Purification of nucleocapsid of tospoviruses from Chenopodium quinoa leaves

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Abstract

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Protocol

Step 1.
Leaves of tospovirus-infected *Chenopodium quinoa* (3-5 days post-inoculation with tospoviruses rely on daily observation) were homogenized with a blender (full speed) for 1 min with the TB buffer [0.01 M Tris-HCl (pH 8.0) containing 0.01 M sodium sulfite and 0.1% cysteine] (300 ml/100 g tissue).

Step 2.
After filtering through four layers of cheescloth, the extract was centrifuged at 15300 *g* (10000 *rpm*) in a Beckman JA-14 rotor for 10 min.

Step 3.
The supernatant was stirred with 1-2% Triton X-100 (final concentration) at 4℃ for 30 min.

Step 4.
Centrifugation at 79700 *g* (25000 *rpm*) in a Beckman 35Ti rotor for 2.5 hr through a 20% (w/w) sucrose cushion (sucrose within TB buffer, 15 ml/tube).

Step 5.
The pellets were then resuspended in the TBG buffer (TB buffer containing 0.01 M glycine) (8 ml/100 g tissue) further purified by isopycnic centrifugation through 35% (w/w) cesium sulfate (cesium sulfate within TBG buffer, 8-9 ml/each tube, and load 2 ml/each tube of samples) at 83500 *g* (30000 *rpm*) in a Beckman SW41 rotor for 18 hr.

Step 6.
The opalescent zones containing virus nucleocapsids were collected in 60 Ti centrifuge tube, fill full with TBG buffer, further centrifugation at 163600 *g* (45000 *rpm*) in a Beckman 70Ti rotor for 1 hr.

Step 7.
The pellets were resuspended in TBG buffer (3 ml/100 g tissue) and regarded as purified nucleocapsids.

Step 8.
Store at -20℃ for further use.