Weed Hosts of Rice Necrosis Mosaic Virus

S. K. GHOSH, Scientist, Division of Plant Pathology, Central Rice Research Institute, Cuttack 753006, Orissa, India

ABSTRACT

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Rice necrosis mosaic virus was transmitted by sap inoculation and through soil to two weeds, *Ludwigia perennis* and *Brachiaria ramosa*. The virus was recovered from these weeds and transmitted to rice plants. The inner epidermal layer of the sheath of infected *B. ramosa* showed granular, oval, intracellular X-bodies, 5.2–7.8 × 5.9–10.4 μm. Natural infection of these weeds in the field further supported their role as hosts of the virus.

Additional key words: alternate hosts

Recently, the occurrence of rice necrosis mosaic virus (RNMV) in India was reported (2). RNMV is sap-transmissible and soilborne (1,3), and characteristic symptoms in cv. TN-1 rice plants include mosaic on the basal portion of the leaf and leaf sheaths, chlorotic streaks parallel to the leaf veins, necrotic spots on leaf sheaths and culm (Fig. 1), and spreading growth of infected plants with no reduction in plant height. Because of widespread RNMV in 1979 in some rice plots on the Central Rice Research Institute farm at Cuttack, a survey of weeds growing in and around rice fields was conducted.

Two weed plants, *Ludwigia perennis* Linn. (=*L. parviflora* Roxb.), family Onagraceae, and *Brachiaria ramosa* (L.) Haines, family Gramineae, showed symptoms resembling those of RNMV on rice. Results of attempts to determine susceptibility of these weeds to RNMV are reported here.

MATERIALS AND METHODS

Seeds from healthy *L. perennis* and *B. ramosa* were collected, germinated, and grown in autoclaved soil. Seeds (F_1) from these plants were used to produce healthy test plants. Rice plants, *Oryza sativa* L. 'TN-1', were used as sources of RNMV inoculum. Unless indicated otherwise, all test and control plants were grown under insectproof conditions.

Mechanical inoculation. Leaves of diseased rice plants were homogenized with a mortar and pestle in chilled sterile distilled water. The crude sap was partially clarified by centrifugation at 3,000 *g* for 10 min.

Apical leaves of healthy weed test plants at the four leaf stage were dusted with 400 mesh Carborundum and inoculated with the freshly extracted sap by using a cheesecloth pad. Weed plants inoculated with sap from healthy rice plants served as controls. Possible RNMV infection in test and control plants was checked by back inoculations to 30-day-old healthy TN-1 rice seedlings.

Soil inoculation. Apical leaves of healthy rice seedlings growing in pots containing thrice autoclaved field soil (20 lb psi, 3 hr) were inoculated with RNMV. After symptoms developed, soil from around the infected plant roots was collected, freed of all root debris, moistened, and planted with seed from healthy *L. perennis* and *B. ramosa* plants. For controls, seeds of the two weeds were planted in soil from pots of rice plants that had been inoculated with sap from healthy plants. Sap from plants with RNMV symptoms and healthy controls was back inoculated to 30-day-old healthy rice plants.

Natural infection. Ten plants of the two weeds, which were thought to be diseased, and surrounding soil were collected from the field and transplanted into individual pots. Sap from leaves with mosaic or streak symptoms was inoculated separately onto healthy 30-day-old rice plants growing in autoclaved soil in pots. Controls were rice seedlings that were inoculated with sap from healthy plants of both weeds grown from seed in autoclaved soil collected around diseased plants in the field.

Soil from around diseased weeds in the field was freed of root debris, moistened, and planted with healthy 30-day-old rice seedlings. For controls, field-collected soils were autoclaved before planting with healthy rice plants.

Anatomic studies. Healthy, naturally infected, and artificially infected *L. perennis* and *B. ramosa* plants were used. The inner epidermis of the stem of *L. perennis* and the inner epidermis of the leaf sheath of *B. ramosa* were peeled with fine point sterilized forceps, stained with 5% iodine for 5 min, and examined under a light microscope.

Table 1. Transmissibility of rice necrosis mosaic virus from rice to two weed hosts

Plant	No. of plants infected/inoculated[a]	
	Mechanical transmission	Soil transmission
Ludwigia perennis	36/50	13/50
Control[b]	0/50	0/50
Brachiaria ramosa	10/50	22/50
Control[b]	0/50	0/50

[a] Average of four replications.
[b] Test plants inoculated with sap or soil from healthy rice plants.

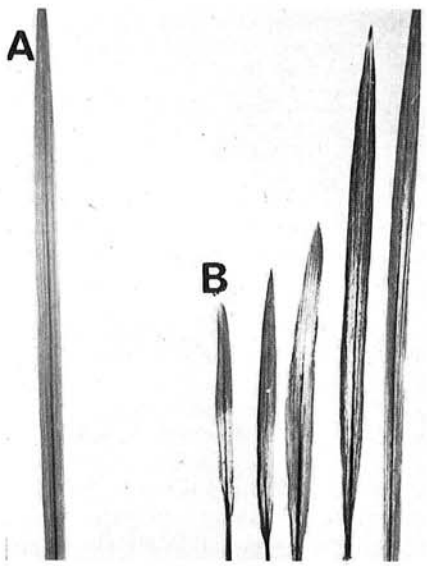

Fig. 1. Rice: **(A)** Healthy. **(B)** Infected by rice necrosis mosaic virus.

RESULTS AND DISCUSSION

Mechanical inoculation. Seventy two percent of the *L. perennis* plants inoculated with sap from RNMV-infected rice developed symptoms (Table 1). Initial symptoms appeared 4 days after inoculation and consisted of pale yellow spots at the base of lamina. As disease progressed, a typical green-yellow mosaic developed throughout the entire lamina. Green vein banding and interveinal chlorosis were prominent on emerging leaves (Fig. 2). No reduction in plant heights was observed. RNMV was recovered from 40% of the diseased *L. perennis* plants in back inoculations to rice.

Twenty percent of the *B. ramosa* plants showed symptoms after inoculation with sap from RNMV-infected rice (Table 1). The initial symptom of mosaic appeared on the emerging leaf sheath 25 days after inoculation. Later, chlorotic or yellow streaks developed parallel to the midrib

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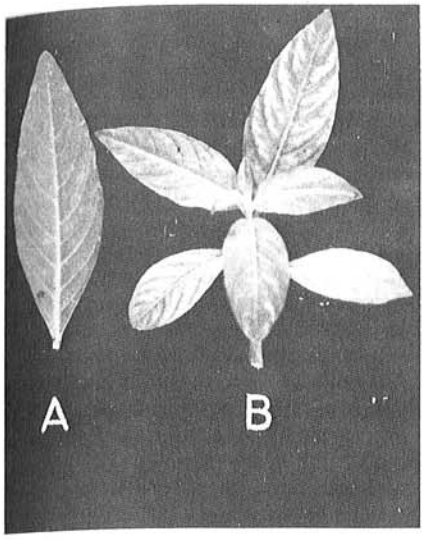

Fig. 2. *Ludwigia perennis*: **(A)** Healthy. **(B)** Infected by rice necrosis mosaic virus.

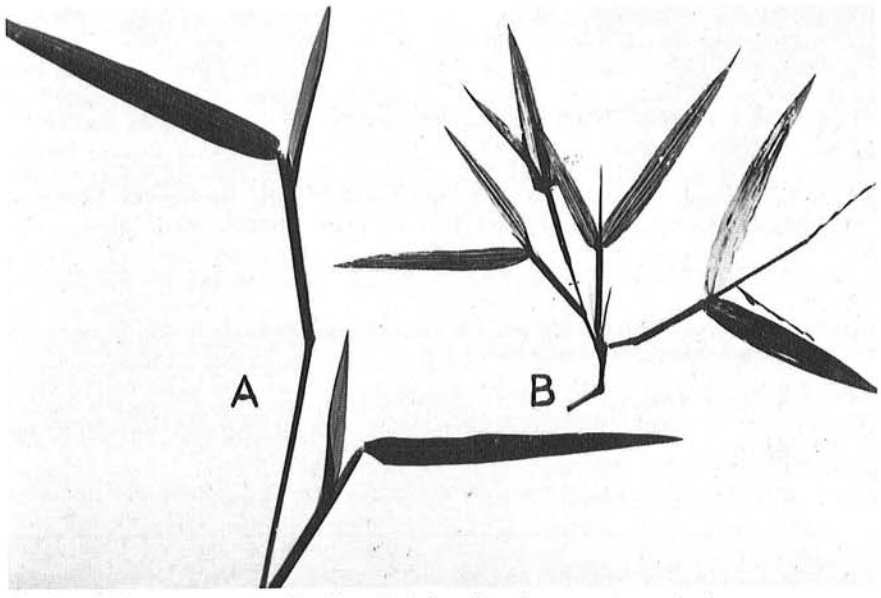

Fig. 3. *Brachiaria ramosa*: **(A)** Healthy. **(B)** Infected by rice necrosis mosaic virus.

Table 2. Transmissibility of rice necrosis mosaic virus from weed hosts to TN-1 rice seedlings

Inoculum source Type	No. of rice seedlings infected/inoculated[a]
Ludwigia perennis	
Sap	12/50
Control[b]	0/50
Soil	1/50
Control[c]	0/50
Brachiaria ramosa	
Sap	23/50
Control[b]	0/50
Soil	30/50
Control[c]	0/50

[a] Averages of four replications.
[b] Sap from healthy plants.
[c] Autoclaved field soil.

(Fig. 3). As disease progressed, streaks on the leaf sheaths and culms became necrotic. Subsequently emerging leaves showed both mosaic and streak. No reduction in plant height was noted. In back inoculations to rice, RNMV was recovered from 30% of the *B. ramosa* plants with symptoms.

Soil transmission. When plants were grown in soil from around RNMV-infected rice, symptoms appeared in 26% of the *L. perennis* plants and 44% of the *B. ramosa* plants (Table 1). RNMV was recovered from 4 and 42% of the *L. perennis* and *B. ramosa* plants, respectively, in back inoculations to rice.

Natural infection. When sap from naturally infected *L. perennis* and *B. ramosa* was used as inoculum, 24–46% of the rice test seedlings showed symptoms of RNMV infection (Table 2). When soil was used as the source of the virus, 60% of the rice seedlings grown in soil from around diseased *B. ramosa* plants and 2% of those in soil from around *L. perennis* became infected. Assays of all 10 samples of each weed collected in the field were positive for RNMV.

Anatomic studies. The cytoplasm of epidermal cells of the leaf sheath of naturally and artificially infected *B. ramosa* plants contained oval, granular, frequently vacuolated X-bodies, 5.2–7.8 × 5.9–10.4 μm. No such bodies were observed in healthy *B. ramosa* plants or in healthy or infected *L. perennis* plants.

This first report of *B. ramosa* and *L. perennis* as hosts of RNMV indicates the potential of these weeds as reservoirs of the virus. RNMV was transmitted by sap inoculation and through soil to both plants, but the mechanism of natural spread of the virus is unknown.

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