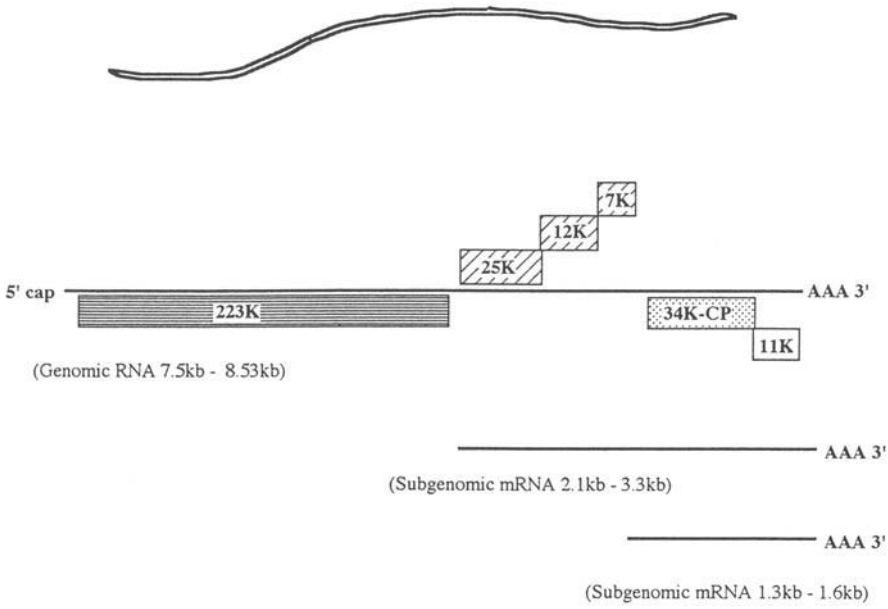

Fig. 1. General gene organization of the carlavirus genome. Particle morphology, sizes of typical RNAs, and encoded ORFs are indicated.

7. Vortexer.
8. Microcentrifuge.

3. Methods

3.1. Virus Isolation

The method described below has been used by the author to purify a wide range of carlaviruses and has been found to generate high yields of pure virus, when compared with a number of purification procedures for carlaviruses. It should be noted, however, that this purification procedure uses the highly volatile diethyl ether as the clarification agent. If diethyl ether cannot be used for safety reasons, then it is recommended that solvent, such as chloroform, butanol, or carbon tetrachloride should be tried.

1. Homogenize infected leaves (*see* **Note 2**) in 0.5*M* borate buffer, pH 7.8, containing EDTA (0.005*M*) and thioglycolic acid (0.1% v/v) at the ratio of 1:2 (w/v).
2. Transfer to a beaker and stir gently with an equal volume of diethyl ether (*see* **Note 3**).
3. Separate phases by centrifugation at 5000*g* for 20 min (at 4°C if possible) and transfer the aqueous phase to a fresh beaker.