Relationship between Concentration of Rice Dwarf Virus and The Corresponding OD₂₆₀ Values of The Virus and Its RNA

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木村郁夫: イネ萎縮病ウイルス濃度と OD₂₆₀ 値との関係について

The relative concentration of rice dwarf virus (RDV) may be expressed in terms of the dilution of virus extract from a given weight of infected leaves and viruliferous insect vectors^{1,2)}. However, experimental results were not comparable, one with another, when such materials often contained different concentrations of virus depending upon the season and other factors.

To determine virus concentration of clover wound tumor virus (WTV) in suspensions, the particle count method was applied by using an electron microscope³⁾. This method gave absolute virus concentrations of WTV and more accurate determinations than other methods available earlier. Ahmed and Black correlated virion counts with the OD₂₆₀ values for WTV-RNA⁴⁾. The particle count method was also applied for RDV virion counts⁵⁾. The note deals with quantitative relationship between number of virus particles and OD₂₆₀ values for RDV-RNA and RDV.

Fresh leaves infected with RDV were used as a source of RDV for purification⁶⁾. In all of experiments, the purified preparations of RDV were used. An improved method was applied for purifying RDV from infected leaves.

The purified RDV samples were centrifugated at 30,000 rpm (RPS-40 rotor) for 90 min. by using a Hitachi model 55-P ultracentrifuge. Each pellet and the part of the tube supporting it was cut out, and was treated with 2 ml of 1N HCl at 30°C for 24 hr in a 10 ml centrifuge tube which was sealed by parafilm. Then the denatured protein of the virus was removed after sedimentation by low speed centrifugation. The supernatant was taken as a sample of RDV-RNA and its OD₂₆₀ value was measured by a Hitachi model 101 spectrophotometer.

The counting method of RDV virions was described previously^{5,7,8)}. The same samples were used for OD₂₆₀ measurements of RDV-RNA and for virion counts.

The OD₂₆₀ values of virus-RNA were compared with RDV concentrations of the corresponding samples of purified RDV. The virus concentration, based on particle counts, was expressed as number of virus particles per ml of the samples. RDV-RNA was obtained by the method described above. The relationship between OD₂₆₀ values of virus-RNA (Y-axis) and concentration of virus particles (X-axis) was linear as shown in Fig. 1. This relationship was expressed as regression line $Y=0.913X+0.024$ (the standard deviation from regression: $S_{yx}=0.04$, the standard error of slope: $S_b=0.0029$). Therefore, the OD₂₆₀ values of RDV-RNA are readily convertible to absolute virus concentrations (Fig. 1).

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Fig. 1. The relationship between OD₂₆₀ values of RDV-RNA and corresponding counts of virions by the electron microscope.

Fig. 2. The relationship between OD₂₆₀ values of RDV and corresponding counts of virions by the electron microscope.

After the OD_{260} values of RDV samples were corrected for light scattering¹⁰⁾, they were plotted in Fig. 2. The regression line for RDV samples was expressed as $Y=0.951X+0.002$ ($S_{yx}=0.04$, $S_b=0.057$).

The two lines relating OD_{260} values for RDV-RNA and for RDV to RDV virion counts were very close. The close agreement between the values of OD_{260} for RDV-RNA samples and the corresponding concentrations of virus particles determined by the particle count method, suggests that the virus particles have a constant RNA content in the purified preparations.

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