ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 5, pp.4252-4256, June, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

NEED FOR RAPID AND NOVEL DIAGNOSTIC TECHNIQUES FOR QUARANTINED FUNGAL PATHOGENS OF WHEAT SUCH AS KARNAL BUNT (TILLETIA INDICA)

Behl.S[1*], Jarrar.M[1] and Gaur.S[2]

[1]Department of Biotechnology, University of Modern Sciences, Dubai, UAE

Received 2nd, April, 2015Received in revised form 10th, April, 2015Accepted 4th, May, 2015Published online 28th, May, 2015

INTRODUCTION

Agriculture continues to be the most predominant sector of the world economy, as 70% of the population is engaged in agriculture and allied activities for their livelihood. The policies and objectives of the Government have been to ensure stability and increment in agricultural production in a sustainable manner to meet the food requirement of growing population and also to meet the raw material needs of agro based industries, thereby providing employment opportunities to the rural population. However, the loss in crop productivity by various pests and diseases and also changes in climatic extremes add to the stress on agricultural resources in a region, where land availability and degradation, food price shocks and population growth are already a major concern. Wheat is grown on more than 218,000,000 hectares (540,000,000 acres) across the world, larger areas than for any other crop. A number of countries grow two wheat crops per year: the winter wheat and spring wheat, named after the period of their planting. China, India, and the United States alone account for 46 percent of global grain production; Europe, including the former Soviet states, grows another 21 percent. Argentina, Australia, Canada, the European Union (EU), and the United States account for 80 percent of wheat exports, while just three nations— Argentina, the EU, and the United States— account for 80 percent of corn exports.

Wide array of microbial diseases highly impact crop products in quality and quantity. Survey of Kumaon and Garhwal regions in India showed prevalence of yellow and brown rusts, loose smut, powdery mildew and hill bunt in wheat; stripe and covered smut in barley; blast, brown leaf spot, and false smut in rice; neck and finger blast in finger millet; *turcicum* leaf blight in maize; powdery mildew, white rot in pea; buck eye rot in tomato, root rot and anthracnose in bean; root rot and wilt in lentil, and frog eye leaf spot, and anthracnose in soybean as the major diseases. Fuscous blight of French bean/raj mash has been reported for the first time from this region. Viral diagnosis based on symptomatology, shows presence of nearly 50 viral diseases affecting different crops grown in hills. Losses caused by major diseases to important crops vary from slight to severe depending on the crop/variety and prevailing climatic conditions. Major pests, like blast and stem borer caused up to 65% and 52% losses respectively in rice, stripe up to 72% in barley, white rot up to 58% in pea, buck eye rot up to 80% in tomato, anthracnose and frog eye leaf spot (combined) up to 20% in soybean and white grub up to 80% in rain fed rice.

Karnal bunt (KB) of wheat ,caused by the smut fungus *Tilletia indica* , is an important disease in the Tarai region and irrigated areas of the north – western plains of India, parts of Bihar, West Bengal, northern parts of Madhya Pradesh and Rajasthan and Junagarh districts of Gujarat. It was first reported in US in Arizona in 1996. Karnal bunt of wheat is a challenge to the grain industry, not due to direct yield loss but to quarantine regulations that may restrict international movement of affected grain. Total damage in terms of quantitative losses due to karnal bunt ranged between 0.2 to0.5 percent of the total value of wheat crop in India during epiphytotic years (Munjal 1975; Joshi *et al*., 1983) and it is estimated that 1.0% of the wheat crop was lost to KB during the epidemic of 1987 in Uttar Pradesh from losses in grain quality and yield (Singh 1994),). In a modern regulatory climate in which the presence of a single teliospore could lead to a costly regulatory action, the mandate for extensive disease surveys requires a fast and simple method for detecting teliospores in grain and seed (Aujla *et al*., 1988).

Ideal crop protection program economically should prevent the damaging effects of pests and diseases safely and without harming the environment or inducing subsequent control problems. Conventional methods, based mainly on pesticides and resistant crop varieties, control many damaging organisms effectively but have important limitations. Vulnerability to the emergence of tolerant strains of pest or pathogen is probably the most severe; chemical methods are also often insufficiently selective and very wasteful. Dependence on these methods will continue, however, and it is therefore essential to seek ways of minimizing their deficiencies. The prospects for improvement are discussed in relation to the need for better intelligence about infestations and their consequences, the need to ensure that control measures remain effective and the need to improve the

Corresponding author:* **Behl.S
Department of Biotechnology, University of Modern Sciences, Dubai, UAE

efficiency of utilization of crop protection agents. Implementation of the suggestions for improvement could require fundamental changes in the organization of crop protection practices. The system should, therefore, be suitably revamped to make it effective in checking this menace. While traditional protection techniques are efficient, they are incapable of ensuring that the final product has all the desired characteristics. Early, fast, accurate, detection methods for identification of such diseases are a paramount for any protection plan or program.

Molecular diagnostic tools in plant disease surveillance

Accurate identification and early detection of pathogens is the cornerstone of disease management in many crops. The detection of harmful viruses and bacteria in plant material, vectors or natural reservoirs is essential to ensure safe and sustainable agriculture. The techniques available have evolved significantly in the last few years to achieve rapid and reliable detection of pathogens. In addition, extraction of the target from the sample is important for optimizing detection. For viruses, sample preparation has been simplified by imprinting or squashing plant material or insect vectors onto membranes. To improve the sensitivity of techniques for bacterial and viral detection, a prior enrichment step in liquid or solid medium is advised. Serological and molecular techniques are currently the most appropriate when high numbers of samples need to be analyzed. Specific monoclonal and/or recombinant antibodies are available for many plant pathogens and have contributed to the specificity of serological detection. Many plant pathogens are difficult to identify using morphological criteria, which can be time consuming and challenging and requires extensive knowledge in taxonomy. Molecular detection techniques can generate accurate results rapidly enough to be useful for disease management decisions. Technologies for the molecular detection of plant pathogens have been a major breakthrough over the past 30 years. Availability of these diagnostic methods provides greater flexibility, increased sensitivity, and specificity for rapid diagnosis of virus diseases in disease surveys, epidemiological studies, and plant quarantine and seed certification as well as in breeding programs. In this review, we survey and contrast a wide range of such techniques that have been frequently used for the identification and detection of diseases.

Nucleic acid based techniques

Nucleic acid–based methods detect organism-specific DNA or RNA sequences extracted from the microorganism. Sequences may or may not be amplified *in vitro*. These methods are generally specific and highly sensitive and can be used for all categories of microbes. Results can be provided rapidly. BIO-PCR has been used for the detection of *Pseudomonas syringae* pv. *Phaseolicola* (Schaad and Hatziloukas., 1997). An IC RT-PCR (Immuno-capture Reverse Transcriptase Polymerase Chain Reaction) has been developed to detect Lettuce Mosaic Virus (Berendsen *et al*., 1997). Polymerase Chain Reaction Amplification of DNA from *Clavibacter michiganensis* sub sp. *michiganensis* has been applied for the detection and identification of this bacterium in tomato seed. Two oligomers, CM_3 and CM_4 were used to amplify a specific 645bp DNA fragment detected by separation of the PCR products on agarose gel when the target bacterium was present in the amplification assay (Sousa Santos *et al*., 1997). *Tilletia indica* is a seed borne pathogen of great regulatory importance. Efforts are on to develop an accurate molecular method to identify teliospores of *T. indica*. Using BIO-PCR and primer pairs T117 M1and T117 M2 (825bp) and T157M1/M2 (118 bp), as few as 5 teliospores could be detected per 50g of wheat seeds. PCR based technique is important in this case because KB teliospores are morphologically similar to *T. barclayana* (Bonde, *et al*., 1997). Polymerase chain reaction (PCR) in conjunction with six short arbitrary primers of random sequences was used to perform RAPD profiling of seven seed borne fungal pathogens of wheat and rice which exhibited distinct polymorphic DNA. A total 117 RAPD loci were observed on polyacrylamide gel which categorized the fungal pathogens in to three different groups and established their relationship of genetic relatedness with 3.0 to 27.0 percent similarity. These six arbitary primers could distinguish *T. indica*, a quarantine fungal pathogen to a non-quarantine fungal pathogen *T. barclayana*. Total 89 RAPDs loci were observed between *T. indica* and *T. barclayana*, out of which, 18 were monomorphic while 71 loci were unique (Mishra *et al*, 2002).

Immunological techniques

Immunoassay techniques to detect plant pathogens have been largely adapted from methods widely used in medical diagnosis (Sikora and Smedleyo, 1984). These techniques are routinely utilized for disease indexing of plant material (Ball and Reeves, 1992; Cassels, 1992; Miller *et al*., 1992). Immunology has been revolutionized by the interdiction of highly specific monoclonal antibodies and enzyme linked immunosorbent assays (ELISAs) as suitable methods to detect only the molecules (antigens) or antibody binding sites (epitopes) that are unique to the infecting organism, or its metabolite. These diagnostic assays are rapid and can be completed in several hours instead of days or even weeks. Single chain Fv (scFv) technology allows the cloning of variable antibody genes, linking them to a flexible peptide as a single chain Fv. These constructs are of great interest in plant pathology because they can be expressed in bacteria as soluble proteins, fused with the capsid proteins of filamentous phages, and expressed in plants. In addition, antibody genes can be expressed fused with other proteins such as alkaline phosphatase or amphipathic helices. The expression of these antigen-binding proteins in bacterial cultures provides standardized diagnostic reagents that are theoretically able to replace conventional monoclonal or polyclonal antibodies and conjugates, providing significant advantages in time and cost.

Microtitre ELISA is being used for early detection of KB pathogen in the host when the infection levels are very low, in-planta proliferation of pathogen, determination of fungal biomass in infected tissues, characterization of genetic races, based on their immune-reactivity pattern (Varshney, 1999).In addition, this technique has been proven to be useful for monitoring the antigenic properties in the process of population shift during sexual development of *T. indica* and for

determination of genetic diversity among pathogens and subsequently used for immunopathotyping (Rai, *et al*.,1998).

Another assay i.e. immunofluorescence staining test is performed to detect the intact teliospores found in the sori of infected wheat seeds or as fungal contaminants on the infested seeds or in soil. This technique is suitable for localization of teliospore associated molecular pattern (TAMP) of KB teliospore, studying the antigenic configuration of the surface of teliospores and could be employed for immunodetection of salvated teliospores present as surface contaminants in wheat lots (Gupta *et al*., 2001).

Recent studies suggest that for detection of solubilized antigen, antibody immobilized latex particles; bentonite or cells of *Staphlococcus aureus* can also be employed. The sensitivity of the agglutination test has been found to correspond with the Howard Mould Colony count for detection of plant fungi in seeds, animal feeds and spices (Notermans and Kamphuis, 1990). Lyons and Taylor (1990) used *Staphylococcus aureus* slide agglutination test for identification of bacteria from plants.

The dyed latex bead agglutination test is considered better for detection of solubilized teliosporic antigens over intact teliospores of KB. The teliosporic antigens are solubilized using sonication and detergent extraction and are allowed to absorb on the surface of blue colour dyed latex beads, which are then immunoprocessed using polyclonal anti-teliospore serum. Positive agglutination reaction reflects the presence of KB infection. The method has the advantage as it is more economical, rapid and user friendly with an absence of pseudo-agglutination which is the most important factor for on-site diagnostics single step test (Kesari, *et al.*, 2005). The test has the sensitivity to detect solubilized teliosporic antigens equivalent to 750 intact teliospores and it is suitable for immunodetection of different grades of infected seeds having teliospores mass ranging from 1000 to more than 10,000. The mechanism of dot blot or immunodipstick assay is essentially the same as that of ELISA. A number of workers have used such rapid formats, which are more sensitive and rapid for onsite detection of pathogens (Dewey *et al*., 1989a; Cahill and Hardham, 1994; Schwick *et al*. 1998 and Moricello *et al*. 1998). Immuno-dipstick test for KB diagnostics includes the use of nitrocellulose padded plastic tags. When teliospores are kept on nitrocellulose pads, the torn-off teliosporic antigens are adsorbed due to the charged nature of the membrane. Immuno-processing of dipsticks using primary (anti-teliospore antibodies) and secondary antibodies (Goat anti-rabbit immunoglobulin IgG conjugated with ALPase) lead to color development and predicts the presence of the pathogen. The advantage associated with the use of this technique is its sensitivity as it can detect a very small number of teliospores i.e., only five and 5ng of antigen (Kesari and Kumar, 2002).

Flowcytometry

Flow cytometry is a technique for rapid identification of cells or other particles as they pass individually through a sensor in a liquid stream. Pathogenic cells are identified by fluorescent dyes conjugated to specific antibodies and detected electronically using a fluorescence-activated cell sorter, which measures several cellular parameters based on light scatter and fluorescence. Multiparameter analysis includes cell sizing, fluorescence imaging, and gating out, or elimination of unwanted background associated with dead cells and debris. Flow cytometry has excellent potential as a research tool and possibly for routine use in seed health testing and other fields. This technique has been adapted to the analysis of viability, metabolic state and antigenic markers of various pathogens.

Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) is a technique applied for pathogenic detection that combines the simplicity of microscopy observation and the specificity of hybridization. Its use in detection of plant pathogenic bacteria is recent and is dependent on the hybridization of DNA probes to species-specific regions of bacterial ribosomes. They are particularly suitable as diagnostic targets because ribosomal RNA contains functional sequences that are common to all species but also sequences that are very specific to individual species, and FISH only needs to recognize this specific information. The probes hybridize with a three-dimensional protein/RNA structure not only with a linear sequence of RNA.

The sensitivity of the FISH technique is equivalent to that of amplification technologies and, in theory, FISH can detect single cells. This high sensitivity is the result of the high affinity and selectivity of DNA probes especially when FISH takes place under very stringent hybridization conditions, where a difference of one nucleotide in a 15–20 oligo nucleotide probe is sufficient to discriminate binding. Furthermore, FISH maintains the structural integrity of the microorganism, confining the reagents in one small vessel and one probe will bind to each of the $1–5 \cdot 10^4$ ribosomes inside. This extremely high signal is responsible for the theoretical ability of the technique to achieve single-cell sensitivity. In practice, however, the detection level is 103 cells /ml (Lopez M. 2003).

Proteomics

The impact of proteomics can be significantly viewed in the development of novel clinical agents. Mass Spectrometry based proteomics has become a powerful and increasingly popular approach to not only identify these pathogens, but also to better understand their biology. There has been a recent push in the proteomics community to make data from large-scale proteomics experiments publicly available in the form of a centralized repository. Such a resource could enable the use of MS as a universal plant pathogen detection technology. Identification of fungal spores based on mass fingerprinting of MALDI–MS spectra of specific secondary metabolites and/or biomacromolecules present on fungal spore surface and testing of the lowest detection limits has been greatly significant. Differential In Gel Electrophoresis (DIGE) coupled with 2-D gel analysis using the DeCyder software package has been used to compare and quantify protein populations from the fruit of wild type and non-ripening rin tomatoes for providing insights into the regulation of fruit development and ripening as well as

suggesting strategies for enhancing fruit quality traits (Beckett *et al*., 2002).

Surface plasmon resonance

Surface Plasmon Resonance (SPR) is an optical detection process that occurs when a polarized light hits a prism covered by a thin (gold) metal layer. Under certain conditions (wavelength, polarization and incidence angle) free electrons at the surface of the biochip absorb incident light photons and convert them into surface plasmon waves.

The interaction between probe molecules immobilized on the chip and captured target molecules, induce a modification of resonance conditions which are in turn seen as a change in reflectivity and which can be measured. This method is sensitive label-free method of visualizing the whole of the biochip via a video CCD camera and also helps to design enables the biochips to be prepared in an array format with each active site (spot) providing SPR information simultaneously. The attempts are made to develop SPR immunosensor for detection of Karnal Bunt in order to identify the presence of teliospores of fungus in wheat lots to be used in seed certification and plant quarantine regulation.

The need for rapid immunodiagnostics

In particular, very few plant diseases can be diagnosed in a laboratory before the symptoms of the diseases become visible in the field. Most such laboratory tests are expensive and time consuming. Consequently, these tests are not normally performed until there is visible evidence in the field that a plant may be infected; and as a result, in most instances, by the time a disease is finally diagnosed, it is quite widespread. Numerous comparatively simple and inexpensive diagnostic tests have been successfully developed for the detection of diseases at very early stages.

Immuno-chromatographic assays, also called lateral flow tests or simply strip tests, have been around for some time. These assays are user friendly, less time consuming, stable over a wide range of climates and relatively inexpensive to make. These features make strip tests ideal for applications such as home testing, rapid point of care testing, and testing in the field for various environmental and agricultural analytes.

CONCLUSION

Karnal bunt of wheat has gained significant importance not only because of qualitative and quantitative losses (0.2-0.5%) but also for reluctance on the part of many countries to accept the wheat from Indian subcontinent. Occurrence and effect of the diseases on plant quarantine has aroused much concern. After reporting of KB at Arizona State in March, 1996, USDA has placed *T. indica* as a quarantined pest. Hence, wheat movement into United States and other Karnal bunt free countries is regulated and subjected to quarantine. Other countries like USA, Canada, Russia and China are also under the threat of spreading this disease. In China, because of fear of Karnal bunt spread, wheat from other countries is only accepted after declaring the wheat lots which have zero tolerance limits against Karnal bunt.

The teliospore of Karnal bunt is an infectious entity and can be detected in grains by simple microscopic techniques, but the teliospores of this fungus closely resemble with teliospores of fungi causing bunts in other crops like rye and rice.

These pathogens have more or less same teliospore texture but different in sizes. Similarity in teliospore configuration makes it difficult to differentiate KB teliospores from the teliospores of other bunt fungi. The presence of rice bunt teliospores which cause the rejection of wheat lots and hence export of wheat is affected. Considering the KB as major barrier in international trade of wheat, a specific and pin-point diagnosis of KB pathogen is required to overcome the problems in export.

In order to determine the correct identity of contaminating fungus, it is essential to develop specific diagnostic probes, which could be used by the seed certification lab and plant quarantine department. The developed immunodiagnostic formats i.e. Seed Immunoblot Binding Assay (SIBA); Indirect Immunofluorescent staining tests, Dyed Latex Bead Agglutination Assay etc. can be employed for the sensitive detection of seed borne inoculums of the fungus present either in bunted seeds or as loose teliospores on the seed surface. However, the major obstacle is specific detection of KB teliospores due to cross reactivity of anti-teliospore antibodies with other bunt and related fungal pathogens.

India's agricultural growth during 60+ year's period of Independence remains impressive at 2.7 percent per annum. Around two – third of this production growth is aided by crop productivity gains (Arunachalam. P, 2009).

The need based strategies followed since independence and intensified since mid – sixties mainly focused on feeding the growing population and making the country self-sufficient in food production. According to the Union Ministry of Agriculture the area under Wheat cultivation in Punjab, Uttar Pradesh and Haryana had increased significantly over the previous year. In Punjab the cropped area had reportedly increased to 33 lakh hectares from 30 lakh hectares. The increase has been indeed very significant in wheat production which has increased from a mere 6.46 million tons in 1950-51 to 68.71 million tons in 1996-97 almost 11times. India has thus emerged as the second highest wheat producer in the world. India is already number two in rice production which has increased from 21 million tons to 81 million tons. Acreage had increased by four lakh hectares to 22.5 lakh hectares in Haryana and from 78 to 81 lakh hectares in Uttar Pradesh. The Minister of State for Agriculture was reported to have estimated a wheat harvest of 67 million tons as compared to estimated 63.5 million tons in the year1997-98. The major destinations for wheat are Bangladesh, Yemen Republic, UAE, Jordan and Turkey.

The major problem during wheat export to these countries is caused by the presence of Karnal bunt disease. This could be overcome if seed lots exported to these countries are declared

pathogen free by adopting suitable diagnostic technologies. With the globalization of trade, there is a need to rationalize quarantine restrictions which hamper the spirit of free market and to develop a rapid immunodiagnostic method for detecting infectious entities of Karnal bunt.

References

Arunachalam. P, (2009) India's Agricultural Products Exports: An Overview, *Journal of Global Economy*, 5(1), 45-72.

Aujla.S.S, Kaur,S. and Mohan.(1998).Correlation between spore load of Neo vossia indica per wheat seed and grade of infection. Seeds and Farms, 14, 39-40.

Ball S and Reeves J, 1992.Application of rapid techniques to seed health testing. In Techniques for Rapid Detection of Plant pathogen.193-207 Blackwell Scientific Publications, Oxford.

Beckett P, J.K.C. Rose, C. Rozanas and R. Asbury (2008) A proteomic study of tomato fruit ripening based on 2D - DIGE analysis, 19(1): 1-72.

Bonde, M.R., Tooley, P.W. and Schaad, N.W. 1997. A PCR technique for accurate identification of teliospores of the Karnal bunt pathogen, Tilletia indica. In: Second ISTA-PDC Symposium Seed Health Testing Towards the 21st century, Cambridge, UK.

Cahill, D.M., Hardham, A.R., 1994, A Dipstick Immunoassay for the specific detection of Phytophthoria cinnamoni in Soils, Phytopathology, 84, 1284-1292.

Dewey, F.M., Twiddy, D.R., Phillips, S.I., Grose, M.J. and Wareing, P.W. (1990). Food Agric. Immunol. 4, 153–67.

Gupta. V., Kumar, A., Singh, A. and Garg, G.K. 2001. Immunodetection of teliospores of Karnal bunt (Tilletia indica) of wheat of viz., fluorescent staining test. Plant Cell Biotech. Mol. Biol. 1: 81-86.

Joshi, L.M., Singh.D.V., Srivastava,K.D. and Wilcoxson,R.D.(1983).Karnal bunt: A minor disease that is now a threat to wheat.Bot.rev.,49,309-330.

Kesari R, Kumar A (2002) Design and optimization of novel immuno-dipstick test for detection of teliospores of Karnal Bunt (Tilletia indica) of wheat. In: Proceedings of new opportunities and challenges for improving crop productivity through biotechnology of SPBB, Hisar, PSIV-2. Pp: 110

Kesari R, Mishra, D.P., Garg, G.K. and.Kumar, A. 2005. Suitability of dyed latex bead agglutination test for immunodiagnosis of Karnal bunt (*Tilletia indica*) teliospores in a single seed of wheat. Food and Agricultural Immunology, 16 (1): 73-81.

Lopez M, (2003) Innovative tools for detection of plant pathogenic viruses and bacteria. Int Microbiology, 6, 233-243.

Lyons and Taylor (1990) Serological detection and identification of bacteria from plants by the conjugated Staphylococcus aureus slide agglutination test. 39 (4), 580-584.

Mishra A, Singh, U.S., Goel R, Kumar, A. 2002. PCR based molecular technique for identification and discrimination of quarantined and non-quarantined Tilletia sps. *Indian Journal of Experimental biology*. 2002; 40: 1137 - 1142.

Munjal, R.L. 1975. Status of Karnal bunt (*N. indica*) of wheat in North India during 1968-69 to 1969-70. *Indian J. Mycol. Plant Pathol*. 5: 185-187.

Notermans, S. and Kamphuis, H. (1990). Food Agricul. Immunol. 2, 37–46.

Rai, G, Kumar, A, Garg, G.K., Singh A and Lakhchaura, B.D. 1998. Development of Microtitre ELISAs for detection and Quantitation of Mycelial Antigens of Karnal, *Indian Journal of Agricultural Biochemistry*, 11, 53-55.

Singh, A.1994. Epidemiology and management of Karnal bunt disease of wheat. Research Bull No. 127, 167 p. Directorate of Experiment Station, G. B. ant Univ. of Agric. & Tech. Pantnagar, India.

Varshney, G.K. 1999. Characterization of genetic races of Karnal bunt (Tilletia indica) of wheat by immunobinding assay procedure. Thesis, M.Sc. G. B. Pant Univ. of Agric. & Tech., Pantnagar.

How to cite this article:

Behl.S *et al*., Need For Rapid and Novel Diagnostic Techniques For Quarantined Fungal Pathogens Of Wheat Such As Karnal Bunt (Tilletia Indica). *International Journal of Recent Scientific Research Vol. 6, Issue, 5, pp.4252-4256, June, 2015*

****