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Production of polyclonal antiserum and development of Enzyme-Linked Immunosorbent Assay for detection of Banana Bract Mosaic Virus

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Banana Bract Mosaic Virus (BBrMV) is widespread disease in banana (*Musa* spp.). Early identification of the disease is important in rapid propagation programmes. The use of commercially available Enzyme-Linked Immunosorbent Assay (ELISA) test kits for indexing the virus is not economical in Sri Lanka. Hence production of polyclonal antiserum and development of indirect ELISA detection technique was the focus of this study. Virus was partially purified by Hammond and Lawson method (1988) with minor modifications. Four purified virus samples were injected to a rabbit at weekly intervals for antibody production. First, containing 1 mg of virus sample in 1 ml of BK buffer (0.1 M Boric acid and 0.1 M potassium chloride pH 8.0) mixed with equal volume of 0.85% sodium chloride solution was injected to the marginal ear vein of the rabbit. Remaining three injections containing 1.5 mg, 2.0 mg, and 2.5 mg of purified virus respectively were given intramuscularly with 1 ml of incomplete Freund's adjuvant. Bleeding of rabbit was done at one week after 2nd injection for titer check and whole blood was collected 10 days after 4th injection for antiserum preparation. Blood was incubated at room temperature for 1 hour and then at 4 ºC overnight. Serum was separated and it was centrifuged at 5000 rpm for 10 minutes to remove blood cells. An equal volume of glycerol was added to the serum and stored under -20 ºC after adding 0.025% sodium azide to the final volume as a preservative. Indirect ELISAs were performed to optimize; sample extraction buffer, antiserum dilution, incubation period after adding para-Nitro Phenyl Phosphate (pNPP) substrate, to detect virus titter of different plant parts and to identify the best plant parts that can be used in ELISA with produced antiserum. Buffer containing 0.1 M PBST buffer pH 7.4 + 0.13% Sodium sulfite + 2% Poly Vinyl Pyrrolidone (PVP) + 0.2% Ovalbumin was identified as the best sample extraction buffer. Best antiserum dilution was at 1:500 in PBS-TPO buffer keeping the Protein A conjugate dilution at 1:2000 in PBS-TPO buffer. One and a half hours after adding pNPP substrate was selected as the best incubation time at 37 ºC to get absorbance values. Comparatively higher virus titers of BBrMV were present in root tips and bract parts. Most suitable plant parts to check the presence of virus by ELISA were bract parts and leaf lamina of flag leaf or 1st leaf.

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