Plant Virus Identification using Transmission Electron Microscopy (TEM)

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Overview

Fig. A. Steps in the process of sample preparation for viral detection by TEM. Icons by Pixabay, Delapouite, and Pypaertv. Photos by Tatiana Lenskaia.

I. Grinding symptomatic plant tissues in buffer

II. Low-speed centrifugation (keep the supernatant)

III. High-speed centrifugation (keep the pellet)

IV. Preparation of grids for TEM

V. TEM screening
I. Grinding plant tissues in buffer

- Grind plant tissues thoroughly in liquid nitrogen: https://www.youtube.com/watch?v=AI1SsJ4JwXc
- Add buffer and continue grinding: https://www.youtube.com/watch?v=OeIRziBNn6c
- Filter through a cloth to remove plant remnants: https://www.youtube.com/watch?v=X4z3hC06wAQ
II. Low-speed centrifugation

This type of centrifugation is used for a crude separation of particles. This step allows us to remove most of the remaining plant tissues and have virus particles released into the liquid buffer.

After the centrifugation,

the **supernatant is kept**
(liquid fraction in the tube),

and the **pellet is discarded**
(solid fraction at the bottom of the tube).
This type of centrifugation is used for a fine separation of particles. This step allows us to remove most of the remaining buffer and have virus particles concentrated as a pellet at the bottom of the tube.

After the centrifugation,

the **pellet is kept**
(solid fraction at the bottom of the tube),

the **supernatant is discarded**
(liquid fraction in the tube).
IV. Grid preparation

**Fig.B.** Example of the TEM grids. Photo by Tatiana Lenskaia.

For detail:
https://www.sciencelearn.org.nz/resources/500-preparing-samples-for-the-electron-microscope
V. TEM screening

**Fig.C.** An Example of a transmission electron microscope. Photo by Tatiana Lenskaia

**Fig.D.** Example of an image of viral particles taken during TEM screening. Microscopist: Tatiana Lenskaia.