Changes in a Nucleic Acid and a Protein Component of Rice Dwarf Virus Particles Associated with an Increase in Symptom Severity

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SUMMARY

Repeated selection of plants with unusually severe symptoms after their inoculation by insect vectors which had been injected with dilute inoculum from crude extracts of a stock culture (O strain) of rice dwarf virus (RDV) resulted in the emergence of a severe isolate (S strain). Of the 12 segments of RDV RNA, the fourth largest RNA of the S strain had an apparent Mr about 20000 larger than that of the corresponding segment of the O strain. The Mr of the protein corresponding to the Mr 43000 protein of the O strain, which is located on the outside of the outer capsid, was 44000 in the S strain. The implication of the differences in the RNA and protein components between the S and O strains is discussed.

Mutants and variants have provided some of the most promising materials for comparative studies of the mechanisms of biological functions of viruses, such as the pathological disorders they induce and their transmissibilities (Nishiguchi et al., 1985; Ohno et al., 1983). Since none of the viruses belonging to the plant reovirus group can be cloned by single local lesion selection, the isolation of mutants of these viruses has generally been considered to be difficult. However, mutants that lost transmissibility by the insect vector were obtained with clover wound tumour virus (Black et al., 1958) and rice dwarf virus (RDV) (Kimura, 1976a; Minobe et al., 1983).

During the course of infectivity assays of purified preparations of RDV from infected rice leaves, a few rice plants showed unusually severe stunting which suggested that a severe strain of RDV had been selected. All the plants had been inoculated by injected insects. This severe (S) strain was selected from the ordinary (O) strain in the present investigation in order to study any deviation in the RNAs and component proteins from those of the O strain.

RDV was maintained and propagated in rice plants (Oryza sativa L., cultivar Norin No. 29) which were inoculated by the viruliferous leafhopper, Nephotettix cincticeps.

Severe symptoms, as judged by exceptionally short plant height, appeared with very low frequency when rice seedlings were inoculated by insects injected with purified preparations (Kimura, 1976b) of the O strain. Leaves showing the severest symptoms were macerated in four times their weight of 0.1 M-sodium phosphate buffer (pH 7.3), and the slurry was clarified in a Hitachi 20PR centrifuge at 1470 g for 15 min. The supernatant fluid was diluted 103-fold and used as the inoculum for the selection test. Second instar nymphs were each injected in the abdomen 2 weeks after hatching with about 0.2 μl of inoculum using a glass capillary with a 50 μm tip diameter. Injected insects were confined individually in glass test tubes each of which contained three healthy rice seedlings and 5 ml tap water. The exposed rice plants were replaced with new seedlings at intervals of a week and the exposed plants were transplanted to a greenhouse where they were observed for expression of symptoms for 60 days. The selection process was repeated 10 more times.

Second instar nymphs were fed on infected plants for acquisition access periods of 10 days, transferred individually to rice seedlings in test tubes and then treated in the same manner as the
injected insects mentioned above. The plants on which they were tested were observed for symptoms for 60 days after the inoculation access period.

The samples used in particle counting were fresh crude extracts of infected leaves diluted fivefold more than the injected inoculum mentioned above. The particle counting method reported by Gamez & Black (1967) and Kimura (1973) was used to compare RDV concentrations in infected leaves with known concentrations of a polystyrene latex suspension (0.126 ± 0.0043 μm in diameter), using a Hitachi model H-500 electron microscope.

The method reported by Omura et al. (1982) was used for the purification of RDV.

RNA was extracted from purified virus particles by adding SDS to 1% and EDTA to 0.1%, and the solution was applied directly to the top of acrylamide gels (Reddy & Black, 1973). Electrophoresis was as described by Omura et al. (1985a). RNAs were designated S1 to S12 in order of descending molecular weight. Dissociation of particles in SDS and electrophoresis of the viral polypeptides were as described by Omura et al. (1985b).

Eighty-five % of the plants after the first selection were severely stunted, whereas about 93 to 96% of the infected plants were severely stunted during the second to sixth passages. All plants inoculated after the 10th passage had severe symptoms. The success of the selection was confirmed by the fact that the stunting of plants infected by the S strain became constant and that ordinary symptoms were no longer observed after the 10th repetition of the process. Although the process is very laborious, this method of selecting the S strain would appear to be generally applicable (as long as the vector is available) for the separation of mutants of plant reoviruses which are not transmissible by sap inoculation.
Fig. 2. Electrophoresis of the RNAs of the S and O strains of RDV in a 10% polyacrylamide gel. (a) Lanes 1 and 4, S strain; lane 2, O strain; lane 3, a mixture of S and O strains. (b) An enlargement of the S4 and S5 bands of lanes 2, 3 and 4 in (a).

Fig. 3. Electrophoresis of the proteins of S (lane 1) and O (lane 2) strains of RDV in a 10% polyacrylamide gel. $M_r \times 10^{-3}$ of the proteins of the O strain are shown to the left.
The average heights of 100 rice plants, inoculated at the one leaf stage and measured 90 days later, were 13.9 ± 0.8 cm for the S strain, 27.6 ± 4.5 cm for the O strain and 63 ± 4.7 cm for the uninoculated controls (Fig. 1). The stunting was attributed to shortening of both the stems and leaves. The panicle never appeared in plants infected before the three leaf stage with the S strain, although small panicles appeared rarely in comparable plants infected with the O strain. Leaves of plants infected with the S strain contained more virus particles than those of plants infected with the O strain. Estimates in three experiments were 4.337 (±0.433) × 10^{12} particles of the S strain and 2.796 (±0.271) × 10^{12} particles of the O strain per gram of leaf tissue. The suppression of the growth of S strain-infected plants (as compared to those infected with the O strain) seems to be due to the more efficient multiplication of the S strain as compared to the O strain in the infected host. Our results seem similar to phenomena reported for other reoviruses where more efficient multiplication of the strain that causes severe symptoms also occurs (Weiner et al., 1977).

The S strain was more readily transmitted by individual insects than the O strain. In four experiments, 43% ± 7.0% of insects fed on the S strain transmitted RDV, compared with only 30% ± 6.1% of those fed on the O strain.

Of the 12 genome segments of viral RNA, only the migration of segment S4 differed between the strains. The S4 segment of the S strain migrated slightly more slowly than that of the O strain (Fig. 2). We estimated the molecular weight difference to be about 20000, that is 1% of the $M_r$ (1.90 × 10^{6}) calculated by Reddy et al. (1974). Electrophoresis of particle proteins in a polyacrylamide gel showed that the molecular weight of the protein corresponding to the $M_r$ 43000 protein of the O strain (Minobe et al., 1984) was 44000 in the S strain (Fig. 3). We are reluctant at present to correlate the differences in nucleic acid and protein shown above, because they are too small to calculate exactly.

The severity of the symptoms induced by a reovirus is controlled by the genome component that codes for the protein located on the outside of the outer capsid (Weiner et al., 1977). Since the 43K protein of the O strain is located on the surface of the outer capsid layer of the RDV particle (Matsuoka et al., 1985), it is probable that such proteins interact more often with host components than do others. Accordingly, it is possible that the 43K component protein has a biological function that affects virus activity in a host cell.

We have added to the strain of RDV with altered transmissibility (Kimura, 1976a) a new strain that may also assist in attempts to understand the pathological functions of the RDV genome.

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REFERENCES
Short communication


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