3.2.2. Rice Black-Streaked Dwarf and Rice Ragged Stunt Viruses

1. Fresh infected leaf and sheath tissue (100 g) (rice for RRSV and corn for RBSDV) are cut into about 1-cm pieces and ground with a meat grinder with 35 mL GMT buffer.
2. The homogenate is further extracted with a ELISA juice press.
3. Squeeze through a double layer of medical gauze.
4. Add 30% (v/v) carbon tetrachloride and 3% (v/v) Triton X-100 while stirring with a magnetic stirrer at 4°C for 1 h.
5. Centrifuge at 1500*g* for 20 min in an angle rotor at 4°C.
6. The aqueous phase is centrifuged at 80,000*g* for 1.5 h at 4°C through one-third vol of a tube capacity of 40% sucrose in GMT buffer in a RPS 27 rotor.
7. Suspend the pellet in 2 mL of GMT buffer.
8. Centrifuge in a microcentrifuge tube at 3500*g* for 5 min at 4°C.
9. The supernatant is layered onto a 10–40% sucrose density gradient tube and centrifuged at 80,000*g* for 1.5 h at 4°C in a RPS 27 rotor.
10. Recover the virus zone at the middle of the tube, using an ISCO density gradient fractionator equipped with an UV monitor.

3.3. Direct Extraction of Viral RNAs from Plants

3.3.1. Direct Extraction of Viral Genomic dsRNAs from Plants

1. Extract the virus with ELISA juice press from 0.05–0.50 g of infected rice leaves, while adding 600 μL of STE buffer into a microcentrifuge tube.
2. Add an equal volume of phenol.
3. Vortex for 3 min.
4. Centrifuge at 10,000*g* for 1 min at room temperature.
5. Transfer the aqueous phase (normally, the aqueous forms the upper phase) to a fresh microcentrifuge tube.
6. Add an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) into the aqueous phase and repeat **steps 3–5**.
7. Add 80 mg of CC41 cellulose (Whatman) powder and 0.2 vol of ethanol into the microcentrifuge tube containing the aqueous phase. Agitate the mixture for 30 min at room temperature.
8. Collect the cellulose by centrifugation at 10,000*g* for 3 min.
9. Add 1.2 mL STE buffer, pH 6.8, containing 15% ethanol, into the pellet of cellulose after removing the supernatant. Vortex for 1 min.
10. Repeat the washing of **steps 8** and **9** once more.
11. Elute dsRNA by adding 150 μL of sterile H_2O and vortex for 1 min.
12. After centrifugation at 10,000*g* for 3 min, transfer aqueous phase to a fresh microcentrifuge tube. Repeat the elusion of **steps 11** and **12** once more. Combine the second aqueous phase with the first.
13. Remove traces of cellulose by centrifuging briefly the combined aqueous phase and transfer the supernatant into a fresh microcentrifuge tube.