

Section 3. Virus and Phytoplasma Diseases

Chapter 5. Other viruses associated with rice diseases

1. Leaf gall and wallaby ear disease

A disease of rice, maize, and other gramineous plants characterized by plantstunting, darker-green leaves with numerous galls along the leaf veins on the leaf blades, and sheaths was noted in 1940 in the Philippines (Agati and Calica 1949, 1950). The leaf gall disease locally caused severe damage in maize but little or none in rice. The disease was postulated as a virus disease based on “transmission tests” using the leafhopper *Cicadulina bipunctella* Matsumura (Agati and Calica 1949, 1950). Rice seedlings exposed to single leafhoppers develop the symptoms at high rates 10 to 20 days after the exposure. The galls are caused by proliferation of the phloem cells, bundle sheaths and other cells surrounding the xylem vessels. The leaf gall is similar to galls induced by feeding of several leafhopper species, indicating that the leaf gall symptoms are caused by the leafhopper toxins rather than a virus (Maramorosch et al 1961). Reactions of upland rice cultivars to leaf gall are variable (Cada and Agati 1958).

On the other hand, “wallaby ear disease” similar to the leaf gall occurs in Queensland, Australia and suspected as of viral origin (**OVA Figure 1**) (Shindler 1942). The disease develops in maize seedlings exposed to the leafhopper *C. bipunctella bimaculata* (Evans). When the leafhoppers are removed from wallaby ear-diseased maize plants, the plants develop new leaves without symptoms (Boccardo et al 1980, Ofori and Francki 1983). The *C. bipunctella bimaculata* (Cbb) in Australia contained leafhopper A virus (LAV) in its salivary glands (Grylls 1975). LAV is 85 nm in diameter with densely stained cores about 50 nm in diameter and contains 10 segments of double-stranded RNA similar to the viruses belonging to the genus *Fijivirus* (Reddy et al 1976).

Similar virus particles are also found in extracts of wallaby ear-diseased maize tissues but the concentration is very low (Boccardo et al 1980). Wallaby ear disease was once attributed to LAV (Grylls 1975). Later, a Cbb colony free of LAV was obtained by mating a male and a female leafhopper raised from surface-sterilized eggs (Ofori and Francki 1983). Maize plants exposed to virus-free Cbb also developed wallaby ear disease, indicating the symptoms are not caused by LAV.



OVA Fig. 1. A maize plant showing wallaby ear caused by leafhopper feeding.

Similar diseases caused by feeding of *C. bipunctata* occur in maize and gramineous weeds in southern China, Indonesia, and Japan (Kawano 1994, Li et al 2004, Matsukura et al 2009). In Kyushu, Japan, the damage in maize caused by wallaby ear disease increased in Kyushu since 1993, after the introduction of double cropping of maize (Matsukura et al 2009, Matsumura et al 2005). The infestation is severe in the second crop maize planted in summer and controlled by using resistant cultivars and early planting of the first crop maize.

Cbb populations collected in Okinawa, Japan, caused wallaby ear symptoms in maize but are not associated with LAV (Kawano 1994). Apparently, LAV is a virus of Cbb and the wallaby ear symptoms are caused by leafhopper toxins as suggested earlier for the leaf gall in the Philippines (Maramorosch et al 1961). *C. bipunctella* and *C. bipunctata* are considered identical, and very close to *C. bimaculata* (Webb 1987).

Occurrence of a few LAV in wallaby ear diseased maize tissues indicated that LAV may circulate in the vascular tissues of maize and other gramineous plants without replication for some time, and is acquired by *C. bipunctella* through feeding the diseased plants (Boccardo et al 1980).

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2. Rice downy mildew fungus

In 1976, virus-like particles of about 30 nm in diameter were isolated in rice plants infected with downy mildew fungus, *Sclerophthora macrospora* (Saccardo) Thirumalachar, Shaw & Narasimhan, collected in Saitama Prefecture, Japan (Hibi et al 1976). The particles are abundant in downy mildew-diseased plants. Virus fractions purified from downy mildew-infected rice plants show UV light adsorption profiles characteristic of a nucleoprotein. Leaf cells of downy mildew diseased plants were reported to contain numerous virus-like particles scattered in the cytoplasm or aggregated in the vacuole. No such particles were found in the fungal cells in rice leaves.

On the other hand, in 1982, two isometric viruses, type A and B, were isolated from rice plants infected with downy mildew in Miyagi Prefecture (Honkura et al 1983). Type A virus is 32 nm in diameter with spikes of 4 nm in height on the surface. The type B virus is 35 nm in diameter without spikes. The type A virus contains three segments of single-stranded (ss) RNA as the genome and two proteins while type B has a single ssRNA and single protein (Shirako and Ehara 1985). The two viruses are separately purified from downy mildew-infected rice plants containing either virus. The antisera obtained from the two viruses are used to detect the viruses in the gel diffusion test. In a survey on the two viruses in downy mildew-infected rice plants in Miyagi, both types A and B were found in 11 fields, A in 9 fields, B in 21 fields, but no virus in 5 fields (Honkura et al 1983). All rice plants without the downy mildew symptoms contained the viruses. In a 1983 survey in Japan, both types A and B were found in fields in nine prefectures, type A in one prefecture, type B in three prefectures, and neither type in six prefectures (Honkura et al 1983).

In downy mildew-infected rice leaves, virus-like particles of about 30 nm in diameter are abundant in oospores and hyphae but none in leaf cells (Ehara et al 1984). When downy mildew-infected rice leaves with either of the viruses were shaken gently in water, zoosporangium were produced on the leaves 5 hours after the treatment and abundant zoospores were released 8 hours after. The amount of viruses in the leaves greatly decreased after treatment (Ehara et al 1986). These results indicate that types A and B infect *S. macrospora* but not rice plants.

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3. Rice tarsonemid mite virus associated with the mite

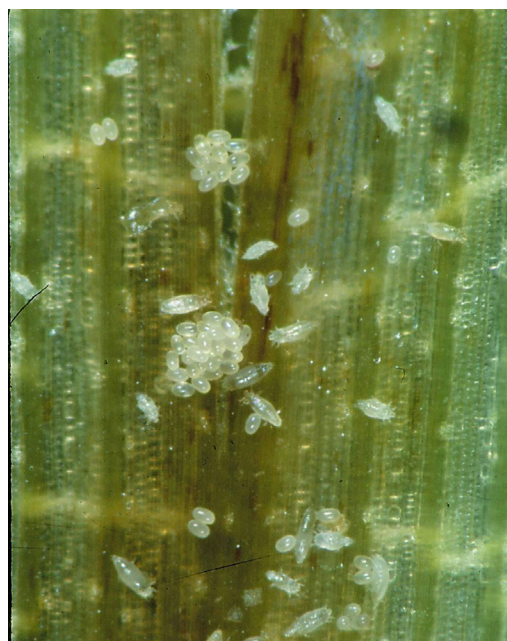
Virus-like particles of 35 nm in diameter were isolated from rice plants grown in the greenhouse at Hokkaido University, Japan (Kawano and Shikata 1983, Shikata et al 1984).

These rice plants showed brown discoloration of the sheaths and hulls (**OVA Figure 2**). The rice tarsonemid mite *Steneotarsonemus spinki* Smiley or *S. madecassus* Gutierrez was found inside the brown discolored sheaths and hulls (**OVA Figure 3**), similar to that reported for rice grain sterility associated with brown or purple sheaths in Taiwan (Chow et al 1976, 1980; Lee and Chow 1977; Chow et al 1980; Lo and Hor 1977; Ou et al 1977) and southern China (Lin et al 1982, Luo et al 1988, Zhang and Pna 1986, Zhang et al 1986). The brown sheath is associated with the sheath rot fungus *Sarocladium oryzae* Sawada Gams & Hawksworth (*Acrocyldrim oryzae* (Sawada) (Shikata et al 1984) in Taiwan and China (Hsieh et al 1977, Shan and Liu 1984, Shan et al 1985). Virus particles of 35 nm occur in the tarsonemid mites, their eggs, and in rice tissues infested with the mites (Shikata et al 1984). It was assumed that *S. spinki* and the 35-nm virus were incidentally introduced into Hokkaido from the Philippines when rice plants infected with rice ragged stunt virus were imported in 1977 (Shikata et al 1984).

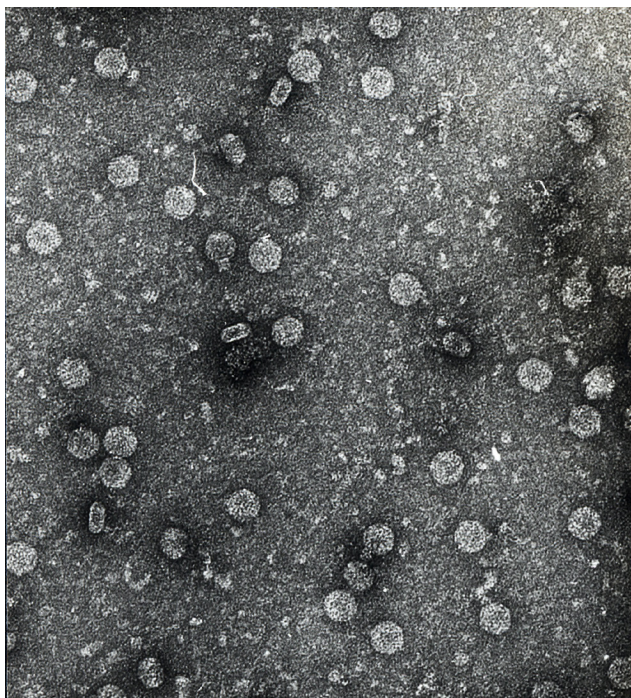
In the Philippines, rice plants grown in screen cages showed brown discoloration of sheaths and hulls and were associated with virus particles of 35 nm in diameter and tarsonemid mites (**OVA Figure 4**) (Duan et al 1993). The 35 nm virus is called rice tarsonemid virus (RTMV). RTMV appears as hexagonal, 35-40 in diameter in flat position, and as oval shaped, 40 nm x 16-20 nm in the standing position, indicating RTMV is disk-shaped (**OVA Figure 3**) (Duan et al 1993; Shikata et al 1984). RTMV contains two single-stranded RNA and a single protein of 19 kDa (Duan et al 1993). RTMV was purified from brown discolored sheaths (Duan et al 1993, Shikata et al 1984) and its antiserum produced. Enzyme-linked immunosorbent assay (ELISA) using the antiserum efficiently detected the virus in mites, their eggs, and in mite-infested rice tissues. ELISA did not detect RTMV in single mites or eggs but detected RTMV in batches of 200 mites or 500 eggs. RTMV was abundant in the tissues of brown sheaths and hulls infested with the mites, but absent in tissues other than sheaths and hulls of the same plants. RTMV was detected in rice plants 25-35 days after confinement in screen cages but absent in rice plants grown outside the cage (Duan et al



OVA Fig. 2. Brown sheath of rice affected with mites.



OVA Fig. 3. Tarsonemid mites, *Steneotarsonemus spinki*, and their eggs on the inner surface of brown discolored sheath.



OVA Fig. 4. Purified tarsonemid mite virus.

1993). In 1992-93 survey in the Philippines, no rice plant infested with tarsonemid mites was found in rice fields (Duan et al 1993).

Numerous RTMV-like particles have been observed in the cytoplasm of the inner epidermal cells and the adjacent parenchymatous cells of brown discolored sheaths (Shikata et al 1984). It is unknown whether RTMV multiplies in rice tissues or if virus particles ejected by mites accumulate in the rice tissues. It is also unknown if RTMV is involved in the development of sheath browning.

In southern Taiwan, grain sterility associated with brown sheaths was observed in 1968 (Ou et al 1977). The areas affected grad-

ually increased and expanded to 17,000 hectares in 1976. Sterility commonly occurred in the second crop rice and was associated with *S. spinki* and *S. oryzae* (Chow et al 1977, 1980; Lo and Hor 1977; Hsieh et al 1977). In the late 1970s, a similar rice disorder (called purple or brown sheath) occurred in southern China and became increasingly important. The purple sheath disease is associated with *S. spinki*, *S. oryzae*, *Xanthomonas* sp. and *Pseudomonas* sp. (Lin et al 1982, Luo et al 1988, Shan and Liu 1984, Shan et al 1985, Zhang and Pna 1986). *S. spinki* likely occurred under warm and humid conditions with weaker sunlight (Ou et al 1977). *S. spinki* is probably carried from rice plants to plants by planthoppers (Ou et al 1977) and *S. oryzae* by *S. spinki* (Hsieh et al 1977). It is not known whether RTMV is associated with *S. spinki* in China and Taiwan. *S. spinki* (rice mite) also occurs in Caribbean Region, Central America and Colombia, causing 5 to 90% yield loss (Smiley et al 1993).

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4. *Nilaparvata lugens* reovirus

Nilaparvata lugens reovirus (NLRV) was found in a colony of the brown planthopper (BPH), *Nilaparvata lugens* Stål in Japan (Noda et al 1991). NLRV is a member of the genus *Fijivirus* of the family *Reoviridae*, 65 nm in diameter, and contains 10 segments of double-stranded (ds) RNA. The electrophoretic profile of NLRV-RNAs differed from those of known rice viruses belonging to *Reoviridae* (Nakashima et al 1996). Ten dsRNA segments of NLRV carry the conserved terminal sequences found in other viruses belonging to *Reoviridae*. NLRV carries 12 spikes each on the outer shell and the core as other viruses belonging to the genus *Fijivirus* (Nakashima and Noda 1996a,b).

NLRV particles are scattered in the cytoplasm or arranged in single row in tubular structure in the cytoplasm of virus-carrying BPH tissues (Noda et al 1991). Immuno-electron microscopy revealed that NLRV occurs in the midgut, fat body, and salivary glands of BPHs. BPHs infected with NLRV showed a sign of negative effects.

BPH female adults injected with NLRV through fine capillary transmitted the virus to their progeny via eggs (Noda et al 1991). The progeny obtained from those females was congenitally infected with NLRV at 15% (Nakashima and Noda 1995). When eggs laid by BPH females were collected from rice plants and surface-sterilized with sodium hypochlorite solution, only 2% of the progeny were congenitally infected with NLRV.

These results indicate that NLRV is largely transmitted to progeny via eggs carrying the virus on the egg surface and probably partly via eggs carrying the virus inside the eggs. When BPH adults carrying NLRV and first instar nymphs from NLRV-free colony were reared on rice seedlings in a cage for 2 weeks, nymphs showed infection with NLRV at 31%, indicating that NLRV is transmitted from BPH adults to nymphs during the mixed

rearing (Nakashima and Noda 1996a,b). NLRV is also transmitted from BPH adults to nymphs of the small brown planthopper (SBPH), *Laodelphax striatellus* Fallen by mixed rearing. SBPH colony exposed to NLRV retain the virus for 3 generations, indicating that NLRV also propagates in SBPH. NLRV was not transmitted by the mixed rearing from BPH adults to nymphs of the rice green leafhoppers *Nephotettix cincticeps* or *N. malayanus*, and to the white back planthopper *Sogatella furcifella*.

NLRV is detected in rice seedlings exposed to NLRV-infected BPHs for 2 days, but not at 3 or 6 weeks after the exposure. BPHs of NLRV-infected colony were confined in a small feeding cage attached on the sheaths of rice seedlings for feeding and BPHs from virus-free colony were placed in two feeding cages attached at upper and lower portions of the same sheaths for 2 days. Some of the BPHs in upper and lower cages gained NLRV, indicating circulation of NLRV injected into the vascular bundles by BPHs in the central cage and acquisition of the virus by BPHs in upper and lower cages. Apparently, NLRV does not multiply in rice plants but is circulated in the vascular bundles of rice plants and can be acquired by other BPHs through feeding. No detectable deleterious effects are observed for NLRV in BPHs.

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5. Himetobi P virus

An isometric virus of 29 nm in diameter was isolated from a colony of small brown planthopper (SBPH), *Laodelphax striatellus* Fallen, maintained on rice seedlings in Japan (Toriyama et al 1992). The virus is called “himetobi P virus” (HiPV) after the Japanese name of *L. striatellus* (Toriyama et al 1990). HiPV is similar to viruses of the family *Picornaviridae* based on the characteristics of coat proteins, structure of the single-stranded (ss)-RNA genome, and physical properties of virus particles (Toriyama et al 1992).

In surveys using ELISA for the occurrence of HiPV on plant- and leafhopper colonies maintained in Japan, HiPV was found in three out of five SBPH colonies, 7 of 10 colonies of the brown planthopper *Nilaparvata lugens*, and one of four colonies of the white back planthopper *Sogatella furcifera*, but none of the colonies of the rice green leafhopper *Nephotettix cincticeps* (Guy et al 1992). The proportion of HiPV-carrying planthoppers in infected colonies was 79-100% for *L. striatellus*, 2-83% for *N. lugens*, and 59% for *S. furcifera*. In a survey in rice fields, HiPV was detected in SBPH populations collected in two of four fields. The percentage of SBPHs carrying HiPV in the two field populations was 6.2 and 3.2%. None of *N. cincticeps*, and aphids *Rhopalosiphum maidis* and *R. padi*, collected in the rice fields had infection with HiPV.

Eggs laid by HiPV-infected female adults were dissected from rice seedlings. Nymphs hatched from the dissected eggs had HiPV at 39% (Guy et al 1992). When eggs dissected were surface sterilized in 2.5% hypochlorite solutions for 3 min, none of nymphs hatched contained HiPV (Guy et al 1992, Suzuki et al 1993). HiPV was not detected in ELISA in batches of 10-40 eggs of the colony. Apparently, HiPV attaches on the surface of SBPH eggs and is transferred to nymphs hatched from the eggs.

When SBPHs from a HiPV-infected colony were confined 50 each with rice seedlings in containers for 2 days, feces collected by washing the containers and seedlings with buffer solution gave strong reactions in ELISA. HiPV was detected at high amounts in rice plants confined with SBPHs carrying HiPV but not in newly developed leaves on the same plants. It is likely that HiPV is transmitted to other SBPHs in contact with feces containing the virus.

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6. Other viruses infecting rice

It is known that some plant viruses infect rice by artificial inoculation by mechanical means or by means of specific vectors in the greenhouse (Ling 1972). Natural infection of rice with these viruses is rare or not observed.

6.1. Barley stripe mosaic virus (BSMV)

BSMV distributes widely in the world. It is rod-shaped, 128 x 20 nm. No vector is known for BSMV. It is seedborne and causes diseases in barley and wheat at varying severities. Rice seedlings inoculated mechanically with BSMV develop systemic symptoms or local lesions depending on rice cultivars (Kahn and Dickerson 1957).

6.2. Barley yellow dwarf virus (BYDV)

BYDV occurs in Australia, Canada, China, Europe, Japan, and the USA (Burnett 1990). It is isometric, 30 nm in diameter. BYDV causes plant stunting and orange or yellow discoloration of leaves in a wide range of gramineous plants. It is not mechanically transmissible but transmitted by aphids, *Rhopalosiphum padi*, *Macrosiphum avenae*, and other species. Rice seedlings infected with BYDV show stunting and yellowing (Watson and Mulligan 1960). Rice giallume virus, which is a strain of BYDV, occurs in Italy and Spain (see **Chapter 3 on Giallume**) but is not known in other rice-growing countries. BYDV infects gramineous plants under cooler temperatures. Rice seedlings may have infection in the late spring or early summer but may recover shortly after the infection, or may not develop clear symptoms under high temperature required for growing rice.

6.3. Brome mosaic virus (BMV)

BMV distributes widely in Europe, South Africa, and the USA. It causes mosaic on brome grass and other gramineous weeds. It is isometric, 25 nm in diameter, and transmitted by the nematodes of the genus *Xiphinema* in the laboratory but not been demonstrated in the field. Rice seedlings of some cultivars showed mosaic symptoms when mechanically inoculated with BMV (Kahn and Dickerson 1957).

6.4. Maize dwarf mosaic virus (MDMV)

MDMV is a strain of sugarcane mosaic virus (SuMV). It is filamentous and 773 nm in length. MDMV is transmitted by the aphid *Schizaphis graminum*. Seedlings of several rice cultivars mechanically infected with MDMV develop local lesions on the inoculated leaves (Brambl and Dale 1967, Ford and Tosic 1972).

6.5. Ryegrass mosaic virus (RMV)

RMV occurs in Canada, Japan, UK, and the USA. It is filamentous and 750 nm in length. RMV is transmitted by the eriophyid mite, *Abacrua hystrix*, and causes chlorotic streaks or mosaic symptoms on the Italian ryegrass *Lolium multiflorum*, *L. perenis* and *Dactylis glomerata*. One out of 10 rice seedlings mechanically inoculated with the British strain of RMV had infection but did not show symptoms (Mulligan 1960).

6.6. Sugarcane mosaic virus (SuMV)

SuMV occurs widely in sugarcane-growing countries. It is filamentous and 650-750 nm in length. SuMV is transmitted in a non-persistent manner by several aphid species including *R. padi*, *Carolinaia cyperi*, *Aphis gossypii*, *S. graminum* and *Hysteroneura setariae*. Rice seedlings of several cultivars mechanically inoculated with SuMV develop mosaic symptoms (Abbott and Tippet 1964, Anzalone 1963, Anzalone and Lamey 1968).

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7. Virus-like diseases in rice

7.1. Mosaic

Mosaic disease of rice characterized by mottling or mosaic symptoms was reported in the Philippines in 1960 (Martinez et al 1960). The putative pathogen was transmitted from rice to maize by mechanical means. No further information is available on the disease. Any similar disease has not been reported thereafter. Mosaic was most likely caused by the SuMV, which occurs in the Philippines and can develop mosaic symptoms on rice when mechanically inoculated (Abbott and Tippet 1964).

7.2. Chlorotic streak

Chlorotic streak characterized by plant stunting and chlorotic streaks of leaves occurred first in 1978 at an experimental field in Cuttack, India (Anjaneyulu et al 1980a,b). Affected plants are associated with the rice mealy bug, *Brevennisia rehi* Lindinger (*Heterococcus rehi*, *Ripersia oryzae*).

The symptoms were not transmitted to rice seedlings by mechanical means but by mealybugs (Anjaneyulu et al 1980a,b). Seedlings of rice cultivars Jaya and IR26 exposed at 3 per seedling for 8 hours to mealybug nymphs collected from rice plants that showed chlorotic streak symptoms exhibited plant stunting, chlorotic streaks, striping, or mottling on newly emerged leaves 7 to 15 days after the exposure. Emergence of leaves and panicles of the rice seedlings was often obstructed. Panicles showed brown discoloration and grain sterility. About 30-40% of the seedlings exposed to mealybugs developed the symptoms but none of the seedlings exposed to mealybugs collected from healthy rice plants developed the symptoms. Rice plants that developed the symptoms may recover from the disease. The percentage of seedlings that developed symptoms increased with the increase in the number of mealybug nymphs from 1 to 10 per seedling for the exposure. Rice seedlings exposed to mealybugs collected from healthy rice seedlings also developed stunting and leaf yellowing and produced less panicles but developed no chlorotic streaks.

In rice fields planted periodically with four rice cultivars, chlorotic streak incidence was higher in plots transplanted in May to July but none in plots transplanted in January to April. The incidence was higher in upland fields than in irrigated fields.

Cultivar reaction to the disease was compared by separately exposing seedlings to three mealybug nymphs collected from rice plants that showed chlorotic streak. Chlorotic streak incidence in exposed seedlings varied from 0 to 100%, with 0% in cultivar ARC 7110 and ARC 13859. In the field, chlorotic streak incidence was 16 to 77% at 22 days after transplanting but 6.5 to 29% at 57 days. The decrease in incidence at 57 days was due to the recovery of diseased plants. The extent of recovery varied among cultivars. Rice cultivars Jhona and CI 8970 showed no such recovery.

7.3. Wrinkled stunt

In 1976, an abnormal growth called wrinkled stunt occurred on the progeny of a cross between rice cultivars IR454 and 71101/9 in experimental fields in Surinam, South America (Ou 1985). It was characterized by pronounced stunting and wrinkled and twisted leaves with darker green color. Panicles of diseased plants were small, poorly exerted, and highly sterile. The abnormal growth was first noticed at the second-leaf stage and appeared on every leaf that developed afterwards. Virus particles were not found in abnormal rice tissues. The abnormality is most likely inherited.

7.4. Witches' broom

Abnormal growth, called witches' broom, characterized by pronounced stunting and proliferation of a large number of tillers was observed on plants of rice cultivar CICA 4 (Ou 1985). The abnormal plants appeared like a bundle of miniature plants and produced small tillers with 10-20 grains.

All plants grown from the seeds of rice plants with witches' broom symptoms showed similar abnormality. The abnormality is most likely inherited.

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