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A comparison of methods to preserve RNA viruses

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The study of virus variability and evolution requires frequently the preservation of infected samples in order to study their serological, molecular or pathogenicity properties several months or years after their isolation. Also, reference virus isolates should be kept on the long term for routine experiments. However, isolates often lose their infectivity after a few years of storage and their particles and/or nucleic acids are degraded.

In this work, we compared different methods to preserve the infectivity of three single-stranded RNA viruses: *Cucumber mosaic virus* (CMV, family Bromoviridae), *Tomato spotted wilt virus* (TSWV, family Bunyaviridae) and *Potato virus Y* (PVY, family Potyviridae). We compared different host plants: (tomato, pepper, *Nicotiana benthamiana* and, for CMV, melon) and different preservation methods (calcium chloride dehydration (Bos, 1977), frozen leaf extracts, freeze-drying and liquid nitrogen).

We tested the infectivity during a fourteen month period. Every two or three months, each virus from each preservation method was inoculated mechanically on *Chenopodium amaranticolor*. This plant produces local lesions in leaves inoculated by the three viruses. Inoculated leaves were observed under UV light to visualize fluorescence spots corresponding to infection foci and pictures were analyzed with the ImageJ software to quantify the density of local lesions, an indicator of virus infectivity.

Pepper was a bad host plant for preservation of the three studied viruses, whatever the method, whereas *N. benthamiana* was the most efficient host. In *N. benthamiana*, the best preservation method depended also on the virus. For CMV, calcium chloride dehydration, freeze-drying and frozen leaf extracts were equivalent methods and produced good results compared to liquid nitrogen or fresh leaf extracts. For TSWV, calcium chloride dehydration and liquid nitrogen performed better than freeze-drying, and frozen leaf extracts were poorly infectious. For PVY, calcium chloride dehydration and freeze-drying performed better than frozen leaf extracts.

This study highlights more favorable hosts and preferential preservation methods for each virus.

Référence :

[1] Bos, L. (1977). Persistence of infectivity of 3 viruses in plant material dried over CaCl₂ and stored under different conditions. *Neth. J. Plant Pathol.* 83:217-220.



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