

Amisa Laprom 2012: Identification and Diagnosis of Canna Viruses and Production of Virus-Free Stocks. Master of Science (Agricultural Research and Development), Major Field: Agricultural Research and Development, Faculty of Agriculture at Kamphaeng Saen. Thesis Advisor: Assistant Professor Pissawan Chiemsombat, Dr. Agr. 125 pages.

A survey on virus diseases of canna plants was conducted and the causal viruses were identified. Virus detection by PCR techniques, and protocol to produce virus free canna stocks were evaluated. Total of 88 canna samples expressing virus-like symptoms, included canna plants collected from public areas at Kasetsart University Bangkhaen and Kamphaeng Saen campuses, commercial canna varieties from Chatuchak market, canna farm, and private company, were used in this study. Virus diagnosis by ELISA, PCR, and RT-PCR indicated that 59.09 and 13.63 % of the samples were infected by *Canna yellow mottle virus* (CaYMV) and *Canna yellow streak virus* (CaYSV), respectively, while 5.68% of canna samples were doubly infected. By using leaf dip preparation and electron microscopy, flexuous rod-shaped virus particles size of 790 nm was observed. Amplification of viral genes by PCR yielded DNA product size of 565 bp resembled partial sequence of CaYMV-ORFIII, while RT-PCR yielded 864 bp DNA product of complete CaYSV coat protein gene. The cloned CaYSV-CP gene was subsequently expressed *in vitro* by using pQE expression system, yielding 34 kDa recombinant 6xHis-CaYSV-CP polypeptide. The expressed protein was injected into a rabbit and polyclonal antibody (PAb-CaYSV) was obtained. The PAb-CaYSV-CP reacted specifically to CaYSV in sap of infected canna leaf by Indirect-ELISA and NCM ELISA. Evaluation of the developed PCR and RT-PCR protocols indicated their efficiency in CaYSV and CaYMV diagnosis from infected leaf, flower, shoot, as well as canna plantlets derived from meristem culture. The production of virus-free canna plant was achieved by culturing 0.5-1 mm. meristem tip of CaYMV and CaYSV-infected plants on 0.5xMS liquid medium supplemented with 0.2 mg/L BA. Regenerated plantlets were 16.67 and 73.33% free from CaYMV and CaYSV, respectively.

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