IMMUNOTECHNIQUES: CONCEPT AND APPLICATION IN PLANT PATHOLOGY

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INTRODUCTION

Proper management of plant diseases requires accurate disease diagnosis and precise identification of the phytopathogens involved. Traditional and conventional methods of disease diagnosis and pathogen detection have several demerits. It takes a lot of time to diagnose a disease and to identify a pathogen accurately by traditional approaches. Moreover, results are not always conclusive because similar symptoms can be caused by different pathogens or physiological conditions and sometimes it is very difficult to distinguish closely related organisms on the basis of their morphological characters only. Thus the traditional methods normally applied for pathogen identification and disease diagnosis are less sensitive and less reliable. The need for rapid and accurate identification and diagnostic methods to identify the organisms that cause plant diseases are essential for the formulation of effective disease control strategies. Much effort has already been devoted to the development of novel methods for detecting and identifying plant pathogens over the last decade. One of the revolutionary steps in plant pathological research is the development of antibody-based diagnostics. although nucleic acid based technologies are also very sensitive for disease diagnosis and pathogen detection (Duncun and Torrance, 1992; Schots *et al.*, 1994; Ward, 1994; Martin *et al.*, 2000; McCartaney *et al.*, 2003). The use of antibody-based techniques in plant pathology is increasing due to its reliabilities, ease of application and producing quick results. Several authors have reviewed the methods of production of antibodies for plant pathogen diagnostics and other purposes (Werres and Steffens 1994; Dewey and Thornton, 1995; Barker, 1996; Dewey *et al.*, 1997, Torrance, 1998; Schots, 1995). The present review analyses the mechanism of some of the major immunotechniques and their role in disease diagnosis and identification of the pathogens involved.

PRODUCTION OF ANTIBODY

Antibodies produced by mammalian immune system are used to identify invading organism or substances. For production of antibodies, vertebrate animals such as mice, rats, guinea pig, rabbit, sheep, goat, horse, donkey, primate and chicken can be considered to immunize with antigens. Out of these, rabbits are being widely used for production of polyclonal antibody in plant pathological research because they are easy to handle and can be bled repeatedly. Moreover, the antibodies produced in rabbits are well characterized and can be easily purified. Young and healthy New Zealand male white rabbits of 4 -12 month old are most suitable for production of polyclonal antibodies. The antibodies are raised through administration of antigen (popularly known as injection) within the body of the rabbit. Repeated administration of antigens over a period of weeks is required to generate the strong immune response against the antigen in rabbit. Such repeated administrations, or boosters,

increase the clonal proliferation of antigen-specific T cells or B cells and thus increases the lymphocyte populations specific for immunogen or antigen. The routes of administration may be varied viz. intravenous (into a vein), intradermal (into the skin), subcutaneous (beneath the skin), intramuscular (into a muscle) and intraperitoneal (into the peritoneal cavity). The administration route strongly influences the organs and cell populations that are to be involved in the immune response.

In order to get potential antibodies, adjuvants are generally mixed with the antigen before immunization. Adjuvants are chemical substances that when mixed with an antigen and injected with it, enhances the immunogenicity (capacity of immunogens/ antigens to produce antibodies) of the antigen. The use of adjuvants is very important when certain antigens have low immunogenicity or when only small amounts of antigens are available (Goldsby *et al.*, 2003). A large number of different adjuvants have been formulated for immunization. Some of the commonly used adjuvants are Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), Aluminium hydroxide or aluminium phosphate (Alum), Bentonite, Quil A, Muramyl dipeptide (MDP), Monophosphoryl lipid A (MPL) and killed *Bacillus pertusis* cells (Kerr and Thorpe, 1994).

A common procedure for raising the polyclonal antibody is to first immunize the rabbit by giving intramuscular injections of antigens emulsified with equal volume of Freund's complete adjuvant. After repeated administration of the antigen with Freund's incomplete adjuvant for six consecutive weeks the blood samples are collected by puncturing marginal ear vein on the fourth day after the last injection. In marginal ear vein puncture, an incision is actually made on the border vein of the ear of rabbit and after collection, the blood samples are incubated for an hour at 35-37^{0}C. After incubation, the antiserum is clarified from the blood by centrifugation.

Although polyclonal antibodies have been used successfully in detecting plant pathogens and cross reactive antigens in the field of plant pathology but they are not always specific enough. To overcome this problem monoclonal antibodies are produced simply by fusing antibody producing cells (lymphocytes) from the spleens of an inoculated animal (usually mice or rats) with cultured myeloma cells. After fusion, many hybrid cell lines are generated, which are commonly known as hybridomas. Each hybridoma produces a single specific antibody. Finally, these individual cell lines are propagated to generate more numbers of monoclonal antibodies that can easily be collected from the culture medium. This particular technique of monoclonal antibody production is commonly known as hybridoma technology. However, production of monoclonal antibodies is expensive and very hard to maintain because occasionally cell lines may die or stop producing the required antibody. It also takes much more time to produce this type of antibodies in comparison to polyclonal one. The use of monoclonal antibodies in plant pathology has been reviewed by Torrance (1995).

The 'phage display' technique (McCafferty *et al.*, 1990) is a recent renovation in the methodology used for antibody production. In this technique, libraries of functional fragments of antibody molecules are amplified by polymerase chain reaction. It produces very species specific antibodies. The advantages of this method over polyclonal and monoclonal antibody production procedures are that the method is generally quicker and cheaper and it does not require any animal system and also an indefinite supply of specific antibodies are restored.

IMMUNOASSAYS

Immunoassays are very sensitive and reliable immunochemical techniques that includes various methods for the detection and quantification of antigens. When appropriately employed, these can be surprisingly easy and produce instant results that are remarkably sensitive as well as specific. Moreover, the information obtained from immunoassays are often difficult if not impossible to determine by other methods. Based on methodology, immunoassays can be broadly categorized into three different types: a) antibody capture assay, b) antigen capture assay and c) two-antibody sandwich assay.

a) ANTIBODY CAPTURE ASSAY: Antibody capture assays are the most versatile among the different immunoassays. They can be used to determine both antigen or antibody levels and to compare antibody binding site. The basis principal is that the antigen is attached to a solid support and labeled antibody is allowed to bind. After washing, assay is quantitated by measuring the amount of antibody retained on the solid support.

b) ANTIGEN CAPTURE ASSAY: This method is mostly useful for antigen detection and quantification. Here the antibody is attached to a solid support and labeled antigen is allowed to bind. The unbound proteins are removed by washing, and the assay is quantitated by measuring the amount of antigen that is bound.

c) TWO-ANTIBODY SANDWICH ASSAY: It is one of the best techniques for determining the presence and quantity of antigens. In this method, the antibody is bound to solid support and antigen is allowed to bind to this first antibody. The assay is quantitated by measuring the amount of labeled second antibody that can bind to the antigen.

Detecting and quantitating antigens

For the detection and quantification of antigens, the most useful method is the two-antibody sandwich assay. These assays are quick and reliable and can be used to determine the relative levels of most protein antigens. They require either two monoclonal antibodies that bind to independent sites on the antigen or affinity purified polyclonal antibody. However, when purified polyclonal antibodies or monoclonal antibodies are not available, competition assays where pure or nearly pure antigens are needed may be used.

In the antigen capture assay, the antigen is labeled and a constant amount of labeled antigen is mixed with the test solution that contains an unknown amount of antigen. The solutions are then allowed to bind to the subsaturating amount of antibody bound to solid phase. High levels of antigen in the test solution will reduce the amount of labeled antigen that can bind. For antibody capture assay, pure or partially pure antigen is bound to the solid support. The antigen in the test solution is mixed with a preparation of labeled antibody and both are added to a solid support. High levels of antigen in the test solution will block the binding of the labeled antibody to the solid phase.

In cases when pure or partially pure antigen is not available, the antigens can be detected by applying a saturating amount of labeled antibody in antibody capture assay. As the quantity of antigen is very low, the use of monoclonal or affinity-purified polyclonal antibodies will be more appropriate than polyclonal antibodies. The specificity of the antibodies becomes less important when there is an increase in antigen concentration.

Several secondary techniques viz. immunoprecipitation, immunoblotting, and cell staining are combined with the antibody capture assay to detect the impure and rare amounts of antigens.

Detecting and quantitating antibodies

Antibody capture assay can be used to detect and quantify antibodies. In this method, purified or semipurified antigen is used. After binding of the antigens to a solid support, the test solution containing antibodies is applied, and allowed to bind to the antigen matrix. A labeled secondary antibody is then applied to measure the amount of primary antibody that bound to the solid phase.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Enzyme linked immunosorbent assay (ELISA) is the most commonly used diagnostic technique that uses antibodies (Clark and Adams, 1977; Voller and Bidwell, 1985). A number of variations of ELISA have been developed, allowing quantitative detection and qualitative measurement of either antigen or antibody. Among the different formats of ELISA, double antibody sandwich - enzyme linked immunosorbent assay (DAS-ELISA) is being widely adopted. DAS-ELISA (Fig.1) is a common format of ELISA in which high affinity antibodies (mainly polyclonal) are coated on the solid phase and allowed to bind with the target antigen. A second antibody supposed to have the similar specificity is then applied to detect and quantify the antigen (Velicheti *et al.*, 1993; Dewey and Priestly, 1994; Holtz *et al.*, 1994; Van de Koppel and Schots, 1995; Yuen *et al.*, 1998). The usefulness, specificity and sensitivity of DAS-ELISA make this method a precise and rapid tool for detection of several phytopathogens. It has also got the excellent potential for the quantification of the fungal pathogen in plant tissues directly and, therefore, has immediate application in research on the pathogen's ecology, epidemiology and host interactions. The procedure of DAS-ELISA starts with the trapping of the antibodies on the wells of a polystyrene microtitre plate. Attaching the antibodies to a solid surface is important as it allows all reactants that do not attach to the antibody and wells to be washed away during washing. Target antigens are then used. Following that a second layer of antibodies conjugated to an enzyme is then used to bind with the antigen. Alkaline phosphatase (ALP) is the most commonly used enzyme, but other enzymes such as horseradish peroxidase (HRP) can also be used. After the final washing, a chromogenic substrate solution is added to each well. Substrates are used because they produce colour after reacting with conjugated enzyme. The coloured product can be visualized and quantified by colorimetry (Langham, 2003). For ALP- conjugates p-nitrophenyl phosphate is used as substrate. It is simple to prepare and has an approximately linear rate of reaction with the enzyme, which can be terminated by simply adding excess alkali. For HRP conjugates, several substrates oxidized by hydrogen peroxide or urea peroxide are available among which o-phenylene diamine (OPD) and 2, 2′ azino-di- [3 ethylbenzothiazolin sulfone] (ABTS) are most sensitive and commonly used. OPD is light sensitive and incubations with this substrate should be done in the dark. Both substrates should be made fresh and used immediately to minimize the auto oxidation of the substrates (Clark, 1981). Another simplest format is plate-trapped antigen - enzyme linked immunosorbent assay (PTA - ELISA). In this procedure, the wells of microtitre plates are directly coated with the test sample (antigen). Plates are then incubated with specific antibody that binds to the target antigen. In some immunoassays the specific antibodies are conjugated

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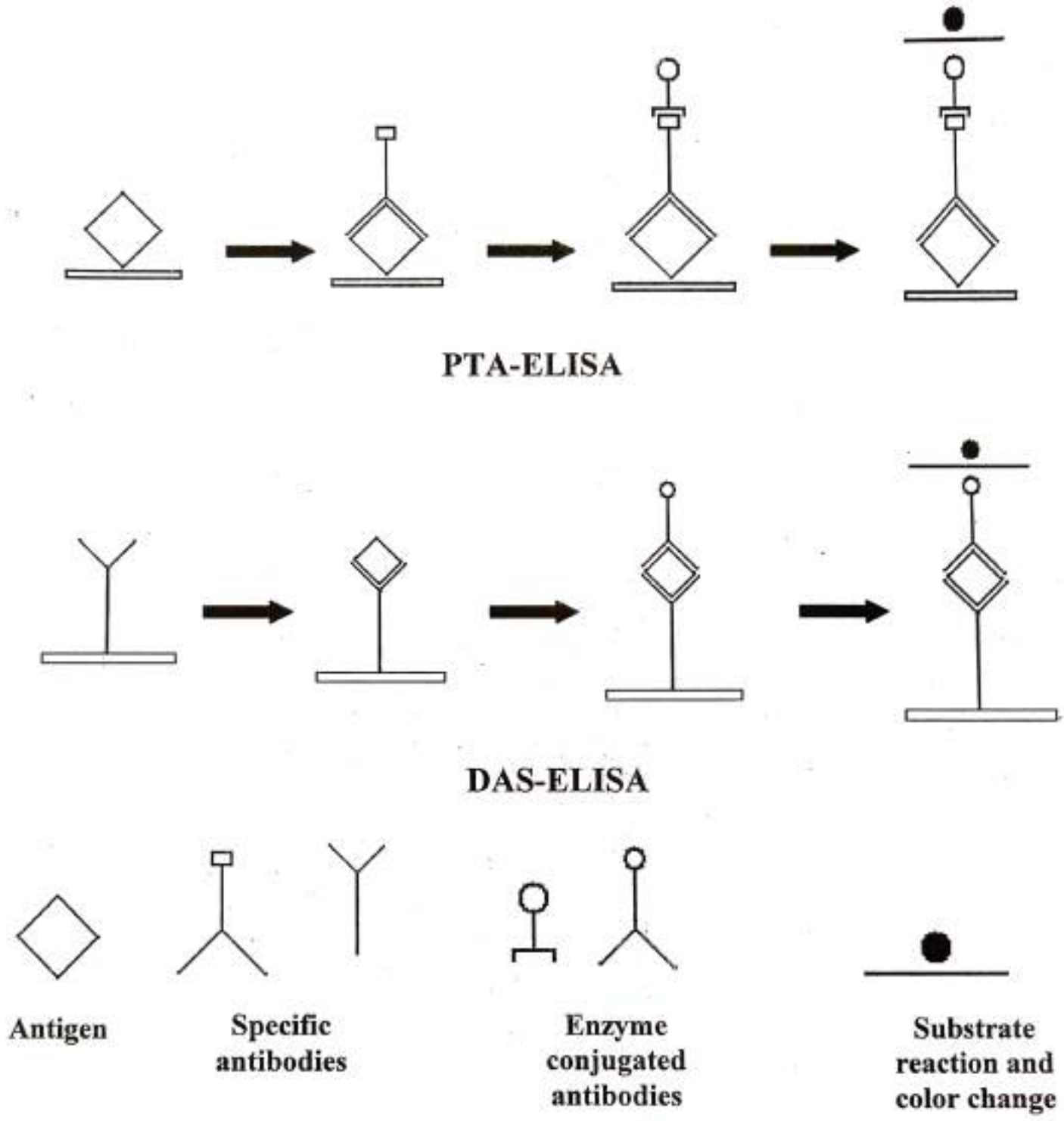

Fig.1. Schematic diagram illustrating two most common ELISA formats: Plate-trapped antigen (PTA-ELISA) and Double antibody sandwich (DAS-ELISA).

to the enzyme (direct detection), and in others the specific antibody is detected by a secondary specific antibodies are conjugated to the enzyme (direct detection), and in others the specific antibody is detected by a secondary antibody viz. anti-rabbit or anti-mouse or anti-goat immunoglobulin, which is conjugated to the enzyme (indirect detection) (Ward *et al.*, 2004). Chromogenic substrates that react with the conjugated enzyme and forms colour are then used to visualize and quantify the antigen – antibody complexes (Fig.1). The indirect detection of ELISA or more commonly indirect ELISA have become increasingly popular in present day

plant pathology to detect the cross reactive antigens (CRAs) shared by the host and the pathogen in compatible host pathogen interaction.

CROSS REACTION

An immunologic reaction in which the immune components, either cells or antibodies react with two molecules that share epitopes but are otherwise dissimilar is called cross-reaction (Coico *et al.*, 2003). When two compounds cross-react immunologically the compounds will have one or more epitopes in common and the immune response to one of the compounds will recognize one or more of the same epitope on the other compound and react with it. Another form of cross-reactivity is seen when antibody or cell with specificity to one epitope bind usually more weakly to another epitope that is not quite identical but has a structural resemblance to the first epitope. Heterologous antigen may or may not react with the immune components. If reaction does take place it may be concluded that the heterologous and homologous antigen exhibit immunologic cross-reactivity. Although the hallmark of immunology is specificity, immunologic cross-reactivity has been observed on many levels. This does not mean that the immunologic specificity has been diminished but rather that the cross-reacting compounds share antigenic determinants. In the cases of cross-reactivity the antigenic determinants of the cross-reacting substances may have identical chemical structures or they may be composed of similar but not identical physicochemical configurations. There are other examples of immunologic cross-reactivity, wherein the two cross-reacting substances are unrelated to each other except that they have one or more areas that have similar three-dimensional characteristics. These substances are referred to as heterophile antigens. For example, human blood group A antigen reacts with antiserum raised against pneumococcal capsules polysaccharide (type XIV). Similarly, human blood group B antigens react with antibody to certain strains of *E. coli*. In this example of cross-reactivity the antigens of the microorganisms are referred to as the heterophile antigens (with respect to the blood group antigen).

IMMUNOFLUORESCENCE

Immunofluorescence is the method for localizing antigens by the use of fluorescent-labeled antibodies (Goldsby *et al.*, 2003). A fluorescent compound has the property of emitting light of certain wavelength when it is excited by exposure to light of shorter wavelength. The emitted light from the antibodies can be viewed with a fluorescence microscope equipped with a UV light source. A wide range of fluorochromes (fluorescent dye generally tagged with antibody molecules) is now available. Some of the common fluorochromes are listed below.

1. Fluorescein, an organic dye that is most widely used in immunofluorescence. It absorbs blue light (490 nm) and emits an intense yellow-green fluorescence (517 nm).
2. Rhodamine, absorbs yellow – green range (515 nm) and emits a deep red fluorescence (546 nm)
3. Phycoerythrine absorbs light efficiently and emits red fluorescence.

Fluorescent-antibody staining of cell or tissue samples can be direct or indirect. In direct staining, the specific antibody (the primary antibody) is directly conjugated with the fluorescein but in indirect staining, the primary antibody is unlabelled and is detected with an additional fluorochrome-labelled reagent. The most common reagent that is in use today is a

fluorochrome-labelled secondary antibody raised in one species against antibodies of another species such as fluorescein labeled goat anti mouse immunoglobulins. The indirect immunofluorescence method is more widely used than the direct method because the primary antibody does not need to be conjugated with a fluorochrome. Indirect method also limits the loss of antibodies that usually occurs during the conjugation reaction. Moreover, indirect methods increase sensitivity of staining because multiple molecules of the fluorochrome reagent bind to each primary antibody molecule, increasing the amount of light emitted at the location of each primary antibody molecule (Goldsby *et al.*, 2003; Coico *et al.*, 2003).

The method of indirect immunofluorescence starts with placing the antigens (mycelia, spores, cells, tissues etc.) to the microscopic slides. The diluted primary antisera are then applied to the antigens. Following proper incubation and washing, a fluorescently labeled secondary antibody is used and the slides are kept for incubation in a dark moist chamber. Finally the slides are washed with phosphate buffered saline (PBS) solution, mounted on glycerol based medium and observed under fluorescence microscope.

IMMUNOGOLD LABELING AND SILVER ENHANCEMENT

Colloidal gold labeling techniques were first introduced by Faulk and Taylor (1971) when they absorbed anti-salmonella rabbit gamma globulins to gold particles for one step identification and localization of salmonella antigens. Indirect labeling techniques with gold probes was subsequently introduced by Romano *et al.* (1974) who also reported gold labeling of Protein A for detection of primary immunoglobulins. The application to thin sections for electron microscopy was described in detail by Roth *et al.* (1980) and since then the use of colloidal gold in transmission electron microscopy has grown at an enormous rate and has become virtually the only method worth considering for ultrastructural studies of cellular antigens. The major reason for this is the high electron density of the gold particles coupled with the ease with which different particle sizes can be used for examination at different magnifications.

Immunogold labeling and electron microscopy is a powerful tool for cellular location of different proteins or antigens (Lee *et al.*, 2000; Trillus *et al.*, 2000; Nahalkova *et al.*, 2001). In this technique, samples (thin sections of plant materials, spores, mycelia, cells of the pathogens etc.) are incubated with blocking buffer. After incubation and washing, antisera are applied to the samples. Antisera treated samples are then reacted with immunogold reagent after proper washing and incubation. Following incubation, the treated samples are fixed with glutaraldehyde solution. To visualize immunogold labels in light microscope, silver enhancement is essential. Silver enhancer enhances the colloidal gold labels by precipitation of metallic silver to give a high contrast signal visible under light microscope. The degree of deposition of metallic silver in the regions bounded with colloidal immunogold particles implies the quantification of the antigen molecules.

IMMUNOSORBENT ELECTRON MICROSCOPY (ISEM)

The development of the techniques of negative staining greatly enhance the use of electron microscope in studying the structure of virus particles, including virus identification and diagnosis of disease (Derrick, 1973, Clark, 1981). Detection and diagnosis of plant viruses use biological, biophysical, biochemical and immunological techniques. For virus

identification immunological techniques, especially immonosorbent electron microscopy (ISEM) and ELISA, are getting importance in plant pathological research continuously for their sensitivity, reliability and readiness over other techniques. Immonosorbent electron microscopy (ISEM) is the most convincing form of immunotechniques used in present day plant science for the identification and detection of plant virus (Beier and Shepherd, 1978; Brlansky and Derrick, 1979; Roberts and Harrison, 1979: Lesemann *et al.*, 1980). The basic steps of ISEM are similar to transmission electron microscopy (TEM) but depending on the type of virus some areas like antisera dilution, incubation period, pH etc can be varied. In this technique, samples (tissue, cell etc.) are macerated in suitable buffer to produce sample slurry and then the macerated slurry are kept for sometime. Diluted antisera (diluted with sodium chloride solution) are then placed on a grid with appropriate specimen substrate support viz. plastic films like collodion (or parlodion), formvar (or butvar) etc. and carbon films. After proper incubation, antiserum coated grid is floated on a piece of parafilm containing few drops of sample slurry and incubated. After proper washing with distilled water and uranyl acetate and air drying, the grid is examined under the TEM. This procedure is known as trapping. In another procedure named decoration, the sample slurry and antisera coated grid are prepared as before and after proper incubation, antiserum coated grid is floated on a piece of parafilm containing few drops of sample slurry and incubated. After washing, the grid is floated on the antisera again and incubated thereafter. Following final washing (with aqueous uranyl acetate), incubation and drying, the grid is examined under TEM. Another format of ISEM, the solid phase immune electron microscopy (SPIEM) is also used for detection and identification of plant virus. It is also known as protein A complemented immune electron microscopy (PAC-IEM). In this method, the trapping is done by floating the carbon-farmvar coated copper grid on protein A solution. After incubation in room temperature the grids are washed with phosphate buffer and then they are again floated on diluted antisera (diluted with sodium chloride). Then the grids are floated on few drops of clarified plant tissue extract and incubated for 1 hour at room temperature and then overnight at 4^0C in humid chamber. Following final washing (with aqueous uranyl acetate), grids are air dried and observed under TEM. In another modification, extraction of the plant tissues is performed first. Then supernatant is taken from the extract by centrifugation and diluted antiserum is added to this and the mixture is incubated. Following incubation, the mixture is centrifuged again and supernatant is discarded. The pellets are resuspended in suitable buffer. Then protein A coated grid is floated on few drops of resuspended pellet. After adequate incubation and washing, the grid is stained with freshly prepared phosphotungstic acid. The grid is then screened under TEM (Nicolaieff *et al.*, 1982; Fox, 1990; Chen *et al.*, 2001).

APPLICATION

The immunotechniques described above are widely used in plant pathological research. A phenomenon of common antigenic relationship has received attention during last three decades. Studies on both animal and plant hosts and their parasites and pathogens suggest that whenever an intimate continuing association of cells of host and pathogen occurs, partners of this association have a unique serological resemblance to one another involving one or more antigenic determinants. In plants, several studies have shown that the possibility of susceptibility is greater when antigenic similarity is greater. Thus the concept of common antigen between a plant and a pathogen is a notable feature in determining resistance or susceptibility. It is believed that the degree of compatibility and susceptibility of a plant

cultivars to a pathogen is correlated to levels of common antigens present in both host and pathogen (Alba *et al.*, 1983; Purkayastha and Banerjee, 1990; Chakraborty and Saha, 1994; Kratka *et al.*, 2002; Ghosh and Purkayastha, 2003; Musetti *et al.*, 2005; Eibel *et al.*, 2005; Dasgupta *et al.*, 2005).

Daniel and Nilson (1991) raised polyclonal antibodies against mycelial extracts of the rot fungus *Phialophora mutabilis* that reacted strongly with its homologous antigen and cross-reacted strongly to moderately with six other *Phialophora* soft rot spp. In ELISA. Brill *et al.* (1994) demonstrated DAS-ELISA technique as 100-fold more sensitive over indirect ELISA while working on soybean and its pathogen *Phomopsis longicolla*. Four polyclonal and two monoclonal antibodies were raised to detect *Colletotrichum acutatum*, a quarantine pathogen of strawberry by Kratka *et al.* (2002). They observed that only one polyclonal antibody was sensitive enough to recognize the pathogen. They also detected *C. acutatum* by PTA-ELISA. Another study performed by Eibel *et al.* (2005) showed cross-reactivity of several *Ustilago* species in DAS-ELISA. The usefulness of commercial ELISA for detecting and quantifying *Pythium* spp in plants has been demonstrated by several authors (MacDonald *et al.*, 1990; Shane, 1991; Yuen *et al.*, 1998).

Besides fungus, virus (Petrunak *et al.*, 1991; Abou-Jawdah *et al.*, 2001; Hema *et al.*, 2001; Devaraja *et al.*, 2005; Chen *et al.*, 2005) and bacterial (Mazerei and kerr, 1990) pathogens of plant could be successfully detected by various ELISA formats. McDaniel and Tsai (1990) did partial purification of geminivirus like particles from pseudo-curly top virus (PCTV) – infected plants and developed a DAS-ELISA for detection of PCTV in insect and plant tissues. They also determined serological relationship between PCTV and beet curly top virus (BCTV) by indirect ELISA. Wang *et al.* (2006) observed that an indirect ELISA is capable of detecting rice black streaked dwarf virus (RBSDV) in very dilute wheat leaf extracts

Immunolocalization is a powerful tool for cellular location of different proteins or antigens. Chakraborty and Saha (1994) labeled polyclonal antiserum with fluorescein isothiocyanate (FITC) and found that cross-reactive antigens between tea leaves and the pathogen *Bipolaris carbonum* was present mainly around epidermal cells and mesophyll tissues of the leaves of host and hyphal tips and in patch like areas on conodia and mycelium of the pathogen. Dasgupta *et al.* (2005) also studied the location of CRAs in tea leaves that were treated with antiserum raised against the pathogen *Curvularia eragrostidis*. Indirect labeling of antibodies with FITC showed that CRA was concentrated mainly in the epidermal cells and also spread throughout the cortical cells.

Present day immunolocalization studies are performed using immunogold labeling, which is successfully used for electron microscopy (Lee *et al.*, 2000; Trillus *et al.*, 2000; Nahalkova *et al.*, 2001; Kang and Buchenauer, 2002 and Wang et al., 2003). For light microscopy, silver enhancement is done after gold labeling (Santen *et al.*, 2005 and Saha *et al.*, 2006). However, immunogold labeling has not yet been utilized for location of CRA in compatible host and pathogens. Kuo (1999) used a gold sol, which was found to be able to localize the extra cellular matrix (ECM) in the conidia of *Colletotrichum gloeosporioides* very well. With gold sol the composition and nature of the ECM could easily be identified using cytochemical and biochemical approaches.

Serological methods are also useful for detecting and quantifying bioparticles (Burge and Solomon, 1987; DeCosemo *et al.*, 1992; McCartney *et al.*, 1997; Flannigan, 1997) that play a major role in spreading plant diseases. Immunofluorescence has been used to identify spores of *Botrytis cinerea* deposited on the trapping surface of a Burkard spore sampler (Dewey, 1996). Kennedy *et al.* (1999) also used immunofluorescence to detect *Mycosphaerella brassicicola* ascospores on Burkard spore traps coated with bovin serum albumin (BSA) as a support medium and blocking agent. Schmechel et al (1994, 1996, 1997) developed a prototype rotating arm sampler, designed for use with ELISA to detect air borne spores. Kennedy et al (2000) demonstrated the potentiality of microtitre immuno spore trap (MTIST) for the rapid detecton of air borne spores of *M. brassicicola* and *B. cinerea* by ELISA. Hence, the immunological techniques play a significant role in the detection and monitoring of air borne plant pathogen inoculum if the suitable antibodies are present.

CONCLUSION

This review has been based on recent researches that utilized different immunotechniques for obtaining important information on plant pathology. Greatest interest has concerned the diagnostic methods for the detection of phytopathogens. Other areas like *in vivo* localization of antigens have been highlighted. In addition fundamental informations on some of the various immunotechniques that are commonly applied in phytopathology research have been included. With the recent advent of modern nucleic acid-based diagnostic methods, the greatest interest in future lies with the appropriate and worthwhile integration of antibody-based and nucleic acid-based methods. Serological methods are not as sensitive and specific as nucleic acid-based methods. Moreover, suitable antibodies may not be available always. On the other hand, Techniques involving DNA extraction and/or PCR amplification are often not suitable for routine use in cultivation programme or disease research in developing countries. Such tests could be too costly and labor intensive considering the number of samples to be tested per field and the risk of obtaining false positives results caused by cross contamination would be too high under these conditions. Thus more efforts are needed to formulate more precise and rapid assay formats in the years to come.

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