Association of Mycoplasmalike Organisms with Rice Orange Leaf in the Philippines

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ABSTRACT

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Thirty four rice plants showing symptoms typical of rice orange leaf disease were collected at several locations in the Philippines. Extracts of each plant were serologically tested, and 17 plants that did not contain either of rice tungro spherical virus or rice tungro bacilliform virus served as orange leaf disease sources. The leafhopper *Recilia dorsalis* recovered the orange leaf disease agent from 13 of 17 plants tested, and rice seedlings infected with each isolate showed similar symptoms. The agent was transmitted by this leafhopper in a persistent manner, and its incubation periods in the leafhopper and rice plant were 15–33 and 10–28 days, respectively. Electron microscopy revealed association of mycoplasmalike organisms with the 13 isolates tested. No similar organisms were observed in uninoculated rice plants.

Orange leaf (7) of rice (*Oryza sativa* L.) is distributed widely in South and Southeast Asia (1,2,7,8,10,11). The disease agent was transmitted by *Recilia dorsalis* Motschulsky in a persistent manner with an incubation period in the leafhopper of 2–15 days (1,2,7,10,11). Infected rice plants develop orange-colored leaves, which later roll inward and desiccate. Infected plants die 2–3 wk after the symptoms appear (1,2,7,10,11). The diseased plants are generally distributed sporadically in the field, and the disease does not cause serious yield loss.

Orange leaf has long been postulated as a virus disease because of its symptoms and its leafhopper vector (3). However, electron microscopy revealed association of mycoplasmalike organisms (MLO) with orange leaf in Thailand and Malaysia (8) and in Indonesia (9). Recently, association with this disease of spherical viruslike particles about 15 nm in diameter was reported in China (2).

To clarify the contradictory findings, we collected orange leaf isolates in the Philippines and used them for transmission tests and electron microscopic studies.

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MATERIALS AND METHODS

Isolates. Thirty-four plants showing orange leaf-like symptoms were collected at seven locations in the provinces of Laguna and Nueva Ecija (Table 1). One leaf was sampled from each plant, and its extract was tested by the latex flocculation test (5) for the presence of rice tungro bacilliform and rice tungro spherical viruses (6). Of 34 plants collected, 17 were free of both viruses. The 17 plants served as sources in the transmission tests with *R. dorsalis*.

Transmission tests. An *R. dorsalis* colony originally begun from leafhoppers collected at Laguna, Philippines, was reared on rice cultivar Taichung Native 1 (TN1) in screened cages. Second-instar nymphs were given an acquisition access period of 2 or 4 days on a source plant and then individually given sequential inoculation access periods of 1 day on 7- to 10-day-old TN1 seedlings until the leafhoppers died. Second-instar nymphs that had not fed on source plants were also given sequential access periods on TN1 seedlings until the leafhoppers died. Seedlings thus exposed to the leafhoppers were transplanted into pots and grown in insect-free cages to maturity for observation of symptom expression.

Electron microscopy. Two to 3 wk after inoculation, leaf pieces about 1.5 × 10 mm were collected from infected seedlings and fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) and postfixed with 2% osmium tetroxide in the same buffer. The samples were dehydrated in an acetone series and then embedded in Spurr's resin. Leaf samples from uninoculated TN1 seedlings were also embedded similarly. Ultrathin sections were obtained with a diamond knife mounted on an LKB ultratome Nova (Sweden). The sections were stained with uranyl acetate and lead citrate and examined in a Philips 410 electron microscope at 80 kV.

RESULTS

Symptoms. First symptoms appeared on the inoculated seedlings 10–28 days after inoculation. Emergence of new leaves was delayed in infected seedlings;

Fig. 1. Taichung Native 1 seedling showing short, ragged, and twisted leaves (arrows) 3 wk after inoculation with rice orange leaf.

such leaves were short and often showed chlorotic stripes, tip twisting, and ragged blades (Fig. 1), as do rice ragged stunt virus-infected leaves (4). Infected seedlings were stunted, and their leaves showed orange discoloration progressing downward from the tips, inward rolling, and eventual desiccation. All infected seedlings died 2–3 wk after the symptoms appeared.

Transmission test. The disease agent was acquired by *R. dorsalis* from 13 of 17 plants tested. The leafhoppers became infective 15–33 days after the initiation of acquisition access (Table 1). Average incubation period in the leafhopper was 22 days. Some leafhoppers transmitted the agent continuously and others transmitted intermittently until they died. A total of 456 leafhoppers were tested for acquisition of the agent from the 13 orange leaf-infected plants, and 13% became infective. A total of 648 seedlings caged with the leafhoppers that had not fed on diseased plants did not develop any symptoms, indicating infection with MLO or virus.

Of 6,974 seedlings inoculated in the transmission test, 502 were infected and about 29% of the infected seedlings showed ragged or twisted leaf symptoms. Plants (one each) showing leaf discoloration with and without ragged leaf symptoms were selected and used as sources for further transmission by *R. dorsalis*. Seedlings inoculated by the leafhoppers fed on either source showed leaf discoloration with or without ragged leaf symptoms.

Electron microscopy. Single seedlings each infected with one of the 13 isolates in the transmission tests were selected for electron microscopy. MLO were observed in the sieve elements of leaf samples infected with all isolates (Fig. 2). MLO were bounded by a unit membrane and contained ribosome particles and DNA-like fibrils. MLO had varying shapes and ranged from 50 to 1,100 nm in diameter. No viruslike particles were observed in any of the samples examined. Leaf samples from infected plants with and without ragged leaf symptoms appeared similar under the microscope and contained MLO. No MLO were observed in the cells of leaf samples from uninoculated rice seedlings.

DISCUSSION

In these experiments, all orange leaf-diseased plants collected and examined in the Philippines had an association with MLO. The incubation period of the disease agent in *R. dorsalis* nymphs was 15–33 days. MLO have been reported in orange leaf-infected plants in Indonesia, Malaysia, and Thailand (8,9), but viruslike particles instead of MLO were reported in China (2). The incubation

Table 1. Transmission of the orange leaf agent by the leafhopper *Recilia dorsalis* from field source rice plants collected at various locations in the provinces of Laguna and Nueva Ecija, Philippines, to susceptible rice seedlings

Location	No. infected plants/ total tested	No. leafhoppers transmitting/ tested	Incubation period (days)	
			In leafhopper	In plant
Laguna				
Los Baños	3/3	12/60	19–33 (27)[a]	10–27
Calauan	1/5	3/36[b]	16–20 (18)	10–24
Sta. Maria	2/2	15/80	15–25 (21)	18–24
Biñan	1/1	3/40	22–27 (24)	16–28
Sta. Rosa	2/2	6/80	17–28 (22)	15–21
Nueva Ecija				
Guimba	4/4	26/160	15–25 (21)	15–28
Total	13/17	65/456		

[a] Numbers in parentheses indicate average incubation period in the leafhopper.
[b] Result of the transmission test from one plant that was infected with orange leaf.

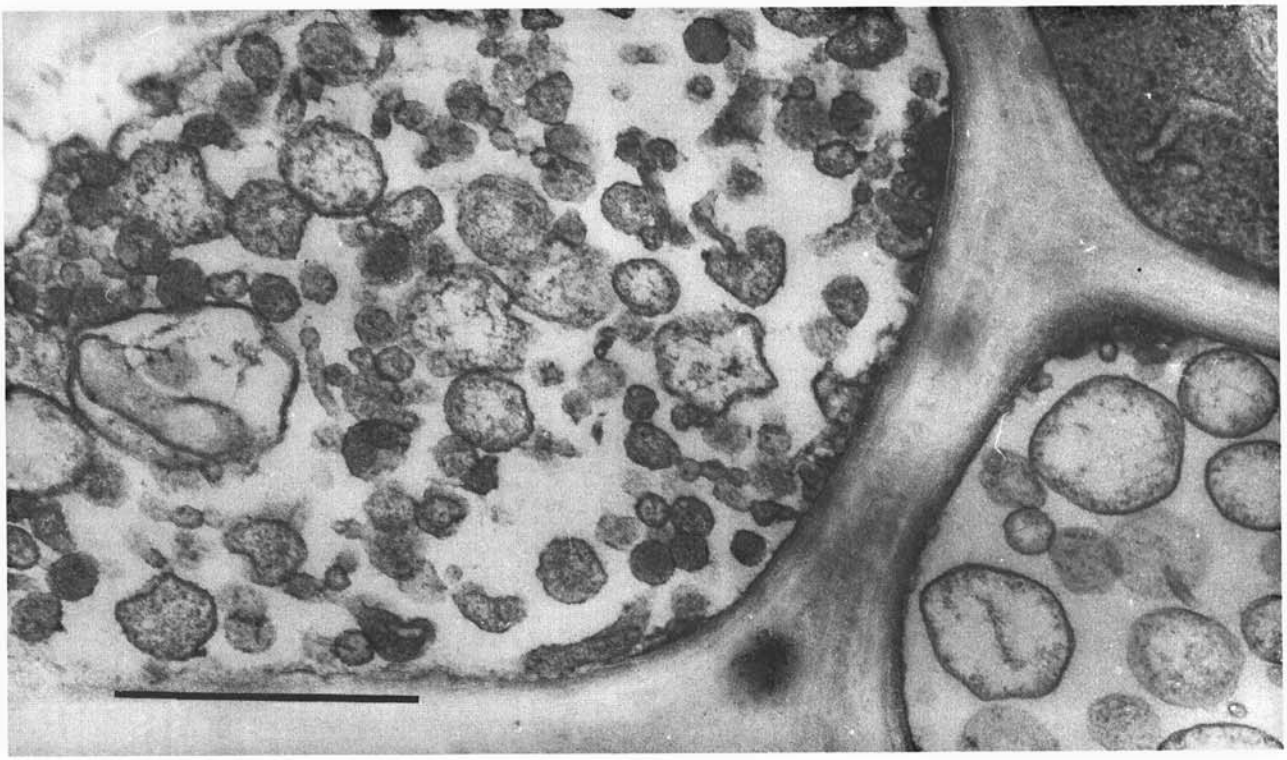

Fig. 2. Electron micrograph of mycoplasmalike organisms in the sieve tubes of an orange leaf-infected rice plant. Scale bar = 1 μm.

period in the leafhopper reported for the orange leaf agent was as short as 2 days in Indonesia, Malaysia, Philippines, and Thailand (7,8,10,11), shorter than 3 days in Sri Lanka (1), and 6–15 days in China (2). Incubation periods of MLO in vector insects are usually longer than those of most plant viruses. Apparently, the incubation periods of 2–6 days are shorter than those of any known MLO.

Although the results of these experiments contradicted those of some previous reports, the major symptoms described for orange leaf in those reports were similar to those of isolates tested in these experiments (1–3,7–11). However, the ragged or twisted leaf symptoms observed in orange leaf-infected seedlings in these experiments have not been described in the previous reports. This may be because seedlings at somewhat advanced age were used for inoculation in the previous reports. The orange leaf agent was transmitted by *R. dorsalis* in a persistent manner, and disease incidence was sporadic in the field in all reports (1,2,8,10,11). These characteristics agree with those of the orange leaf isolates tested in these experiments. These facts suggest to us that the diseases previously described as orange leaf were identical or very close to the disease in our experiments.

The contradictory results may indicate possible presence of both MLO and virus agents that cause similar symptoms and are transmitted by *R. dorsalis*. This leafhopper is known as a vector of rice dwarf virus and rice tungro-associated viruses (3,6). Rice dwarf causes symptoms distinct from orange leaf disease (3). Tungro symptoms are similar in some aspects to those caused by orange leaf, and the geographical distribution of the two diseases overlaps (3).

In these experiments, only plants that were free of both of the tungro-associated viruses were used in the transmission test. In all previous investigations (1,2,7–11), however, precautions to eliminate the tungro-associated viruses from source plants were not taken. There is a great possibility that those sources were infected with tungro or with both tungro and orange leaf. In these experiments, 17 of 34 plants collected were infected with either or both of the tungro-associated viruses. The tungro-associated viruses are transmitted in a semipersistent manner and the incubation period in leafhopper vectors is as short as 1 day, if any (3). Therefore, if sources were infected with tungro as well as orange leaf disease agent, *R. dorsalis* might transmit the tungro virus agents immediately after an acquisition access and the orange leaf agent after its incubation period is over.

These experiments confirm previous reports (8,9), which indicated association of MLO with rice orange leaf. Although there is still a possibility that a virus that is transmitted by *R. dorsalis* and causes orange leaf-like symptoms on rice is present, the virus disease should be given a name other than "orange leaf" if such a virus is isolated.

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