Evolving Paradigms in Biotechnology for Management of Crop Diseases

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ABSTRACT

Disease management of crops significantly contributes to increase in food production to keep pace with the growing human population. The applications of recent developments in biotechnology are imperative for detection, identification, prevention and control of plant diseases. The use of biotechnology also opens up the possibility to produce disease free plants by tissue culture, besides conferring resistance against several diseases that affect important crops through conventional breeding and/or genetic engineering. Although the debate about various facets of biotechnology often circle around the economics, risks and consequences associated with use of various approaches, but what matters most is how they are used.

Highlights

- The review provides application and overview of biotechnology for priming disease management of crops.

Keywords: Biotechnology, disease diagnosis, genetic engineering, plant diseases, resistance, tissue culture

Protection of crops against plant diseases contributes significantly in meeting the growing demand for food quality and quantity. Crop losses due to pests and pathogens have both short and long-term negative consequences in terms of crop losses, food availability, public health, environments, trade, economics and livelihood of farmers. Disease management practices can contribute to sustainability by protecting crop yields, maintaining and improving profitability for crop producers, reducing losses along the distribution chain, and reducing the incomprehensible environmental impacts of diseases and their management (Vincelli 2016). Traditionally, control of plant diseases has been either attempted by using conventional plant breeding to develop cultivars resistant to various diseases or usually handled with applications of chemicals, which is often non-specific in its effects, killing beneficial organisms as well as pathogens. The process of development of disease resistant cultivars is time-consuming and requires availability of large base of genetic resources for most of the crops for continued improvement, besides losses of gene pools during the process of domestication and plant breeding have restricted its application (Mehrotra and Aggarwal 2003). Further, it is well established that the crops introduced to a new area far from their centers of origin may be poorly equipped to resist pathogenic organisms of the introduced area as they may be far from the pathogens that have coevolved with them(Strange and Scott 2005). As a result, they are unlikely to have evolved resistance to new strains of the pathogen that may have subsequently arisen in the center of origin. Similarly, chemical control of diseases may have undesirable effects on health, safety and cause environmental risks. In this context, the developments in Plant biotechnology have ushered in a new era for agricultural scientists working to maintain disease free plants, increase
crop yields, and minimize usage of chemicals to achieve sustainability goals. Biotechnological applications of plant cell and tissue culture, serology and molecular biology are contributing to plant disease control through diagnosis of existing and emerging diseases, development of disease resistant or pathogen free plants, production of biological control agents and interpretation of host-parasite relations. The paper reviews in brief the major developments and application of biotechnology in management of plant diseases.

CURRENT METHODS OF DISEASE DIAGNOSIS

Advanced disease detection and prevention in crops are vital to minimize the disease induced damage in crops during growth, harvest and post-harvest processing, maximize productivity and ensure sustainable production. Monitoring plant health and detecting pathogen could be realized either through direct or indirect methods. Traditional molecular methods of disease detection comprise serological assays and nucleic acid based techniques that could be used for high-throughput analysis when large numbers of samples need to be analyzed. The disease causing pathogens such as bacteria, fungi and viruses may be directly detected by these methods. Many of these techniques were first developed in the medical sciences before being applied to the plant sciences including plant pathology. On the other hand, indirect methods rely on parameters like change in morphology, temperature, transpiration rate and volatile organic compounds released by infected plants for identification of diseases.

Serological diagnostic techniques

Serological assays were initially developed to detect viruses as they cannot be cultivated ad hoc. However, more than a thousand other pathogens, bacteria, and fungi (Caruso et al. 2002; Martinelli et al. 2015) can now be detected using polyclonal and monoclonal antiserum, and techniques like enzyme-linked immunosorbent assay (ELISA), western blots, immunostrip assays, dot-blot immune-binding assays, and serologically specific electron microscopy. Diagnosis using serological methods has many advantages. Although antibodies may take several weeks to produce, they are generally stable for long periods, if stored correctly and produce results quickly. They have wide application for general and specific recognition of unique epitopes of many micro-organisms but have been under-utilized in the diagnosis of plant pathogens other than viruses. Tests using antibodies have improved greatly. They are now suitable for both laboratory and field conditions, can identify strains within species with sensitivity to the nano-gram level and take less time to carry out.

Serology has found many uses in the detection and identification of viruses but less so with other pathogens since relatively more complex composition of these organisms makes the identification of specific antibodies difficult. However, the Nucleic acid based methods now have much wider application for all plant pathogens.

Enzyme-linked immunosorbenent assay (ELISA)

Amplification through the binding of an enzyme to the antibody has led to the development of a large range of sensitive methods for pathogen diagnosis. Among various immunodiagnostic techniques, the double-antibody sandwich (DAS) ELISA is by far the most widely used molecular technique because of its low cost and high throughput potential for identification of diseases based on antibodies. It also has the advantage of using visual colour change as a result of interaction between the substrate and the immobilized enzyme for disease detection. However, the sensitivity of ELISA varies depending on the organism, sample freshness, and titre; for instance, it has relatively low sensitivity for bacteria ($10^5-10^6$ CFU/mL), which can now be detected at 100 CFU/mL (Schaad et al. 2001; Schaad et al. 2003). Antibodies need to be stored at lower than $-20^\circ$C and cannot be defrosted several times. Polyclonal antisera for many viruses and bacteria have been developed for commercial use or research labs and have been used in numerous protocols (Nolasco et al. 2002), but their frequent cross-reactivity inspired the development of more effective monoclonal antisera using hybridoma technology (Holzloehner et al. 2013), with cell lines with specificity to single epitopes. To date, ELISA procedures using both polyclonal antibodies are available for numerous taxa of phyto-pathogenic bacteria; likewise, rapid detection kits are commercially available. Monoclonal antibodies recognize one epitome only and are generally more expensive.
Polyclonal antibodies recognize multiple epitomes on an individual antigen and are less expensive. This method has good reliability, although time consuming with low potential for spatialization and possibility of false negatives.

**Immuno-fluorescence microscopy**

The immuno-fluorescence (IF) based optical technique can be utilized to detect pathogen infections in plant tissues. For this technique, plant samples are fixed to microscope slides in thin tissue sections and the detection is achieved by conjoining a fluorescent dye to the specific antibody to visualize the distribution of target molecule in the sample (Ward et al. 2004). A significant problem with the use of IF is photo-bleaching that results in false negative results. However, the decrease of sensitivity due to photo-bleaching can be controlled by reducing the intensity and duration of light exposure, increasing the concentration of fluorophores, and use of more robust fluorophores.

**Nucleic acid based detection**

Nucleic acids are responsible for information content and transfer in all organisms. All molecular methods for detecting plant pathogens are based on the accurate design of oligonucleotides and probes. Nucleic acid-based methods can distinguish the class, family, genus, species and even pathotype of a pathogen. These pathogen detection techniques are either DNA based like fluorescence in situ hybridization (FISH) and the many PCR variants (PCR, nested PCR (nPCR), cooperative PCR (Co-PCR), multiplex PCR (M-PCR), real-time PCR (RT-PCR), and DNA fingerprinting) or RNA based (reverse transcriptase-PCR, nucleic acid sequence-based amplification (NASBA), and AmpliDet RNA). All of these methods can overcome uncertain diagnosis or pathogen taxonomy, enabling a rapid and accurate detection and quantification of pathogens (López et al. 2009). Modern techniques for analysing nucleic acids are so sensitive that under ideal conditions, a single cell can be detected and identified and in the case of genes such as the ribosomal DNA genes, which are present in many copies in the genome of a single cell, less than one cell may be necessary for detection and identification.

**Fluorescence in situ hybridization (FISH)**

Hybridization refers to the hydrogen bonding of complementary sequences of DNA and/or RNA to form a stable duplex molecule. Hybridization methods also allow the amount of similarity between the nucleic acids of related pathogens to be estimated so that sequence similarities can be recognized without sequencing being carried out. The components of hybridization-based diagnostic systems are nucleic acid target, a nucleic acid probe and a method to detect hybridization between probe and target.

The fluorescence in-situ hybridization (FISH) technique has the advantage of high sensitivity (10^3 CFU/mL) and is applied for bacterial detection in combination with microscopy and hybridization of DNA probes and target gene from plant samples (Kempf et al. 2000). Due to the presence of pathogen-specific ribosomal RNA (rRNA) sequences in plants, recognizing this specific information by FISH can help detect the pathogen infections in plants. In addition to bacterial pathogens, FISH could also be used to detect fungi and viruses and other endosymbiotic bacteria that infect the plant (Hijri, 2009; Kliot et al. 2014). The high affinity and specificity of DNA probes provide high single-cell sensitivity in FISH. In addition to the detection of culturable microorganisms responsible for plant diseases, this technique could also be used to detect unculturable or yet-to-be cultured organisms to investigate complex microbiome. However, FISH also has some disadvantages like false positive results with auto-fluorescence materials that often lowers the specificity that limit the technique's potency for plant disease detection. Besides, insufficient penetration, higher order structure of target or probe (e.g., three-dimensional rRNA, loop and hairpin formation and rRNA-protein interactions), low rRNA content, photo-bleaching etc. could also cause false negative results and may compromise the limit of detection (Fang and Ramasamy 2015).

**Polymerase chain reaction**

The limitations of the hybridization approach have largely been overcome by the polymerase chain reaction (PCR). In the short time since its inception, PCR has become an almost indispensable part of
medical and diagnostic sciences. It is now being widely used for the detection of plant pathogens as well. In addition to the basic PCR technology used for pathogens with DNA as their nucleic acid, advanced PCR methods, such as reverse-transcription PCR (RT-PCR) has also been used for identification of plant pathogen (López et al. 2009). Although RT-PCR and the traditional PCR both produce multiple copies of particular DNA isolates through amplification, the applications of the two techniques are fundamentally different. The traditional PCR is used to exponentially amplify target DNA sequences whereas RT-PCR is used to clone expressed genes by reverse transcribing the RNA of interest into its DNA complement through the use of reverse transcriptase. Subsequently, the newly synthesized cDNA is amplified using traditional PCR. Nested polymerase chain reaction (Nested PCR) is a modification of polymerase chain reaction intended to reduce non-specific binding in products due to the amplification of unexpected primer binding sites. Nested polymerase chain reaction involves two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product. This allows amplification for a low number of runs in the first round, limiting non-specific products. The second nested primer set should only amplify the intended product from the first round of amplification and not non-specific product. This allows running more total cycles while minimizing non-specific products. This is useful for very rare templates or PCR with high background. Multiplex (M) PCR, a variant of PCR, enables simultaneous detection of different DNA or RNA by running a single reaction. Although The Real-time PCR,now known as quantitative polymerase chain reaction (qPCR), measuring the amplification of DNA using fluorescent dyes,is most practical for plant virus detection, although the platforms have been used also for on-site, rapid diagnosis of plant diseases based on the bacterial, fungal and viral nucleic acids, it (Bustin et al. 2009; Lievens et al. 2006; Schaad and Federick 2002). The PCR technique may provide high sensitivity and specificity due to the fidelity of DNA amplification, but it is also limited by lack of operational robustness. PCR depends on the efficacy of DNA extraction and the performance is affected by inhibitors present in the sample assay, polymerase activity, PCR buffer and concentration of deoxynucleoside triphosphate (Van der Wolf et al. 2001). In addition, application of PCR for pathogen detection requires designing a primer to initiate DNA replication, which could limit the practical applicability of this technique for field sampling of diseases (Schaad and Federick 2002).

**DNA finger printing**

DNA fingerprinting usually used for identification of individuals based on unique patterns (polymorphisms) in their DNA, was adapted to detect plant disease. Different DNA fingerprinting methods use either restriction fragment length polymorphism (RFLP), PCR, or both, to target areas of DNA with known variations in single nucleotides (single-nucleotide polymorphisms (SNP), short tandem repeats (STR), or other repeating polymorphic regions. Several finger printing methods are in use; most use PCR for fragment detection. They include pulsed-field gel electrophoresis (PFGE), RFLP, random amplified polymorphic DNA (RAPD), repetitive-sequence PCR (rep-PCR), amplified 16S ribosomal DNA restriction analysis (ARDRA) (Scortichini et al. 2001), and amplified fragment length polymorphism (AFLP) (Manceau and Brin 2003). The choice of technique depends on the application, such as identification, DNA marker mapping, the organism under study, and the plant/pathogen system. Ideally, a fingerprinting technique should require no prior investment in sequence analysis, primer synthesis, or characterization of DNA probes. Presently, AFLP analysis is one of the most discriminating genomic methods to detect genomic restriction fragments by PCR amplification, and can be used for DNA of any origin or complexity. Fingerprints are produced without prior sequence knowledge using a limited set of generic primers. The AFLP technique is robust and reliable because stringent reaction conditions are used for primer annealing: the reliability of RFLP is combined with the power of PCR. To reduce the complexity of the original AFLP technique, the protocol was recently modified by the introduction of fluorescent dye-labeled primers and automated DNA sequencers for data capture. This improved method, Fluorescent Amplified Fragment Length Polymorphism (fAFLP), has been used successfully to identify and/or type bacterial species (Manceau and Brin 2003; Cirvilleri et al. 2007 a, b; Martinelli et al. 2015).
Nucleic acid sequence-based amplification (NASBA) is commonly used to amplify RNA sequences. It was developed in the early 1990s for continuous amplification of nucleic acids in a single mixture at a single temperature. NASBA does not require a thermal cycler, only a water bath. This technique has been used to detect viruses (Klerks et al. 2001, Olmos et al. 2005) and bacteria (Rodriguez-Lázaro et al. 2006; Scuderi et al. 2010). As NASBA amplifies only RNA, so no DNase treatments are needed. This technique can selectively amplify mRNA sequences in a background of genomic DNA and can be used to specifically detect viable cells. The use of a hybridization device system (i.e., Hybrimax, Hybrio Limited) can simplify the time and reagents employed to develop the amplicons (Olmos et al. 2005). Target sequences were detected with good speed and specificity (Martinelli et al. 2015).

Loop-mediated isothermal amplification (LAMP) detects amplicons via photometry for solution turbidity (Mori et al. 2001). With SYBR Green, a color change can be seen without equipment. LAMP can be used easily as a simple, rugged screening assay and eliminates the need for expensive thermocyclers. It has been widely used to detect plant viruses, besides several bacterial and fungal pathogens such as Plum Pox Virus (Varga and James 2006; Martinelli et al. 2015). The technique has the potential to be used as screening method even at the field level.

**DNA micro-arrays**

DNA arrays caused a revolution in nucleic acid detection in the early 1990s. Further, improved image scanning allowed support miniaturization and increased spot density in the last few years. The resulting “microarrays” or chips are only a few square centimeters and can include hundreds of thousands of probes representing an organism’s entire genome or transcriptome. Oligo DNA microarrays have been used extensively for disease detection in plants, using different oligo-lengths and techniques to print the spots. Microarrays can also multiplex diagnosis of multiple pathogens. The technique allows different steps in molecular diagnosis such as nucleic acid extraction, PCR reactions, and detection to be performed directly on the array (Lui et al. 2007; van Doorn et al. 2007), with the potential for automation.

It must be understood that many factors drive the choice of a molecular technique: the available budget, time of analysis, and the number of investigated species (López et al. 2009). Such methods being highly efficient and specific have weaknesses too that includes uneven pathogen distribution inside plants, particularly woody perennials, can render molecular tools unreliable, particularly at pre-symptomatic stages. In materials such as seeds, insect vectors, water, and soil, pathogen titers are often below the sensitivity limit of these methods that is usually 10–100 pg. There are also chances of false negatives to occur when the DNA target sequence is degraded or reagents are of insufficient quality. Small sample sizes may misrepresent the real situation and sample cross-contamination can give false positives. The sensitivity of PCR-based methods is often lower than expected due to inhibitors of transcriptases and/or polymerases. PCR can amplify nonspecific products and artifacts due to mispriming or primer dimer formation and dead pathogens can give nonspecific results, which is particularly relevant when analyzing quarantine pathogens. It is desirable that the cost of equipment and reagents used must be considered while selecting a molecular detection method. In applications where total absence of a pathogen is required, such as quarantine, it may be prudent to use a number of tests in conjunction with one another.

**Nano Diagnostics**

Nanotechnology is one of the most fascinating and rapidly advancing sciences and possesses potential to revolutionize many disciplines of science, technology, medicine and agriculture. Current technologies, such as quantitative polymerase chain reaction (qPCR), require a relatively large amount of target tissue, time consuming, lack desired sensitivity and rely on multiple assays to accurately identify distinct plant pathogens. Consequently, developing low-cost methods to improve the accuracy and rapidity of diagnosis of plant pathogens is needed. Nanotechnology, nanoparticles and quantum dots (QDs) have emerged as essential tools for fast detection of a particular biological marker with extreme accuracy. Biosensor, QDs, nanostructured platforms, nanoimaging and nanopore DNA sequencing tools have the potential
to raise sensitivity, specificity and speed of the pathogen detection, facilitate high-throughput analysis, and to be used for high-quality monitoring of crop protection. Furthermore, nano-diagnostic kit equipment can easily and quickly detect potential serious plant pathogens and toxins, allowing experts to help farmers in the prevention of epidemic diseases. Such an accurate technology may help to design a proper integrated disease management system which may modify crop environments to adversely affect crop pathogens. A good example is the micro-PCR where 40 cycles of PCR can be performed in less than 6 minutes. In the near future, nano-scale devices with novel properties could be used to identify plant health issues before these become observable to the grower. Such devices may be capable of responding to special situations, identifying the problem and taking an appropriate disease management action. In this way, nano-smart devices will act as both a protective and an early warning system. During the next decade, nano-devices, which can make thousands of measurements speedily and very cheaply, will become available. Future prospects in plant disease diagnostic will continue in miniaturization of biochip technology to the nano-scale range. Specific nano-devices and DNA nano-devices could enable accurate tracking, detection and diagnosis of plant pathogens in the early stages of plant disease (Khiyamia et al. 2014).

TISSUE CULTURE FOR PRODUCTION OF DISEASE FREE PLANTS

Tissue culture has been playing a significant role for rapid propagation of disease free plants of vegetative propagated crops, especially in horticultural crops in many countries. Among these crops potato, sweet potato, banana and citrus are the major crops being propagated intensively to produce disease free planting materials. Tissue culture technology makes it also possible to produce a large number of disease-free and uniform plants of medicinally important species. Such plants can be used for the extraction of medicinally important compounds, or for pharmacological studies. Many research works have being conducted on the potential of in vitro plantlets over conventional one on productively per unit area particularly in horticultural crops. Almost all research results revealed that in vitro plantlets significantly perform better than conventional in terms of uniformity, earliness, yield, and quality due to free from disease load (Habtamu and Mohammed 2016). This technique has made available different unique commercial plant species such as medicinal, ornamentals and foliages in large scale, which were not produced earlier by the conventional methods. They can also help in germplasm conservation, and can be reintroduced into protected habitats such as National Parks. Pathogen-free plants maintained under in- vitro conditions can also be used for the safe exchange of germplasm across national borders. The first limiting factor of using in vitro plantlets from the point of view of farmers is the higher price of the material if compared to conventional. TC plants require additional care and improved management. Since they have no nutrient reserves when transplanted, external stress is particularly harmful in the first months after transplanting and most of the pathogens are removed in the tissue culture procedure, viruses can still be transmitted through in vitro plants in some crops like banana (Singh and Shetty 2011). However, the higher cost is justified by high economic returns. In addition, the technology has created several employment opportunities and opened up many entrepreneurial fields.

Almost all tissue culture techniques are used in plant pathology. The propagation from meristematic tissue generally provides a method of cleaning up material from viruses and other systemic pathogen infections. In-vitro micro-propagation is used for rapid and true to type multiplication of plants on artificial nutrient media under controlled environment. Micro propagation is the most commercially exploited area of plant tissue culture, having been widely used for production of quality planting material in vegetative propagated species (Singh and Shetty 2011). The most significant advantages offered by micro propagation are large numbers of disease free propagates obtained from a single plant in a short period, besides convenience of year-round propagation, requirement of small space and reduced labour costs (Mtui 2011). According to Chadha and Choudhary, 2010, various steps involved in production of pathogen free plantlets by meristem tip culture include testing of parent material for the presence of viruses and similar pathogens, thermotherapy/chemotherapy
of parent material in the absence of disease-free material, excision of meristem tip under aseptic conditions, culture of apical dome plus one or two leaf primordia on suitable medium to produce plantlets, indexing of plantlets for presence or absence of viruses, plantlets transferred to soil, maintenance of pathogen free nuclear plant stocks, meristem culture is then followed by in-vitro mass propagation of the virus-free plants thus obtained.

**BREEDING AND BIOTECHNOLOGY FOR IMPROVED DISEASE RESISTANCE**

One of the great challenges for food security is to improve yield stability through the development of disease-resistant crops. A major goal of plant research in the 21st century is to increase understanding of the plant immune system and unravel how this is manipulated by pathogens, in order to engineer durable resistance against pathogens and increased yields (Dangl et al. 2013). The plant breeders have fast-paced the dramatic improvement of crops for resistance to biotic stresses, enhanced yield and quality with increasing knowledge of genetics. These new techniques are supplementing and extending traditional breeding methods to enhance the production of food, fiber, and other agricultural products. The genetic improvement of plants can be accomplished by more precise breeding techniques, by genetic engineering to introduce desirable traits and by other newer improvement technologies.

Plants have their own networks of defense against plant pathogens that include a vast array of proteins and other organic molecules produced prior to infection or during pathogen attack. Not all pathogens can attack all plants and a single plant is not susceptible to the whole plethora of plant pathogenic fungi, viruses, bacteria or nematodes. Recombinant DNA technology allows the enhancement of inherent plant responses against a pathogen by either using single dominant resistance genes not normally present in the susceptible plant or by choosing plant genes that intensify or trigger the expressions of existing defense mechanisms. Many biotechnological tools facilitate the discovery and elucidation of the molecular interactions between plants and pathogens and facilitate direct selection of plant resistance genes from virtually any plant.

**Marker Assisted Selection for Resistance Breeding**

Introgression of desired genes using traditional protocols is lengthy and complicated. Therefore, DNA markers are being widely used in breeding crop varieties having optimal combinations of desirable genes and also for transferring disease resistance genes. Molecular markers linked to resistance genes can obviate the need for testing to identify resistant individuals from early generations, leading to an effective improvement of the breeding procedure. Today, the most successful applications of marker assisted selection (MAS) in plant breeding have been those for major disease resistance genes assisting backcrossing into elite cultivars and selecting alleles with major effects on high-value traits with relatively simple inheritance (Torres, 2009). By contrast, the approach has not been used as widely for the improvement of polygenic traits, due to the insufficient precision of QTL mapping techniques and the unreliable extrapolation of QTL information across multiple populations. Commonly used markers are restricted fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphism (SNP) with predilection of PCR based markers. Improvements in marker technologies and major investments in economically important crops have extended the major benefits of MAS in private sector. However, there has been very limited work in India involving application of MAS in plant breeding for disease resistance (Kumar 2014). Further genomic research and reductions in the costs associated with molecular markers are required to provide new opportunities to deploy it as well in minor crops and public breeding programmes (Torres 2009).

**Marker assisted pyramiding of disease resistance genes**

Marker assisted pyramiding of disease resistance genes termed as ‘Breeding by Design’ can help to control the pathogen which recurrently and rapidly develop their new virulence (Kumar 2014). Rice is among the first crops where marker assisted pyramiding of disease resistance genes was initiated in India and varieties developed by using MAS were released for commercial cultivation. The variety amend as Improved Pusa Basmati-1 was
developed by using conventional plant breeding approach integrated with MAS and two bacterial blight resistance genes Xa13 and Xa21 incorporated in Pusa Basmati-1 (Gopalakrishnan et al. 2008). Another variety of rice resistant to bacterial blight was developed in non basmati type rice in India by using MAS. The PCR-based molecular markers were used in a backcross-breeding program to introgress three major bacterial blight resistance genes (Xa21, Xa13 and Xa5) into Samba Mashuri from a donor line (SS1113) in which all the three genes are present in a homozygous condition (Sundaram et al. 2008). These two reports successfully demonstrate the application of marker assisted selection for targeted introgression of BLB resistance genes into Basmati type and non-basmati types varieties of rice. Therefore, it is imperative to strengthen work for other host-pathogen system for control of other serious diseases that adversely affect yield and economic gain. Molecular markers can also help in assaying the germplasm for presence or absence of a particular disease resistance gene. Cloning of disease resistance genes by tagging approaches can identify the function of a specific gene by uncovering a specific pathotype (Kumar 2014).

Transformation techniques

Genetic engineering (GE) offers an array of techniques for enhancing disease resistance of crop plants. Certain GE applications involve transgenesis, in some cases creating a metabolic pathway novel to the GE crop. In other cases, only cisgenesis is employed. In yet other cases, engineered genetic changes can be so minimal as to be indistinguishable from natural mutations. Thus, GE crops vary substantially and need to be evaluated for risks, benefits, and societal considerations on a case-by-case basis (Vincelli 2016). Genetic engineering or transformation techniques make it possible to isolate particular gene from one organism, insert them into the genome of another organism and make them to express at right time. Genetic engineering has the potential to provide a large number of beneficial plant traits, particularly an enhanced ability to withstand or resist attack by plant pathogens. New approaches to plant disease control are particularly important for pathogens that are difficult to control by existing methods. Genetically engineered plants resistant to plant pathogens can prevent crop losses and reduce pesticide usage. Currently, major areas of research and application of plant genetic engineering for resistance to plant pathogens include identification of plant resistance genes and the genes involved in resistance reactions to engineer into crop plants to protect them against diseases, pathogen derived resistance by transforming genes derived from the pathogens themselves into plants, use of antimicrobials peptides/proteins to improve plant resistance to pathogens, engineering plants to express an antibody against a protein crucial for pathogenesis resulting in a level of immunity or resistance to the pathogen.

Boosting Plant recognition of infection

A common feature of the immune system of many eukaryotes is the ability to recognize particular patterns on pathogens (PAMPs). The patterns are conserved across species of pathogens and, once recognized by immune cells as they examine the cells present in their host, they trigger an immune response. All plants have the ability to recognize a range of PAMPs, however, they may not recognize all of them. Therefore, if one species of plant has developed the ability to recognize a particular pathogen and defend against it, identifying the requisite gene and transplanting it into another plant will quickly enable it to muster its own immune defense against it (Tripathi et al. 2014; Schwessinger et al. 2015).

Mining Resistance Genes

Resistance genes, or ‘R genes’, allow a plant to overcome effector molecules used by pathogens to increase their chances of successfully invading a host. In a never-ending arms race, a pathogen will develop an effector molecule to enhance susceptibility of the host to infection, while the host will in develop the ability to recognise the effect or and induce the immune reaction again. In response, the pathogen may develop a new effector molecule, and the plant must again develop the ability to recognize the effector and respond when it is present. The DNA encoding these new proteins developed by plants to detect new effectors are termed R genes. There are a multitude of R genes relating to a several pathogens throughout the plant kingdom. Therefore, transferring R genes from a resistant plant to a susceptible plant will transfer resistance.
Conventional breeding techniques are often suitable for introgressing cisgenes into new varieties in which case GE is unnecessary. However, in crops like potato, grape, banana, apple and strawberry etc conventional breeding is exceptionally difficult or may be time consuming (Dangl et al. 2013; Jo et al. 2016). In such crops, cisgenics will be quicker, and more effective to enable greater precise insertion of the genes. Further, it will reduce the inheritance of unwanted genes along with the R genes. However, the obvious disadvantage of conferring resistance in this way is that the pathogen will again develop a new virulence method which will again need to be addressed, resulting in only a temporary resistance (Jones and Dangl 2006). However, helping crops quickly adapt to a new infection will help ensure short-term yields while also allowing the engineering of specific resistance to specific pathogens as new effectors and R gene couples are discovered (Kim et al. 2016). The ability to recognize genes from plants outside of a crop’s breeding pool may be important to ensure sustainability by opening a vast pool of R genes potentially useful for breeding.

Boosting Defense Responses

Molecules involved in defense signaling, defense regulation or other processes can be upregulated, boosting general defense responses (Vincelli 2016). This strategy takes advantage of the plant’s own natural immune system and does not introduce new metabolic pathways. This approach has been successful against bacterial pathogens attacking several host species (Tripathi et al. 2014; Huang et al. 2004).

Changing DNA Sequences Responsible for Susceptibility

Some pathogens have the ability to exploit some required host protein to give itself a route of infection. The susceptibility genes encoding these proteins are problematic to deal with given the necessity of the gene product. However, modification to the gene, either natural or synthetic, that alters the protein to reduce their ability to be exploited by the pathogen without rendering the efficacy of the protein for its required role, has been shown to be effective in increasing resistance (Van Schie and Takken 2014).

Plants producing their own Antimicrobials

The crops producing their own antimicrobials provide a potential basis for sustainable protection. The advantage of transforming crops with genes for natural antimicrobial substances is that it can be achieved by in-vitro techniques of molecular evolution to broaden the range of molecular targets of such antimicrobials (Badran et al. 2016). Such techniques potentially can be employed to reverse the buildup of pathogen resistance to the antimicrobial. Although public acceptance of transgenes from microorganisms is not encouraging, microbes could potentially serve as a source of many antimicrobial compounds (Lusk and Rozan 2006).

Silencing Pathogen Genes RNAi

The discovery that double-stranded RNA results in the silencing of genes with a complementary sequence has made it possible to insert genes into an organism coding to silence a specific gene to improve plant immunity or reduce pathogenicity of invading organism. RNA interference, or RNAi (Carthew and Sontheimer 2010) can be elicited in plants to silence pathogen genes, resulting in reduced disease through the use of genetic constructs. Recent research clearly highlights the substantial potential which RNA silencing offers for management of diseases caused by biotrophic fungi, necrotrophic fungi, and oomycetes (Andrade et al. 2015; Govindarajulu et al. 2015).

Removing Host Virulence Factors

Removal or modification of host virulence factors such as a particular protein that allows strong binding of the pathogen is a potential method of reducing infection rates. It is similar to removing or modifying susceptibility genes to remove a target route of infection used by pathogens. Genetic modification of targets of pathogen virulence factors increases host resistance without introducing an exogenous biochemical pathway into the plant, and also can be achieved without transgene insertion.

Detoxifying the toxins

Many pathogens produce toxins that attack particular targets of plant cells to allow easier invasion to render the toxin ineffective. This in
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turn reduces infection rate of many pathogens. The gene constructs used so far in the GE for this purpose have been transgenic and associated with societal concerns. However, these potential concerns may be addressed by employing native promoters derived from the engineered crop and marker-free transformation (Yang et al. 2014).

**Use of CRISPR/Cas 9**

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a prokaryotic defense system that targets the DNA of invading viruses and plasmids (Ali et al. 2015, Shan et al. 2013). The ability of CRISPR to make target endonuclease activity to specific parts of a DNA sequence apparently has limitless uses in disease management by transforming plants to produce both a target specific CRISPR associate protein 9 (Cas9) and a target specific RNA guide strand (gRNA). A variety of viral genetic elements can be successfully targeted (Ali et al. 2015, Shan et al. 2013), which would confer long-term utility to this strategy to give significant host resistance. Crops engineered to express a CRISPR/Cas immune system may constrain public acceptance due to their transgenic character.

It is apparent that the potential risks of the GE need to be balanced against its proven or potential benefits. Scientists, research institutions, and non-governmental organizations should take an active role to promote the holistic development of biotechnology. Development of disease resistance through transformation is still in infancy in India. Private corporations and research institutions should make the technology and its products available to at relatively low or no cost where they are urgently needed. A recent review by Vincelli, 2016 over viewed the possible targets of genetic modification in detail to increase pest control, modalities of modifications to increase immunity and the possible risks that must be addressed if engineering resistance is to be sustainable.

**NANOPARTICLES IN PLANT DISEASE MANAGEMENT**

The nanotechnology has potential prospects of use in plant disease management in different ways. The most simple and obvious way is direct application of nanoparticles (NPs) in the soil, on seeds or foliage to protect plants from pathogen invasion by suppressing the pathogens in a way comparable to chemical pesticides. Hence, to reckon the scope and application of NPs in plant disease management, the effects can be discussed through two major point of views i.e. direct effect of NPs on pathogens and use of nanomaterials in formulating the pesticides i.e., nanopesticides (Khan and Rizvi 2014). It is imperative to examine the effect of NPs on microorganisms to harness the benefit of this technology in the plant protection, especially against phytopathogens. However, the ultra-small sizes that make the nanoparticles of immense usefulness, unfortunately the same characteristic is a basic cause of several adverse effects that represent hazards to environment, animals, human beings and plants. Therefore, detailed studies are warranted before their widespread use to control diseases in plants.

**CONCLUSION**

Recent Advances in Plant biotechnology offer more efficient, sensitive, reliable and rapid methods in detection of plant diseases, obtain pathogen-free mother plants, disease resistance and control of plant diseases. It will be prudent to strengthen use of these tools as keystones to devise pathogen specific holistic disease management of crops and sustain food security.

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