Tungroo Virus

ORIGIN:

Rice tungro disease was described for the first time by Anon in 1964 and Rivera and Ou in 1965. The disease is distributed throughout the South and Southeast Asian countries and Japan. The disease has been reported under different names such as yellow orange leaf disease in Thailand, penyakit habang in Indonesia, penyakit merah in Malaysia, waika disease in Japan and tungro disease in the Philippines and India. This disease is one of the major rice virus diseases and an important potential threat to rice production in this area due to its occasional severe outbreaks. Previously, the causal agent of the disease was thought to be rice tungro virus, a spherical virus about 30nm in diameter. However, it became clear recently that tungro disease is associated with a virus complex composed of two different viruses and these viruses were described as rice tungro spherical virus and rice tungro bacilliform virus .

SYMPTOMS:

The symptoms of the disease in the rice plant are characterized by slight-to-severe stunting of the plants, yellow-orange to orange-red discoloration of the leaf blades usually starting from the leaf tip and gradually moving downwards. Very small rustycolored necrotic spots may be found in the discolored areas of the older leaves. Infected plan ts, especially those of a susceptible cultivar produce few tillers. The symptoms of the disease were severe in double infection with RTBV and RTSV, moderately severe in single infection with RTBV, while clear symptoms were detected in single infection with RTSV. Taichung (Native) 1 seedlings show typical symptoms of tungro disease and are suitable sources of virus for purification.

TRANSMISSION:

Six leafhopper species are known as vectors of tungro disease, namely Nephotettix virescens (Rivera and Ou, 1965), N. cincticeps (Hibino, 1983), N. nigropictus (Rivera and Ling, 1968), N. parvus (IRRI, 1972), N. malayanus (IRRI, 1973) and Recilia dorsalis (Rivera et al., 1969). N. parvus and N. malayanus have little biological relationship with rice and N. cincticeps is not distributed in South and Southeast Asia where tungro is predominant. Of the three leafhopper

species, vectors of tungro, N. virescens is the most efficient vector. N. nigropictus and R. dorsalis are less efficient and their transmission efficiency of tungro variable depending on the colonies and locations tested. Percentage of active transmitters was 50-90 for N. virescens, 0-27 for N. nigropictus and 0-8 for R. dorsalis (Sogawa, 1976). Recent tungro transmission studies based on the presence of RTBV and RTSV in inoculated plants indicated the complex nature of the relationships between the green leafhopper and the two tungro associated viruses (Hibino et al., 1978, 1979; Hibino, 1983ab). N. virescens exposed to both RTBV and RTSV predominantly transmit both viruses together, while some of them may transmit RTBV alone and a few may transmit RTSV alone. The leafhopper can transmit RTSV from plants infected with RTSV alone but cannot transmit RTBV from plants infected with RTBV alone. N. cincticeps also transmit both viruses together or one virus alone from plants infected with RTBV and RTSV, though the transmission efficiency is lower than that of N. virescens. In the case of N. nigropictus, transmission ability of RTBV and RTSV depended on the colony. Of the plants infected with RTBV and RTSV, N. nigropictus transmitted RTSV alone in one case in Indonesia (Hibino et al., 1979), and transmitted either RTBV or RTSV in Japan (Hibino, 1983a), while it transmitted RTBV and/or RTSV in the Philippines, though its transmission efficiency was low (Cabauatan and Hibino, unpublished data). However, the three N. nigropictus colonies transmitted the virus efficiently from plants infected with RTSV alone. R. dorsalis has been tested in Indonesia and Japan (Hibino et al., 1979; Hibino, 1983a). None of the leafhoppers transmitted either RTBV or RTSV. Rice waika virus (RWV) (Furuta, 1977), a virus identical or very close to RTSV occurring in Kyushu, Japan, is transmitted similarly as RTSV by the Nephotettix spp. but not by R. dorsalis (Hirao and Inoue, 1978). RTBV is dependent on RTSV for its transmission by N. virescens (Hibino et al., 1978; Hibino 1983b). RTBV was transmitted by leafhoppers which had been given acquisition access to RTSV at the same time or before. Both RTBV and RTSV are transmitted in a semipersistent manner by the leafhopper and their retention periods in the leafhopper are 4 and 3 days, respectively.

PURIFICATION:

As described previously, RTSV was transmitted by the vector Nephotettix virescens, but RTBV was transmitted concomitantly only when RTSV was acquired previously or simultaneously, i.e., the propagation of RTBV using plants solely infected with RTBV as inoculum was impossible. Because large quantities

of doubly-infected plants were available, such plants were used for virus purification. Infected plants were harvested about 40 days after inoculation and were stored at -80°C. Clarification and partial purification were carried out according to the method applied for rice waika virus (RWV) (Usugi and Saito, 1975). Leaf extracts in 0.01 M ethylenediaminetetraacetic acid (EDT A), pH 6.0 were heated at 40°C for 1 hr. Virus particles were precipitated with 7% polyethylene glycol 6,000 plus 0.2 M sodium chloride (NaCl) containing 1% Triton X-100. The resuspended virus particles were treated with 20% carbon tetrachloride, and were subjected to differential centrifugation (96,000 x g for 60 min at 4°C in a Hitachi RP-40 rotor, then 10 min at 3,000 x g). Supernatant was layered on 10-40% linear sucrose density gradients (prepared in 0.01 M borate buffer pH 9.0 (BB)) and centrifuged at 60,000 x g for 3 hr at 4°C in a Hitachi RPS-25 rotor. Tube contents were scanned at A2,4 and fractionated through an ISCO model UA5 ultraviolet analyzer. Typical density-gradient scanning pattern presented in Fig. 2A showed a peak (arrow with "a") containing RTSV, and a shoulder (arrow with "b") on the descending slope containing RTBV. Peak and shoulder fractions were separately collected, virus particles were pelletted by centrifugation, and were subjected to two additional density-gradient centrifugations (Fig. 2B). After three sucrose densitygradient cycles, RTSV were further purified by equilibrium centrifugation in CsCI. When 3.273 g Cs Cl was dissolved in 4 ml (45% W IV) of the peak material in 0.01 M phosphate buffer (PB), at pH 7.0 and the suspension was centrifuged at 114,5000 x g for 40 hr at 4°C in a Hitachi RPS-40 rotor, a single band was formed at about twothirds of the distance below the meniscus. The peak material was recovered, and CsCl was removed by two cycles of differential centrifugation. Final pellets were resuspended in 0.01 M EDTA, pH 6.0 and such preparations contained isometric particles about 30 nm in diameter (Fig. 3). Preparations were negatively stained with neutralized phosphotungstic acid (PA) and were examined under a Hitachi H-500 electron microscope. The sedimentation coefficient of the virus determined by analytical centrifugation was 173 S. Equilibrium centrifugation in CsCl was not appropriate for the purification of RTBV, because the particles were degraded during the two cycles of differential centrifugation applied to remove CsCl after the equilibrium centrifugation. After three cycles of density-gradient centrifugation, RTBV was further purified using antiserum to RWV whose titer was 1:640 in the precipitin ring interface test. Partially purified RTBV fraction was mixed with equal volume of the serum which was diluted to 1/100 with 0.01 M PB, pH 7.0 containing 0.85% NaCl. The mixture was incubated for 1 hr at 37°C,

followed by overnight incubation at 4°C. Contaminating RTSV particles were clumped and easily sedimented by low speed centrifugation. After two additional serum treatments, final supernatant was layered on sucrose density-gradient, centrifuged, and fractionated as mentioned above. Virus was concentrated by high speed centrifugation and the pellets were resuspended in 0.01 M EDT A, pH 6.0. Purified RTBV were 30-35 nm in width with varying length (Fig. 4). Ratios of absorbance (260/280) for purified RTSV and RTBV were 1.75 and 1.11, respectively. Buoyant densities of the particles were estimated by equilibrium centrifugation in CsCl. For RTBV, 2.154 g CsCl was dissolved in 4 ml (35%, W/V) of the purified preparation in 0.01 M PB, pH 7.0 and centrifuged at 114,500 x g for 40 hr at 4°C in a Hitachi RPS-40 rotor. A single band was formed at about one-third of the distance below the meniscus. The density of the fraction was determined from the refractive index using the method of Brakke (1967). Buoyant densities of RTSV and RTBV were 1.551 g/cm3 and 1.312 g/cm3 , respectively. The fact that RTBV formed a single band indicated that the composition of RTBV was even, though the virus contained particles of various sizes. Rabbits were immunized separately against RTSV and RTBV by an intramuscular injection with purified preparations emulsified with an equal volume of Freund's complete adjuvant as well as by repeated intravenous injections. In the precipitin ring interface test, antiserum titers were 1/320 and 1/2048 against RTSV and RTBV, respectively. For double gel diffusion test, 0.8% agar in 0.01 M PB, pH 7.6 containing 0.85% NaCl, 0.001 M EDTA and 0.05% sodium azide was used. No heterologous reactions occurred between these antisera and the two kinds of particles. RTSV and rice waika virus were serologically identical both in agar gel-diffusion test and complement fixation test.