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Review

Biotechnological tools for detection, identification and management of plant diseases

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Detection and identification of plant pathogens is one of the most important strategies for sustainable plant diseases management. For this reason, the availability of fast, sensitive and accurate methods for detection and identification of plant pathogens is increasingly necessary to improve disease control decision making process. In other words, new technologies and improved methods with reduced/fair cost and improved speed, throughput, multiplexing, accuracy and sensitivity have emerged as an essential strategy for the control of both fungal and bacterial diseases. The development of recombinant DNA technology is also possible to isolate individual genes and incorporate resistance genes into otherwise agronomically acceptable cultivars to develop genetically resistance variety for a particular disease. These advances have been complemented by the development of new nucleic acids extraction methods, increased automation, reliable internal controls, multiplexing assays, online information and on site molecular diagnostics. The different types of polymerase chain reaction (PCR) are the most common DNA amplification technology used for detecting various plant pathogens. With the applications of bioinformatics as a modern technology in plant pathology, identification of specific motifs, DNA sequences has become possible, which ultimately increase the accuracy of modern techniques in plant disease diagnosis. The newly emerged proteomic technology is also a promising tool for providing information about pathogenicity and virulence factors that will open up new possibilities for plant disease diagnosis and appropriate protection measures.

Key words: Biotechnology, molecular markers, marker assisted selection, quantitative trait loci (QTL), polymerase chain reaction (PCR), proteomics.

INTRODUCTION

Biotechnology is broadly defined as set of biological techniques developed through basic research and now applied to research and product development. In other words, it is the genetic manipulation and multiplication of any living organism through new technologies resulting in the production of improved and new organism and products can be used in a variety of ways (Agrios, 2005; Fagwalawa et al., 2013). It is an applied science in the field of agriculture and known as agricultural biotechnology. Based on knowledge of DNA, scientists have developed solutions to increase agricultural productivity (Agrios, 2005). New biotechnological tools enhance pathologists' ability to make improvements in crops regarding to their respective diseases. It can be

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applied as genetic engineering, molecular markers, molecular diagnosis and tissue culture (James, 2003).

Chemical control of plant diseases is often non-specific in its effects, killing beneficial organisms as well as pathogens, and it may have undesirable health, safety, and environmental risks. Because of these factors, nowadays, control of plant disease is a subject of great interest for biotechnologists. Biotechnology will enhance our understanding of the mechanisms that control a plant ability to recognize and defend itself against disease causing organisms like fungi (Haggag, 2008) and the future of sustainable agriculture will increasingly rely on the integration of biotechnology with traditional agricultural practices.

Biotechnology is an important discipline for accurate diagnosis of plant disease. Enzyme-linked immunosorbent assays (ELISA) and nucleic acid techniques are used in the identification of fungal, viral and bacterial diseases. New techniques in plant genetic engineering strategies for the management of plant diseases are now available and the application of genetic engineering for plant disease resistance has been discussed and presented by many scholars. The different tools of biotechnology and genomic approaches have enhanced the process of identifying plant pathogens with greater accuracy. Plant genes and their products that interact with plant pathogens have been identified and either inserted into plants or used as specific molecular markers to improve plant breeding for disease resistance. Therefore, acquiring noble information from applied biotechnology to plant-pathogen interactions is providing new knowledge and new approaches to improve plant health, yield and quality of plants contributing to food (Fagwalawa et al., 2013).

Accurate identification and diagnosis of plant diseases are very important for sustainable food security as well as prevention of the spread of invasive pathogens (Balodi et al., 2017). In this regard, special interest should be taken in the early detection of pathogens in plant propagating materials (seeds, mother plants and vegetative propagative plant material) to avoid the introduction and further dispersal of new pathogens in a growing area where it is not present earlier. Therefore, rapid detection and identification of plant pathogens is strongly necessary by using fast, sensitive and accurate methods so as to come to last decision for appropriate control strategies. Some of the most important molecular methods for detection of plant pathogenic disease are the PCR isothermal amplification methods, fingerprinting [restriction fragment length polymorphism [RFLP], random-amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites], DNA hybridization technology and sequencing (McCartney et al., 2003; Barnes and Szabo, 2007; Kang et al., 2010). Successful management of plant diseases is primarily dependent on the accuracy and efficient detection of the pathogens, knowing the amount of genetic and pathogenic variability present in a pathogen population, development of disease resistant cultivars and quantifying disease resistance genes in different epidemiological regions. Beside conventional methods of pathogen detection and breeding resistant cultivars, recent development in molecular biology techniques particularly the advent of various DNA based markers have greatly influenced the plant protection methods. Therefore, the objective of this article is to review the major application of biotechnological tools to detect, identify and control of plant diseases as well as to give an overview of proteomics studies in bacterial diseases.

ADVANCED TOOLS FOR DETECTION AND IDENTIFICATION OF PATHOGENS

Detection protocols used for the diagnosis or quarantine measures should be reproducible, repeatable and should have minimum false results. All molecular detection methods should be sensitive to pathogen concentration, genetic variability within a target pathogen population, and similarities between the target and other organisms (Martin et al., 2016; Balodi et al., 2017).

Bacterial diseases

Detection of pathogenic bacteria in seed and other plant tissues (particularly in latent infections) is challenging because the target bacteria are often irregularly distributed and present as a small component of a much larger bacterial population. Moreover, it is often difficult to distinguish and identify pathogenic bacteria from all the soil-associated and other saprophytic bacteria normally present on plant surfaces. In addition to epiphytic and casual surface contaminants, non-detrimental or beneficial endophytic bacteria may also be present (Punja et al., 2008).

Traditional techniques to detect the presence of pathogenic bacteria involved in field inspection for symptoms and signs of disease as well as laboratory tests (Figure 1). Laboratory procedures for detection of the bacteria may involve grow-out assays, serological tests such as ELISA and immune-fluorescence microscopy. In addition, isolation of the bacteria on selective or semi-selective media is also done. Following isolation, strains need to be characterized by physiological, biochemical and pathogenicity tests. Use of traditional methods is reliable and efficient for some of the bacterial plant pathogens, but for many others they lack adequate sensitivity and specificity. Another major disadvantage is the long times required for grow-out assays, bacterial isolation and pathogenicity tests. Therefore, new and modern molecular techniques are the best option to diagnosis of bacterial pathogens (Punja et al., 2008).

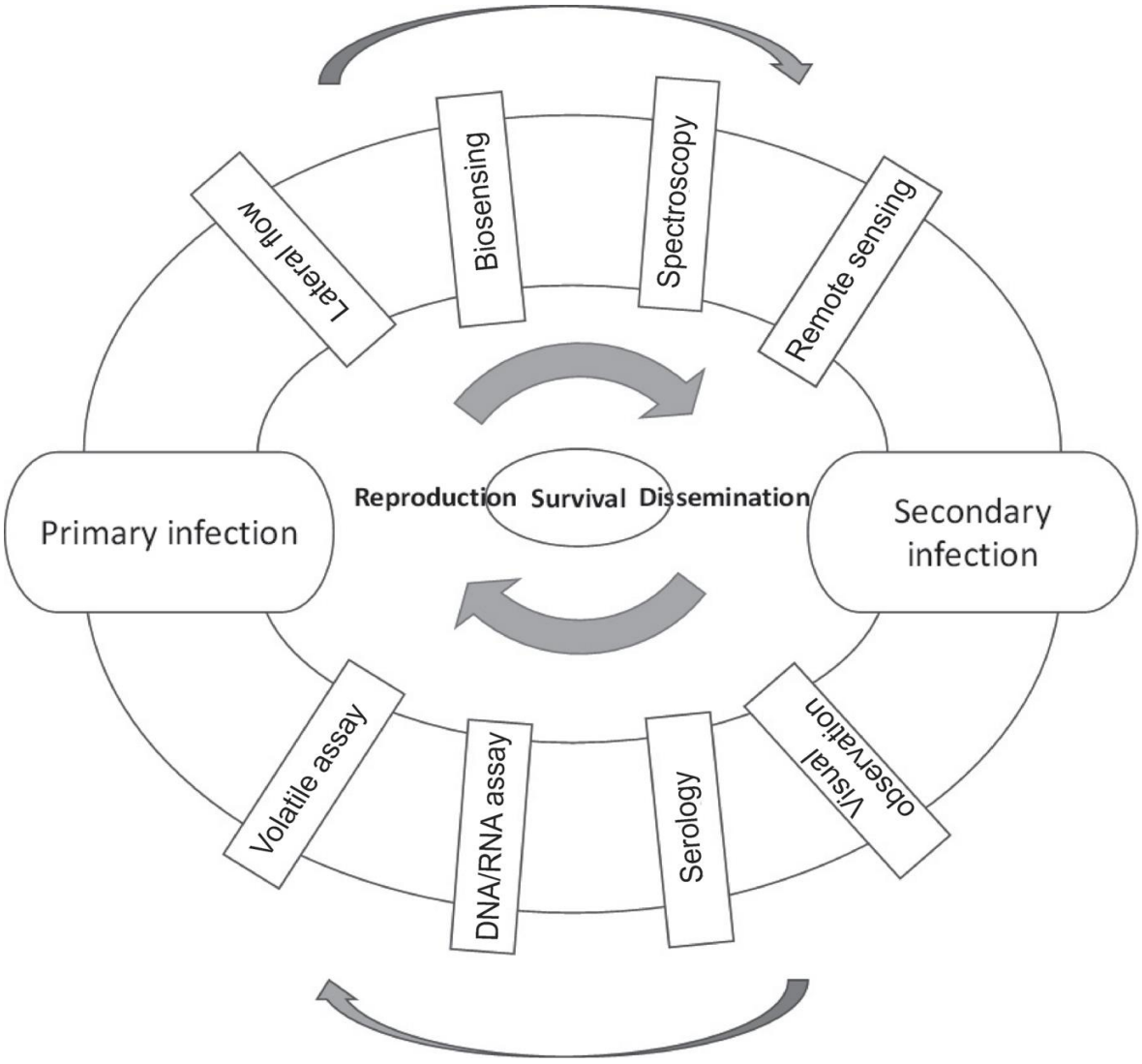

Figure 1. Detection methods of plant pathogens at various stages of disease development (adapted from Balodi et al., 2017).

Nucleic acid (DNA)-based techniques have been demonstrated to be generally more sensitive, specific and reliable for the detection, identification and quantification of bacterial plant pathogens than other methods. Among the nucleic acid-based diagnostic techniques, PCR assay or its variants have been used very widely for detection of bacterial pathogens in pure cultures or in single/multiple infections of plant hosts (Narayanasamy, 2011). For example, a PCR-based assay using the primers from DNA sequences of the phaseolotoxin gene was able to detect efficiently the pathogen *Pseudomonas savastanoi* pv. *phaseolicola*, causing bean halo blight disease, even in the presence of high populations of non-target bacteria. Similarly, *Xanthomonas axonopodis* pv. *manihotis* and *Ralstonia solanacearum* (from soil) can be detected by PCR, on amplifying an 898 and 288-bp fragment, respectively (Alvarez et al., 2008). In another study by Audy et al. (1994), *X. axonopodis* pv. *phaseoli* (*Xap*) was detected in the first time by employing primers from plasmid DNA, in PCR assay which had a detection limit of 10 to 100 fg of *Xcp* DNA (equivalent of 1 to 10 cfu).

To date, real-time PCR seed detection assays have been reported for *Acidovorax avenae* subsp. *citrulli* in watermelon seeds, *R. solanacearum* race 3, biovar 2, in asymptomatic potato tubers, *Xanthomonas arboricola* pv. *pruni* in *Prunus* species and *Xanthomonas oryzae* pv *oryzae* in rice by Real time Bio-PCR (Balodi et al., 2017).

Fungal diseases

Plant pathogenic fungi are the causal agents of the most detrimental diseases in plants, provoking considerable

yield losses worldwide (Aslam et al., 2017). Some of the basic methods used to detect fungal pathogens mostly rely on microscopic, morphological and cultural approaches that require extensive time, labor and classical taxonomy knowledge (Nilsson et al., 2011). Even though, these approaches are the cornerstone of fungal diagnostics, they can lead to the unreliable results due to the problems in weak identification. Therefore, advances in the development of molecular methods, like immunological methods, nucleic acid-based probe technology and PCR technology have provided diagnostic laboratories with powerful tools for detection and identification of phytopathogenic fungi (Aslam et al., 2017). These methods are much faster, more specific, sensitive, and accurate, and can be performed and interpreted by personnel with no specialized taxonomical expertise (Badali and Nabili, 2012). Additionally, these techniques allow the detection and identification of non-culturable microorganisms, and due to its high degree of specificity, molecular techniques can distinguish closely related organisms at different taxonomic levels (Capote et al., 2012). PCR technology includes multiplex PCR, nested PCR, real-time PCR and reverse transcriptase (RT)-PCR and DNA barcoding has been recently used as a molecular tool for detection and identification of fungal pathogens (Table 1).

PCR methods for identification of *Sclerotium rolfsii* and *Colletotrichum capsici* have been developed based on specific sequences of the internal transcribed spacer (ITS) region. Multiplex PCR technique has been used for the simultaneous detection and differentiation of fungal pathogens in different crops like in sunflower (*Podosphaera xanthii* and *Golovinomyces cichoracearum)*, in cedar trees and water samples (*Phytophthora lateralis).* It is also very important for determining the mating type of the pathogens *Tapesia yallundae* and *Tapesia acuformis*; for differentiating two pathotypes of *Verticillun alboatrum* infecting hop and for distinguishing among eleven taxons of wood decay fungi infecting hardwood trees (Guglielmo et al., 2007; Chen et al., 2008; Jeeva et al., 2010; Torres-Calzada et al., 2011; Capote et al., 2012). Padlock probes have been used for the simultaneous detection of *Phytophthora cactorum*, *Phytophthora nicotianae*, *Pythium ultimum*, *Pythium aphanidermatum*, *Pythium undulatum*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *radicis-lycopersici*, *Fusarium solani*, *Myrothecium roridum*, *Myrothecium verrucaria*, *Verticillium dahliae* and *Verticillium alboatrum* in samples collected from horticultural water circulation systems in a single assay (Van Doorn et al., 2009; Capote et al., 2012). ISSR and SRAP markers were also used for molecular characterization of *Venturia inaequalis* isolates for the first time and they were more informative, easily applicable, reproducible, and specific (Kaymak et al., 2016).

A polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) detection tool based on the amplification of the ITS region has been recently applied to detect multiple *Phytophthora* species from plant material and environmental samples (Shamim et al., 2017). Real-time PCR is currently considered the gold standard method for detection of plant pathogens. This technique allows the monitoring of the reaction during the amplification process by the use of a fluorescent signal that increases proportionally to the number of amplicons generated and to the number of targets present in the sample (Alemu, 2014). Another advantage of real-time PCR is the capability to perform multiplex detection of two or more pathogens in the same reaction. *Microdochium nivale* in wheat seeds and *Fusarium circinatum* in pine seed were detected by real time-PCR assay (Balodi et al., 2017). SYBR Green real-time PCR assays can detect so many pathogenic fungi like *Botrytis cinerea, Fusarium oxysporum* f. sp. *vasinfectum, Colletotrichum acutatum, Phoma sclerotioides, Pythium irregular, Rhizoctonia solani, Verticillium dahliae,* etc. (Capote et al., 2012).

APPLICATION OF MOLECULAR MARKERS FOR RESISTANCE BREEDING

Disease resistance in crop plants is a major challenge in plant breeding. Conventional breeding for disease resistance is based on phenotypic identification and crossing with agronomically desirable but susceptible plants. It has made great progress in incorporating natural defense genes. This is performed based on a backcross program which takes more than 7 years to reach the final goal. However, the modern molecular techniques make it possible to use markers and probes to track the introgression of several resistance genes into a single cultivar from various sources during a crossing program. The advent of new biotechnology techniques such as marker-assisted selection provides new opportunities to enhance plant disease resistance (Torres, 2010; Torres-Calzada et al., 2011).

Different types of molecular markers (DNA based markers) have been developed and used for developing of disease resistance varieties. Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeat (ISSR), Microsatellites or Simple Sequence Repeat (SSR), Allele Specific Associated primers (ASAP), Expressed Sequence Tag (EST), Cleaved Amplified Polymorphic Sequence (CAPS), Diversity Arrays Technology (DArT), Sequence Characterized Amplified Regions (SCARs) and Single Nucleotide Polymorphism (SNP) are some of molecular markers and have been used in several crops (Table 2) (Doveri et al., 2008; Singh et al., 2013; Ragimekula et al., 2013). Application of molecular markers for breeding disease resistant varieties is especially interesting when breeding for

Table 1. The advantage and disadvantage of molecular detection and identification tools in fungi.

Molecular tools	Advantages	Disadvantages
Conventional PCR	Gives rapid and precise results when use the primer of specific species	Required much labor and cost
Nested PCR	Use of two sets of primer increase the yield and specificity of amplification of the target DNA	Risk of contamination, because of two cycles of amplification
Multiplex PCR	Time and money saver by using the several pairs of primers in a same reaction	Interference of primers and probes, reduce sensitivity
Reverse transcriptase (RT) PCR	Gives quantitative data about pathogens, more sensitive than conventional PCR	Formation of each assay is time consuming and requires the expensive equipment and the reagents
Real-time PCR (q PCR)	Automated and no need of post amplification analysis	Cost and complexity due to simultaneous thermal cycling and fluorescence detection
In situ hybridization	Maximum use of the short supply tissue	Difficulty in identifying targets that have low DNA and RNA copies
FISH	Can be used for non-dividing cells	Probe-preparing method is very difficult because it is necessary to tailor the probes to identify the particular sequences of DNA
Microarray	Easy to use because it does not require the large-scale DNA sequencing	Large amount of mRNA is required
LAMP	Rapid, sensitive and highly specific	Primer design is complex; recognizes only one specific pathogen; risk of sample contamination
NASBA	Expensive equipment is not needed. Better than RTPCR	Specificity of the reactions is dependent on thermolabile enzymes. Reaction temperature cannot be exceeded than 42°C without compromising it
RNA interference (RNAi)	Ability to simultaneously interrogate thousands of genes	Variability and incompleteness of knockdowns and the potential non specificity of reagents
Northern blotting	Detection of RNA size	Applied only on a small sample of the genes
SAGE	Prior knowledge of the subject's genome is not requisite	Specificity of tag sequence
RNA-Seq	Increased specificity and sensitivity	Needs expensive equipment. Bioinformatics knowledge requisite for data analysis

resistance traits that are difficult or expensive to assess phenotypically.

Resistance to *Turcicum* leaf blight in sorghum accession G-118 was found to segregate as a single dominant trait in a cross with susceptible cultivar HC-136. By using SSR markers coupled with bulk segregant analysis, a molecular marker linked to the locus for resistance to *Turcicum* leaf blight was identified (Mittal and Boora, 2005). In the same population, an SSR marker, Xtxp 309, produced amplification of a 450 bp band. This was found to be located at a distance of 3.12 cM away from the locus governing resistance to leaf blight which was considered to be closely linked and 7.95 cM away from the locus governing susceptibility to leaf blight (Mittal and Boora, 2005). By the RAPD technique with bulk-segregant analysis, it was possible to identify

Table 2. Some applications of molecular marker assisted selection (MAS) in four crops.

Character/Trait	Target genes	Types of marker used	References
Rice			
Bacterial blight resistance	*xa5, xa13 & Xa21*	CAPS for *xa5* (RG556+DraI), CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248)	Sundaram et al. (2008)
Bacterial blight (BB) resistance + Grain quality	*xa13 & Xa21*	CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248)	Gopalakrishnan et al. (2008)
Bacterial blight (BB) resistance	*Xa4, X17 & Xa21*	STS for *Xa4 & Xa7,* STS for *Xa21* (pTA248)	Perez et al. (2008)
Bacterial blight (BB) resistance	*xa5* and *xa13*	CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248)	Sundaram et al. (2009)
Blast resistance	*Pi-9(t)*	*pB8*	Wen and Gao (2011)
Bacterial blight (BB) resistance	*Xa4, xa5, xa13 & Xa21*	STS for Xa4, CAPS for *xa5* (RG556+DraI), CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248)	Shanti et al. (2010)
Bacterial blight (BB) resistance + Blast resistance	*xa5, xa13, Xa21 &Pi25*	CAPS for *xa5* (RG556+DraI), CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248) and STS for *Pi25* (SA7)	Zhan et al. (2012)
Bacterial blight (BB) resistance + Blast resistance + sheath blight (ShB)	*xa13, Xa21, Pi54 & qSBR11-1*	CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248), SSR for *Pi54* (RM206), SSR for *qSBR11-1* (flanking markers RM224 and RM7443)	Singh et al. (2012)
Bacterial blight (BB) resistance	*xa13 & Xa21*	CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248)	Pandey et al. (2013)
Bacterial blight (BB) resistance + Blast resistance	*Xa21& Pi54*	STS for *Xa21* (pTA248), SSR for *Pi54* (RM206)	
Bacterial blight (BB) resistance	*Xa4, xa5, xa13 & Xa21*	STS for Xa4, CAPS for *xa5* (RG556+DraI), CAPS for *xa13* (RG136+HinfI), STS for *Xa21* (pTA248)	Dokku et al. (2013)
Barley			
Barley yellow mosaic virus I-III	-	RFLP	Okada et al. (2003)
Resistance to cereal cyst nematode	-	RFLP	Barr et al. (2000)
Barley stripe rust	-	RFLP, RAPD, AFLP	Hayes et al. (2003)
Barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2)	-	RAPD, SSR, STS	Werner et al. (2005)
Resistance to BYDV	-	CAPS, SSR, STS	Scholz et al. (2009)

Table 2. Contd.

Wheat			
Fusarium head blight (FHB) resistance	-	SSR	Miedaner et al. (2006)
Fusarium head blight QTL	-	SSR	Wilde et al. (2008)
Leaf rust (*Puccinia triticina*) resistance gene *Lr47*	-	SSR	Bainotti et al. (2009)
Powdery mildew (*Erysiphe graminis* f.sp. *tritici*) resistance genes	-	RFLP	Liu et al. (2000)
Soybean			
Soybean mosaic virus (SMV)	-	SSR	Shi et al. (2009)
SCN resistance	-	SSR	Arelli et al. (2007)
Resistance to frogeye leaf spot (Cercospora sojina)	-	SNP, SSR	Shannon et al. (2009)

PCR amplification products segregated with the response to Turcicum leaf blight (Boora et al., 1999). A three-gene pyramid line of rice was identified by MAS to possess broad-spectrum bacterial blight resistance and excellent grain quality (Sundaram et al., 2008). Pandey et al. (2013) improved the two traditional bacterial blight (BB) susceptible rice varieties through the strategy of limited marker-assisted backcrossing for introgression of two major BB resistance genes coupled with phenotype-based selection for improvement of their plant type and yield. Generally, many scholars identified new QTLs related to resistance genes, however, very few of them reported (shown in Table 2) have been used for MAS in breeding programs. Manulis et al. (1994) were able to identify the specific banding patterns that were subsequently used as probes to distinguish between the races of the carnation wilt fungal pathogen F. oxysporum f. sp. dianthi by using RAPDs. In another study, RAPD markers were used to infer the genetic relationships among the wheat bunt fungi. Globally, RAPD markers are also reported to be useful in diagnostic studies of many plant pathogens (Singh et al., 2013). Billard et al. (2012) also identified fungal pathogen using ASAP marker system. In addition, they also identified resistance gene by molecular markers, knowing the genetic variability and diversity of the disease causing agents are also imperative. Kaymak et al. (2016) used RAPD, ISSR, SSR and SRAP markers to evaluate the genetic divergences and the relationships of Venturia inaequalis isolates. They reported that SSR and SRAP markers were found to be more informative and consistent than other marker techniques during their study.

PROTEOMIC STUDIES AS A TOOL IN PATHOGENIC BACTERIA

Proteomics is the large-scale study of the whole set of proteins present in a cell, tissue or organism at a specific time point under specific conditions. In recent years, proteomics has played a key role in identifying changes in protein levels in plant hosts upon infection by pathogenic fungal and bacterial organisms and in characterizing cellular and extracellular virulence and pathogenicity factors produced by pathogens (Lodha et al., 2013). Mehta and Rosato (2001) reported the analysis of X. axonopodis pv. citri cultivated in the presence of the host Citrus sinensis leaf extract and recognized differentially expressed proteins, including a sulfate-binding protein, by NH2 terminal sequencing. The same authors suggested that the induction of this enzyme may have been caused by the amino acids or different sugars present in the leaf extract. Tahara et al. (2003) also analyzed the expressed proteins of X. axonopodis during interaction with the host *Passiflorae edulis* leaf extract, and identified an inorganic pyrophosphatase and an outer membrane protein up regulated in the presence of leaf extract, also by NH2 terminal sequencing. It was proposed that the protein which was identified in outer membrane may have an important role in pathogenicity (Tahara et al., 2003).

In a 2DE-mediated proteomic study of Xylella fastidiosa, the causal agent of citrus variegated chlorosis, showed that this pathogen did not produce significant changes in heat shock protein expression when compared with X. axonopodis pv. citri (Martins et al., 2007). However, it was found that X. fastidiosa constitutively expressed several stress-inducible proteins such as heat

shock protein A (HspA) and GroeS, which were induced in *X. citri* under stress conditions. The authors suggested that the constitutive expression of these proteins may help *X. fastidiosa* cope with sudden environmental changes and stresses.

With regard to plant defense responses, direct evidence of the involvement of target proteins has also been provided by proteomic studies (Table 3). Although few of the reports outlined clearly show the importance of proteomic approaches, which can aid significantly in the understanding of plant bacterium interactions. A detail understanding of plant defense response using successful combination of proteomic techniques is needed for practical application to secure and stabilize yield of many crop plants (Lodha et al., 2013). Jones et al. (2004) analyzed the proteomic and transcriptomic profiles of *Arabidopsis thaliana* leaves during early responses (1 to 6 h post-inoculation) to the challenge by *Pseudomonas syringae* pv. *tomato*. They compared the proteomic changes in *A. thaliana* in response to the *P. syringae* pv. *tomato* highly virulent strain DC3000, which results in successful parasitism, a DC3000 hrp mutant, which induces basal resistance, and a trans-conjugant of DC3000 expressing avrRpm1, which triggers a gene-for-gene-based resistance. As a follow-up study, Jones et al. (2006), examined the global proteomic profile in three sub-cellular fractions (soluble protein, chloroplast and mitochondria enriched) of *A. thaliana* responding to the same three *P. syringae* pv. *tomato* DC3000 strains. This was the first report to associate post-translational events (1 to 6 h post-inoculation) occurring before significant transcriptional re-programming. The results showed that several chloroplast systems are modified during all aspects of the defense response.

Many study was conducted between rice and bacterial association, some are pathogenic and cause severe damages to the crop, such as *X. oryzae* pv *oryzae*, *Burkholderia glumae, Burkholderia kururiensis* and *Pseudomonas fuscovaginae.* Among them, *B. kururiensis* is very often isolated from rice and has been studied recently for its potential beneficial effects on the plant and the mechanisms of interaction (Suarez-Moreno et al., 2012). The role of defense responsive proteins in the *X. oryzae* pv. o*ryzae* interaction was investigated through a proteomic approach (Mahmood et al., 2006). Cytosolic and membrane proteins were fractionated from the rice leaf blades 3 days post-inoculation with incompatible and compatible *X. oryzae* pv. *oryzae* races. From 366 proteins analyzed by 2DE, 20 were differentially expressed in response to bacterial inoculation. Analyses clearly revealed that the four defense related proteins [PR-5, probenazole-inducible protein (PBZ1), superoxide dismutase (SOD) and Prx] were induced for both compatible and incompatible *X. oryzae* pv. *oryzae* races, wherein PR-5 and PBZ1 were more rapid and showed higher induction in incompatible interactions and in the presence of jasmonic acid (JA). Study in the same rice *X. oryzae* pv. *oryzae* interaction, Chen et al. (2007) analyzed proteins from rice plasma membrane to investigate the early defense responses proteins involved in XA21-mediated resistance. XA21 is a rice receptor kinase, predicted to perceive the *X. oryzae* pv. *oryzae* signal at the cell surface, leading to the 'gene-for-gene' resistance response. At the same time, a total of 20 proteins were differentially regulated by pathogen challenge at 12 and 24 h post-inoculation and identified at least eight putative plasma membrane-associated and two non-plasma membrane-associated proteins with potential functions in rice defense were observed by the same authors.

By comparing two partially resistant lines and a susceptible line in a time course (72 and 144 h post-inoculation) experiment, proteins from the wild tomato species *Lycopersicon hirsutum* that are regulated in response to the causal agent of bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) were identified. Twenty six differentially regulated tomato proteins were identified by using 2DE and ESI-MS/MS, 12 of which were directly related to defense mechanisms.

Proteomic analysis was also used to detect the responses of the model legume *Medicago truncatula* to the pathogenic bacterium *Pseudomonas aeruginosa* in the presence of known bacterial quorum sensing signals, such as N-acyl homoserine lactone (AHL) (Mathesius et al., 2003). To make appropriate responses to the pathogen, the fast and reliable detection of bacterial AHL signals by plant hosts is essential. Therefore, *M. truncatula* is able to detect very low concentrations of AHL from *P. aeruginosa* and response in a global manner by significant changes in the accumulation of 154 proteins, 21 of which are related to defense and stress responses.

CONCLUSIONS

The science of plant disease**s** diagnostics and management has progressed in technological advancements from the visual inspection of signs and symptoms of diseases to molecular level detection of the pathogens. Currently, precise identification and diagnosis of plant pathogens to the species or strain level, information during their early stages of infection, and better understanding of pathogenicity factors are the crucial prerequisite for disease surveillance and development of novel disease control strategies. Therefore, the advancements in the field of plant pathology, coupled with biotechnology, bioinformatics and molecular biology have opened new avenues for development of specific and sensitive procedures of diagnosis. And currently more sensitive methods like Real Time PCR and Microarrays are being used. Genetic engineering is also one of the potential tools to provide an abundance of beneficial plant traits, particularly an

Table 3. Some examples of proteins expressed in plant-bacterial interactions and identified in plants using proteomic approaches (Jones et al., 2004, 2006; Mahmood et al., 2006; Chen et al*.,* 2007).

Protein	Studied organism	Pathogen
lutathione S-transferase	*A. thaliana*	*P. syringae*
Peroxiredoxin	*A. thaliana*	*P. syringae*
Peroxiredoxin, chloroplast	*O. sativa*	*X. oryzae pv. oryzae*
Glyceraldehyde 3-phosphate dehydrogenase	*O. sativa*	*X. oryzae pv. oryzae*
Triosephosphate isomerase, cytosolic (EC 5.3.1.1)	*O. sativa*	*X. oryzae pv. oryzae*
Thaumatin-like protein X	*O. sativa*	*X. oryzae pv. oryzae*
Superoxide dismutase	*O. sativa*	*X. oryzae pv. oryzae*
Alcohol dehydrogenase 1 a	*O. sativ*	*X. oryzae pv. oryzae*
Quinone reductase	*O. sativa*	*X. oryzae pv. oryzae*
Prohibitin	*O. sativa*	*X. oryzae pv. oryzae*
Ascorbate peroxidase	*O. sativa*	*X. oryzae pv. oryz**ae***
Remorin 1	*L. hirsutum*	*C. michiganensis* subsp *michiganensis*
Ascorbate peroxidase	*L. hirsutum*	*C. michiganensis* subsp. *michiganensis*
Glutathione S-transferase	*L. hirsutum*	*C. michiganensis* subsp *michiganensis*
Pathogenesis-related 3 (endochitinase precursor)	*L. hirsutum*	*C. michiganensis* subsp *michiganensis*

enhanced ability to withstand or resist attack by plant pathogens. In addition, developments in DNA marker technology together with the concept of marker-assisted selection provide new solutions for selecting and maintaining desirable genotypes. Marker assisted selection can be performed in early segregating populations and at early stages of plant development for pyramiding the resistance genes, with the ultimate goal of producing varieties with durable or multiple disease resistance. Generally, a timely detection of resistance levels in populations of phytopathogenic pathogens in a field would help the growers formulate proper decisions on resistance management programs to control plant diseases. Finally, future studies will be focused on the practical application of each traditional or innovative method, their cost and availability of instruments, specialization level, rapidity of analysis, and the stage of disease at which detection is possible.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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