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Development of an ELISA kit through chicken egg antibody production for Banana Streak Virus (BSV) of AAB-Mys banana

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Banana streak virus (BSV) that causes banana streak disease is a dsDNA virus (genus Badnavirus). It has been shown that complete and incomplete BSV sequences naturally inherited in the banana genome. Disease symptoms include yellow and necrotic leaf streaks, and in some situations pseudostem necrosis. Appearance of free virions is known to be dependent on genetic and environmental factors. BSV is mainly spread by vegetatively propagated and micropropagated infected planting material. Disease management requires accurate detection of the virus. But, BSV has high genomic and serological variability, which makes reliable detection difficult. BSV specific broad-spectrum polyclonal antibodies for BSV isolates from AAB-Mys banana were obtained by immunizing a hen once with purified [several cycles of Cs_2SO_4 density gradient centrifugation, followed by Immunosorbent Electron Microscopy (ISEM)] BSV-Mys virus isolates. IgY antibodies were extracted from egg yolk using Polyethylene glycol (PEG) and purified by cryo-ethanol treatment (a modified procedure). Egg yolk extracts (before and every 5 days after Immunization) consisting 15-27 mg/mL of total IgY were tested for BSV specific IgY using a specifically developed DAS-ELISA kit for BSV, and by ISEM. Egg yolk extracts containing highest concentration of BSV specific IgY were pooled according to DAS-ELISA results (Fig 1). Pooled IgY extracts were further purified using DEAE-Cellulose ion exchange chromatography and the purity assessed by SDS-PAGE (non-reducing conditions). Part of the purified IgY was conjugated to Alkaline phosphatase enzyme to produce enzyme linked conjugate, while the rest was used as coating antibody in DAS-ELISA. Lower dilutions of ELISA reagents (coating antibody 1:100, conjugated antibody 1:50) compared to the commercial DAS-ELISA kit for BSV (Agdia®) were obtained. However, a large quantity of purified antibody was produced from 5 eggs (120 mL) at low cost avoiding bleeding method. The technique and the kit developed have great potential for commercial scale application even for other viruses as well as for BSV-Mys.

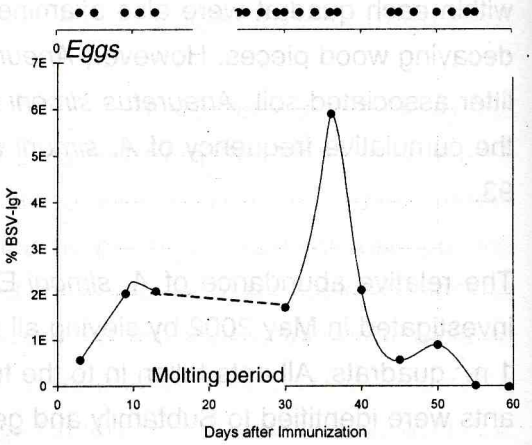

Fig 1. Variation of BSV-IgY% with time

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