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Antibody based techniques in detection and diagnosis of plant pathogens

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Abstract

Antibodies are molecules produced by mammalian immune systems that are known to help identify invading organisms or substance. If antibodies will be generated that recognize specific antigens related to a given plant pathogen, they can be used in the form of a diagnostic tool .The serological methods of diagnosis, detection and identification of pathogens in plants play an important role for the diagnosis of plant pathogens. A wide range of pathogens can be detected using polyclonal and monoclonal antisera and techniques like ELISA, DBIA, western blots, etc.

Keywords:-ELISA, Immunostrips, western blots, gel electrophoresis.

Introduction

Plant diseases are one of the main constraints in production of agricultural crops. All types of plant, either wild or cultivated are attacked by a wide variety of pathogens which can belong to one or more than one of the given groups, i.e, Fungi, Bacteria, Viruses, Viroids, Plant parasitic nematodes and Fastidious prokaryotes. Proper and timely detection of the causal agents is the most crucial factor in management of plant diseases.Earlier the diagnostics techniques heavily relied on morphological observations of the affected plant and plant parts.Traditional methods are not sensitive enough and can sometimes be misleading, so, much effort has been devoted in the development of techniques that are less time consuming and more reliable.Antibody based techniques(Serological assays) is one such example where specific antigens produced by the pathogen is recognized by the artificially excised antibodies and is used in the identification of plant pathogens.

Different serological assays employed in plant pathology

- Enzyme linked immunosorbent assay:-The enzyme linked immunosorbent assay(ELISA) is a serology based method for identification of diseases based on colour change in the assay due to interaction between epitomes(antigens) and antibody.

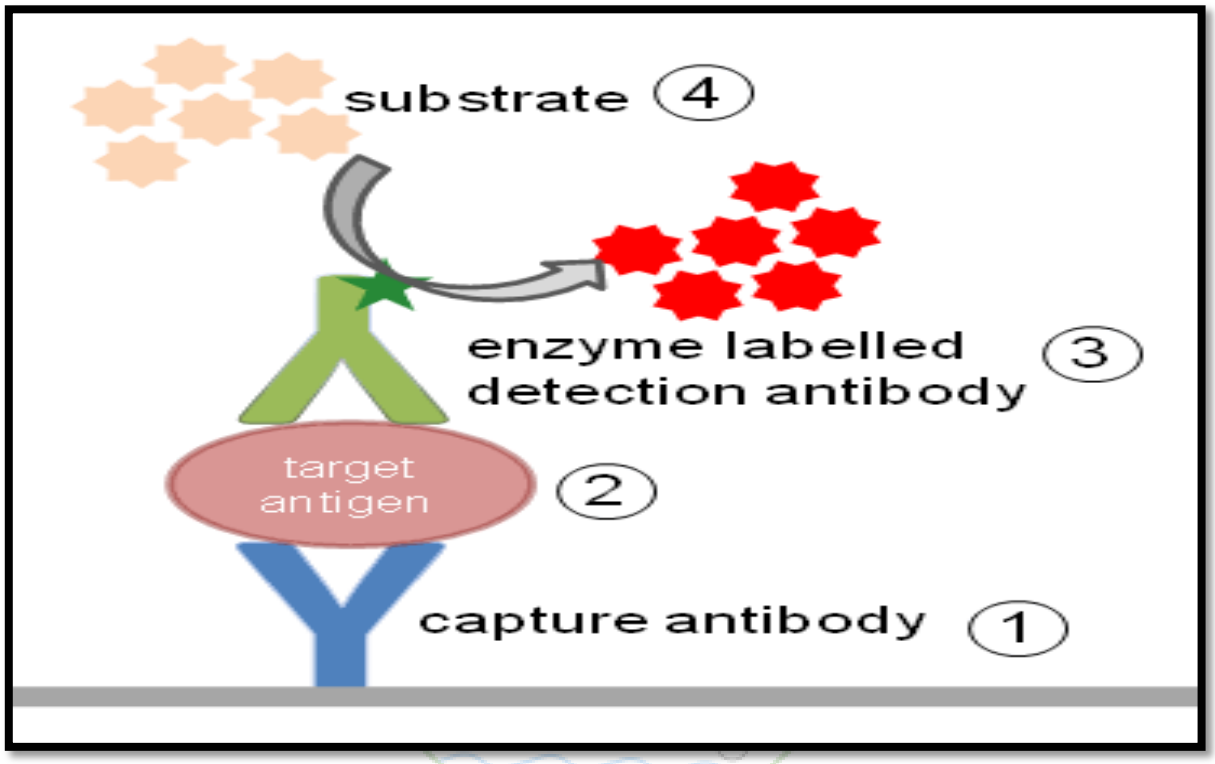

Fig. 1:-Principle of ELISA.

Image Reference:- Horlock C.;Enzyme Linked Immunosorbent Assay;Imperial College of London.

- Depending on the antigen antibody combination the ELISA assays can be classified as:-

1. *Direct ELISA*:-A target protein is immobilised on the surface of microplate wells and incubated with an enzyme labelled antibody to the target protein.
2. *Indirect ELISA*:-A target protein is immobilized on the surface of microplate wells and incubated with an antibody to the target protein followed by a second antibody against the first antibody.
3. *Sandwich ELISA*:-An antibody to a target protein is immobilized on the surface of microplate wells and incubated first with the target protein and then with another target protein-specific antibody which is labelled with an enzyme.

4. *Competitive ELISA*:-An antibody specific for a target protein is immobilized on the surface of microlplate wells and incubated with samples containing the target protein and a known amount of enzyme labelled target protein. When the antigen level with in the sample is high, the level of antibody bound enzyme labelled antigen is lower and also the colour is lighter and vice-versa.

- Western blots:-The western blot (immunoblot) enables the identification and quantification of individual proteins from a complex mixture. In general, the techniques involves separating proteins under denaturing conditions by gel electrophoresis and then transfer of proteins from the gel to solid support. This is followed by probing the membrane with an antibody specific for a target protein which is then visualized.

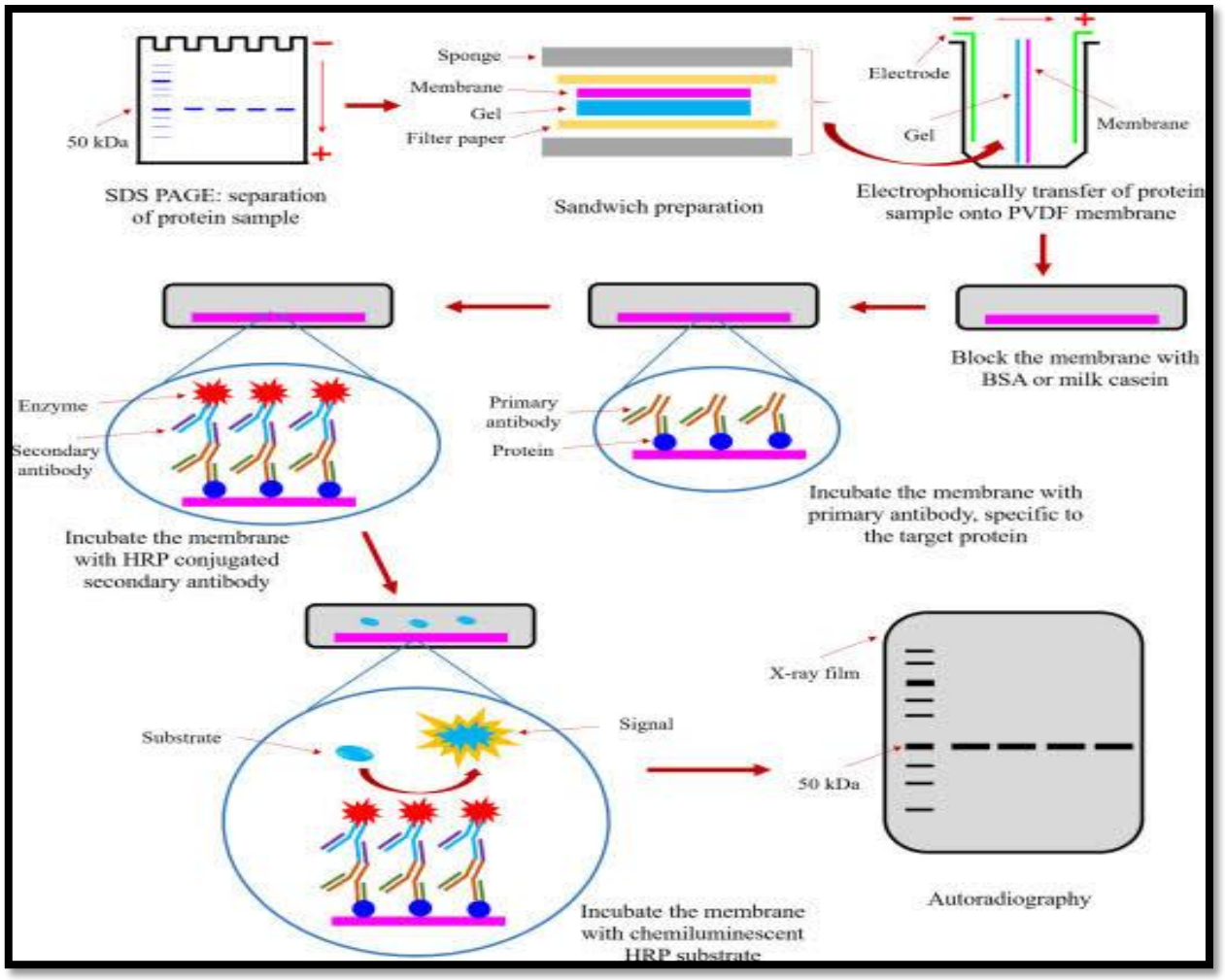

Fig.2:-Procedure of Western Blot

Image Reference:-Animal Biotechnology (2014), Science Direct.

- Dot-blot immune binding assays:-Blotting techniques are widely used for specific identification of nucleotides and proteins.In this assay proteins are detected by spotting the antigens on a nitrocellulose membrane and then incubating in secondary antibody to the primary antibody.

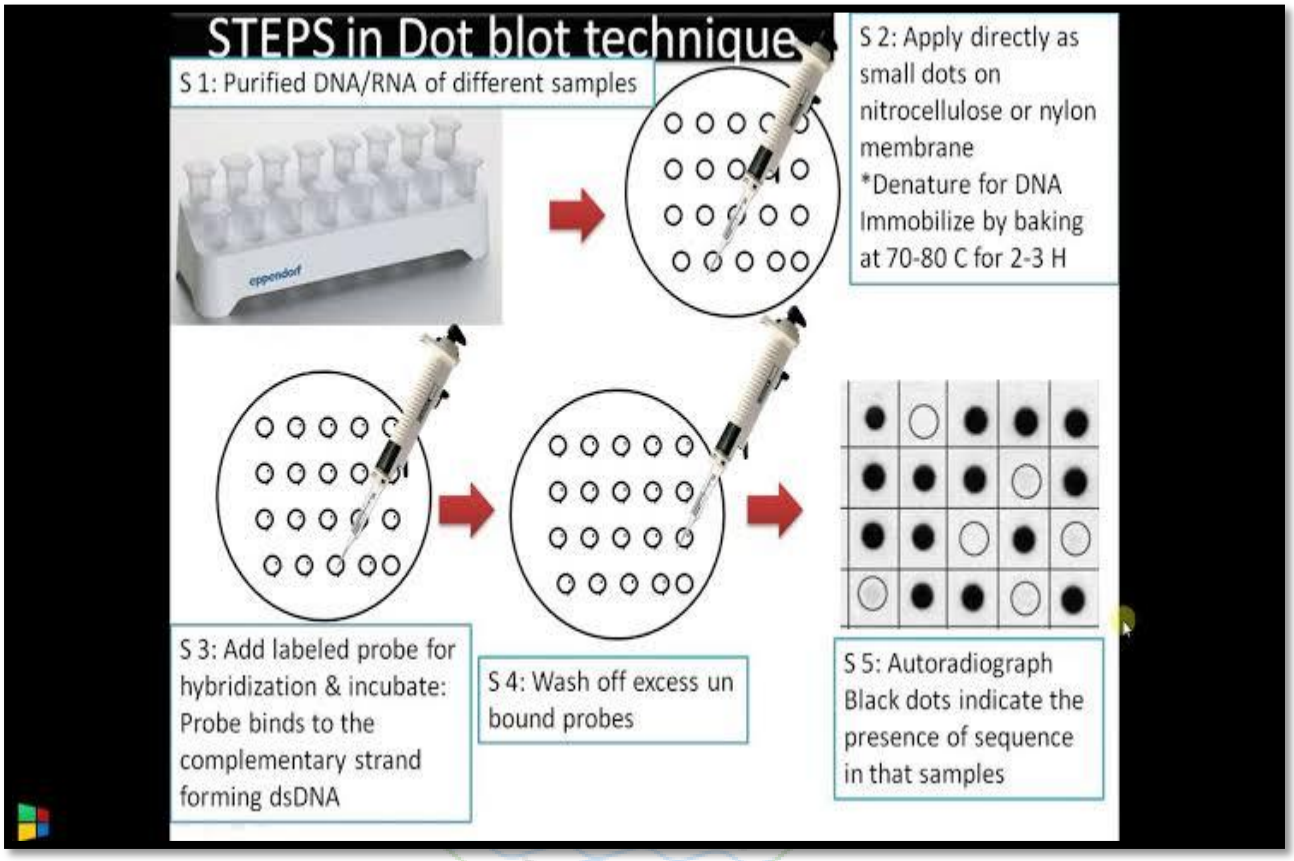

Fig.3:-Dot blot Immunobinding Assay.

- Tissue blotting immunoassay:-This technique is principally applied in the detection of plant viruses. In this method, leaf samples from plants are rolled into tight bundles, sliced with a razor blade and then the cut area is then lightly blotted onto 0.45um Nitro E nitrocellulose membranes for 3-5s.Antigens were then detected by an enzyme labelled immunological probes.
- Immunosorbent electron microscopy:-This technique involves the coating of electron microscope grids with specific antibodies and then incubating these grids with extracts of test plants. The particles are observed after negative staining.
- Quartz crystal microbalance immunosensors:-This technique measures mass based on vibrations and frequency change in real time. Antigen-antibody binding reaction causes decreased quartz crystal oscillation frequency in positive reaction.

Recent advances: immunostrips (lateral flow devices)

Lateral flow devices are used for on-site rapid detection of plant pathogens. This assay comes as a whole kit and doesn't require any additional equipment. The extraction bag in which the sample is placed already has the extraction solution. Upon the insertion of the immunostrips, the extracted leaf material flows up into the immunostrip. One line(red colour) indicates a negative test and two lines indicates a positive test for the presence of pathogen.

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