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Optimization of an ELISA kit for testing Banana Bract Mosaic Virus (BBrMV) in Sri Lanka using purchased antiserum, and TEM observations of the virus for antigen

Work on development of a Double Antibody Sandwich ELISA (DAS ELISA) kit for local Banana Bract Mosaic Virus (BBrMV) using purchased antiserum, to cut-down the high cost on imported commercial ELISA kits is reported. At the same time, isolation and purification of BBrMV was done to produce antiserum locally at a later stage. Initial step of virus extraction from local 'Embul' (AAB Mysore) banana leaves was done with two different extraction buffers *ie.* standard procedure with Borate buffer for one set and Phosphate buffer as the modification for another set. Purification of the two different extracts was done by equilibrium centrifugation with CsCl, and virus containing fractions were determined at absorbance ratio A260/280 nm, which was further confirmed by commercial DAS ELISA (Agdia™) kit. Purity of the extract was determined by TEM to clarify the absence of plant contaminants before injecting it to selected animals for antiserum production. Phosphate extraction buffer resulted in relatively higher concentration of the virus than the use of Borate buffer, as detected by TEM (Fig 1). However, Virus concentration of purified extracts was low in this study compared to those of reported work.

To develop an ELISA kit, Immunoglobulin G (IgG) was purified from the BBrMV antiserum purchased (from QDPI laboratory, Australia). Part of this IgG was conjugated with alkaline phosphates enzyme (AP), according to a standard protocol with some modifications. DAS ELISA procedure was standardized to use purified IgG as coating antibody (which was reported previously) and the IgG-AP as the conjugated antibody. Performance of this kit was compared with that of Agdia™ commercial DAS ELISA kit. The optimum dilution factor of IgG-AP and IgG for new kit was x1000 as detected by the ELISA plate reader. The color intensity of the reaction was visible even with increasing dilutions of the samples. This showed that the modified ELISA kit was more efficient (and also economical) than the commercial kit for which the required dilution factor for above reagents was x200. The developed DAS ELISA kit is being used for routine indexing of tissue cultured banana plants. This kit is also suitable for indexing even other strains of banana plant material because there are no significant variants reported so far for BBrMV which is a potyvirus. The potential to produce an efficient complete local DAS ELISA kit with local antiserum was confirmed by this study.