3.3. Luteovirus RNA Extraction

1. Dilute virus particle suspension with 10X extraction buffer to make the final concentration 1X (*see* **Note 16**).
2. Heat at 60°C for 15 min.
3. Add an equal volume of phenol containing *m*-cresol and 8-OH and vortex thoroughly.
4. Centrifuge at room temperature in a microcentrifuge at full speed (12,000*g*) for 10 min.
5. Remove the upper aqueous phase and retain it on ice. Avoid material at the interface between the upper and lower phases.
6. To the original mix, add an equal volume of 1X extraction buffer, vortex and centrifuge in a microcentrifuge at full speed (12,000*g*) for 10 min.
7. Remove the aqueous phase and add it to that already on ice.
8. Repeat **steps 3–5**, using the combined aqueous phases.
9. To the final aqueous phase, add 0.1 vol of 3*M* sodium acetate, pH 6.5, and 2.5 vol of 100% ethanol. Store at –20°C overnight.
10. Allow the tubes to warm at room temperature for a few minutes, then sediment the RNA by centrifugation in a microcentrifuge at 12,000*g* for 10 min.
11. Discard the supernatant and add 1 mL of 70% ethanol (v/v) to the pellet. This should remove all traces of phenol and SDS. Recentrifuge in a microcentrifuge at 12,000*g* for 10 min.
12. Drain the tube and dry the pellet using a vacuum desiccator.
13. Resuspend the pellet in a convenient volume of sterile distilled water. Repeat **step 9**.
14. To ensure that the RNA is free from contaminants, **steps 10–13** can be repeated several times.
15. Determine the RNA concentration by UV spectroscopy. At 260 nm, an optical density of 1 indicates an RNA concentration of 40 mg/mL ***(6)***. The 260/280 nm absorption ratio should be greater than 1.8, indicating sufficient purity to assess the concentration.

4. Notes

1. The two most important considerations in purifying BYDV are addressed at this stage: the choice of starting material, and the method of tissue disruption ***(3,5,8)***. The concentration of virus in plant parts can vary radically, depending on the virus strain involved, host, and growing conditions. For example, though leaves of various oat cultivars (e.g., Coast Black and Clintland 64) have proved to be a convenient source for many isolates, roots of barley (cv. Moore) were far more preferable with an SGV isolate ***(4)***. Culture temperatures of about 20°C generally give superior yields. Purification from roots may yield more virus than from leaves, but the latter are easier to produce in quantity. Virus content usually peaks in recently infected material (10–21 d postinoculation). The use of tissue that has been stored frozen is preferred, but fresh or dried tissue may be used ***(9)***. The virus is phloem-limited, so recovery depends on thoroughly disrupting the tissue to free the virus. Alternatives to ensure this, especially with older, fibrous tissue,