2. Purification of Sonchus Yellow Net Virus

2.1. Introduction

The procedure used for SYNV has been adapted for use with several other rhabdoviruses. This method was initially developed by Jackson and Christie *(8)* in conjunction with an infectivity assay using the local lesion host *Chenopodium quinoa* (*see* **Subheading 2.4., Note 1**). The local lesions formed after inoculation permitted us to evaluate the most appropriate host, the optimum greenhouse conditions for virus replication, and the relative levels of infectivity at different times after inoculation of plant tissue, as a prelude to development of the purification protocol. These bioassays revealed that *N. edwardsonii* is a suitable host for virus maintenance and recovery, and subsequent experiments have shown that equal or better yields can be recovered from *N. benthamiana*. Local lesion assays were also valuable for assessing virus recovery after each purification step. As the procedure developed, particular attention was directed to reproducible removal of host components.

2.2. Materials

1. Inoculation buffer: Shortly before use, prepare 40 m*M* sodium sulfite (Na_2SO_3), containing Celite as a mild abrasive by adding 125 mg of Na_2SO_3 and 500 mg (2%) of Celite Analytical Filter Aid (Johns-Mansville, Denver, CO) to 25 mL of H_2O. Store on ice and mix well immediately before use.
2. Extraction buffer: Add 60 g of Tris base, 1.1 g of Mg acetate, and 120 mg of $MnCl_2$ to 450 mL of H_2O. Adjust the pH to 8.4 with HCl. Store at 4°C until just before use, then add 2.5 g of Na_2SO_3 and bring the volume to 500 mL. The final extraction buffer is 100 m*M* Tris-HCl, pH 8.4, 10 m*M* Mg acetate, 1 m*M* $MnCl_2$, and 40 m*M* Na_2SO_3.
3. Maintenance buffer: Maintenance buffer is identical to extraction buffer, except that the pH is adjusted to 7.5.

2.3. SYNV Purification Method

1. Transfer SYNV at 14-d intervals by inoculating *N. benthamiana* or *N. edwardsonii* plants grown in 15-cm clay pots containing autoclaved loam soil. Prepare inoculum by macerating 2 g of infected leaves in a chilled mortar containing 5 mL of freshly prepared cold (~4°C) inoculation buffer (*see* **Subheading 2.4., Note 2**). Gently rub the leaves with cheesecloth dipped into the leaf extract. Under optimum growth conditions in the greenhouse (~25°C) and normal summer sunlight, the light yellow netting symptoms characteristic of SYNV begin to appear on the leaves by 8–10 d after inoculation.
2. Harvest systemically infected leaves, with the midribs and petioles, including the youngest rosette leaves, where the most intense symptoms are normally found. The inoculated leaves generally contain much lower titers and are not harvested. Either extract immediately or store for several days at 4°C.