.20 ij
TH24	21.75 ij

[a]Means are average of three replications. Means followed by the same letters are not significantly different using Duncan's multiple range test, $P < 0.0001$.

development (Table 4). Bactericides showed no effect on the disease.

Effects of fertilizer applications and cultural practices on red stripe

Since the outbreak of the disease, many experiments have been conducted at the Cuu Long Delta Rice Research Institute (CLRRI) to understand the role of fertilizer application in disease development. Some field trials were conducted in three successive crop seasons:

- Long-term effect of NPK application on yield and yield components of IR64, 1990 wet season (9th)
- Residual effect of different P_2O_5 levels on yield and yield components of IR64 (12th), 1990 wet season, 1991 dry season
- Effect of NP combined application on some promising varieties, 1991 wet season
- Effect of N source applied on yield and yield components of OM86, 1990 late wet season (Fig. 5)
- Effect of N application and plant spacing on pest and disease occurrence, yield and yield components of two varieties, IR64 and OM606, 1991 wet season
- Effect of N application on diseases, yield, and yield components of some leading short-duration varieties, 1991 dry season

Table 3. Red stripe symptoms recorded on promising varieties of the Mekong Delta, 1998 dry season.

Variety	Duration (d)	Percent severity				
		59-61 DAT[a]	66-68 DAT	73-75 DAT	78-80 DAT	Yield (t ha^{-1})
OM1701-2	90	0.0	12.5	29.3	33.1	5.17
OMCS1490	90	14.0	47.5	59.7	68.5	*[b]
H1	90	0.0	27.5	29.9	28.3	5.44
OM2058-17	90	0.0	11.3	13.9	21.7	5.99
OM2057-20	90	0.0	20.0	33.8	40.8	5.13
OM2053	91	0.0	11.3	28.5	40.7	5.05
L24	93	0.0	10.0	30.9	35.1	5.87
OM2048	93	0.0	25.0	27.3	36.1	5.28
ML65	94	20.0	35.5	44.5	33.1	4.77
ML62	95	0.0	23.3	50.0	32.1	5.95
OM1633	96	5.0	25.0	42.9	54.7	6.28
VND95	97	0.0	20.3	33.1	64.9	6.73
OMCS97-1	97	0.0	15.1	43.2	41.8	3.20
OM997	98	0.0	27.4	52.3	55.2	5.56
OMCS 95-5	98	8.0	12.0	29.6	34.1	3.10
OMCS97-25	98	18.8	8.0	31.4	34.9	3.20
OMCS96	98	24.0	5.5	71.1	50.2	8.99
OM1995	98	11.7	17.1	54.3	52.6	3.10
OM1308	98	22.5	9.1	49.6	27.3	8.00
IR70140	98	0.0	0.0	32.3	43.1	3.12
AC1007	98	56.0	81.0	19.8	20.0	3.10
OM1490	98	7.3	30.0	26.2	51.1	3.80
TNDB (check)	99	0.0	10.0	33.2	53.6	5.47
KMD40-1	99	0.0	0.0	18.0	39.6	5.30
Mäüt buûi-1	100	0.0	16.3	36.0	53.8	5.00
OM1033	100	16.7	23.3	27.5	66.5	3.30
OM2008	100	0.0	37.5	27.7	19.4	2.75
NANJING	100	1.0	0.0	13.3	17.1	2.44
NÃÚP2065	100	0.0	5.0	22.5	37.9	3.40
OM1271-1	100	5.0	9.5	10.0	20.9	2.78
OM1314	103	0.0	36.7	33.2	53.7	6.30
OM1723-62	105	0.0	6.2	27.8	51.4	3.28
OM1269	105	0.0	24.5	31.8	54.1	2.92
IR65596	105	2.0	3.7	14.8	58.5	4.22
IR62126	105	3.3	41.4	40.2	52.5	3.43
OM1647-8	105	1.0	3.5	18.6	45.4	3.32
OM2014	105	3.5	8.0	32.9	44.7	3.40
IR50404NCM	105	1.0	36.3	12.2	31.9	3.22
IR64	105	0.0	2.0	19.8	31.8	4.40
KSB288	106	0.0	22.1	32.0	49.4	6.20
OM1704	108	0.0	6.6	49.3	57.6	6.16
IR62030	108	0.0	18.3	35.7	68.6	6.76
IR62126	108	0.0	13.3	43.1	45.5	5.01
IR64	108	0.0	17.6	40.6	56.1	5.98
IR62032	108	0.0	7.5	32.6	47.8	6.26
MTL147	110	15.0	27.4	64.3	47.1	5.83
MTL150	110	0.0	13.0	31.6	53.7	5.09
OM1493	110	2.0	5.1	15.3	40.5	2.73
ÂS96	110	1.0	0.0	30.2	35.8	2.63
ÂS20	110	2.0	15.0	24.7	29.6	2.10
OM1643	110	5.0	41.8	25.1	50.5	3.45
OM1726	110	3.0	32.5	36.9	35.9	6.50
OM1570	110	0.0	30.0	58.5	49.1	6.40
MTL152	111	20.0	32.5	33.8	41.2	5.40

[a]DAT = days after transplanting. [b]*Missing.

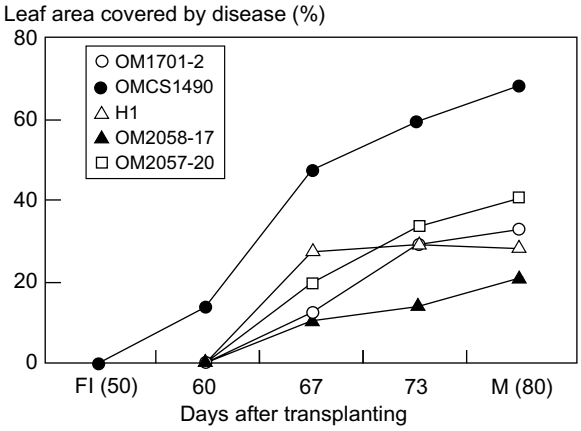

Fig. 1. Reaction of varieties with different levels of severity against red stripe syndrome.

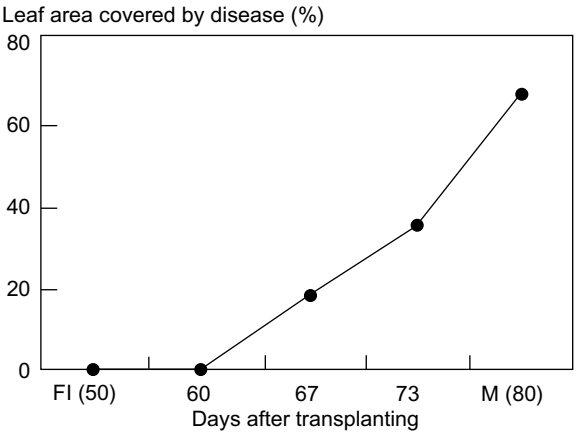

Fig. 2. Typical red stripe disease progress curve of variety IR62030, 1998 dry season.

Table 4. Effect of fungicides and bactericides against red stripe disease under field conditions.

Name of product (common or trade name)	Source	Controlling effect[a]
Fungicides		
Fosetyl/Efosite-Al (Aliette)	Rhoâne-Poulenc	–
Benomyl (benlate)	Dupont	+ +
Fundazol	Hungary	+
Cu + benlate (copper-B, benlate-C)	Can Tho University	+
Edifenfos (Hinosan)	Bayer	–
Hexaconazol (Anvil)	Zeneca	–
Iprofenfos (Kitazin)	Kumiai Chem Inc.	–
Iprodione (Rovral)	Rhoâne-Poulenc	–
Iprothiolane (Fuji-one)	Nihon Noyaku Co.	–
Kasugamycin + Rabcide (Kasai)	Hokko Chem Co.	–
Propiconazole (Tilt)	Ciba Geigy	+ +
Thioplanate methyl (Topsin-M)	Nippon Soda Co.	–
Validamycin (Validacin)	Takeda Chem Co.	–
Zineb	Dupont	–
Thioplanate (Cercosin)	ICI	–
Monceren	Bayer	–
Carbendazim (Derosal)	Hoechst	+ +
Triphenyl tin hydroxide (Brestan)	Hoechst	–
Bactericides		
Kasuran	Hokko Chem Co.	–
Probenazole (Oryzemate)	Meiji Kaisha	–
S 0208 (Starner)		–
Penicillin + streptomycin		–

[a]Results of field tests from 1990 to 1998, Plant Pathology Department, CLRRI. – = no effect, + = effective.

- Effect of N application and seeding rate on pest and disease occurrence, yield, and yield components of two varieties, IR64 and OM606, 1991 wet season

Results from these experiments can be summarized as follows:

- Without NPK application for a long time (9th crop), red stripe also occurs (Fig. 6)
- Potassium (K_2O) reduces red stripe severity (Fig. 6)
- P_2O_5 enhances red stripe severity (Fig. 7)
- Nitrogen enhances red stripe severity (Figs. 3, 4)
- High planting density and seeding rate increase red stripe severity (Figs. 3, 4)
- NP combined enhances red stripe severity

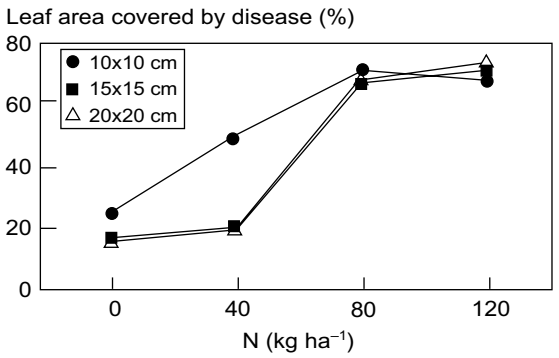

Fig. 3. Effect of N and planting density (transplanting) on red stripe disease, CLRRI, O Mon, Can Tho.

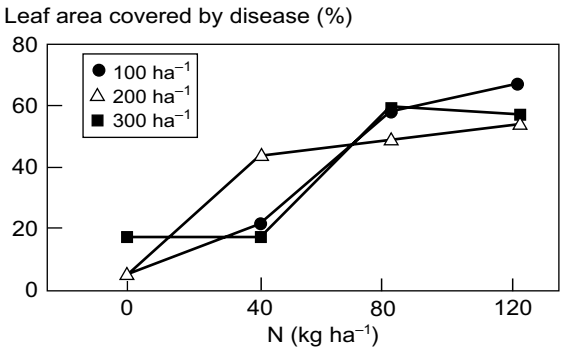

Fig. 4. Effect of N application and planting density (direct seeding, 100, 200, and 300 kg ha^{-1}) on red stripe disease, CLRRI, O Mon, Can Tho.

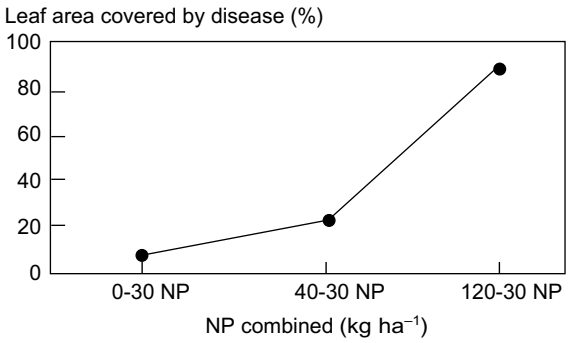

Fig. 5. Effect of NP combined application on red stripe disease, CLRRI, O Mon, Can Tho.

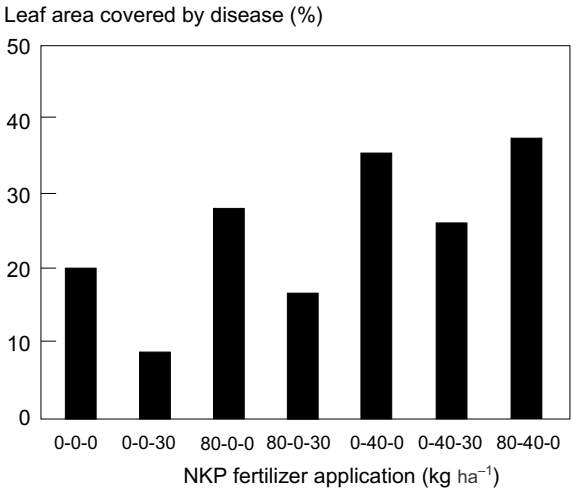

Fig. 6. Effect of NPK application on red stripe disease, CLRRI, O Mon, Can Tho.

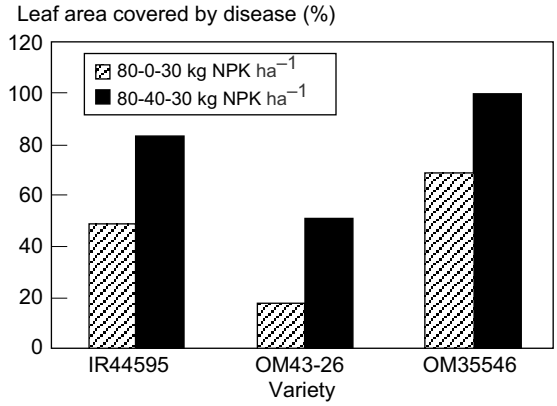

Fig. 7. Effect of NPK fertilizer application on red stripe disease in different varieties, CLRRI, O Mon, Can Tho.

Etiology with hypothesis testing

The causal agent of the disease is still unknown. Several hypotheses on the origin of the disease had been studied by many scientists with no conclusion results. Some of the issues that need to be addressed by future research follow:

- Disease appearance after milky stage. Lesions are first found in older leaves of rice plants. In the flag leaf, more lesions are found concentrated in the leaf angle.
- Disease can be observed at the very early stage, even in 20-d-old seedlings.
- Disease severity and spread occur under lowland conditions. Standing water also enhances severity. Under upland conditions, appearance of the disease in the same variety is not observed.
- Healthy leaves will be contaminated when they make contact with red stripe lesions.
- Surfaces of lesions are sometimes very clean; the red stripe lesion seems to develop fast.
- Cultural practices seem to play an important role in disease development.
- Benomyl, Derosal, and Tilt appear to suppress lesion development, but some bactericides have no effect.

Red stripe disease appears to be associated with a kind of toxin development that might be produced by an organism started with initial type of very small yellowing spots. Rice plants could be infected from very early stages. The appearance of the red stripe "toxin" is observed with a lot of "dirty-pollen" on the leaf surface from the heading stage onward.

Future research should address the following concerns:

- What is the causal agent of the disease?
- What biochemical factors are related to the appearance of typical symptoms (red stripe toxic, upward expansion)?
- How can research funds be assured?

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Mogi S, Sugandhi Z, Baskoro SW. 1988. A newly discovered disease (bacterial red stripe) on rice in Indonesia, its symptoms and distribution. In: Proceedings of the 5th International Congress of Plant Pathology, Kyoto, Japan. Abstr. p 388.

Notes

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Effects of soil and nutrients on red stripe symptoms of rice

Cao Van Phung and Luu Hong Man

Rice covers large areas in Vietnam, especially in the Mekong Delta where rice production contributes more than 50% to the country's total and makes up about 80% for export. Under intensive rice farming (more than two rice crops per year), rice cultivation in the Mekong Delta now faces a new threat—red stripe— which reduces rice yield.

Red stripe symptoms were observed first in Indonesia and were reported to occur in Vietnam in 1987. The etiology of red stripe is still unknown, despite much effort spent by many scientists.

The symptoms are often observed in fields under intensive cultivation during both the dry and wet seasons. They are more likely to appear at the time of flowering. Severity depends on early or late occurrence, leading to heavy or light yield losses (Phung 1992). Red stripe symptoms first occur on the leaf as a pin-size spot, gradually enlarging and moving up to the tip. In serious cases, all basal leaves become dry and turn orange-red; the flag leaf also dries up.

Materials and methods

A series of experiments have been conducted at the experimental farm of the Cuu Long Delta Rice Research Institute to identify the causal agent of red stripe. The experimental plot has the following soil characteristics:

Soil type: Fluvaquentic Humaquept
Soil texture: 88% clay, 10% silt, 1–2% sand–heavy clay soil
Infiltration rate (<5 mm h^{-1})
CEC: 21.5 meq 100 g^{-1}
pH: 4.4-5.2
EC: 0.14 mS cm^{-1}
Organic C: 2.2%
Total N: 0.24%
Total P: 0.06% Available P (Bray 2): <2 ppm
Total K: 0.18%

1. Fertilizer experiment (1997-98 dry season)
   Rice variety: IR64
   Experimental design: split plot
   Main plot is lime application of 200 kg Ca ha^{-1} and no amendment
   Split plot is fertilizer treatment (kg NPK ha^{-1}) as follows:

60-60-60	80-80-100
80-60-60	100-80-80
80-80-80	100-100-100

   Plot size: 6 m × 6 m

2. Control of red stripe by plant separation in nylon net cages (1998 wet season)
   Rice variety: AS833
   Experimental design: split plot
   Main plot is lime application of 400 kg Ca ha^{-1} and no amendment
   Split plot is fertilizer treatment—60 and 100 kg N ha^{-1} in combination with 60 kg P_2O_5 and 60 kg K_2O ha^{-1}
   Plot size: 6 m × 6 m

3. Effect of water management and chemicals on red stripe symptoms (1998-99 dry season)
   Rice variety: AS833
   Experimental design: split plot
   Main plot is water management: flooding (W1) and intermittent wet and dry (W2)
   Split plot is fertilizer treatment (kg NPK ha^{-1}) as follows:
   80-40-30 (F1)
   80-60-60 (F2)
   100-80-60 (F3)
   Plot size: 6 m × 6 m

Rice was transplanted in all three experi-ments and the red stripe spots were scored in terms of percentage of the rice plant having red stripe spots on the flag leaf. Four chemicals were applied before flowering: Tilt (C1), butyl (C2), Cypran (C3), and copper sulfate (C4).

Results and discussion

Results of the first experiment showed that red stripe symptoms were more with the high N treatment (Table 1). Lime amendment seemed to reduce infection, but it was not significantly different from no-lime application. Red stripe spots are quite high under low N (60-60-60) and in the plots without-lime application perhaps due to the water level in the rice field.

Since rice was not heavily infected with red stripe, the occurrence of the symptoms late after flowering explains why rice yields were not significantly different among treatments (Table 2).

An analysis of the NPK content of rice straw and seed (Tables 3-8) revealed no statistical differences among treatments. These indicate that N, P, and K uptake in rice plants was sufficient to ensure normal growth and hence rice yields.

Table 1. Red stripe index on rice.

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	12.3 b	28.0 ab	20.2 b
80-60-60	11.7 b	11.7 b	11.7 b
80-80-80	4.0 b	29.7 ab	16.8 b
80-80-100	17.0 ab	22.0 ab	19.5 b
100-80-80	22.0 ab	27.0 ab	24.5 ab
100-100-100	35.0 a	41.7 a	38.3 a
Av	17.0	21.8	19.4 a

Table 2. Rice yields (t ha^{-1}).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	7.0	6.8	6.9
80-60-60	6.7	6.7	6.7
80-80-80	6.1	6.5	6.3
80-80-100	6.8	6.5	6.6
100-80-80	7.0	6.2	6.6
100-100-100	6.6	6.7	6.6
Av	6.7	6.5	6.6

Table 3. Nitrogen content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	0.63 b	0.78 a	0.75
80-60-60	0.60 b	0.71 a	0.65
80-80-80	0.75b	0.69 a	0.72
80-80-100	0.8ab	0.70 a	0.69
100-80-80	0.74 a	0.68 a	0.71
100-100-100	0.73 a	0.73 a	0.73
Av	0.69	0.72	0.70

Table 4. Nitrogen content in rice seed (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	1.17 b	1.21 b	1.19
80-60-60	1.26 ab	1.2 b	1.24
80-80-80	1.20 b	1.26 b	1.23
80-80-100	1.37 a	1.23 b	1.30
100-80-80	1.36 a	1.26 b	1.31
100-100-100	1.28 ab	1.43 a	1.36
Av	1.27	1.2	1.27

Table 5. Phosphorus content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	0.12	0.15	0.13
80-60-60	0.12	0.12	0.12
80-80-80	0.13	0.13	0.13
80-80-100	0.12	0.13	0.13
100-80-80	0.13	0.13	0.13
100-100-100	0.13	0.13	0.13
Av	0.123	0.13	0.13

Table 6. Phosphorus content in rice seed (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	0.15	0.15	0.15
80-60-60	0.18	0.16	0.17
80-80-80	0.17	0.15	0.16
80-80-100	0.19	0.17	0.18
100-80-80	0.18	0.15	0.17
100-100-100	0.18	0.18	0.18
Av	0.17	0.16	0.17

Table 7. Potassium content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	1.67	1.64	1.65
80-60-60	1.67	1.63	1.64
80-80-80	1.66	1.92	1.79
80-80-100	1.71	1.71	1.71
100-80-80	1.74	1.83	1.79
100-100-100	1.75	1.76	1.75
Av	1.70	1.74	1.72

Table 8. Potassium content in rice seed (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	0.21	0.23	0.22
80-60-60	0.23	0.21	0.22
80-80-80	0.22	0.22	0.22
80-80-100	0.28	0.25	0.27
100-80-80	0.27	0.21	0.24
100-100-100	0.26	0.27	0.27
Av	0.25	0.23	0.24

Table 9. Calcium content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	0.015	0.019	0.017
80-60-60	0.016	0.014	0.015
80-80-80	0.019	0.019	0.019
80-80-100	0.020	0.018	0.019
100-80-80	0.027	0.021	0.002
100-100-100	0.021	0.019	0.020
Av	0.019	0.018	0.019

Table 10. Magnesium content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	0.017	0.018a	0.017ab
80-60-60	0.014	0.014b	0.015c
80-80-80	0.016	0.017ab	0.016abc
80-80-100	0.016	0.0144b	0.015bc
100-80-80	0.017	0.019a	0.018a
100-100-100	0.017	0.017a	0.017a
Av	0.016	0.017	0.017

Table 11. Iron content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	262.7	285.3	274.0
80-60-60	291.3	260.0	275.7
80-80-80	266.0	267.3	266.7
80-80-100	250.0	244.7	250.3
100-80-80	258.0	258.0	258.0
100-100-100	277.3	265.3	271.3
Av	268.6	263.4	266.0

Table 12. Manganese content in rice straw (%).

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	200	0	Av
60-60-60	358.7	403.3	381.0
80-60-60	428.7	402.7	415.7
80-80-80	422.7	483.3	453.0
80-80-100	458.7	361.3	409.0
100-80-80	494.0	456.7	475.3
100-100-100	470.7	467.3	469.0
Av	438.6	429.1	433.8

Table 13. Fe-Mn ratio in rice straw.

Fertilizer treatment (kg NPK ha^{-1})	Av
60-60-60	0.71c
80-60-60	0.66bc
80-80-80	0.59ab
80-80-100	0.61ab
100-80-80	0.54a
100-100-100	0.58a
Av	0.62

Calcium content in rice straw also did not show any statistical difference among treatments. Magnesium content in rice straw, however, differed among treatments in no amendment with lime (Tables 9 and 10).

It seemed that application of 200 kg Ca ha^{-1} was not adequate to cause any difference in iron and manganese uptakes in the rice plant (Tables 11 and 12).

The results of Fe/Mn shown in Table 13 is interesting. The high N application tended to reduce this ratio. An increase in P and K application also seemed to decrease Fe uptake in the rice plant.

In the 1998 wet season, an increase in lime application (400 kg Ca ha^{-1}) significantly increased rice yield in the amended plot (Table 14).

Insects were prevented from attacking the rice plant by using nylon net cages; unfortunately, all rice plants inside the cages were heavily infected with red stripe (Table 15). We also observed that the temperature and humidity inside the cage were always higher than those outside, so these may have contributed to the development of red stripe.

The measurements of pH and redox potential (Eh) did not reveal any significant differences in these parameters (Tables 16 and 17).

The data in Table 18 clearly showed that water management in the 1998-99 dry season had strongly influenced the development of red stripe symptoms. The ANOVA table also indicated an interaction between N dosage and water management.

All chemicals (fungicides and insecticides) did not effectively control red stripe on rice (Table 19).

Table 14. Rice yield (t ha^{-1}) in the 1998 wet season.

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	400	0	Av
60-60-60	2.77	3.02	2.89
100-60-60	2.89	3.20	3.04
Av	2.83	3.11	2.97
LSD (5%) 2-Ca mean = 0.253			

Table 15. Percentage of rice plants infected with red stripe.

Fertilizer treatment (kg NPK ha^{-1})	% infected plants	
	Inside	Outside
60-60-60	98	24
60-60-60	99	16
60-60-60	97	5
100-60-60	100	38
100-60-60	100	25
100-60-60	100	5
Av	99	19

Table 16. Soil pH measurement at panicle initiation under different treatments.

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	400	0	Av
60-60-60	6.72	6.59	6.65
100-60-60	6.68	6.47	6.58
Av	6.70	6.53	6.62

Table 17. Redox potential (mV) measured at panicle initiation under different treatments.

Fertilizer treatment (kg NPK ha^{-1})	Calcium (kg ha^{-1})		
	400	0	Av
60-60-60	–306.7	–300.7	–303.7
100-60-60	–218.3	–300.3	–259.3
Av	–262.5	–300.5	–281.5

Table 18. Effect of water management on red stripe symptoms.

Fertilizer treatment (kg NPK ha^{-1})	Water management		
	W1	W2	Av
60-60-60	4.8	2.0	3.4
100-60-60	55.0	1.0	28.0
Av	29.9	1.5	15.7
LSD (1%) 2-fert mean at each W = 12.45			

Table 19. Effects of chemicals on red stripe.

Chemical	Fertilizers			
	F1	F2	F3	Av
C1	17.33 a	8.00 a	16.33 a	13.89 a
C2	6.33 a	2.33 a	23.67 a	10.78 a
C3	5.33 a	5.33 a	27.00 a	12.56 a
C4	17.33 a	8.67 a	22.00 a	16.00 a

Conclusions

A high dosage of N, high temperature, and high humidity favor the appearance of red stripe symptoms. Some insecticides and fungicides could not effectively control this disease. Future work should concentrate on finding out the abiotic causal agent of these symptoms.

Notes

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Red stripe and associated diseases in Vietnam

Nguyen Van Tuat

Rice is the world's leading food crop and the major source of livelihood for the majority of rural people in Asia, the most densely populated region in the world.

During the last few years of Vietnam's economic renovation, agricultural production, particularly food production, has recorded important increases in yield. The data collected by MARD point to Vietnam's great achievement in food grain production. In 1996, it produced 29.2 million t; of this, rice accounted for 26.4 million t. In 1997, it produced 30.6 million t of food grain and 27.7 million t of rice grain. The 1998 figures were 31.7 million t and 29.8 million t, respectively.

One of the important factors that enhanced rice production was the use of intensive farming, new high-yielding varieties, more rice cropping seasons per year, and larger doses of fertilizer.

International and local experience, however, had shown that intensive farming often breaks up the biological balance between agricultural crops and their pests, resulting in greater pest emergence and greater damage. In the last few years, the areas infected by such important pests as brown planthopper (BPH), leaf roller (LR), stem borer (SB), rice blast (RB), and sheath blight (ShB) have obviously expanded. In addition to these pests, several new diseases appeared on a fairly large area such as leaf yellow syndrome or red stripe (in the Mekong Delta), bacterial grain rot, grain discoloration, and tungro virus disease.

This paper presents the initial results of research on leaf yellowing syndrome and other associated diseases in Vietnam.

Current status of leaf yellowing syndrome

Leaf yellowing syndrome (LYS) was first recorded in the Mekong Delta in 1998. The local people called this newly emerging symptom "premature ripening disease."

Prompt action to control this newly emerging disease in terms of disease surveys and control method studies was taken in 1990-93 in the Mekong Delta by the National Institute of Plant Protection (NIPP) and the Southern Regional Plant Protection Center in Long Dinh-Tien Giang.

Nevertheless, the disease has continued to spread, causing damage to rice production in the Mekong Delta (Table 1).

Research on seedborne diseases of rice

Studies on components of the seedborne diseases have been conducted by NIPP in the last few years. Samples were collected periodically in different growth stages starting from the milky stage up to 15

Table 1. Status of leaf yellowing symptom infection in Vietnam.[a]

Year	Crop season						Total (ha)
	Winter-spring		Summer-autumn		Monsoon		
	Infected area (ha)	% incidence	Infected area (ha)	% incidence	Infected area (ha)	% incidence	
1996	132,413		41,780		25,051		199,244 (22,395)
1997	–	–	–	–	–	–	256,675
1998	38,171	2.81	21,821	1.12	3,510	0.46	63,502 (540)

[a]Heavily infected areas shown in parentheses.

Table 2. Seedborne diseases found in rice grain in Vietnam.

Common name	Causal agent	Distribution[a]	
		North Vietnam	Central Vietnam
Brown spot	*Bipolaris oryzae*	++	+++
Black gray	*Curvularia lunata*	+++	++
Pink mold	*Fusarium moniliforme*	++	++
Black smut	*Tilletia horrida*	+	+
White spot	*Phyllosticta*	+	+
False smut	*Ustilaginoidea virens*	–	–
Grain discoloration	*Alternaria* sp.	++	–
Narrow brown leaf spot	*Cercospora oryzae*	+	+
	Nigrospora sp.	+	+
Blast	*Pyricularia oryzae*	+	+
	Rhizoctonia oryzae	+	+
	Penicillium sp.	+	+
Grain rot	*Pseudomonas glumae*	+++	+++
	Xanthomonas sp.	+	+

[a]+++ = high frequency of availability, ++ = average availability, + = low availability, – = no availability.

d before harvest. The microorganisms obtained from these samples have been identified (Table 2).

Twelve species of fungi and two of bacteria were found in rice grain in north and central Vietnam.

Identification and characterization of *Pseudomonas avenae*

Rice grains collected from diseased LYS plants were examined for the presence of microorganisms associated with LYS. Varieties CR203, MTL 58, and A10 were assessed. Several microorganisms were identified.

Examination of induced LYS symptoms in seed

Seeds with typical LYS symptoms were examined in the laboratory. The results showed that seedlings derived from these seeds also produced the same symptoms. Stripes were found in the leaf blade and the leaf turned yellow and developed poorly. The leaves appeared water-soaked and became rotten. These plantlets were isolated on NA; 3 d after inoculation, colonies were formed and identified as *Pseudomonas avenae* (Table 3).

Chemotaxonomy of *P. avenae*

A similar study in terms of identification of the pathogen that causes LYS in Vietnam has been done at the IMI, UK. The chemotaxonomy method aimed to confirm the results derived from the biochemical tests (Table 4).

The data given indicated the existence of *P. avenae* upon comparison with the type isolate of IMI (number NCPPB 1011) (Table 4). The new approved name for this bacterium is *P. avenae* subsp. *avenae* (Mann) (Williams-Goor 1992), with 17 synonym names documented in the literature.

Cluster analysis of FAME profiles of *P. avenae*

Examination of quantitative fatty acid data by cluster analysis revealed that the 13 strains of *P. avenae* could be placed in two subgroups (A and B) based

Table 3. Biochemical tests to evaluate the presence of *P. avenae.*

Test	Bacterial strains[a]						
	Viet 58	Viet 84	Viet 134	Viet 136	Viet 137	Viet 201	IMI351968
Gram stain	–	–	–	–	–	–	–
KOH	–	–	–	–	–	–	–
Oxidase	+	+	+	+	+	+	–
NO_3	+	+	+	+	+	+	+
P_5F	–	–	–	–	–	–	–
Anaerobic COH	–	–	–	–	–	–	–
Aerobic COH	+	+	+	+	+	+	+
Potato rot	–/+	–	+	–	–	–	–
Arginin	–	+	+	+	+	+	+
Levan	–	–	–	–	–	–	–
SSA	–	–	–	–	–	–	–
Catalase	+	+	+	+	+	+	+
Urease	+	+	+	+	+	+	+
Milk	+	+	+	+	+	+	+
H_2S	–	–	–	–	–	–	–
AÉS	–	–	–	–	–	–	–
NaCl 2%	+	+	+	+	+	+	+
5%	–	–	–	–	–	–	–
Gelatin	–	+	+	+	+	+	+

[a]Isolates Viet 58, 84, and 201 were collected from Ha Tay, Hanoi Province, Viet 136 from Dong Nai Province, and Viet 134 and 137 from Long An Province.

Table 4. Fatty acid profiles of *P. avenae* isolated from Vietnam.

Fatty acid	Value in profile (%)						
	Viet 58	Viet 84	Viet 134	Viet 136	Viet 137	*C. acido-vorans*	*P. avenae*
10:0	0.23	0.27	0.21	0.25	0.22	–	0.20
10:0 30H	2.86	2.93	3.47	3.29	3.54	2.8	3.1
12:0	2.41	2.54	2.85	2.59	2.9	2.6	2.5
14:0	2.63	2.71	0.86	2.4	0.79	0.7	2.4
15:0	0.43	0.46	2.3	0.24	0.17	1.9	0.3
16:1cis 9	39.15	37.48	39.15	42.29	42.23	36.4	41.4
16:0	34.7	36.58	33.51	33.61	33.43	32.0	31.9
17:0 cyclo	1.37	6.13	5.06	1.69	2.19	6.3	0.8
18:1 cis 11	12.88	10.45	10.46	12.62	13.0	16.0	15.5
18:0	0.39	0.23	0.29	0.26	0.34	V	0.2

mainly on the relative amounts of some acid compounds (C10: 30H, C16:1, cis 9, C16:0, C17: cyclo, and C18:1 cis 11). The relationships of the FAME profiles of the *P. avenae* isolates to each other are depicted in the dendrogram (Fig. 1) based on the coefficient of similarity generated by the Euclidean distance between pairs of bacteria. At a Euclidean distance of about 7.2, all strains of *P. avenae* were grouped together as a single cluster. At a distance of about 6.4, the two different clusters were well separated into subgroups A and B of *P. avenae*.

Discussion

According to Bradbury (1986) and Saddler (1994), *P. avenae* Mann 1909 has several synonym names. The pathogen can cause bacterial leaf blight in maize and sorghum, brown stripe in rice, and red stripe in sugarcane. The pathogen can also cause streak and stripe symptoms on leaves, and occasionally on sheaths, and discoloration of rice grains. The symptoms are more severe on seedlings and immature plants of rice. Occasionally, stalk rot develops in many crops of the family *Graminaceae*.

The results of our identification confirmed that the causative pathogen of yellow stripe in Vietnam was associated with *P. avenae* subsp. *avenae*. The bacterium can be easily isolated from discolored grains of rice. Six strains were identified; they were closely related to each other (coefficient of similarity of 92.8%). The dendrogram (Fig. 1) generated by cluster analysis of fatty acid components clearly showed two distinct subgroups of the bacterium. Subgroup A includes all strains of *P. avenae*, whereas subgroup B includes strains of *P. avenae* subsp. *citrulli*. The bacterium *P. rubrilineans* isolated from *Saccharum officinarum*, which is causing red stripe in sugarcane, is closely related to strains of *P. avenae* subsp. *avenae* (coefficient of similarity is 93.6%). It is possible that the causative agent of red stripe in sugarcane is also associated with yellowing stripe disease of rice (Fig. 1). The remaining questions that need to be answered concern tests on host pathogenicity and frequency of bacteria isolated from diseased lesions.

Proposals for further research

- Conduct studies on etiology of LYS in various areas where similar symptoms were observed.
- Determine the host range of *P. avenae* and the factors involved in the outbreak of LYS (biology, ecology, rice varieties, etc.).
- Study LYS and nutrient interaction that results in disease occurrence (microorganisms involved in grain discoloration, nutrient factors resulting in disease development).
- Test the integrated pest management approach to manage LYS (pilot demonstration, learn from other countries' experiences, cropping patterns, etc.).

Notes

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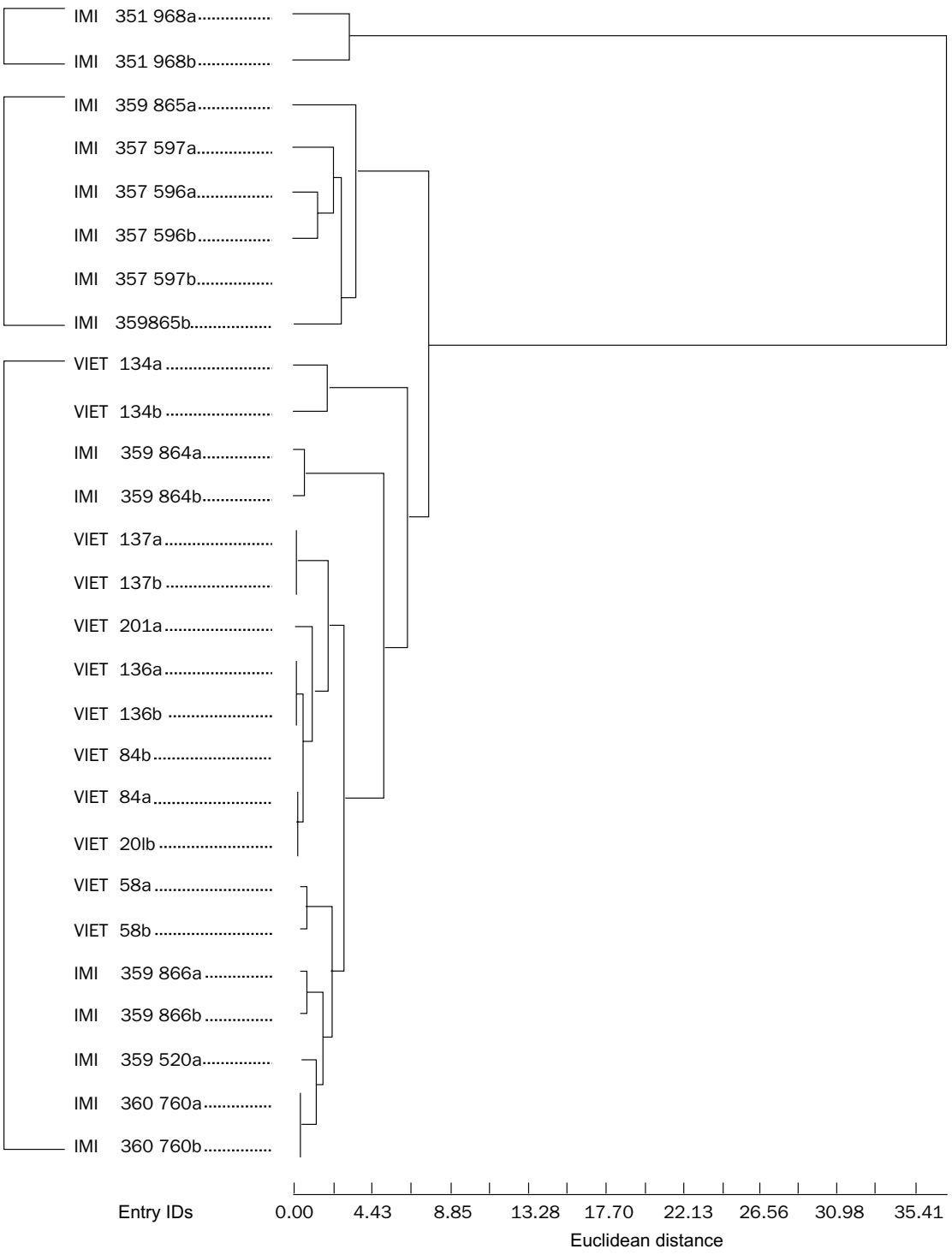

Fig. 1. Dendrogram of isolates generated by cluster analysis of FAME profiles showing subgroups of *Pseudomonas avenae*.

Micro-fungus closely associated with lesions of red stripe disease of rice

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A serious rice disease, which shows reddish or yellowish blight on the leaves, is widely distributed in many tropical Asian countries. The disease was first reported as bacterial red stripe disease of rice by Mogi and others in 1988. However, since the pathogenicity of this bacterium could not be confirmed, experiments to find the pathogen of the disease were carried out.

The disease was readily transmitted to healthy rice plants when healthy plants were planted together with a diseased plant in the same pot, covered with mylar insect cage, and sprayed with sterilized water to maintain high humidity. In this case, fresh lesions appeared 10~14 d after inoculation.

The transmission test was successful in Indonesia, Vietnam, Thailand, and Japan, but not in Thailand.

These results suggest that the disease is caused by some biotic agent.

The young lesions collected from the field and/or greenhouse were ultrathin-sectioned and observed under an electron microscope. We could not find any fungal, bacterial, or viral structures in the diseased host tissues.

The lesions collected from the rice field and/or greenhouse were cut by scissors into small pieces, directly stained with a mixture of lactophenol and cotton blue on the glass slide, and inspected under a microscope. Extremely thin mycelia, sometimes with conidia, were observed. The thickness of the mycelium is less than 1.0 mm (usually 0.2~0.3 mm), and the size is 16.2 ¥ 3.2 mm on average. All conidia are divided into four cells by septa. Some other unknown fungal structures looking like ascospore, zoosporangium, or chlamydospore connected with mycelia were observed on the lesions.

Under a scanning electron microscope, the mycelia were stretched, adhering tightly to both the upper and under surfaces of the lesions. The surface of the spherical or elliptical unknown structures (10~50 mm diameter) connected with the mycelia showed the specific appearance of fruiting bodies on the chlamydospore.

When both the upper and under surfaces of the lesion were washed strongly with an artificial sponge ball in distilled water, the micro-fungus was eradicated almost completely. This suggests that a majority of the micro-fungi are growing on the leaf surface.

Disease transmission was markedly prevented by spraying benomyl, suggesting that the causal pathogen of red stripe disease of rice is a kind of fungus.

To isolate the micro-fungus, many trials were carried out with various techniques, using many kinds of media. Up to now, however, no micro-fungus showing pathogenicity could be isolated.

Notes

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Histopathological observation of red stripe of rice with special reference to finding bacterial masses in xylem

H. Kaku and T. Noda

Red stripe disease has been reported in some countries of Southeast Asia. The disease was first reported in Indonesia in 1987, and it occurred on cultivar IR64. Since then, the disease has been reported from many areas in Indonesia, occurring not only on IR64 but also on local varieties such as Cisadane and Krueng Aceh. The disease has caused severe damage in rice production in the dry season in several countries. To date, however, the causal agent has not been identified. The disease was designated as bacterial red stripe, but the pathogen has not been isolated. Some researchers speculate that the causal organism is a fungus. The symptoms of red stripe suggest the existence of a causal organism because the halo spreads lengthwise along the veins. A histopathology was therefore conducted to observe the causal organism and histological changes in the host tissues using naturally occurring lesions of red stripe.

Rice leaves showing typical symptoms of red stripe (cultivar IR64) were collected in Indonesia in 1998.

Histological observation showed that, in the spot area, bacterial masses were observed in the xylem vessels of the vascular bundle. The bacterial colonies were frequently detected in the xylem vessels of connecting strands (transverse bundles), especially in samples from Vietnam. They remained, in many cases, as a small mass stained in purple in a joint of longitudinal and transverse vascular bundles. In thin sections, the bacterial mass was observed as an aggregate of bacterial cells. In most cases, bacterial growth was poor in the longitudinal vascular bundle, though rather long and thick bacterial colonies were formed in some areas of the lesions. In some cases, however, the lumen of all three kinds of vessels of the large vascular bundle was filled with the bacterial mass.

In the reddish-brown spot, many large granules stained dark blue to black appeared in the parenchyma cells. In severely affected areas, the cells became necrotic and the protoplasm was stained brown. Necrosis was also common in the vascular bundle sheath and mestome sheath. The reaction materials, appearing to be of host origin, were frequently detected in the lumen of the vascular bundle where the bacterial masses were present. Most of them were recognized as granules stained in yellow. In the halo area, the denatured chloroplast was a common feature.

The presence of bacterial masses and the histological changes in host tissues were observed in all samples showing red stripe symptoms collected from both Indonesia and Vietnam. In the leaf samples of rice leaves without red stripe, such bacterial masses were not found, and the host tissues appeared to be intact. These facts suggest the possibility that a bacterium is responsible for inducing red stripe disease.

Notes

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Bacterial orange leaf blight of rice in Indonesia

Suparyono

The disease was first observed in Subang District during the 1987 dry-season crop. At that time, the disease was called bacterial red stripe (BRS) (Mogi et al 1988). Based on the color of the infected tissue, type of symptom, and plant part infected, Suparyono (1989) proposed a new name for the disease—bacterial orange leaf blight (BOLB). The infected leaves turn orange, the final symptoms of the disease are those of blight, and it occurs mostly on the leaves. Since then, research has been done in Indonesia to determine the symptoms, the distribution of the disease, the causal organism, the transmission mechanism, factors affecting disease develop-ment, the effect of fertilizers on BOLB development, and responses of several rice genotypes to the disease.

This report summarizes the results obtained from this research.

Symptoms and distribution

A preliminary study on BOLB distribution was done during the 1989 dry season in West Java. A survey was conducted across the coastal rice-growing areas of West Java up to the higher elevation areas in the southern parts of the region. The study involved four plant pathologists and one plant physiologist. When the disease is severe, it is similar to that caused by *Xanthomonas oryzae* pv. *oryzae*. The symptoms start with minute orange spot(s) appearing at any point on the leaf surface. From such a spot develops a straight transparent yellow stripe upward (never downward). At a later stage, the disease produces blight symptoms and finally the infected leaves dry up.

Several factors significantly affect the development of BOLB: variety, fertilizers, and plant spacing. In West Java, the disease occurred mostly in the northern coastal areas (0–100 m above sea level) (Suparyono et al 1989). In this ecosystem, BOLB intensity ranged from low to severe, depending on the rice variety planted. In the higher altitude ecosystem, the range was from trace to none, with sporadic spread. During the 1990 dry season, the disease was observed in North Sumatra (Suparyono, unpubl.). The disease has also been reported in West Sumatra and South Kalimantan (Anonymous 1992).

Causal organism and transmission

Two types of bacterial colonies (white and yellow) were frequently isolated from the leaf tissue showing BOLB symptoms. Inoculation tests showed inconsistent results; at times, BOLB symptoms were clear, other times, they were not. Clear BOLB symptoms were observed on inoculated plant(s) covered with a plastic bag. Symptoms did not develop when the plastic bag was removed, indicating that high humidity and high temperature were required for the infection process to occur and BOLB to develop (Suparyono et al 1989, Anonymous 1992). The disease was easily transmitted mechanically. When diseased hills were placed next to healthy hills, the healthy hills were infected. Mechanical transmission was also evaluated on five rice varieties during the 1996 wet season in a greenhouse at the Research Institute for Rice. Disease incidence ranged from 13.64% to 62.22% (Table 1). The lowest disease incidence was noted in IR72, while the highest was on Ciliwung. Under normal conditions, Ciliwung and IR72 were the varieties most and least susceptible, respectively, to BOLB (Suparyono, unpubl.).

Table 1. Means of disease incidence of BOLB on five rice varieties mechanically transmitted by diseased leaves, Sukamandi, 1996-97 wet season.

Variety	Disease incidence (%)
IR64	8/23 (34.78)
Cisadane	9/26 (34.62)
Ciliwung	15/23 (62.22)
IR42	10/22 (45.45)
IR72	3/22 (13.64)

BOLB development under different cultural practices

Trials to evaluate the effects of several agronomic practices on disease development were conducted at the Sukamandi Research Station during the1989 dry season. The following treatments were evaluated: (1) plant spacing of 25 × 25 cm, (2) plant spacing of 15 × 30 cm, (3) plant spacing of 25 × 25 cm + intermittent drainage, (4) plant spacing of 15 × 30 cm + intermittent drainage, and (5) intermittent drainage. Observations on disease incidence and disease severity were made at 2-wk intervals starting at 30 d after transplanting (DAT), using a scale developed for bacterial leaf blight (IRRI 1983). The data indicated that BOLB started to appear at 80 DAT, and no disease symptoms were observed before that. At 80 DAT, disease severity ranged from 0.4% to 20.37% and disease incidence ranged from 0.35 to 4.50. The disease developed fast up to 90 DAT, then declined around harvest time (Fig. 1). Intermittent drainage consistently decreased BOLB, as indicated by disease development in plots receiving treatments 3, 4, and 5 (Suparyono and Sudir 1989b).

Development of BOLB under different fertilizer applications

Trials to evaluate the effect of fertilizers on BOLB development were conducted at the Sukamandi Research Station during the 1994-95 wet seasons, 1995 dry season, and 1995-96 wet seasons, and at Plumbon (Cirebon) during the 1995 dry season. In 1994-95, IR64 was planted at Sukamandi and Plumbon at a plant spacing of 22 × 22 cm in 7 × 7-m plots. Treatments were (1) 300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl; (2) 300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl + 100 kg ha^{-1} of urea at panicle initiation (PI); (3) 300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl + 75 kg P_2O_5 ha^{-1} at planting; (4) 300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl + 75 kg KCl ha^{-1} at planting; (5) 300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl + 75 kg P_2O_5 and 75 kg KCl ha^{-1} at planting; and (6) 300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl + 100 kg urea ha^{-1} with split applications (1/3 as basal, 1/3 applied at mid-tillering, and another 1/3 at PI). In 1995-96 at Sukamandi, IR64 and Cisadane were planted in 4 × 5-m plots with the following treatments: (1) 0 N + 0 P + 0 K, (2) 150 N + 0 P + 0 K, (3) 150 N + 75 P + 0 K, (4) 150 N + 0 P + 75 K, (5) 150 N + 75 P + 75 K, and (6) 150 N + 5 t straw compost ha^{-1}. Disease severity was observed at maturity using the standard evaluation system for bacterial leaf blight (IRRI 1983). Results indicated that fertilizers significantly affected BOLB development. The lowest disease severity was observed in crops fertilized using the recommended rate (300 + 75 + 75 kg ha^{-1} of urea, P_2O_5, and KCl), whereas the highest severity was noted in crops fertilized by a higher urea rate with no P_2O_5 and KCl (Suparyono and Sudir 1989b).

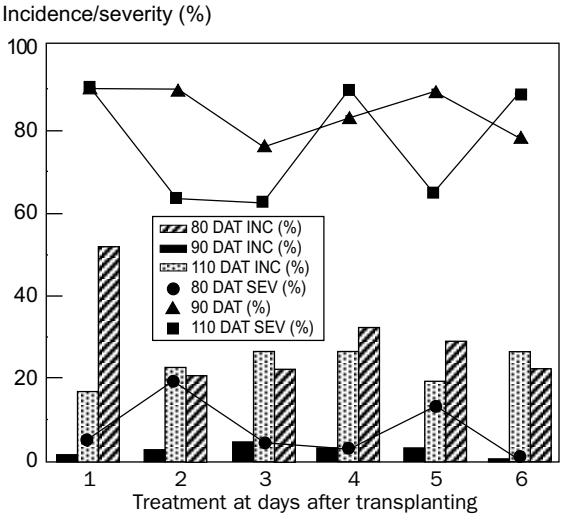

Fig. 1. Effects of different technology inputs on the development of BOLB (Suparyono and Sudir 1989b).

Response of several breeding lines and cultivars to BOLB

In the 1989 dry season, 150 genotypes were evaluated under natural conditions at the Pusakanegara and Sukamandi experimental stations. In Pusakanegara, infected leaves per hill were observed at about 80 DAT; those in Sukamandi were observed at about 90 DAT. BOLB was more severe in Pusakanegara (disease incidence ranged from 6.3 to 26.6%) than in Sukamandi (1.8 t–9.6%) (Suparyono and Sudir 1989a).

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Notes

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Occurrence of red stripe disease in Malaysia

A. Saad

The rice diseases of economic importance in Peninsular Malaysia are blast, penyakit merah virus (tungro), bacterial leaf blight, and sheath blight. Other diseases of lesser importance are brown spot, sheath rot, bakanae, narrow brown leaf spot, and bacterial leaf streak (Chin and Supaad 1986). Rice blast was the most important disease in the 1970s and early 1980s. Tungro was a major disease in the Muda area in the early 1980s. Other diseases such as bacterial leaf blight and sheath blight gained prominence in the 1980s and 1990s (Fig. 1). Various changes in rice cultivation and varietal adaptation played a major role in changing the disease scenario. Red stripe disease (the origin and causal pathogen of which are still not well understood) gained importance in the Muda area in the 1990s (Yazid et al 1996).

Red stripe disease syndrome

The first incidence of red stripe in Malaysia was observed in Projek Barat Laut Selangor on MR84 in the 1987 main season. The disease was also found in other granary areas: Seberang Perai and Seberang Perak in the 1988 off-season and Muda and Kerian areas in the 1989 main season (Fig. 2). In the Muda Irrigation Scheme, the disease affected MR84 in the 1994 main season (Fig. 3).

Disease symptoms

Typical leaf lesions are comet-striped. The disease begins as orange water-soaked stripes that subsequently coalesce with each other and cover most of the leaf blade. The disease, which is similar to

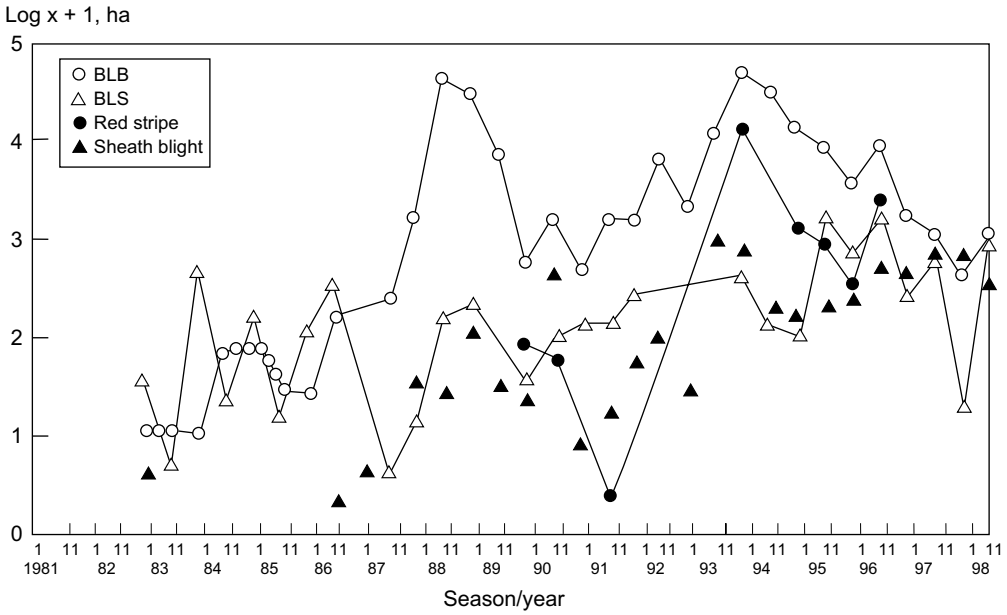

Fig. 1. Incidence of BLB, BLS, red stripe, and sheath blight in the Muda Irrigation Scheme, 1981-98.

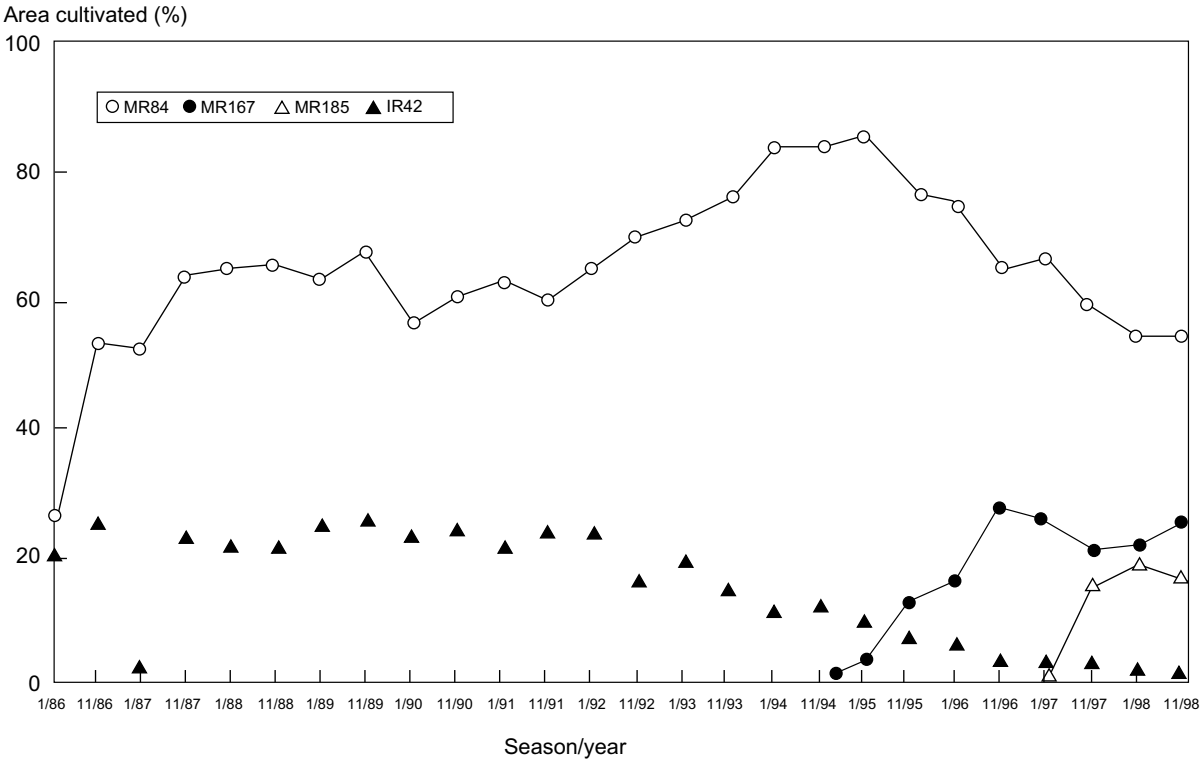

Fig. 2. Percentage of areas planted to MR84, MR167, MR185, and IR42, Muda Irrigation Scheme, 1986-98.

vascular bacterial disease but without the ooze, normally appears at the dough-ripe stage. It was also observed on the ratoon crop after being maintained in the greenhouse.

Economic importance

Etiological studies and inoculation methods failed to get enough data about the syndrome. Assessment of yield loss was first conducted during breeding trials at Permatang Bendahari, Seberang Perai, in the 1993 off-season. Healthy hills (five hills per plot) were compared with infected hills using varieties MR77, MR84, MR164, MR166, MR169, and MR170. There were three replications. The disease scores ranged between 6.3 and 7.8 and yield losses ranged from 9.0% to 18.5% (mean: 11.5%) (Table 1). The second yield evaluation was done on naturally infected rice variety MR84 at Padang Langgar and Titi Besi, Kedah (Muda area) during the 1998 off-season. Disease reaction (% severity of 1st and 2nd leaf) was plotted against yield (filled grain weight of observed tillers). The results showed that yield reduction was 22.5% at Padang Langgar (234 tillers) and 25.4% at Titi Besi (170 tillers), suggesting substantial yield losses in Malaysia.

Table 1. Yield reduction due to red stripe disease at Permatang Bendahari, Seberang Perai, Malaysia, 1993 off-season.

Variety	Mean[a]
MR77	9.03 a
MR84	12.28 a
MR159	7.72 a
MR164	10.37 a
MR166	9.13 a
MR169	13.16 a
MR170	18.54 a
Mean	11.46

[a]Means followed by a common letter are not significantly different at the 5% level by DMRT.

Varietal reaction

Reaction of varieties to the disease was obtained from breeding lines/varieties planted in major rice-growing areas. The first record came from adaptability trials of breeding lines at Guar Jering, Seberang Perai, in the 1998 off-season. MR84 was susceptible (60.2% leaf area infection), along with MR106, MR107, and MR105. MR103 (8.2% infection), MR104, and MR77 were resistant (mean disease reaction of 36.0%) (Table 2).

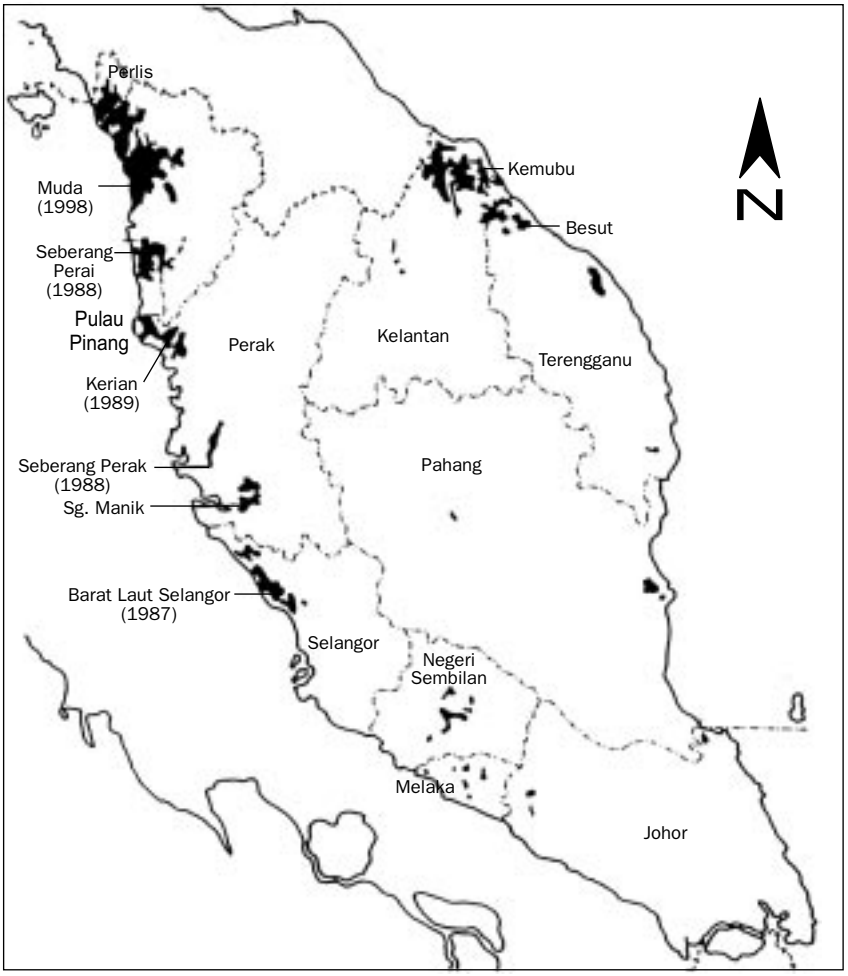

Fig. 3. Granary areas in Peninsular Malaysia.

The second observation was during the adaptability trials of breeding lines at Kerian, Perak, in the 1995 off-season. Among the varieties, MR84 was recorded to have 66.0% infection (Table 2).

The rain-shelter data were on MR84, MR166, MR167, and MR185 in the 1997 off-season. The rain shelter was equipped with a sprinkler to keep the humidity at >80% and temperature at 35–40 °C. MR166 was the most susceptible and MR185 the most resistant among the varieties (Table 2). This suggests various varietal reactions to red stripe disease in Malaysia. The resistant varieties are MR181, MR103, MR104, MR167, and MR185 and the susceptible varieties are MR84, MR106, MR107, MR166, and MR175.

Etiology of the pathogen

Isolation of the pathogen was done in several ways: sterilized 5 × 5-mm pieces of infected leaves, macerated infected leaves, planted and cultured on NA, PSA, PDA, or King's medium B. A total of 33 bacterial isolates and 11 fungal isolates were selected for identification. Among the bacterial isolates, *Xanthomonas maltophilia* (8), *Pseudomonas syzygii* (6), and *Ervinia tracheiphila* (3) were the most prevalent. Most fungi isolates were saprophytic—i.e., *Monosporium* sp. (6), *Nigrospora* sp. (2), *Curvularia* sp. (2), and *Helminthosporium* sp. (1).

Ten bacterial isolates—*X. maltophilia* (4), *P. syzygii* (3), and *E. tracheiphila* (3)—were selected for

Table 2. Percentage leaf area infected by red stripe disease of rice.[a]

Variety	Guar Jering (S. Perai) 1998 off-season	Kerian 1995 off-season	Rain shelter (S. Perai) 1997 off-season
MR10		50.0 bc	
MR77	12.5 ab	44.7 bc	
MR84	60.2 d	66.0 c	15.0 ab
MR103	8.2 a		
MR104	8.7 a		
MR105	61.3		
MR106	65.3 d		
MR107	62.0 d		
MR108	26.0 bc		
MR109	31.2 c		
MR110	24.5 abc		
MR166			34.0 b
MR167			9.0 a
MR175		58.0 bc	
MR176		43.3 bc	
MR177		38.3 bc	
MR178		52.7 bc	
MR179		44.3 bc	
MR180		31.3 b	
MR181		5.2 a	
MR182		34.0 b	
MR183		48.7 bc	
MR185			6.7 a
Y 1157		46.0 bc	
YEP 119		44.0 bc	
Mean	36.0	43.4	16.2

[a]Means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

inoculation studies on MR84, MR166, MR167, and MR185. The bacterial suspension was pin-pricked, sheared, sprayed, or injected on the leaves/leaf sheaths of the plants. All methods formed no symptoms as they occur naturally.

Negative results were also obtained from the contact studies where infected leaves were made to come in contact with healthy leaves.

Rain-shelter studies showed that healthy plants surrounded by infected plants resulted in lesion development similar to natural infection, but the pathogen is still not well understood.

Extensive studies on fungi isolates were not done because most of them were saprophytic and the symptoms of red stripe were similar to those of vascular bacterial diseases.

Some of the isolates and infected leaves were also sent for testing of virus and mycoplasma-like organisms at the MARDI central laboratory in Kuala Lumpur. But results were still negative. Recent data suggest that bacteria, fungi virus, and mycoplasma-like organisms are not the pathogen of the disease. There is a possibility that the organisms involved are obligate parasites/fungi.

Effect of environment

Disease incidence was more severe in the rain shelter equipped with the sprinkler (RH: >80% and temperature of 35 °C). Incidence of red stripe was nil in the cabin with 90% RH and a temperature of 24 °C. Incidence of red stripe was found to be severe with higher plant density and with application of nitrogenous fertilizer of more than 120 kg ha^{-1}. The close planting density causes higher humidity in the rice field (RH at >80%) and the higher temperature (35 °C) influences the severity of the disease.

Conclusions

Red stripe has become an important rice disease. Because the etiology of the pathogen is not understood, studies on host-pathogen interaction cannot recommend cultural practices or control and surveillance strategies that can ensure effective control of the disease.

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Rice red stripe in Thailand

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The disease syndrome called red stripe or bacterial red stripe was first reported in Indonesia in 1987 by Mogi et al (1988). At that time, the causal agent of the disease was found to be *Pseudomonas* sp. Symptoms of the disease appear on the leaf or upper part of the leaf sheath as circular or oval-shaped spots with yellow-reddish or yellow-reddish brown color. The lesion expands upward along the leaf blade and develops as a long stripe with the same color to the tip of the leaf. Usually, the stripes are not found to develop from the spot to the end of the leaf (downward development). The lesions will develop very fast on the uppermost leaf of the rice plant at heading. In severe cases, drying of the entire blade of the flag leaf and second and third leaves is common. Similar symptoms were found in Tien Giang Province, Vietnam, in 1988 by Du et al (1991). They isolated *Curvularia lunata* from new spots and demonstrated typical symptoms on inoculated plants with the fungus in the vascular system. In the Philippines, similar symptoms of red stripe appeared in rice plants in many rice-growing areas. Symptoms of this disease may be caused by interactions between soil fertility and a weak pathogen (Barroga and Mew 1994).

This paper reports on the distribution of rice red stripe in Thailand and some advanced studies related to it.

Distribution of rice red stripe

In 1992, Dr. Somkid Disthaporn went to Vietnam and observed this disease spreading in a large area in Vietnam. When he came back to Thailand, he started to survey this disease. Red stripe of rice was first reported in Thailand in the 1992 rainy season. Damaging an area of around 320 ha, the disease was observed during the tillering stage in Lumlugka District, Pathum Thani Province, in the central plain region of Thailand. Subsequently, it appeared in the northern and northeastern regions. Red stripe was found in Pathum Thani, Nonthaburi, Suphan Buri, Chai Nat, Sing Buri, and Chachoengsao in the central plain region. In the northern region, it was found in Phitsanulok, Phichit, and Sukhothai provinces. In the northeastern region, it was found in Nong Khai, Yasothon, Surin, Maha Sarakham, Roi Et, Buri Ram, and Si Sa Ket provinces. Both lowland and upland rice varieties including recommended and local varieties such as RD23, Supan Buri 60, Supan Buri 90, Chai Naht 1, and Sew mae Jan were infected by red stripe.

In late February 1998, a team of plant pathologists from the rice pathology research group (RPRG), Department of Agriculture and Department of Agricultural Extension, assessed variety Chai Naht 1. The symptoms of the disease were reddish or yellowish or orange bright spots on the leaves and these symptoms were observed throughout the field. The disease affected the flag leaf at harvesting stage. The panicles seem abnormal and have more unfilled grains at the harvesting time.

One year later, red stripe was found to be very severe on Chai Naht 1 and Klong Leaung 1 in Chai Nat and Nonthaburi provinces. The damaged area was about half the total rice-growing area in Nonthaburi Province. Yield loss from red stripe, however, has not been studied yet.

Red stripe and fungicides

Rice fields sprayed with fungicides such as benomyl showed resistance to red stripe, whereas nearby rice fields untreated with the same rice variety at the same age showed severe symptoms.

Bacteriological study

Different kinds of bacteria from the diseased leaf were isolated and used for inoculating healthy TN1 rice plants. Clipping, pin pricking, spraying, and wounding with **carborundum** were done but inoculation was not successful.

Fungi appearing on the surface of infected leaves

Infected leaves of Chai Naht 1 were cut, sterilized, and incubated on moist filter paper. After incubation, the mycelia and a lot of sclerotia of *Sclerotium oryzae* were observed on the lesion surface under a light microscope. In infected leaves that were not sterilized, some genera of fungi were observed—*Fusarium* sp., *Curvularia* sp., and *Nigrospora* sp.

Furthermore, lesions of infected leaves were cut and directly stained with a mixture of lactophenol cotton blue and then observed under a microscope. The thin mycelia and a group of small spores were found on the surface of the lesion.

Collaboration with Japan

In 1995 and 1996, Japanese researchers Dr. S. Wakimoto and Dr. K. Suyama studied rice red stripe in Thailand together with RPRG plant pathologists. They surveyed the disease and collected diseased samples. The Thai researchers gained experience in identifying symptoms of the disease. An experiment to control red stripe with benomyl was conducted. Artificial inoculation was done by inserting diseased leaves into rice tillers, covering the whole plant with a mylar's insect cage, and frequently spraying it with distilled water. The experiment was not successful because the benomyl-treated and check plants were not infected.

Inoculation by injection method with sap extraction

In early 1998, inoculation by injection method with sap extraction from infected leaves was tried. Rice variety RD23 at tillering stage was used. The rice leaf showed red stripe symptoms 1 wk after inoculation, but the symptoms disappeared 2 wk later.

The experiment was repeated in 1999. Again, the rice plant showed symptoms 1 wk after inoculation, and they remained even 2 wk after inoculation. Reisolation and reinoculation will be done to confirm these results.

Transmission electron microscopy

Leaves of Chai Naht 1 that were infected with red stripe in 1998 were used in this study. Under a transmission electron microscope, the morphological changes at the cell levels were observed. The malformation of the parenchyma cell wall, stomata, and bulliform cell was noted. The structures or cells of microorganisms,which were expected to appear in the infected leaves, could not be observed.

Ongoing experiments

Meristem injection

The leaves with red stripe symptoms were cut and homogenized in phosphate buffer 1:10 (w/v) after being separated from the plant tissue by centrifugation at 7,000 rpm. After 15 min, the supernatant is used to inject the meristem of a 1-mo-old seedling. There were no symptoms up to 3 wk after inoculation.

Seed transmission

Seeds from infected plants were sown in the greenhouse to observe symptoms until the harvesting stage.

Conclusions

Red stripe of rice has occurred in both lowland and upland rice in the northern, northeastern, and central plain regions of Thailand. Many local rice varieties and recommended varieties can be infected by this disease. Rice variety Chai Naht 1 showed severe symptoms at the harvesting stage. At present, the cause of red stripe is unknown. Although thin mycelia with spores of micro-fungi on surface lesions were observed under a scanning microscope, isolation of the fungus was not successful (Wakimoto et al 1998).

Because red stripe can be prevented by spraying fungicides, the causal agent of the disease may be a fungus. Other microorganisms such as virus or viroid or phytoplasma are also suspected to be causal agents. It is possible that these microorganisms are carried by the fungi found on the surface of lesions.

Sap extraction from infected leaves can be used to inject rice plants at the tillering stage and the symptoms on rice leaves can be observed 1 wk after inoculation.

Further research should find the cause of red stripe so an effective control strategy can be implemented.

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Red stripe disease of rice: the Philippine experience

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Red stripe disease (RSD), also known as orange leaf and yellow spot of rice, was first observed in the Philippines in 1988 in the IRRI screenhouse and experimental farm in Calauan, Laguna (C. Vera Cruz, pers. commun.). The early symptoms of the disease were first thought to be young lesions of bacterial blight. The bacteria isolated from the chlorotic lesions, however, did not induce any symptom similar to what had been first noted. Conidia of *Alternaria* sp. were also observed RSD on rice plants on the lesions, but further testing was not pursued. In 1993, IRRI scientists again observed in southwestern Philippines (Tulungatong, Ayala, Zamboanga del Sur). Farmers and plant pathologists likewise confirmed the occurrence of the disease in Davao del Sur, Davao del Norte, North Cotabato, Tarlac, and Sta. Barbara in Pangasinan. In 1998, RSD was observed in rice plants in a small plot in Calinan, Davao City (Tisalona 1998). And, in December 1998, RSD was observed in the very same plot in the farm in Calauan where RSD occurrence was first reported.

A notable field observation was the "nonspread" of RSD in all the provinces surveyed in the Philippines. Disease occurrence was limited to only one or two plots in the infected area, in contrast to the more severe and more widespread distribution of RSD in countries such as Vietnam. Moreover, RSD was not observed in any two adjacent municipalities in the provinces surveyed.

Symptomatology

RSD starts, as noted by others, as pinpoint yellow specks with a regular outline and the margin stays regular even as the lesion enlarges. The lesions are circular to oval. As the lesion grows older, it darkens in color and becomes yellow-orange, sometimes with the center becoming markedly darker. A yellow streak then extends from the lesion to the leaf tip. The lesion then turns reddish brown and the streaks become orange to rusty. Old lesions usually become gray at the center, become necrotic, and eventually the whole leaf dries up.

The lesions are classified into five types:

Type 1: The pinpoint lesion ranges from 1 to 2 mm in length and 0.5 to 1.5 mm in width (at the widest point of the lesion). The pinpoint lesion type is light yellow to light yellow-orange.

Type 2: The young lesion is also light yellow-orange, but larger than the pinpoint lesions. Its length ranges from 4 to 5 mm and its width from 1 to 2 mm. This lesion still has a uniform yellow color, with no distinct center.

Type 3: The medium lesion has a brownish tinge, sometimes already showing a distinct center. Its length ranges from 8 to 10 mm and its width from 2 to 4 mm. This lesion has no necrotic streak.

Type 4: This medium lesion has a necrotic streak extending from the lesion. The origin of the streak is distinct.

Type 5: The old lesion is longer than 13 mm. Where the lesion ended and where the necrotic streak began can no longer be differentiated. Often, the lesion is dark yellow at the margin, with brownish inner tissues and a gray center. Sometimes lesions are observed to be water-soaked.

The etiology of RSD was determined through experiments that include (1) isolation of possible causal agent, (2) pathogenicity tests, (3) transmission tests to know the mode of transfer within plant populations, and (4) microscopic examinations of tissues to study the causal organism, i.e., whole-leaf clearing to determine the ingress of the organism and electron microscopy.

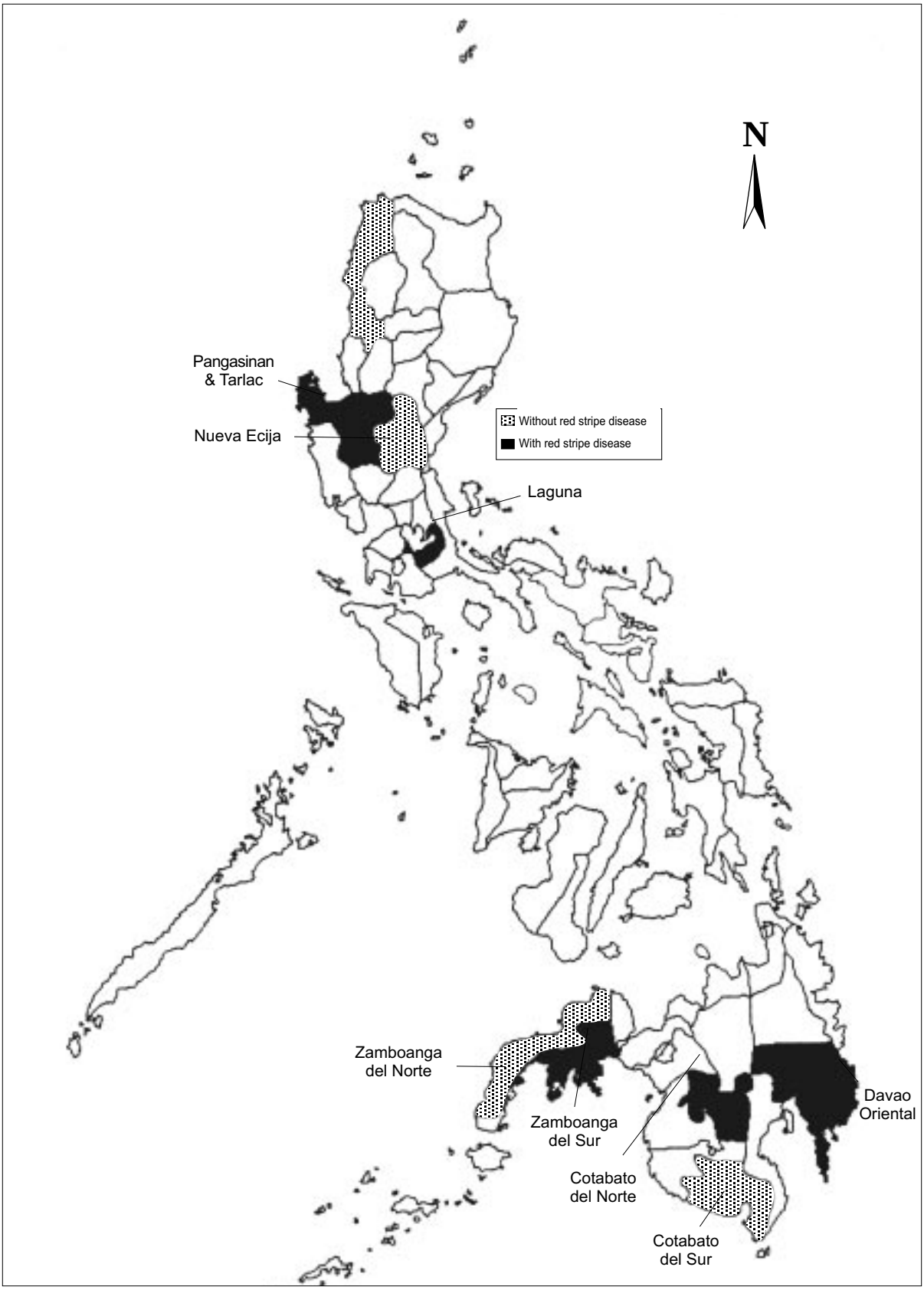

Fig. 1. Occurrence of RSD, by province, in the Philippines.

Methodology

Tissue and soil analyses

Field-collected samples from RSD-infected Bordagul and IR74 from Tulungatong were brought to IRRI for analysis. Soil samples from the field (1 kg) where RSD infection was observed were randomly selected in the field. Infected flag leaves were also collected and classified into low infection (types 1 and 2), medium infection (types 3 and 4), and high infection (type 5).

Isolation of causal organism

Lesions were segregated according to types. The lesions were surface-sterilized and tissue-plated onto potato dextrose agar (PDA). Type 1 lesions were also plated to different agar media, including rice leaf decoction agar, prune agar, peptone sucrose agar, and V-8 agar. Serial dilutions were also done for bacterial isolation. Mechanical inoculations of the crude sap of infected leaves were done.

Blotter tests of RSD-infected seeds

Four hundred seeds of RSD-infected and healthy IR64 were plated onto moist Whatman filter paper and incubated under light. Seven days after incubation, fungal and bacterial growth on the seeds was identified and recorded.

Pathogenicity tests

The isolated organisms were inoculated to healthy test plants. Several treatment combinations were done to induce symptom development, including

1. the use of fungal and bacterial isolates singly or in combination inoculated at the same time,
2. staggered inoculation for the inoculated organisms to test for opportunistic characteristics of the organism,
3. the use of "infected" and healthy soils to test for the effect of soilborne organisms/content,
4. organism and potassium amendment in the soil to affect K excess or deficiency, and
5. organism and herbicide singly or in combination to test for the effect of herbicide injury.

Transmission tests

Transmission experiments by leaf-to-leaf contact were carried out in the greenhouses of the Institute of Agricultural Sciences of South Vietnam, Ho Chi Minh City, Vietnam, and IRRI, Philippines, in August 1998 and January 1999, respectively.

Vietnam experiment. The experiments were done following the procedure of Vinh (pers. commun., 1998). The transmission test conducted in Vietnam aimed to evaluate the effect of treatments applied—i.e., whether symptom development and transmission of RSD were enhanced or hindered with the treatment. Infected plants from Hoc Mon were potted and placed in the greenhouse. A plastic mylar film was used as a cylindrical enclosure and relative humidity was maintained at 80–90%. The source plants were left until the balled RSD-infected plant, which served as the inoculum source, was established. Four greenhouse-grown, tillering, and healthy IR64 (susceptible variety) were placed beside the inoculum source with the leaves of both infected and healthy plants in direct contact. The treatments were as follows—treatment 1 = RSD-infected against healthy; treatment 2 = healthy against healthy plant (control); treatment 3 = RSD + *Sclerotium oryzae*; and treatment 4 = benomyl-sprayed, RSD-infected source plant against healthy test plants. There were four subsamples per replication per treatment. Each replication was enclosed in the cylindrical chamber, with relative humidity maintained. The experiment was carried out in a complete randomized design. Assessments for leaf area infected and % infection were at 2-wk intervals using the following rating scale:

Scale	Lesion based on leaf area
0	No incidence
1	Less than 10%
2	11–25%
3	6–50%
4	51–75%
5	76–100%

Percent infection was computed as the number of lesions multiplied by the number of infected leaves over the total number of leaves × 100:

$$\% \text{ infection} = \frac{\text{Number of infected leaves per hill}}{\text{Total number of leaves per hill}} \times 100$$

Philippine experiment. Transmission tests were also conducted at IRRI. The study determined the effect of plant age (AGE) and number of days the inoculum source and healthy test plants were in contact with each other (DAI) on % infection. The experiment was carried out in a split-plot design. The same incubation procedure was followed with the following treatments: main plot factor was days of inoculation/days in direct contact: 7, 14, 28, and 42 DAI, and the subplot factor was the age of test plant: 3, 4, and 5 wk.

Microscopy

Light microscopy. To determine the organisms thriving on infected leaves and to observe the relationship of these organisms with infection, the whole leaf clearing method was followed. Lesioned leaves were fixed-cleared in 70:30 absolute alcohol:acetic acid. Cleared tissues were then stained in lactofuchsin for 1–3 min to stain the fungal structures. To remove excess stain, tissues were rinsed with plain lactophenol. Stained samples were mounted in 50% glycerine and ingress was examined under the microscope. The types of organisms present were documented.

Electron microscopy. Diseased leaves with type 1 lesions from Vietnam and the IRRI greenhouse were cut with a sharp razor (1 + 10 mm) and fixed in 3% gluteraldehyde and osmium tetroxide. Dehydration of the specimen was achieved by immersion in increasing concentration of acetone; the Spurr mixture was used in infiltration and embedding. Thin sectioning was done using a diamond glass cutter and sections were mounted on collodion-filmed, carbon-coated copper grids. Staining with 1% uranyl acetate and lead citrate completed the process and the grids were then viewed under the transmission electron microscope.

Results

Plant and soil analysis

Soil and flag leaf samples brought back to the laboratory for analysis allowed us to associate nutrient deficiency or nutrient unavailability with RSD. Nutrient analysis of flag leaves of Bordagul and IR64 with RSD symptoms showed that P, Ca, Mg, and Cu content of the flag leaves did not fall below critical concentrations. However, the N content of both varieties was below the critical concentration, and Zn on Burdagol was found to be way below the desired range and was therefore considered deficient. IR74's Zn content, though higher, is still regarded as borderline deficient. The soil samples analyzed revealed deficiencies in N, P, K, Fe, Mn, and available Cu (Table 1). Moreover, the more severe the symptoms were on the plot, the more K-deficient the soil.

Table 1. Nutrient analysis of flag leaves of Bordagul and IR74 infected with RSD in Tulungatong, Zamboanga, Philippines.

Cultivar	Degree of RSD infection	Sample weight (kg)	Nutrient content (g kg^{-1})		
			N	P	K
Bordagul	Low	2.59	16.2	1.13	7.16
	Medium	2.38	15.4	1.06	8.23
	High	4.44	22.0	1.51	6.05
IR74	Low	3.40	21.2	1.48	10.70
	High	6.48	24.8	1.64	10.80

Isolation of causal organism

Various fungal and bacterial organisms were isolated from the different lesion types of RSD on PDA. *Nigrospora* sp. and *Bacillus* were consistently the most frequently isolated organisms from lesion types 1 to 4 for Bordagul and IR74, respectively (Table 2). Type 5 lesions yielded *Trichoconis* and *Bacillus* in the greatest amount for Bordagul and IR74, respectively. Blotter tests conducted on seeds from RSD-infected and healthy IR64 showed that RSD affected seed viability as indicated by the very low germination rate (Table 3). All the seeds tested had at least

Table 2. Frequency of occurrence of organisms isolated from five lesion types from leaves of Bordagul (Bor) and IR74 growing in Tulungatong, Zamboanga, Philippines.

Organism	Organisms (%) isolated from lesions									
	Pinpoint		Young		Medium		Medium with streak		Old	
	Bor	IR74	Bor	IR74	Bor	IR74	Bor	IR74	Bor	IR74
Nigrospora	8	0	7	0	13	1	3	0	3	0
Trichoconis	0	0	3	5	4	24	3	13	17	25
Colletotrichum	3	0	1	0	11	1	2	0.7	0	0
Bipolaris	2		1		7		1		0	
Helminthosporium	0	0	0	0	4	0	3	0.7	0	0
Curvularia	2	0	1	1	0	1	1	2	3	0
Pestalotia sp.	0		0		2		0		3	
Fusarium sp.	0		0		1		1		0	
Dactylosporium	0		0		2		0		0	
Phaeotrichoconis	0		0		0		0		0	
Bacillus		8		27		25		16		94

Table 3. The effect on RSD-infected leaf in contact with a healthy leaf of IR64 planted in soils previously observed to be with and without RSD-infected plants.

Lesion type	Soil with RS-infected plants (%)		Soil without RS-infected plants (%)	
Pinpoint	66	X	100	X
Young	100	X	100	X
Medium	100	X	100	X
Medium with streak	100	X	100	X
Old	100	X	100	X

?? = symptoms observed were not similar to RSD symptoms. Reisolation resulted in various fungal isolates. X = no symptom manifested.

one organism associated with them. The most frequently isolated fungus was *Tilletia barclayana* (80%), which was not associated with healthy seeds. *Alternaria longissima* was present in 10% of the RSD-infected seeds. *Nigrospora* sp., *Cercospora oryzae*, *Nakateae* sp., *Fusarium semitectum*, *Cladosporium* sp., *Phacotrichoconis* sp., *Pyrenochaeta* sp., *Penicilium, Phoma,* and *Streptomyces* were present in 3% or less of the RSD-infected population and were not isolated in healthy seeds. *Sarocladium oryzae* was present in both healthy and RSD-infected seeds, whereas *Bipolaris oryzae* and *Trichoconis padwickii* were higher in RSD-infected seeds than in healthy seeds.

The organisms isolated on the different agar media did not differ from those isolated on PDA. One unidentified fungus was observed on rice leaf decoction agar. The fungus had a characteristic hyphal fusion and/or branching of older mycelium. It was not determined whether it was branching or fusion.

Pathogenicity tests

The isolated organisms were tested on how well they induce RSD symptoms. Table 3 shows the effect of combining different organisms, rice varieties, and soil sources on the symptoms produced on IR74 and a Vietnamese traditional variety. However, the symptoms observed on the inoculated plants were not similar to the RSD symptoms observed in the field. An RSD-infected leaf attached to a healthy leaf also failed to produce the desired RSD symptom. The isolated organisms in combination with amendment in K levels of the soil used to grow the healthy test plant also failed to induce RSD symptoms (Table 4). The lesions from the test plants growing on "infected" soil and inoculated with the isolated organisms were not similar to the RSD lesions observed in the field (Table 5). *Pseudomonas* and other bacterial isolates, when used as inoculum, failed to induce

Table 4. Effect of different K levels and fungi associated with RSD on RSD symptom induction.

Isolate	K levels[a]	
	.023 meq[b]	.063 meq[c]
Botryodiplodia sp.	+[d]	+
Curvularia sp.	+	+
Fusarium sp.	–[e]	–
Pestalotia sp.	+	+
Colletotrichum sp.	+	+
Nigropora sp.	+	+
Trichoconis sp.	+	+
Macrophoma sp.	+	+

[a]Av of 4 trials. [b]Low level of K. [c]High level of K. [d]+ = symptom observed not the same as RSD symptom. [e]– = no infection observed.

Table 5. Effect of soil from fields previously infected with RSD on RSD symptom induction.[a]

Isolate	Soil source		
	G9	Calauan	Zamboanga
Botryodiplodia	+	+	+
Curvularia	+	+	+
Fusarium	–	–	–
Pestalotia	+	+	+
Colletotrichum	+	+	+
Nigrospora	+	+	+
Alternaria	+	+	+

[a]Av of 2 trials.

Table 6. *Pseudomonas* isolates used in the inoculation of IR64 using the pin-pricking method.

Cluster	Isolate number	Identity
A4	7174	*P. putida* bv. A
	6717	*P. fulva*
	7470	*P. putida* bv. A
A6	6535	*P. fluorescens* bv. c
A5	7343	*P. aeruginosa*
A3	6239	*P. fluorescens* bv. c
B2	5229	*P. marginalis*
	4846	*P. marginalis*
	5200	*P. marginalis*
B1	6031	*P. fuscovaginae*
	6235	*P. fuscovaginae*
	7008	*P. fuscovaginae*
E2	1851	*P. avenae*
	7015	*P. avenae*
	1840	*P. avenae*
D1	2057	*P. glumae*
	2051	*P. glumae*
	1857	*P. glumae*
	2056	*P. glumae*
	4956	*Erwinia*
	3548	Fermentatives
	5446	Fermentatives

RSD symptoms (Table 6). Finally, even the staggered schedule of inoculating combinations of fungal isolates did not induce RSD symptoms on the test plants (Table 7).

Transmission tests

Vietnam experiment. Lesions began to appear at the start of the second week after the initial leaf-to-leaf contact. Lesions were assessed until the 6th week, at 2-wk intervals. The lesions, which developed from test plants inoculated with RSD by leaf-to-leaf contact, were similar to RSD lesions observed in the field. An analysis of variance (ANOVA) performed for % infection. ANOVA on % infection at 42 d, however, showed that the effect of treatments on RSD infection was significant (Table 8). Comparison among treatment means using the LSD test showed that the effect of benomyl on % infection on the test plants and RSD transmission was not significantly different from that of the control. However, by Duncan's multiple range test (DMRT), the effect of benomyl was not significantly different from the other treatments. Therefore, the ability of benomyl to control RSD was not confirmed in this study. The effect of the leaf-to-leaf contact of RSD-infected plants with the healthy test plants was significantly different from the negative control (healthy + healthy) both by LSD and DMRT at the 5% level. Moreover, the effect of inoculating the test plant with *S. oryzae* right before leaf contact was highly significant by LSD (1%) and significant by DMRT (5%) tests. *S. oryzae* therefore enhanced infection of RSD in this experiment. ANOVA on LAI at 42 d, however, showed that the effect of treatments on leaf area infected with RSD was significant (Table 9). Comparison among treatment means using the LSD test showed that LAI from leaf-to-leaf contact between RSD infected and healthy plants was significantly different from that of the control plants. Spraying benomyl on RSD-infected plants before leaf-to-leaf contact also had a significant effect on the test plants' LAI. Moreover, the effect of inoculating test plants with *S. oryzae* right before leaf contact was highly significant by LSD (1%). Treatment means of LAI at 42 d for RSD + healthy, RSD + healthy inoculated with *S. oryzae*, and RSD sprayed with benomyl + healthy were not significantly different from each other. However, the treatments were significantly different from the control at the 5% level by DMRT.

Philippine experiment. Lesions appeared on test plants when RSD-infected IR72 plants were placed beside them to effect leaf-to-leaf contact. Symptoms began to appear in the 3rd week from initial contact. These symptoms were similar to those observed in the field. ANOVA for LAI at 14 and 21 d showed a highly significant effect of age of test plants on LAI (Table 10). For 3-wk-old test plants, LAI at 14 d showed a significant difference from results of 28 d leaf-to-leaf contact between RSD infected and healthy plants. For 5-wk-old test plants, LAI at 21 d showed a significant difference from 21 d leaf-to-leaf contact between RSD infected and healthy plants.

Table 7. Inoculation scheme for single and combined inoculations of fungi associated with RSD on symptom induction in IR64.

1st inoculum	2nd inoculum	3rd inoculum
Trichoconis	*Bipolaris*	*Curvularia*
Trichoconis	*Curvularia*	*Phoma*
Phoma	*Trichoconis*	*Curvularia*
Phoma	*Curvularia*	*Bipolaris*
Curvularia	*Trichoconis*	*Phoma*
Curvularia	*Drechslera*	*Phoma*
Trichoconis	–	*Nigrospora*
Phoma	–	*Nigrospora*
Curvularia	–	*Nigrospora*
Trichoconis	–	–
Phoma	–	–
Curvularia	–	–
Control	Control	Control

Table 8. ANOVA for RSD infection (%) 42 d after initial leaf-to-leaf contact of IR64 to RSD-infected MTL 199, Ho Chi Minh City, Vietnam, 1998.

SV	DF	SS	MS	F
Trt	3	1.875	0.625	3.16*
Error	60	11.875	0.19791667	
Total	60	13.7500000		

CV = 142.4%. * = significant at 5% level.

Table 9. Treatment means for RSD infection (%) on IR64 42 d after leaf-to-leaf contact with RSD-infected MTL 199, Ho Chi Minh City, Vietnam, 1998.

Treatment	Means	Difference[a]
RSD × healthy	0.4	0.4*
Healthy × healthy	0	–
RSD + *S. oryzae* × healthy	0.5	0.4**
RSD + benomyl × healthy	0.3	0.2 ns
Mean	0.3	

[a]** = significant at 1% level, * = significant at 5% level, ns = not significant.

Table 10. ANOVA for leaf area infected with RSD by leaf-to-leaf contact method in IR72, IRRI, 1999.

SV	DF	SS	MS	F[a]
DAI	3	198.494854	66.16495	3.17 ns
Rep	2	40.88043	20.44022	<1
Error (a)	6	125.20288	20.86715	
Age	2	346.25375	173.1268	6.38**
DAI × Age	6	29.77316	46.62886	1.72 ns
Error (b)	16	434.17306	27.135816	
Total	35	1424.77814		

[a]** = significant at 1% level, ns = not significant.

Microscopy

Light microscopy. Conidia and other fungal structures of common pathogens were observed on diseased tissues coming from type 1 lesions examined under the light microscope. Spores of *Nigrospora*, *Botryodiplodia* sp., *Alternaria* sp., *Helminthosporium* sp., and *Curvularia* sp. were clearly seen at 400X magnification. An unidentified fungus was observed at 1,000X magnification.

Electron microscopy. Observations at 10,000–15,000X yielded no fungus-, bacterium-, virus-, and mycoplasma-like entities within and outside the cells comprising the diseased tissues. Starch accumulation, one of the degenerative changes in chloroplast often associated with destructive effects of virus infection (Esau 1968), was not observed in the cells. However, electron-dense accumulations resembling inclusion bodies were observed in the mesophyll cells of the sections examined. The inclusion-like bodies took no definite shape. No virus particles, inclusion bodies, and mycoplasma-like organisms reported on rice resembled these inclusion-like bodies. Fine filaments, which were nonmembrane bound, were also observed in the phloem cells but these did not resemble the filaments reported on rice grassy stunt.

Conclusions and recommendations

Although the occurrence of RSD in the Philippines is not as severe as that reported in Vietnam and Malaysia, this should not deter us from doing further studies on RSD. Establishing the RSD etiology at the very least will equip us with enough knowledge to combat the disease if and when it reaches an epidemic level.

The extensive pathogenicity testing that we have done proved futile in inducing healthy plants to produce RSD symptoms. But the effectiveness of leaf-to-leaf contact in trans-mission of RSD certainly helped in under-standing RSD. However, mode of transmission is only one aspect of etiology. The question of the identity of the causal organism remains unanswered. Our results indicate that we have to consider other options as we tackle the RSD problem. The unconventional approach of Hanold and Randles (1991) in isolating the *cadang-cadang* causal agent and of Haber et al (1992) in producing RNA probes to detect flame chlorosis of cereals, for example, may point to another alternative: the use of biotechnology tools in our future research.

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Notes

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Workshop summary

Current situation

Occurrence and effect

- Wide occurrence in Indonesia, Malaysia, Thailand, and Vietnam, but limited occurrence in the Philippines
- Often occurs at panicle initiation and flowering stages until maturity
- When it occurs at panicle initiation and flowering stages and when spread is fast, grain filing is affected, thus causing yield losses. If it occurs at late flowering stage, there is no effect on grain filling, thus a limited or no yield loss is expected
- Normally occurs under high humidity, high temperature, and high N fertilizer conditions
- Has not been observed and reported in subtropical environments
- Occurs in both dry and wet seasons, but more in the dry (spring season in Vietnam) than in the wet season (summer season)
- Farmers in Vietnam spray fungicides, Tilt, Topcin M, and benomyl to control red stripe. It is reported to reduce symptoms.
- A relationship seems to exist between pollen grains on the leaf surface and occurrence of red stripe. Experiments are needed to prove it.
- Do we need to establish a database on occurrence and yield loss, and do we need to conduct more farmer surveys?

Conditions for red stripe development

Factors to consider in conducting experiments to test hypotheses

1. Isolation—Why can we not isolate the causal agent? We may not have the correct hypothesis. We should therefore be more focused on and more committed to the isolation from the right tissues (initial lesion of red stripe) and use the right techniques. Need to do more isolation focusing on different stages of lesions to prove transmission by injection method or other methods of inoculation.
2. Inoculation/transmission—Are we confident with the results we have obtained so far to draw conclusion? Majority of the participants believe that the red stripe is caused by a biotic agent. From the results of many experiments conducted so far, it appears that the red stripe could be transmitted to healthy plants grown side by side with source plants, so it is likely that there is a biotic agent involved here. However, confirmation is still needed. The experimental setup consists of a diseased plant and a healthy plant placed together in the same pot and caged with a Mylar film. The nontreated diseased plant is potted together with a healthy plant as check. Plants grown in soil from a different origin produced no red stripe, suggesting that red stripe is not soil-borne.
3. Incubation period—Results indicate very long incubation period (1–2 wk) by sap injection. Why is the incubation period so long? Could this be attributed to the inoculum being too low to cause red stripe? Inoculum concentration should be checked in the inoculation test.
4. Conditions for red stripe development and incubation under artificial inoculation are well established. In conducting any of the experiments to test the hypothesis, these conditions should be standardized and maintained.
5. In the process of establishing the etiology, it appears that red stripe is a response to antibiotic or fungicide treatment. Are we confident with the results about the response of the red stripe to the different chemical treatments? Fungicides and antibiotics should be used to test the hypothesis that red stripe is caused by biotic agent(s).
6. Use a standard set of rice cultivars for the research on the red stripe etiology: susceptible and resistant cultivars.

7. Susceptible varieties are IR64, MR84, and IR (line used in Vietnam); resistant varieties are IR72 and MR185.

Working hypotheses on the etiology of red stripe rice

Hypothesis 1: Red stripe is caused by a biotic agent.

The first step to assess the problem is to review the types of disease symptoms known to be caused by biotic agents on rice and on other crops.

Red stripe lesions appear to be very different from all symptoms of known rice diseases.

The second step is to ask whether red stripe is cause by a biotic agent that has not been reported in the literature. Another question is "Does a biotic agent cause red stripe?"

If red stripe is caused by a biotic agent that has not been reported in the literature, then this new biotic agent could be either one of those known classes of plant pathogens.

Are these viruses, viroids, MLOs, or RLOs? If there is involvement in a disease of insect transmission of MLOs or RLOs, the effect of antibiotics such as tetracycline and penicillin in controlling the syndrome should be observed. Transmission studies including seed, fungal, and mechanical transmission can prove or disprove this.

Nematodes are not known to cause this kind of symptoms.

Both fungal and bacterial pathogens may cause leaf spot (stripes, streaks, etc. with different color) on rice.

Is red stripe caused by a toxin?

The stripe formed in red stripe lesions extends only toward the tips of the leaves. It appears, therefore, that there is some kind of toxin that is water-soluble and translocated upward through the xylems of the leaves.

If a biotic agent among the group of fungi or bacteria causes red stripe, two possibilities may be considered: they are either facultative parasites or obligate parasites.

If a fungal pathogen is a causal agent of red stripe, then sporulation of diseased leaf tissues incubated under humid conditions and isolation from red stripe lesions on an artificial medium will produce the likely microorganisms responsible for the symptom.

Thus, Koch's postulates may be tested completely with standard steps from isolation to inoculation with single or a combination of cultures, reproducing the typical symptom and reisolating the pathogen.

Supposing red stripe is induced by obligate parasites, leaf contact inoculation between healthy and infected leaves will reproduce red stripe lesions under favorable conditions. This means transmission through contact inoculation will give positive results.

Hypothesis 2: Red stripe is caused by an abiotic agent.

If red stripe is caused by nutrient deficiency or mineral imbalance, then we should be able to reproduce the syndrome by growing rice plants in soil collected from red stripe-infected rice fields.

Hypothesis 3: Red stripe is caused by an interaction between a biotic agent and abiotic stress.

Should this be the case, both information from hypothesis 1 and 2 should reveal some possibilities.

Hypothesis 4: Red stripe is caused by an abiotic stress.

The abiotic stress could be due to mineral deficiency or toxicity associated with the soil of the rice field. Then the symptom should be reproducible.

Hypothesis 5: Red stripe is induced by a pesticide alone or in interaction with high temperature and heat.

New working hypotheses

Based on the discussion and the work done so far, the hypothesis regarding red stripe etiology is redefined:

1. No concluding agents—further research is still needed but what type of research?
2. Micro-fungi
   — No such fungi have been isolated yet—although fine hyphae of micro-fungi were observed on the surface of the lesions. A unique fungus was isolated at IRRI, but it appeared to be not related to red stripe by pathogenicity test.
   — Intact—cannot be isolated, obligate

3. Bacteria
   — Related to halo blight or *Pseudomonas syringae* pv. o*ryzae*
   — *Acidovora avenae* was isolated and tested to show stripe lesions but relation to red stripe is not established

4. Toxin
   — Results of a toxin produced by a causal agent (the toxin theory)

5. Indirect involvement of organisms
   — A possible vector-cause relationship
   — *Sclerotium oryzae* was tested with inclusive results
   — Why was only *Sclerotium oryzae* tested for this purpose? Why not the other fungal pathogens?
   — Plants with red stripe were also found to have a high incidence of sheath rot and sheath blight.

6. Unknown agent but similar in nature to a viroid; potentially transmitted through leaf-associated microorganisms such as micro-fungi.
   — Leaf extract for inoculation by injection into leaf sheath, lesions appeared to be produced.
   — Confirmation is needed.
   — What was the check? Leaf extract should be subjected to different treatments (e.g., heat treatment).

7. Interaction between pesticides and biotic factors.

Experimental protocols

Isolation of potential pathogens

1. Place exudates from the cut edge of red stripe leaves in a test tube with sterile distilled water.
2. Collect exudates and streak on PCA (potato-carrot agar medium) for potential bacteria and fungi.
3. Isolate potential pathogens from red stripe lesions at different developmental stages.
4. Pathogenicity tests with these microorganisms revealed that no distinct microorganisms were isolated to show positive results of red stripe lesion induction.
5. A unique and slow-growing fungus was frequently isolated but not always from type 1 lesions. There is no proof that it causes red stripe lesions.
6. Do we need to continue isolations for potential pathogens?
7. As part of a transmission study, do we need to establish if fungi may be vectors of red stripe-inducing agents?

Inoculation transmission

1. Experimental setup in pots with diseased and healthy plants in different arrangements to test the hypothesis that red stripe is air-borne and transmitted from source plant to healthy plants placed in the same pot or different pots separated by Mylar cages
2. Mobile seedling box with seedlings developed in greenhouse and placed amidst plants with red stripe in the field
3. Injection method with sap extraction from infected leaves
4. Modified leaf sap method as proposed by Dr. Noda and presented by Dr. Du
5. Injection method to the meristem point
6. Seed transmission
7. Transmission/inoculation via vectors
   – Insects (Mylar cages) (mites)
   – Plant surface-associated micro-fungi

Words of caution

1. Methodology (how it is being done) is important, not the results.
2. Use healthy seedlings or plants grown in the greenhouse to avoid potential contamination with plants having red stripe lesions.
3. For field tests, use 1–2-wk-old seedlings raised in the greenhouse. Care must be taken to avoid any contamination with the source red stripe plants—bring these seedling boxes to the field (source inoculum at mature stage) for 7–10 d, then bring them back to the greenhouse for red stripe development. To test potential insect transmission, seedling box may be placed in a Mylar cage.
4. Avoid mite infestation to maintain leaf wetness.

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