16. RICE NECROSIS MOSAIC, A SOIL-BORNE VIRUS DISEASE

Tadao INOUYE*

Since 1959, an unusual disorder of rice confronted farmers especially at reclaimed paddy field in the southern part of Okayama Prefecture. The causal agent of the rice disorder remained a mystery until 1966. Fujii et al. (1966, 1967) first found that the disorder was caused by a soil-borne virus and named it rice necrosis mosaic virus (RNMV).

Occurrence and economic importance

Occurrence of the rice necrosis mosaic is so far recorded in at least 9 prefectures in the Kanto districts and westward of Japan. According to a 1964 survey, occurrence of the disease was observed in about 270 ha of paddy field in the southern part of Okayama Prefecture (Fujii, 1967). The disease usually occurs in the paddy field of transplanted rice seedlings grown in upland rice nursery and also in direct seedling culture of paddy rice on upland field.

The disease causes an appreciable loss of the yield in some fields due to reduction of the number of tillers and grains and inferior ripening (Fujii, 1967). Necrosis mosaic diseased paddy rice often suffered also from inveterate rice blast especially on panicles and nodes, resulting in a great reduction of the yield (Fujii, 1967).

Symptoms

The first symptoms of the disease in paddy rice, leaf mottling, usually become visible on a few lower leaves at maximum tillering stage about 70 days after sowing. The mosaic symptoms are characterized by spindle shaped yellow flecks and streaks and are usually observed only on the lower leaves. With the growth of plants, leaf mottling successively spreads to the upper leaves. However, no mosaic symptoms are visible on the newly growing leaves.

Necrotic fleck lesions are observed on the basal portion of stems and sheaths of diseased plants in some cases. Infected rice plants are moderately stunted and reduce the number of tillers, and appear in somewhat decumbent growth. In epidermal strip of inside surface of sheath of infected rice, X-bodies are observed. Both leaf mottling and X-bodies are useful characteristics for simple diagnosis of the disease.

Causal virus

The only known host of RNMV is rice. RNMV is transmitted through infective soil and with difficulty by plant sap, but probably not by insects. RNMV is not likely transmitted through seeds, though two contrary results on seed transmission of the virus have been reported. Fujikawa (1972) reported 2.6–5.3% of seed transmission of RNMV. On the other hand, Fujii (1975) observed no seed transmission of RNMV in about 16,000 seedlings.

Properties of the virus in crude sap were reported by Fujikawa et al. (1970), i.e. thermal inactivation point was between 60 and 65°C (10 min), dilution end point was between 1 : 5,000 and 1 : 10,000, and longevity *in vitro* was between 7 and 14 days (20°C).

* Associate Professor, College of Agriculture, University of Osaka Prefecture, Sakai, Osaka, Japan

Particle morphology of the virus is very similar to those of the other two soil-borne cereal viruses in Japan, barley yellow mosaic virus (BYMV) and wheat yellow mosaic virus (WYMV). RNMV particles are slightly flexuous rods having at least two different lengths, *c.* 275 and 550 nm, and 13–14 nm in widths (Inouye, 1968).

In infected rice plant cells, characteristic laminated aggregate inclusions and membraneous structures are observed in cytoplasm. Virus particles are observed in cytoplasm scattering or loosely aggregating, and associating with laminated aggregate inclusions (Inoue, 1970; Fuji, 1975).

Fujii (1975) obtained partially purified virus preparation from frozen leaf tissue of infected rice by chloroform-butanol clarification followed by two cycles of differential ultracentrifugation and a sucrose density gradient centrifugation.

Inouye (unpublished data) also purified the virus partially for antiserum production by pulverization of infected tissue by using liquid nitrogen followed by chloroform clarification and two cycles of differential ultracentrifugation. The antiserum collected from immunized rabbit represented a titer of 1 : 10 (crude sap) in microprecipitin test.

RNMV is serologically related to BYMV and WYMV. Usugi and Saito (1976) reported positive serological reactions between RNMV and both of the antisera to BYMV and WYMV in complement fixation test.

Concerning the affinity of RNMV, Inouye (1968) proposed that the three soil-borne viruses of cereals, RNMV, BYMV and WYMV, would be classified into the same virus group under the tentative name of the RNMV-group on the basis of the similarities in patricle morphology and transmissibility through soil. These three viruses have an additional similarity in cytology of infected plant cells, and also have close serological relationships.

Classification of soil-borne cereal viruses including RNMV having elongated particles and inducing cylindrical inclusions in infected plant cells would be considered to be a different virus group from the aphid-borne Potyvirus group at the present time, though Edwardson (1974) placed some of these cereal viruses into the Potato virus Y-group.

Soil transmission

Natural occurrence of the disease is usually observed in paddy field transplanted seedlings grown in upland rice nursery, whereas it is extremely rare in those grown in lowland rice nursery. Different frequencies of the disease by different types of cultivation was also observed experimentally by Fujii (1975).

Frequent occurrence of the disease was observed in transplanting culture with seedlings grown in upland rice nursery and also in direct seeding culture of paddy rice on upland field, but infrequent occurrence was observed in direct sowing in flooded paddy field.

Soil transmission of the disease was studied in detail by Fujii and his staff (1967–1975). Soil moisture content of the rice nursery was the most important limiting factor in infection of rice seedlings to the virus: Percentage of plants which was diseased totaled 31 when seedlings were grown in the soil in which moisture content was as low as 15%, whereas those of diseased plants were only 7 and 0 in the soils in which the moisture content was 51–54% and 74–94%, respectively.

Besides the low content of soil moisture, the optimum conditions for virus infection examined were as follows: 25–30°C of soil temperature and 6–8 of pH level in the soil. The 3–5 cm layer of soil from the surface of the nursery bed was responsible for seedling infection, though the infectivity of soil was detectable even at 30 cm layer from the surface.

Many of the seedling infections in diseased soil occurred within 15 days after sowing, and almost all of them were found within 50 days, while extremely rare infection did occur in the seedlings after transplanting in paddy field.

Infectivity of diseased soil was not lost for at least several years under natural conditions. The soil of paddy field, in which only very low infectivity had been observed became highly infective when the field was changed to upland rice nursery for two successive years.

Fujii (1975) suggested that soil fungus, *Polymyxa graminis*, was the probable vector of RNMV because (1) steamed soil became infective on addition of extract of rice roots naturally infected with the virus, (2) *P. graminis* was frequently found in the roots of naturally infected rice, (3) invasion of zoospores of the fungus into root hairs of germinated rice seed was observed on the 2nd day after sowing in diseased soil, (4) steamed soil became infective on addition of resting spores of *P. graminis* collected from rice roots naturally infected with the virus, and the fungus was consistently found in the roots of rice experimentally infected, (5) higher population of the fungus in roots of the seedlings grown in upland rice nursery.

Varietal reactions

Fujii (1975) surveyed varietal reactions of rice in the field using a number of varieties (236 vars of Japanese and 193 vars of foreign source) during 8 years. Most of the Japanese paddy field rice varieties were found to be 'most' or 'moderately susceptible' to the virus, and only 4 vars including Kanto No. 52 out of 161 were found to be 'most resistant'.

On the other hand, Japanese upland rice varieties were separated largely into two groups in susceptibility to the virus, 'most susceptible' and 'most resistant', and 29 vars of ordinary upland rice out of 50 and 8 vars of glutinous upland rice out of 25 were found to be 'most resistant'. Many of C-type (indica type), 37 vars out of 72 examined, were found to be 'most resistant' to the virus among rice varieties of foreign source.

Control

A simple, effective measure to escape from virus infection in the infective field change from upland rice nursery to lowland nursery is recommended. However, it is necessary to note that soil infectivity may still remain for a long period at least over several years.

Treatment of diseased soil by using methyl bromide or PCNB (pentachloronitrobenzene) was found effective to control the disease. According to Fujii's experiment (1975), no diseased plants were observed in rice grown in infective soil which had previously been fumigated by 3–6 g/m^2 of methyl bromide, while 44% of plants became diseased in non-treated soil. Mixing PCNB (8–12 g/m^2) with infective soil before sowing or treatment with PCNB dust (5% of seed wt) on wet seed was also an effective means to reduce occurrence of the disease.

References

1. FUJII, S. (1967). *Shokubutsu Boeki* **21**, 188–190.
2. ——— (1971). *Shokubutsu Boeki* **25**, 267–270.
3. ——— (1975). Thesis, Tokyo University.
4. FUJII, S., OKAMOTO, Y., YAMAMOTO, H. & INOUYE, T. (1966). *Ann. Phytopathol. Soc. Japan* **32**, 82.
5. FUJII, S., OKAMOTO, Y., IDEI, T. & INOUYE, T. (1966). *Ann. Phytopathol. Soc. Japan* **32**, 325.
6. FUJII, S. *et al.* (1967). *Ann. Phytopathol. Soc. Japan* **33**, 105.
7. FUJIKAWA, T., TOMIKI, T. & SATO, S. (1971). *Ann. Phytopathol. Soc. Japan* **37**, 373–374.
8. ———, ———, ——— (1971). *Proc. Assoc. Pl. Protect. Kyushu* **17**, 15–16.
9. FUJIKAWA, T., TOMIKI, T., SATO, S. & ANDO, S. (1976). *Ann. Phytopathol. Soc. Japan* **42**.

10. INOUYE, T. (1968). *Nogaku Kenkyu* **52,** 31–45.
11. ——— (1968). *Ann. Phytopathol. Soc. Japan* **34,** 301–304.
12. ——— (1970). *Ann. Phytopathol. Soc. Japan* **36,** 186.
13. USUGI, T. & SAITO, Y. (1976). *Ann. Phytopathol. Soc. Japan* **42,** 12–20.

Discussion

K. C. Ling, IRRI: Could you include the following; (1) Mechanical transmission (2) Positive transmission through seed by Fujikawa and negative results by Fujii in your paper?

Answer: Mechanical transmissibility and Fujikawa's positive data on seed transmission are included in the paper.

E. W. Kitajima, Brazil: (1) You mentioned that 2 types of particle do occur in preparation from RNMV infected plants. Have you any evidence that RNMV might be another multicomponent virus?

(2) What is the rice variety do you use commonly as test plant for RNMV?

Answer: (1) No information has been obtained on the function of 2 different types of particle.

(2) We used a variety "Akebono".