Methods for detection of Virus

1. ELISA (Enzyme-linked Immunosorbent Assay)

ELISA is a diagnostic method that uses proteins called antibodies to detect plant pathogens. This assay is based on the ability of an antibody to recognize and bind to a specific antigen, a substance associated with a plant pathogen. The antibodies used in the diagnostics are highly purified proteins produced by injecting a warm-blooded animal (like a rabbit) with an antigen associated with one particular plant disease. The animal reacts to the antigen and produces antibodies. The antibodies produced recognize and react only with the proteins associated with the causal agent of that plant disease. Colour changes on the unit's surface indicate a positive (disease present) reaction.

General procedure using Antigen Coated Plate (ACP)-ELISA

1. Harvest fresh samples of leaf of controls and samples. Weigh 0.2 g of each. Grind each sample in 2 ml of coating buffer (0.05 M carbonate buffer)+ 2% w/v PVP and 0.2 % w/v Na2SO3. Transfer the sap to a labelled eppendorf tube. Spin 5 min at 10,000 rpm.
2. Label the microtitre plate and load the samples, 100 µl per well. Cover the plate with parafilm lightly and incubate at 4oC overnight.
3. Weigh 4 g of the healthy sample and grind in 10 ml Phosphate buffered saline solution with Tween (PBST used as blocking solution). Filter through cotton wool and make the volume of the filtrate up to 80 ml. Use this to make dilutions of the antiserum. Allow to absorb at 4°C overnight.
4. Make a 0.1% solution of BSA in PBST.
5. Rinse your plate in a gentle stream of tap water and wash three times with PBST.
6. Add the blocking solution to all wells of your plate, 150 µl per well. Cover the plate with parafilm and incubate for 30 min at room temperature.
7. Empty the blocking solution from your plate completely. Without washing, add the diluted antiserum, use 100 µl per well. Cover your plate with parafilm and incubate 3-4 hrs at RT.
8. Make the dilution of the alkaline phosphatase enzyme conjugate in PBST (1/1000)(made fresh).
9. Wash your plate three times with PBST. Then add the diluted conjugate according to the pattern on the loading diagram, starting with the most diluted one. Use 100 µl per well. Cover the plate with parafilm and incubate at 4°C overnight.
10. Wash your plate with PBST three times.
11. Dissolve the substrate tablet (p-NPP, Sigma) in substrate buffer (1mg/ml) (diethanolamine 10%, pH 9.8, stored in fridge).
12. Add the dissolved substrate to all wells, 150 µl per well. Incubate at room temperature for 30 min. Evaluate and score colour intensity of each well with the ELISA reader.

2. Tissue-blot immunoassay (TBIA) on nitrocellulose membranes

General procedure

1. Collect tissues (leaves, petioles, stems, etc...).
2. For thin tissues such as leaves, roll them into a tight core. For batch samples bind together with Parafilm.
3. Prepare nitrocellulose membrane (NCM), cut top left corner, mark grid with Bic biro.
4. Hold tissues in one hand and cut with a new razor blade in a steady motion with the other hand to obtain a single plane cut surface.
5. Press, with a firm but gentle force, the newly cut surface onto NCM.
6. Wash NCM 3 times with PBST at 5 min interval.
7. Dilute antiserum in healthy sap in PBS (dilution 1/500-1/2000), allow absorbing at room temperature for 2 hours or 4^{o} C overnight.
8. Block NCM in 2 µg/ml PVA (Polyvinyl Alcohol) in PBST and incubate one min at room temperature.
9. Wash as in step 6.
10. Add diluted antiserum and incubate for one hour at room temperature.
11. Wash as in step 6.
12. Add anti-rabbit conjugate (dilution 1/1000 - 1/5000 in conjugate buffer), and incubate for one hour at room temperature.
13. Wash as in step 6.
14. Add substrate solution (NBT/BCIP).
15. To stop reaction wash with deionized H2O.