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Reovirus Isolation and RNA Extraction

Ichiro Uyeda, Bong-Choon Lee, Yuko Ando, Haruhisa Suga, Yun-Kun He, and Masamichi Isogai

1. Introduction

Plant reoviruses are classified in three genera in the family *Reoviridae*: *Phytoreovirus*, *Fijivirus*, and *Oryzavirus.* Fifteen viruses, including possible members, are described ***(1)***. With two exceptions, all of them infect plants in *gramineae*. They possess 10–12 segmented double-stranded RNAs (dsRNAs) as a genome and are transmitted propagatively by leafhoppers or planthoppers. Rice dwarf *Phytoreovirus* (RDV) is the only plant reovirus whose complete nucleotide sequence is known ***(2)***. Rice dwarf virus has 12 genomic segments separated by PAGE. They are numbered from S1 to S12, from the slowest migrating segment, and all the structural and nonstructural proteins of RDV have been assigned ***(3)*** (**Fig. 1**). Genome characterization of other plant reoviruses is reviewed by Uyeda et al. ***(4)***.

Although methods described in this chapter are mostly for RDV, those for virus isolation and genome extraction should be applicable to other viruses, since most plant reoviruses have gramineae hosts, and all of them have dsRNAs as a genome. However, purification of the virus particles must be carefully chosen and there seems to be no universal or general methods. We describe those for RDV, rice black-streaked dwarf virus (RBSDV), and rice ragged-stunt virus (RRSV). The most difficult part of the plant reovirus study is to obtain good plant material to start with. The best plant material is fresh and young infected plants grown under an appropriate greenhouse or growth chamber condition. In order to do so, one has to maintain the virus culture by frequent transfers through the vector insect, because they often lose vector transmissibility after prolonged culturing in a plant host. Field-grown plant material contains a genomically heterogeneous population of viruses ***(5)*** and a

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