### 3.4. Yields of Virus and Viral RNA

ACLSV is known to replicate at a relatively low level in the infected plants. Yields of virus typically range from 100 μg to 400 μg/100 g tissue. This corresponds to about 5–20 μg of viral RNA/100 g tissue. This yield can vary, depending on the experiment, but rarely exceeds 20 μg. In the case of the Japanese apple strain of ACLSV, the reported yield was higher: 50 μg of viral RNA/100 g tissue ***(12)***. Similar virus yields are obtained for PVT: 300 μg to 1 mg of virus/100 g tissue, corresponding to 15 μg to 50 μg of RNA ***(3)***.

The purification yield is reportedly higher for HLV: Yields of virus average 5 mg/100 g tissue, which is about 250 μg of viral RNA ***(4)***.

### 3.5. Purification of Other Trichoviruses

GVB is purified from leaves of *N. occidentalis*. The method also includes clarification by bentonite, followed by differential centrifugation and sucrose density gradient centrifugation ***(6)***. GVA is purified from leaves of *N. clevelandii*. The method includes either clarification by bentonite or chloroform extraction, before PEG precipitation ***(5)***.

A protocol very similar to the one given for ACLSV is used for HLV ***(4)***.

For PVT, the method does not include the bentonite clarification step, but uses carbon tetrachloride clarification instead ***(13)***.

## 4. Notes

1. Inoculation of *C. quinoa* (young plants at the four-node stage) (*see* **Subheading 3.2., step 1**) is done by dusting leaves with Carborundum (600-mesh), then rubbing pairs of leaves at the third node with infectious *C. quinoa* sap. This sap is prepared by grinding 10 g of infected leaves of *C. quinoa* in 30 mL of buffer B, and by adding activated coal (90 mg/mL) in this homogenate just before the inoculation. The infectious *C. quinoa* sap must be kept at 4°C. It must be noted that ACLSV is very susceptible to high temperatures; therefore, inoculation should not be done during the summer period. Grinding the leaves in the blender should be done carefuly and should not last too long, because excessive blending will result in virus degradation. The homogenate obtained should be thick and not too liquid. The choice of the Fisher Scientific brand to prepare the bentonite solution seems to make a difference in the purification issue.
2. The choice of the Merck brand for PEG also seems to make a difference for the effective viral particles precipitation (*see* **Subheading 3.2., step 3**).
3. The virus pellet after PEG precipitation (*see* **Subheading 3.2., step 3**) may be contaminated by plant components and appear very green. Because excess bentonite can lead to the loss of virus particles, it is better not to exceed four steps of clarification. To remove any noticeable green color from the pellet, additional differential centrifugation steps can be done before loading onto the gradients, to avoid interference in the UV absorption analysis of the gradients. The virus pellet