

Mycoviral double-stranded RNA extraction from plant leaf with Minilys

Tokyo University of Agricultural and Technology, Institute of Agriculture, division of Bio-regulation and Bio-interaction, Japan

CONTEXT

This laboratory develops bio-molecular solutions for plant biomass production. Extraction of mycoviral double-stranded RNA (dsRNA) from plant leaves for the investigation of attenuation of *Alternaria* and *Magnaporthe* (Ascomycete fungi) infection is a part of the laboratory's daily work.

MATERIAL

- Minilys homogenizer.
- Precellys kit: 03961-1-002 (2.8 mm ceramic beads).
- Samples: 20 mg of green pepper (*Capsicum annum*) leaf and 20 mg of Malabar spinach (*Basella alba*) leaf.
- Buffer: 500µL of STE buffer 2X.

PROTOCOL

- Minilys: 5000 rpm, 60 sec x 1, 2 or 3 cycles with no time break on ice between each cycles.
- Addition of SDS ; phenol-chloroform extraction.
- Analysis: Agarose gel electrophoresis stained with EtBr.

RESULTS

A protocol of 5000 rpm x 60 sec with CK28 gave the best results for both green pepper and Malabar spinach leaves homogenization. Extension of the time homogenization attenuated the dsRNA band of mycoviral especially in the Malabar spinach sample.

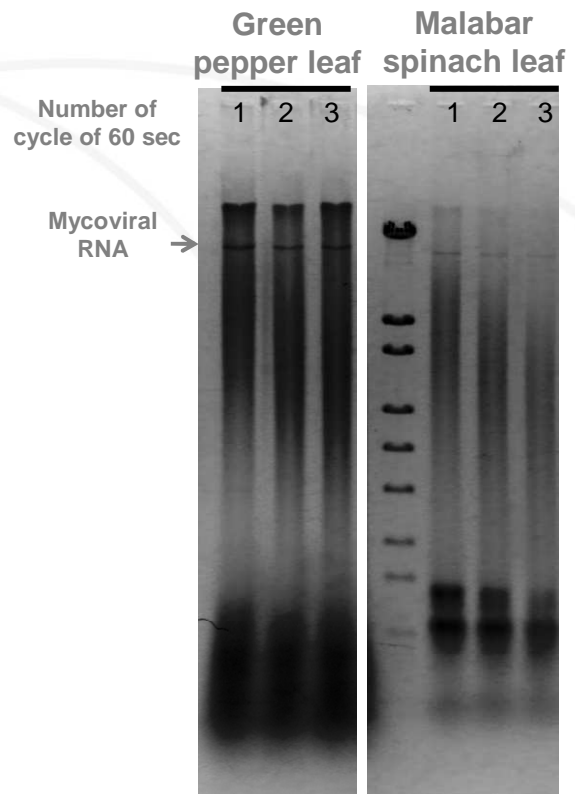
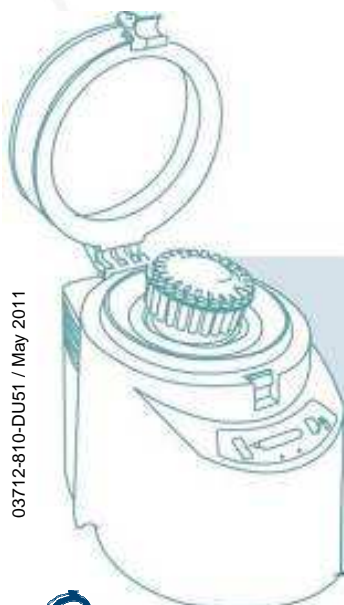


Figure 1: Agarose gel electrophoresis of 5 mg extracted plant



東京農工大学

CONCLUSION

A short homogenization allowed to have good dsRNA yield where as a longer one lead to a lower RNA yield.

Minilys is a suitable homogenizer for extracting mycoviral dsRNA from plants. Minilys provides a way of standardizing sample preparation for laboratories with a low or medium throughput.

