Section 3. Virus and Phytoplasma Diseases

Chapter 4. Fungus-borne virus diseases

1. Necrosis mosaic (RNM); pathogen: RNMV

1.1. History and distribution

1.1.1. Japan. An unknown disorder of rice (**RNM Figure 1**) characterized by striping symptoms occurred around 1959 in the southern part of Okayama Prefecture in western Japan (Fujii 1965; Fujii et al 1966a,b). The disorder occurred locally and damaged rice only in certain fields. Areas affected with the disorder expanded to 269 hectares in Okayama in 1964. The disorder was first called "waika" (stunting) disease (Fujii et al 1977a,b) and then necrosis mosaic (RNM) (Fujii 1978, Inoue and Fujii 1977). RNM is associated with filamentous virus called *Rice necrosis mosaic virus* (RNMV) (**RNM Figure 2**) (Fujii 1967, 1978; Inoue 1968; Inoue and Fujii 1977). RNMV is soilborne and transmitted by the soilborne fungus *Polymyxa graminis* Ledingham (Ledigham, 1939) of *Plasmodiaphoraceae* (Fujii 1967; Fujii et al 1966a,b, 1967b; Fujikawa et al 1969; Inouye 1968, 1970). RNMV is grouped in the genus *Bymovirus* (Usugi et al., 1989). However, unlike other viruses in the genus (Usugi et al 1976, 1984), the RNMV Okayama isolate is not mechanically transmissible or transmissible with difficulty (Fujii 1978, Inoue and Fujii 1977).

RNM occurs in localized areas in the southwestern, western, and central regions of Japan (Fujii 1978, Fujii and Okamoto 1969, Fujikawa et al 1969, Hibino et al 1981, Momota

RNM Fig. 1. Rice plants infected with *Rice necrosis mosaic virus* (RNMV) showing striping symptoms. Courtesy of Tadao Inoue.

RNM Fig. 2. Purified *Rice necrosis mosaic virus* (RNMV) in a shadow-casted preparation (x 30,000). Courtesy of Tadao Inoue

1974, Mori et al 1970, Shibata et al 1971, Takahashi et al 1972). Infection of rice plants with RNM occurs largely in seedbeds under upland conditions but limited in transplanted fields (Fujii 1978). Indeed, RNM incidence increased in Japan after the use of upland nurseries protected with oiled papers became popular around 1960. The upland nursery stabilizes rice production and allows early planting of rice especially in cool regions. RNM practically disappeared after the use of seedling boxes for machine transplanters became popular around 1975.

An RNMV isolate that occurred in Ooita Prefecture in southern Japan was different from the Okayama and other isolates of RNMV in Japan. It was reported transmissible in rice by mechanical means and through seeds (Fujikawa et al 1969, 1970a,b, 1971a,b, 1972a,b,c, 1974).

1.1.2. India. In 1979 in Orissa, India, a rice disease showing symptoms similar to RNM was found in the experimental farm of the Central Rice Research Institute at Cuttack (Ghosh 1979 1980). The disease was identified as RNM based on symptomatology, soil-borne nature, and association of inclusion-like bodies in infected rice cells. Rod-shaped, virus-like particles were reported in rice plants infected with the isolate (Ghosh 1982). In the slide agglutination test using an antiserum to partially purified RNMV Okayama isolate (Inouye and Fujii 1977), extracts of diseased rice plants formed aggregation of chloroplasts and cellular components (Ghosh 1984). It was reported that two monocotyledonous weeds and some dicotyledonous fiber crops were sap inoculated with the isolate (Ghosh 1981, 1982). Further confirmation is also required on the identity of the disease with RNM. Thereafter, no report has confirmed the occurrence of a similar disease in India.

1.2. Symptoms

Infection of rice seedlings with RNM occurs about 30 days after seeding in RNM-affected soil (Fujii 1967, Fujikawa et al 1969). Primary symptoms that include faint chlorotic or yellow spindle-shaped lesions or flecks, 1 mm in width and 1-2 cm in length, at upper portion of lower leaves (**RNM Figure 1**) occur in infected seedlings at 30 to 45 days after transplanting. Infected plants show moderate stunting and spreading growth habit, and are associated with filamentous virus particles (**RNM Figure 2**). Faint lesions on infected leaves increase their number and elongate to form chlorotic or yellowish streaks with the growth of plants. At the heading stage, mottling and streaking appear on the upper and flag leaves. Elongated necrotic lesions occur at the basal part of culms and sheaths of primary tillers. Later, necrotic lesions also occur in upper stems and sheaths. Generally, susceptible cultivars show clear symptoms, while some resistant cultivars show very mild symptoms.

The rice disease reported as RNM in Cuttack, India, appears different in properties from RNM (Ghosh 1981, 1982). The disease agent was reported to be mechanically transmissible to rice, weed species ***Ludwigia perenis*** and ***Brachiaria ramose***, and four fiber crops and to show mosaic symptoms and growth promotion in infected plants (Ghosh 1979, 1980, 1981, 1982).

In RNMV-infected rice plants, large, round or oval shaped inclusions (X-bodies) occur in the epidermal cells of inner surface of the sheaths (Fujii 1978, Inouye 1968, Inouye and Fujii 1977). X-bodies are stained yellow or brownish in iodine-iodate solution and pink or red with pyronine-methyl green stain (Fujii 1978). X-bodies may change appearance to diffused forms at later stage of infection.

Rice cells infected with RNMV contain filamentous virus particles, pinwheel or bundle inclusions, and masses of vesicles and membrane bodies in the cytoplasm (**RNM Figure 3**) (Fujii 1978, Hibino et al 1981, Inouye 1970, Inouye and Fujii 1977, Nagao et al 1976). Virus particles are scattered or aggregated in the cytoplasm, or in association with

the pin-wheel or bundle inclusions (**RNM Figure 3**). Membrane bodies that consist of tangled tubules or vesicles are often arranged in lattice-like structures, or in honeycomb or floral patterns.

In the field, RNM-infected rice plants show more severe infection in the presence of panicle blast and neck blast (Fujii 1967).

RNM Fig. 3, Part of a parenchyma cell infected with *Rice necrosis mosaic virus* (RNMV) showing bundle or pin-wheel inclusions associated with virus particles (x 30,000). Courtesy of Tadao Inoue.

1.3.Virus

RNMV is a member of the genus ***Baymovirus*** of the family ***Potyviridae***. Other members of ***Bymovirus*** are also soilborne, transmitted by the fungus ***P. graminis***, and infect cereal crops (Usugi et al 1989). Interestingly, RNMV, upon inoculation, can induce higher growth and yield in some plants of commercial importance (Ghosh et al 2012).

RNMV is a flexuous rod, 13-14 nm in width, with two modal lengths of 275 and 550 nm (Fuji 1978, Inouye 1968) (**RNM Figure 2**). Except for one isolate from Ooita Prefecture, the RNMV isolates reported in Japan are not mechanically transmissible or transmissible with difficulty (Fujii 1978, Inoue and Fujii 1977, Momota 1974). The RNMV isolate from Ooita and the RNM-like disease reported in India are readily transmissible by mechanical means (Fujikawa et al 1969, 1970; Ghosh 1979). Unlike the RNM Okayama isolate, the Ooita isolate is reported to be seedborne in rice at the rate of 0 to 22%, largely 2 to 10% (Fujikawa et al 1971a,b, 1972a,b).

RNMV is serologically distantly related to Barley yellow mosaic virus (BYMV) and Wheat yellow mosaic virus (WYMV) of the genus ***Bymovirus***, both of which are also transmitted by ***P. graminis*** (Usugi and Saito 1976, 1984). RNMV is assumed to be incorporated into the plasmodia, and zoospores of ***P. graminis***. Zoospores are released from root cells, move to rice roots, attach at the root cells, and inject protoplasm into the root cells (Fujii 1978, Hiruki and Teakles 1987). Infection of rice plants is achieved by RNMV released from the zoospores into the rice cells.

Based on an infectivity assay by mechanical inoculation on RNMV Ooita isolate, the infectivity of RNMV in rice extracts is inactivated at 60°C in 10 minutes and at 20°C in 14 days. RNMV infectivity is detected up to 10^{-4} dilution in extracts of rice leaves (Fujikawa et al 1970).

Purification of RNMV is difficult because virus content in infected rice tissues is low and virus particles tend to aggregate during the purification process. RNMV is purified from infected rice leaves by macerating in 0.2 M phosphate buffer,pH7.4 containing 0.1% thioglycollic acid and 2% triton X-100 (Fujii 1978). Extracts are filtered through gauze. The filtrate is clarified by macerating with 2 volumes of chloroform-butanol (1:1) mixture and by low speed centrifugation. The supernatant is subjected to differential and sucrose density gradient centrifugations. Purified fraction obtained contains RNMV particles with lengths of from 100 to 1800 nm. RNMV particles observed in dip preparations directly from

infected rice leaves have two modal lengths at about 275 and 550 nm. RNMV can also be purified from small amounts of rice leaves by pulverizing tissues in liquid nitrogen and 3 volumes of 0.05% borax that contains 0.001 M ethyelenediamine-tetraacetate (EDTA) (Inouye and Fujii 1977). The homogenate is filtered through cheesecloth, clarified with 0.2 volume of chloroform, and subjected to three cycles of differential centrifugations. The final pellet is suspended in 0.05 M borate buffer, pH 7.6. Antiserum is obtained by immunizing rabbits with the purified virus fractions.

RNMV contains single-stranded, positive-sense RNA and a coat protein. The nuclear inclusion body b gene (Nib) of RNMV is amplified from RNMV-RNA in extracts of RNMV infected plants by reverse transcriptase-polymerase chain reactions (RT-PCR) using a primer set specific for viruses belonging to the genus ***Bymovirus*** (Badge et al 1997). The sequence of a 1.4-kb DNA fragment obtained by RT-PCR contains the coat protein gene with high sequence homology to viruses of the genus ***Bymovirus***.

The variability of RNMV is not well understood. The RNMV Ooita isolate is reported to be mechanically transmissible and seedborne (Fujikawa et al 1969, 1972, 1974). All RNMV isolates so far tested in Japan are not mechanically transmissible nor seedborne. The Ooita isolate has not been well characterized and requires confirmatory studies. The RNM-like disease in India was reported to infect two weeds and four fiber crops by mechanical means (Ghosh 1981, 1982). Confirmatory studies are also required on this disorder.

1.4. Host range

Rice is the primary host of RNMV. In host range studies on the RNMV Okayama isolate, none of gramineous and nongramineous plants grown in RNM-affected soil or sap-inoculated with RNMV showed symptoms (Inouye and Fujii 1977). In Ooita, Japan, plants of ***Echynochloa crus-galli*** var. ***orizicola*** showing symptoms similar to those of RNM-infected rice plants were found in RNM-affected rice fields (Fujikawa et al 1974). When rice and ***E. crus-galli*** seedlings are grown with RNM-infected rice stubble under upland conditions in pots filled with RNM-infected soil, the seedlings develop RNM-like symptoms. Rice and ***E. crus-galli*** seedlings mechanically inoculated with sap of symptomatic ***E. crus-galli*** plants showed symptoms. ***E. crus-galli*** is common in rice fields in Japan and may be an important disease source.

In Cuttack, India, mosaic symptoms were observed in two weeds, ***Ludwigia perenis*** of ***Onagaceae*** and ***Brachiaria ramose*** of ***Gramineae***, in RNM-exposed fields (Ghosh 1979, 1981). Seedlings of these weeds mechanically inoculated with extracts of diseased rice plants developed similar mosaic symptoms. When grown in pots filled with soil from RNM-exposed fields, these weeds developed the symptoms at 26% for ***L. perenis*** and at 44% for ***B. ramosa***. Back inoculation from the symptomatic plants to rice seedlings revealed infection with the disease. Infected ***L. perenis*** plants showed increase in shoot growth and leaf size (Ghosh 1982). Fiber plants ***Corcholus olitorius***, ***C. capsularis***, ***Hibiscus sabdariffa*** and ***H. cannabinus*** inoculated with extracts of infected rice plants were reported to show growth promotion in plants (Ghosh 1982).

1.5. Transmission by vectors

RNM is soilborne and transmitted by the soilborne fungus ***P. graminis*** (Fujii 1967, 1978; Fujii and Okamoto 1968; Fujii et al 1967b; Inouye 1968). Clusters of resting spores of ***P. graminis*** are always found in the root tissues of RNM-infected rice plants (**RNM Figure 4**), but not always in healthy root tissues. RNM infectivity in soil is retained for more than 8 years under natural conditions without growing rice and for more than 6 years under air-dried conditions at room temperature (Fujikawa et al 1976a,b), indicating that the transmission

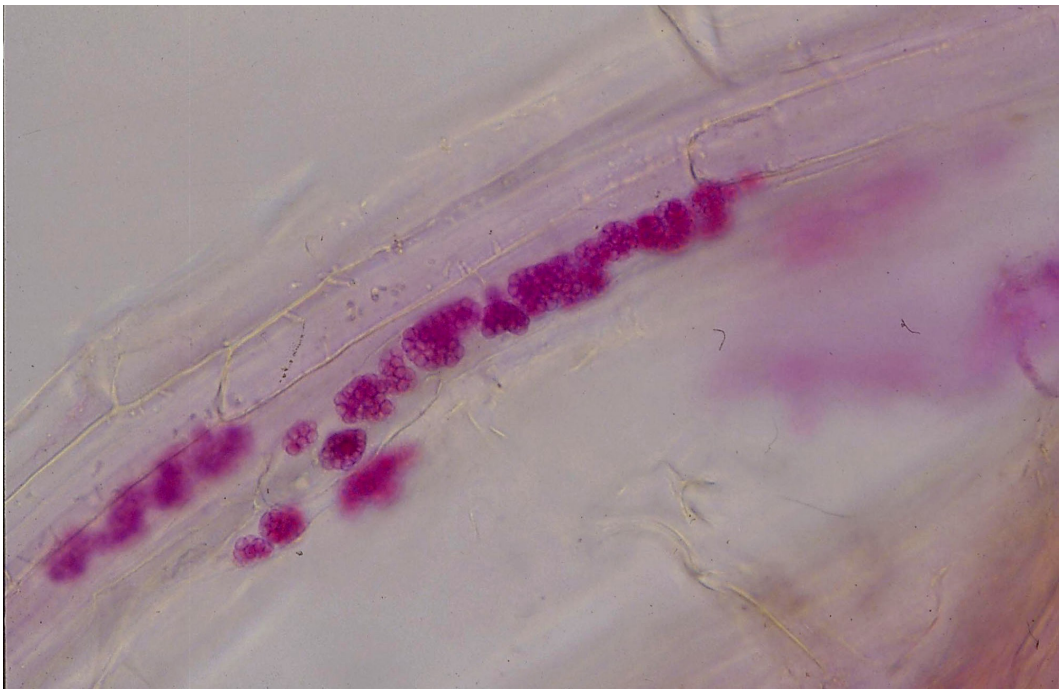
of RNMV with ***P. graminis*** is in a "persistent manner" (Adams 1991). RNM-affected soil looses its infectivity by heating at 55°C for 30 min (Fujii 1978) or 60°C for 20 min (Fujikawa et al 1974) and greatly reduces its infectivity after application of fungicides (Fujii 1978; Fujikawa et al 1970b, 1971b, 1972c, 1975).

When roots of RNM-infected rice plants are incubated in water with germinated rice seeds for 10 or 20 days, seedlings show infection with RNM (Fujii 1978). When RNM-infected rice roots are incubated for 1 day with sterilized water, the wash water obtained has RNM infectivity (Fujii 1978). In other studies, fractions containing ***P. graminis*** resting spores collected from RNM-affected rice roots are infective of RNM (Momota 1974). Rice seedlings incubated with extracts of RNM-infected rice roots have infection with RNMV and ***P. graminis***. When RNM-infected rice roots are incubated with water for 1 day, consecutively 4 times, the first and second wash waters are infective of RNM. The infectivity in water is retained when fresh rice seedlings and water are supplied every 10 days (Momota 1974).

P. graminis formed a cluster of resting spores in infected rice root cells (**RNM Fig. 4**) and developed zoospores, which swam to the rice roots, attached at the surface of roots, and injected zoospore protoplasts into the root cells. RNMV is assumed to be released from plasmodial protoplast into the host cell at the plasmodium stage. Plants infected with ***P. graminis*** alone do not develop visible symptoms.

Soil texture has substantial effects on the infectivity of RNM-infected soils (Fujikawa et al 1974). Infectivity is highest in sandy soils but lower in silt loam, loam, clay loam, and clay. When seedlings from rice seeds raised in pots filled with RNM-infected soil for various periods were washed and transplanted in RNM-free pots, infection of the seedlings occurred in as short as 30 minutes (Fujikawa et al 1974). When seedlings grown in RNM-free soil were transplanted at different ages in pots with RNM-infected soil, seedlings at 10 to 50 days old were most sensitive to infection (Fujikawa 1980).

The RNM isolate in Ooita was sap-transmissible and seedborne. By contrast, all other RNM isolates tested in Japan were usually not sap-transmissible or transmitted with difficulty. An RNM-like disease reported in India was mechanically transmissible. Further



RNM Fig. 4. Clusters of resting spores of *Polymyxa graminis* in a rice root stained in Giemsa solution.

confirmation is required for these inconsistencies. However, dispersal of RNMV through mechanical transmission is unlikely important in RNM epidemics.

P. graminis (Ledingham 1939) is an intracellular parasite, infects gramineous roots, and transmits several viruses in barley, oat, rice, and wheat (Adams 1991, Rao 1968, Kusaba et al 1971). Plants infected with ***P. graminis*** alone do not show any symptoms. Fujii (1978) studied the life cycle of ***P. graminis*** in relation to the transmission of RNMV. The optimum temperature for growth of ***P. graminis*** is around 15-18°C, lower than the temperatures required for rice and RNMV. In rice, there may be strains of ***P. graminis*** adapted to higher temperature. The rice stripe necrosis virus in Côte d'Ivoire, Africa, and Colombia, South America, is also believed to be transmitted by ***P. graminis*** (Fauquet and Thouvenel 1983, Morales et al 1999).

1.6. Diagnosis and serology

The diagnosis of RNM in the field is primarily based on plant stunting, spindle-shaped lesions or mottling on leaves, and a spreading growth habit (Fujii 1967, 1971; Inouye and Fujii 1977). The leaf symptoms and necrotic lesions on the basal parts of stems and sheaths may not be distinct in the fields. The presence of clusters of resting spores in root cells is only a necessary indicator for the occurrence of RNM.

Diagnosis may be based on association of rod-shaped virus particles in dip preparation and occurrence of X-bodies in the epidermal cells of the inner surface of sheaths. Although extremely difficult, mechanical transmission of RNMV to rice seedlings using sap of diseased plants may still be possible (Fujii 1978; Fujikawa et al 1969, 1970).

Antiserum to RNMV has been obtained by immunizing rabbits with partially purified RNMV fractions (Inouye and Fujii 1977). The antiserum has a low titer (1:10) in the micro-precipitin test. The antisera to BYMV and WYMV reacted with RNMV in the complement fixation test (Usugi and Saito 1976) but did not react in ELISA (Usugi et al 1984), indicating that RNMV is serologically distantly related to BYMV and WYMV.

1.7. Disease cycle and epidemiology

The disease source for RNM was primarily RNM-infected soil in nursery beds (Fujii 1978; Fujii et al 1967b, 1977). RNM occurred in fields transplanted with seedlings grown in nurseries in RNM-infected soils. Infection of rice seedlings after transplanting was limited. Major infection occurred at the seedling stage in nursery beds and dispersal of RNM after transplanting from infected plants to surrounding plants was limited (Fujii 1978; Fujii et al 1967b, 1977b; Fujikawa 1980; Okamoto 1969). Seedling infection was high in upland nursery beds but limited in flooded nursery beds. Seedling infection occurred within 30 days, largely in 15 days after seeding. When rice seeds were grown in pots under upland conditions, infection was observed even at 70 days after seeding (Fujikawa et al 1974).

RNM incidence was high in rice fields raised by direct-seeding in drained fields but scarce in fields raised by direct seeding in flooded fields (Fujii and Okamoto 1969, Fujii and Idei 1974). RNM incidence was 52% in direct tillage seeding in drained fields, 18% in direct nontillage seeding in drained fields, 3% in direct tillage seeding in flooded fields, and 2% in direct nontillage seeding in flooded fields, but 81% in fields transplanted with seedlings raised in upland nursery beds. When direct seeding in drained conditions was practiced repeatedly in the same field, RNM incidence increased year after year (Fujii and Idei 1977).

In RNM-infected fields, infectivity was found largely in soils 0-5 cm deep, a little at 5-10 cm, and none at deeper than 10 cm (Fujii 1978). The soil temperature effective for seedling infection with RNM was 16-28°C. Soil moisture greatly affected seedling infection with RNM (Fujii 1978). Drained soil gave highest infection while soil with higher moisture gave fewer infections. Seedling infection was high in soil adjusted to pH at 5.6-7.8.

1.8. Losses

RNM causes appreciable yield loss in infected fields. Yield reduction was largely due to reduction in number of tillers and grains and inferior ripening (Inouye and Fujii 1977). RNM-infected fields often suffered from rice blast especially on panicles and nodes, resulting in even greater yield reduction (Fujii 1967).

Plants of cultivar Akebono with severe RNM infection produced biomass at 40-60% of that produced by healthy plants, grain weights per hills at about 55-80%, 1,000-kernel weight at 50%, panicle weight at 46%, and grain number per head at 62% (Fujii 1978, Fujii et al 1967a). Reduction in grain yield was indicated as:

$$Y = 100 - (100n_0 + 85\,n_1 + 70n_2 + 55n_3) / N$$

where Y is grain yield reduction; N, number of hills; n_0, number of hills with mild infection; n_1: number of hills with moderate infection; n_2: number of hills with severe infection.

1.9. Cultivar resistance

In 1966-72, 429 rice cultivars were tested for resistance to RNM by transplanting seedlings grown in RNM-affected upland nurseries (Fujii 1978, Fujii et al 1977). Scoring is based on percentage infection as follows: 0-5%, resistant; 5.1-20%, moderately resistant; 20.1-40%, intermediate; and 40.1-60%, susceptible. In nonglutinous rice, cultivars Norin No. 20, Kantou No. 52, and Kuuiku No. 9 showed strong resistance while six cultivars were moderately resistant. In glutinous rice, cultivar Iwaimochi showed strong resistance while two cultivars were moderately resistant. In nonglutinous upland rice, 29 cultivars showed strong resistance while two showed moderate resistance. In glutinous upland rice, eight showed strong resistances while two were moderately resistant.

Fifty of 193 cultivars from outside Japan showed strong resistance while 27 showed moderate resistance. Many indica cultivars showed moderate resistance to RNM. In a field test in Shizuoka, Japan, all 100 Japanese cultivars tested showed RNM infection (Mori et al 1970). Some rice cultivars from the Middle East, France, Italy, Korea, and the Philippines showed no infection but many cultivars from Taiwan and the USA had high levels of infection. In Ooita, Japan, seeds of 28 rice cultivars were raised in pots with RNM-infected soil. Nine cultivars had low infection (Fujikawa 1982). These results were somewhat different from the results obtained in Okayama, Japan (Fujii 1978, Fujii et al 1977).

Upland cultivars Norin Mochi 4 and Norin 21 with strong resistance to RNM were crossed with susceptible cultivars (Fujii 1978). F_2 and F_3 generations showed segregation in resistance levels, indicating the resistance is inherited. In the resistant cultivars, RNM symptoms appeared after transplanting at about the same time as in susceptible cultivars. However, the development of the symptoms in resistant cultivars appeared slower than in susceptible cultivars (Fujii 1978, Fujii and Idei 1973). The resistant cultivars infected with RNM did not show significant differences in plant height and panicle length, but produced less panicles than do susceptible cultivars.

Reactions to RNM of selected cultivars were tested using RNM-infected soils from 10 locations in central and western Japan (Fujii 1978). Cultivar reactions appeared different among soil samples used, indicating variability in pathogenicity among RNM isolates from different locations.

1.10. Control

The most simple and effective measure to control RNM is to raise rice seedlings in RNM-free nurseries or under flooded or wet-bed conditions (Fujii 1978, 1971; Fujii 1967; Fujii and Okamoto 1969; Inoue and Fujii 1977). RNM incidence was less in direct seeding in flooded fields. Treatment of nursery beds with soil fumigants or fungicides effectively reduced

RNM incidences in transplanted fields (Fujii 1978; Fujikawa et al 1970b, 1971b, 1972c). Disinfection of rice seeds using fungicides, H_3PO_4 (phosphoric acid), or pentachloro-nitrobenzene (PCNB) reduced seedling infection in nursery beds (Fujikawa et al 1971b, 1975). Application of fertilizer, especially nitrogen fertilizer, also reduced RNM incidence (Fujikawa et al 1976c).

In Japan, RNM became a problem after upland nursery beds protected with oiled paper or plastic seats became popular in central, western, and southern Japan. The protected nursery beds availed early planting of rice and stabilized yields. On the other hand, RNM was greatly reduced and practically disappeared after the use of machine transplanters and seedling boxes using commercial soils became popular.

The recommended control measures for RNM are as follows:

- raise seedlings in RNM-free nursery beds;
- if use of RNM-affected nursery is inevitable, raise seedlings in wet or flooded nursery beds;
- if upland nursery is inevitable, apply fumigant methyl bromide at 3-6 g per m^2 in nursery beds and use rice seeds disinfected with fungicides;
- select rice cultivars with resistance to RNM;
- apply sufficient amounts of fertilizer;
- avoid keeping excess seedlings for a long time in the fields (Fujii 1971, 1978; Fujikawa et al 1975).

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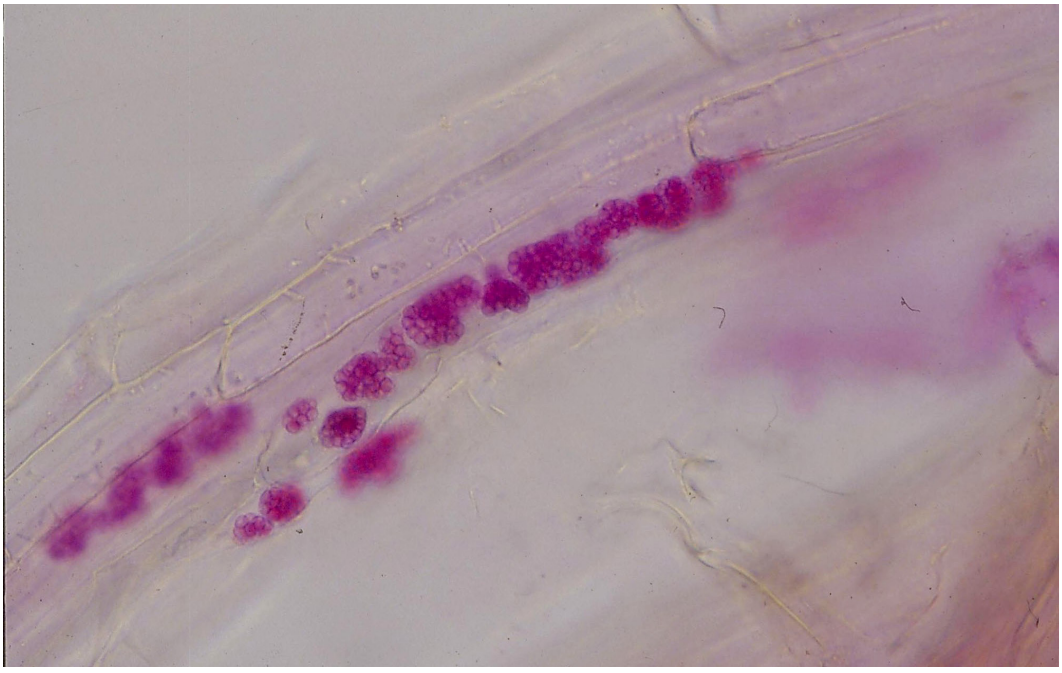
2. Stripe necrosis (RSN); synonym: Crinkling disease; pathogen: RSNV

2.1. History and distribution

Stripe necrosis of rice (RSN) occurs in Côte d'Ivoire, Nigeria, Liberia, and Sierra Leone in West Africa (Fauquet et al 1988), and in Brazil, Colombia, Costa Rica, and Ecuador in South America and in Panama in Central America (Morales et al 1999, Lozqnol and Morales 2009).

A disease of rice called "crinkling disease" was first found in upland rice fields in Sierra Leone in 1976 (Ou 1985). A similar disease has been known for many years in the upland rice fields in Côte d'Ivoire (Louvel and Bidaux 1977, Ou 1985). The disease is characterized by chlorotic or necrotic stripes and crinkling of leaves and premature plant death (**RSN Figure 1**) and initially thought to be caused by nematodes (Vuong 1968) or a bacterial agent (Fauquet et al 1988). Later, the disease was identified as a soilborne disease associated with a rod-shaped virus, rice stripe necrosis virus (RSNV) (Fauquet and Thouvenel 1983, Fauquet et al 1988). RSNV is associated with the fungus *Polymyxa graminis* Ledingham of Plasmodiophoromycetes (**RSN Figure 2**) and mechanically transmissible from rice to

RSN Fig. 1. A rice plant infected with RSNV showing crinkling of young leaves. Courtesy of F.J. Morales.



RSN Fig. 2. Clusters of resting spores of *Polymyxa graminis* in rice root, stained in Giemsa solution.

Chenopodium amaranticolor and ***Nicotiana benthamiana.*** Mechanical transmission from rice to rice was achieved only in one plant out of more than 10,000 tested (Fauquet and Thouvenel 1983).

In 1991, a disease of rice called "entorchaminento" (crinkling) occurred in the Eastern Plains of Colombia, South America (Morales et al 1999). The disease is characterized by striping and malformation of leaves and seedling death, similar to RSN in West Africa. By 1994, the disease spread to most rice fields in the region, causing yield losses of more than 20% (Pardo and Munoz 1994). It was initially attributed to aphids (Tapiero 1994) or nematodes (Pardo and Munoz 1994). Later, it was found to be soilborne and associated with ***P. graminis*** and rod-shaped virus particles (Morales et al 1995a,b, 1999). The virus agent is serologically related to RSNV in West Africa and identified as RSNV (Morales et al 1999). RSN has spread rather rapidly in the main rice-producing regions in Colombia and in Brazil, Ecuador, and Panama. Indeed, it is a potential threat to upland rice cultivation in countries on the South American continent.

2.2. Symptoms

RSN symptoms in rice appear 21 to 39 days after seeding in upland fields. Infected rice seedlings show chlorotic or yellow stripes along the leaf veins, crinkling and malformation of leaves, and plant stunting (**RSN Figure 1**) (Fauquet and Thouvenel 1983, Fauquet et al 1988). Later, the stripes become necrotic. Infected plants develop reduced number of tillers, maybe only one tiller. Often, whole plants become necrotic and wilt around 30 days after seeding. Plants infected at the later growth stage may show reduced tillering, and develop only one distorted and almost sterile panicle. Often, infected plants showing severe stunting occur in patch in upland fields.

Plants of ***Chenopodium amaranticolor*** inoculated mechanically with RSN-infected rice sap develop yellow spots on the inoculated leaves, which enlarge to show oak-leaf patterns, which eventually occupy whole leaves (Fauquet and Thouvenel 1983, Fauquet et al 1988). ***Nicotiana bentamiana*** plants inoculated with RSN-infected rice sap also develop yellow spots on the inoculated leaves.

In the leaf parenchyma cells of RSN-affected rice plants, rod-shaped virus particles are scattered or aggregated in the cytoplasm (Fauquet et al 1988). Paracrystalline aggregates of virus particles occur in the cytoplasm. In plants infected with RSNV in Colombia, virus aggregates and inclusions that consist of convoluted masses of endoplasmic reticulum and abnormal mitochodria occur in the cells (Morales et al 1999).

2.3. Virus

RSNV was once grouped in the genus ***Furovirus,*** then later as a tentative member in the newly defined genus ***Bynyvirus.*** The RSNV in West Africa is rod-shaped with a modal length of 110-160 nm, 270 nm, and 380 nm long and 20 nm in wide (Fauquet and Thouvenel 1983, Fauquet et al 1988). It has coat protein of 24 kDa. The RSNV isolate in Colombia has similar particles with bimodal length of 260 and 360 nm and additional shorter particles of 95-250 nm in length and longer particles of 370-450 nm at low frequencies (**RSN Figure 3**) (Morales et al 1999). The shorter and longer particles are considered to be fragments or end-to-end aggregates of particles. The RSNV in Colombia contains a coat protein of 22.500 kDa. Gel electrophoresis on double-stranded (ds) RNAs extracted from rice plants infected with Colombian isolate revealed four distinct bands corresponding to 6,300, 4,600, 2,700, and 1,800 base pairs (Morales et al 1999). RSNV is assumed to be incorporated into ***P. graminis*** cells, though virus particles are not observed in the fungal plasmodia, cistosori, or zoosporangia in infected rice tissues (Hiruki and Teakle 1987).

RSN Fig. 3. Purified RSNV (x 50,000). Courtesy of F.J. Morales.

RSNV can be purified from infected rice leaves or roots by macerating in 0.1 M phosphate buffer containing 0.4 % thioglycolic acid, clarification with chloroform-butanol mixture, and differential and sucrose density gradient centrifugations (Fauquet et al 1988, Fauquet and Thouvenel 1983). Three virus bands are formed in the tubes after density gradient centrifugation. Each of the fractions contains rod-shaped virus particles and nucleic acid at 5%. The three virus fractions contains corresponding particles of 110-160, , and 380 nm in length. RSNV is 20 nm in width and shows a hollow center in negative staining for electron microscopy. The purified virus fraction shows infectivity when inoculated to the two local lesion hosts. The virus yield is 10-15 mg/kg of infected rice tissues.

The RSNV in Colombia is also purified by macerating in 0.5 M potassium phosphate buffer (pH 7.2) containing 0.75% sodium sulfite, incubating in 1 M urea containing 2.5% Triton X-100, and centrifugation at low speed (Morales et al 1999). The supernatant is subjected to high-speed centrifugation over 20% sucrose cushion. The obtained pellet is suspended in phosphate buffer and subjected to 10-40% sucrose density gradient centrifugation. The purified virus preparation shows maximum UV adsorption at 260 nm and $A_{260/280}$ ratio of 1.5. The virus yield is estimated at 32 mg/kg of infected rice tissues.

Antiserum to RSNV in West Africa with a titre of 1/250 is obtained by immunizing rabbits with purified virus fractions (Fauquet et al 1999, Fauquet and Thouvenel 1983). The antiserum reacts with RSNV in Colombia in serologically specific electron microscopy (SSEM) and Western blotting (Morales et al 1999), but does not react with seven other species of rod-shaped fungal-borne viruses (Fauquet et al 1988).

RSNV contains two single-stranded (ss) RNAs (King et al 2012) and a single protein of 24 kDa or 22.5 kDa (Fauquet et al 1988, Morales et al 1999). The amino acid composition of the coat protein is characteristic of rod-shaped viruses transmitted by soil fungi (Fauquet et al 1988). RSNV–ssRNAs are poly-adenylated at the 3' end, as those of fungal-borne viruses classified in the newly defined genus ***Benyvirus*** (King et al 2012, Morales et al 1999). The nucleotide sequence of RSNV has been determined for RNA 1 and RNA 2 (Lozano and Morales 2009). The genome organization of the 2 RNAs are nearly identical to those of ***Beet necrotic vein virus*** and Beet soilborne mosaic virus belonging to the genus ***Bennyvirus***.

2.4. Host range

RSNV has a narrow host range. Rice (***Oryza sativa***) and ***O. glaberrima*** are the only known hosts naturally infected with RSN (Fauquet et al 1988, Fauquet and Thouvenel 1983). ***C.***

amalanticolor and *N. benthamiana* develop local lesions when mechanically inoculation using RSN-infected rice sap. In Colombia, extracts of RNM-affected rice plants in 0.05 M KPO4 buffer (pH 7.2) were mechanically inoculated to several indicator plants (Morales et al 1999). Inoculated *C. album*, *C. amlanticolor* and *C. murale* plants developed chlorotic local lesions on the inoculated leaves.

2.5. Transmission by vectors

RSNV is not mechanically transmissible from rice to rice, but is transmitted from rice to *C. amalanticolor* and *N. bentahamiana* and develops local lesions on the inoculated leaves. RSNV is successfully transmitted from the inoculated leaves of *C. amaranticolor* or *N. benthamiana* to leaves of other plants, but not to rice plants. RSNV in West Africa is not transmitted through seeds in six rice cultivars susceptible to RSN (Fauquet et al 1988). The RSNV in Colombia is also not seedborne (Morales et al 1999).

RSNV is soilborne and believed to be transmitted by the soilborne fungus *P. graminis* (Fauquet and Thouvenel 1983, Fauquet et al 1988, Morales et al 1999) though scientific proof is lacking. The cistosory (resting spores) and plasmodia of the fungus are always observed in infected rice roots. It is assumed that RSNV is carried inside the zoospores, which can move to rice roots and inject the protoplast including the virus into rice cells (Hiruki and Teakle 1987). RSNV has not been observed in fungal cells. In Colombia, fungal DNAs extracted from two root samples collected from RSN-infected rice plants were subjected to polymerase chain reaction (PCR) to amplify ribosomal DNA using fungal consensus primers (Morales et al 1999). The sequence of DNA fragments obtained showed high homology with Type II *P. graminis* isolates.

2.6. Diagnosis

RSNV is poorly immunogenic (Fauquet et al 1988). Rabbit antiserum having a titer of 1/250 was obtained in rabbits immunized with RSNV in Côte d'Ivoire. The antiserum reacted with RSNV in Colombia in serologically specific electron microscopy and Western blotting (Morales et al 1999) for the coat protein gene. Purified RSNV did not react with any of antisera against seven fungal-borne rod-shaped viruses and some other soilborne rod-shaped viruses tested (Fauquet and Thouvenel 1983, Fauquet et al 1988).

2.7. Disease cycle and epidemiology

The disease source is primarily soil infected with RSN. In RSN-infected upland fields, RSN symptoms appear at from 21 to 39 days after seeding, depending on the cultivars planted. Infected plants generally occur in patches of varying sizes in the field. At from 39 to 45 days after seeding, RSN incidence assessed by visual observation appeared to have decreased as many of RSN-infected plants were covered with surrounding healthy plants. When 30 cultivars were planted in RSNV-affected fields in 1977 and 73 cultivars in 1981 and 1983, RSN incidence was 0-48% and 0-100%, respectively.

RSN is not economically important in upland culture in West Africa. It occurs locally in many places but generally at low incidences (Fauquet et al 1988, Johnson et al 1993). In RSN-affected upland fields, RSN occurs infrequently and reaches epidemic levels from time to time after short periods of rainfall following long dry periods (Fauquet et al 1988). Such situations occur often at the beginning of the rainy season when planting of upland rice starts. In Colombia, RSN became important in rice cultivation soon after it was observed. It was newly found in Meta, Colombia, in 1991, spread by 1994 to most of rice-producing areas in the region, and caused yield losses at an average of from 20 to 50% (Morales et al 1999). Rapid dispersal of RSN in Colombia is suspected to be due to the use of contam-

inated agricultural machinery shared by rice growers. Seeds contaminated with RSN-infective soil may also be involved in the rapid dispersal of the disease. The scarcity of RSN in West Africa may be due to lower degree of mechanization and to planting of cultivars having resistance derived from ***O. glaberrima*** lines (Johnson et al 1998).

In Côte d'Ivoire, RSN incidence appeared to be correlated with the level of inhabited weeds (Johnson et al 1998). In upland rice fields where weeding is practiced, RSN incidence was lower than in fields where no weeding is practiced.

2.8. Losses

When 103 rice cultivars were planted in RSNV-infected fields in Côte d'Ivoire in 1977, 1981, and 1983, the yield reduction in RSN-infected plants, based on number of panicles produced, was on the average from 13 to 16% but from 76 to 92% in highly susceptible cultivars (Fauquet et al 1988, Louvel and Bidaux 1977). Yield loss due to RSN in Colombia was over 20% on average (Morales et al 1999).

2.9. Cultivar resistance

In Côte d'Ivoire, all six rice cultivars planted in RSN-affected upland fields with 3 ***O. glaberrima*** lines (Johnson et al 1998) showed infection with RSN. However, none of the ***O. glaberrima*** lines had infection.

In Colombia, high levels of resistance were identified in ***O. glaberrima*** lines but the tested rice cultivars showed infection with RSN at varying rates. The resistance in ***O. glaberrima*** lines were transferred to hybrid lines after crossing with rice cultivars Caiapo and Bg90-2. Four lines obtained show no infection while seven lines had from 1 to 10% infection in the field. Three resistant lines selected from the crosses and the ***O. glaberrima*** lines had fewer ***P. graminis*** resting spores in rootlets while susceptible rice cultivars had numerous resting spores. The resistance may be, at least, partly for the vector ***P. graminis***.

2.10. Control

The use of resistant cultivars is the most efficient control measure for RSN. Strong levels of resistance to RSN have been identified in ***O. glaberrima*** lines (Johnson et al 1998), which can be transferred to hybrid lines (Correa et al 2008). The use of fungicides or fumigants to control RSN is not economically and environmentally feasible.

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