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Review

Application of molecular and biotechnological techniques in plant disease management: A review

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Plant diseases are a major challenge in crop production. They are caused by nematodes, bacteria, fungi, viruses as well as plant nutritional factors. Diseases interfere with the normal physiological and metabolic processes of plants. This results in various effects including wilting, stunting, yellowing and death of plant tissues and organs. Crop losses due to diseases manifest in form of reduced yield, poor quality produce, and reduced post-harvest storage. Past research has brought to the limelight the continuous capacity of pathogens to revert to new pathotypes and strains, some that break resistant varieties or are less sensitive to chemical control products. Currently, farmers are advised to combine several plant disease management practices, a strategy known as integrated plant disease management. Such strategies include crop rotation, use of disease free planting materials, field sanitation, and chemical methods as well as use of resistant varieties. However, some of these methods are expensive and substantially increase the cost of production. Development in molecular biology and biotechnology found application in plant disease management. This ranges from identification, diagnosis to control through gene transfer, mutation breeding and RNA interference, among others. In this paper, the current developments in the application of molecular techniques and biotechnology to manage plant diseases, outlining their possible future application and potential for enhanced plant disease management.

Key words: Phytopathogens, genetics, molecular biology, plant transformation, control options.

INTRODUCTION

Crop production traditionally depends on several inputs including certified planting materials, irrigation, fertilizers, and pesticides among others. However, recently, devastating cases of pathogen attacks have increased and are feared to worsen with the increasing variability in weather patterns and environmental conditions due to global climate change. Loses to farmers are significant, including low yields of reduced quality (FAO, 2017). Important examples include cassava mosaic and cassava brown streak virus (Legg et al., 2015), maize lethal

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necrosis outbreaks in Eastern and Southern Africa (Mahuku et al., 2015), and banana Xanthomonas wilt in Africa (Biruma et al., 2007; Kebede et al., 2017). There are also increased cases of crop damage by nematodes, for example potato cyst nematode (Globodera rostochiensis) in Kenya (Onkendi et al., 2014).

The impact of pathogens on agricultural crops has been wide. Pathogens release enzymes, growth regulators, toxins and other substances which manifests in the affected plants through a myriad of symptoms. The symptoms may be in form of destroyed vascular bundles, yellowing and drying of leaves, witling, and necrosis among others. These developments inhibit absorption and movement of water and minerals from the soil to plant tissues, leading to reduced photosynthesis and death of plants (De Werra et al., 2015; Prince et al., 2015). For the case of cassava brown streak virus, there is a

development of necrotic spots on the roots and rotting, which has a direct impact on the yields (Patil et al., 2015; Legg et al., 2015; Anjanappa et al., 2017). Maize lethal necrosis leads to stunted plants that could turn yellow and die causing 40% or higher loss of yields. The cyst nematodes in potato (*Solanum tuberosum*) growing areas attack tubers, which results in discoloration and sometimes rotting, which directly affect yields (Adams et al., 2014; Thorpe et al., 2014; Mahuku et al., 2015). In tomatoes, *Ralstonia solanacerum* attacks cause accumulation of bacterial mucilage and exudates in the vascular bundles that blocks flow of water and minerals to other parts of the plant (Sarkar and Chaudhuri, 2016). Furthermore, sometimes the bacteria act synergistically with *Fusarium* species as well as the root knot nematodes in an infection complex. In such a case the wilting is severe and impacts adversely on total yields (Lamichhane and Venturi, 2015). Some fungal pathogens lead to development of spots on leaves or fruits which substantially decrease their market value (Hayes et al., 2014).

The common plant disease management strategies can be categorized into cultural, mechanical, biological and chemical approaches. In cultural methods, there is maintenance of good hygiene at the field through removal and destruction of diseased plants, selection and use of clean planting materials and planting resistant crops (Katan, 2000, 2010; Mehta, 2014). Mechanical methods include heat treatments to kill pathogens in planting materials, in the soils borne pathogens (Bruez et al., 2017) and in reducing postharvest pathogens (Wisniewski et al., 2016). Chemicals are used widely in soil fumigation as well as in control of foliar pathogens and their vectors (Li et al., 2016; Gao et al., 2016). Biological methods involve use of viral, bacterial and fungal organisms as biopesticides in control of plant disease causing microorganisms (Tjamos et al., 2013; Mach, 2016).

Examples include bacteriophages (Balogh et al., 2003; Jones et al., 2007; Iriarte et al., 2007), baculoviruses (Lincoln et al., 2002; Del Pozo and Lam, 2003). *Xanthomonas campestris* pv. *pruni* phage 1 was successfully tested against *Agrobacterium tumefaciens,* while *X. oryzae* phage reduced the incidence of leaf blight (Jones et al., 2007).

Integrated plant disease management combines several of the aforementioned methods to control particular plant diseases or the pathogen vectors (Fry, 1982). To complement the approaches described earlier, molecular tools have lately found wide applications in the study, diagnosis and management of plant diseases. This paper reviews the application of current molecular tools as well as some tissue cultures techniques in the management of plant diseases.

GENETIC ENGINEERING

Gene transfer

The initial work by Flor (1955) that gave rise to the gene-for-gene concept has been very instrumental in the study of resistance as well as spearheading development of disease resistant plants (Jones and Dangl, 2006). Genetically, this requires a virulence (Avr) gene in the parasite and a resistance gene (R-gene) in the host plant. Several R genes have been identified to date and characterization of the *Arabidopsis* resistance gene complement managed to provide better understanding of the r genes structure (Meyers, 2003). Identification of resistant plants initially provided sources for resistance genes; this has always been quickly followed by breeding efforts aimed at introgression of the resistance genes to cultivated crops. However, normal classical breeding faced many drawbacks including low success and carryover of unwanted traits to crops (Miklas et al., 2006; Fry, 1982).

Over time, new more precise molecular approaches came into play. These include proteomics, metabolomics, transcriptomics, plant tissue culture and genetic engineering. Genetic engineering involves gene transfer, gene silencing, mutation breeding, and regulation of transcriptional factors (Sankaran et al., 2010; Ocsoy et al., 2013; Mahlein, 2016). Gene transfer method of developing disease resistant plants has been used for several decades. Some of the earlier achievements include *Agrobacterium* mediated introduction of rice chitinase gene in strawberry (*Fragaria ananasa*), which led to resistance to powdery mildew, done using the CaMV 35S viral promoter. Expression of rice *chitinase*-3 gene in transgenic peanut through *Agrobacterium* mediated gene transfer was also found to be effective in conferring resistance against many soil borne fungal pathogens. This transformation involved *Agrobacterium tumefaciens* strain LB4404 having the binary vector (pB1333-EN4-RCG3) containing the chitinase (chit) and hygromycin resistance (hpt) gene as selectable marker

(Iqbal et al., 2012). Studies on chitinase genes have led to their wide utilization against many other fungal pathogens (Jabeen et al., 2015; Richa et al., 2016, 2017; Munir et al., 2016).

Embryogenic tissue transformation through particles bombardments using tungsten particles coated with DNA of the PRSV HA 5-1 coat protein gene was able to produce ringspot virus resistance in pawpaw. Polyethylene glycol (PEG) method was also successfully used to transfer Stilbene synthase gene to rice to increase resistance to blast caused by *Pyricularia oryzae* (Stark-Lorenzen et al., 1997).

CRISPR/Cas9 is a new technique in genome editing that has enabled manipulation of plants allowing study of resistance genes, and has been used in mutational breeding in rice (Zhou et al., 2014; Lu et al., 2017), tomato (Brooks et al., 2014) and tobacco (Gao et al., 2015). In *Arabidopsis*, CRISPR/cas9 was used to introduce mutation on elF (iso) 4E locus enabling acquisition of resistance against Turnip mosaic virus (Pyott et al., 2016). CRISPR/cas9 was also used to modify elF4E gene in cucumber thereby creating resistance to a number of viral diseases including cucumber yellowing virus, zucchini yellow virus and papaya ring spot virus (Chandrasekaran et al., 2016). The technique has also been used against fungal diseases (Wang et al., 2016). Currently, gene transfer is being widely applied in crop improvement techniques to introduce resistance mechanisms to plants (Vleeshouwers and Oliver, 2014).

RNA interference

RNA interference (RNAi) is a molecular technology that uses gene down regulation principle via transcriptional gene silencing (TGS) or posttranscriptional gene silencing. Three types of RNA silencing have been investigated in plants. These are cytoplasmic small interfering RNA (SiRNAs), micro RNA (miRNAs) in down regulating endogenous mRNA and DNA methylation-suppression of transcription. In these different RNA interference pathways, long double stranded RNA (dsRNA) precursors are cleaved by DICER enzyme (DCL2 or DCL3) into approximately short 21-nt length of SiRNAs and miRNAs (Bernstein et al., 20101). Once constructed, based on the fact that large and small RNA molecules are mobile between organisms especially in plant-pathogen interactions (Castel and Martienssen, 2013; Kim et al., 2014), SiRNAs and miRNAs will down regulate plant pathogen mRNA and chromatin modification. RNAi has been investigated as a powerful approach in developing disease-resistant crops. It has been used in combating plant fungi, for example *Sclerotinia sclerotiorum,* the causal agent of white mould, a devasting plant disease that causes up to 100% yield loss. RNAi approach has been reported to be more efficient against white mould as compared to conventional methods. Transgenic tobacco plants were used to construct a hairpin RNA in order to down regulate Chsgene, the gene controlling chitin synthesis in the fungus. A reduction of 55.5 to 80% in disease severity was observed as compared to non-transgenic tobacco (Andrade et al., 2016).

Fusarium oxysporum is a soil-borne fungus responsible for significant economic damages in potato, bean, wheat and bananas, among other crops. Studies have shown that Fox can cause 30 to 70% yield losses in different host crops. Host-induced RNAi has been used in silencing the pathogenicity genes (FOW2, FRP1, and OPR) that allow *F. oxysporum* to counteract its host resistance mechanism (Hu et al., 2015). RNA silencing has also been used in protecting crops from viral infection such as tomato leaf curl virus, potato virus X (PVX) and citrus tristeza virus (CTV) (Soliman et al., 2008; Praveen et al., 2010; Soler et al., 2011). Despite being a powerful method for switching off expression of pathogen genes during infection, RNAi has some drawbacks to the plant as well as in the environment. RNA silencing could result in host genome modification which might interfere with gene flow between plants and their relatives leading to biodiversity reduction. In addition, RNAi construction is difficult for some plant species (Rodrigues et al., 2009).

Transcriptomics as an approach of managing plant diseases

Transcriptomics entails the study of RNA transcripts produced by the genome within a specific cell using high throughput approaches such as Illumina sequencing. Improved understanding of the cell genome has enabled various techniques such as genome editing which plays a vital role in plant disease elimination, besides improving plant immunity. Through transcriptomics, many disease resistance genes have been identified leading to significant breakthrough in the management of plant diseases (Horgan et al., 2011; Lowe et al., 2017).

Transcriptomics has been used successfully in the management of *Xanthomonas oryzae* on rice (Cheng et al., 2016). Genome editing technologies have been used successfully in enhancing plant resistance to phytopathogens (Andolfo et al., 2016). The advent of this technology has envisioned the use of RNA-sequencing for transcripts or genes expression profile in the management of various plant diseases (Prabha et al., 2013). Furthermore, manipulation of the key plant immunity modulators such as the R-genes can boost the generation of disease free plants. This technique has also improved understanding of the interaction of various diseases and the plant host such as *Phytophthora nicotianae* infecting *Nicotiana tabacum* (Yang et al., 2017). Understanding plant response to infections is important in the development of effective plant disease control measures. Investigation of gene expression profiles during viral infections would shed more light in

ascertaining significant components of the resistance alleyways (Yang et al., 2017).

Proteomics

Various proteins and their functions as well as their interaction in an organism can be studied through the mean of proteomics analysis (Zulkarnain et al., 2015). This can be useful in determining the pattern and the specificity of a particular protein released in plants when there is a pathogenic stress. Common techniques used in proteomics analysis are Two-dimensional Electrophoresis (2DE), Fluorescence 2D Difference Gel Electrophoresis (2D-DGE), Mass Spectrometry (MS) and Multidimensional Protein Identification Technology (MudPIT) also known as ''shotgun'' approach (Chandramouli and Qian, 2009).

About 1,500 proteins were identified in rice during bacterial leaf streak (BLS) infection with 23 up-regulated proteins that were potentially associated with BLS resistance in rice (Li et al., 2012). Brown root honey caused by *Monilinia laxa* proteins was investigated from apple and apricot, up to 800 proteins were expressed and around 10 proteins isolated from apple showed potential use in developing *M. laxa* host specific diagnostic marker (Bregar et al., 2012). In order to understand the host response mechanism against *Alternaria alternate* infection, both resistant and susceptible apple varieties were subjected to comparative proteomics analysis using Two-Dimension (2-DE) and Mass Spectrometry (MS). A total of 43 differentially expressed proteins were detected which included pathogenesis-related proteins beta-1,3 glucanase, mald 1 and ascorbate perioxidase. The pattern of mald1 in resistant, as well as in susceptible apple contributed to understanding the mechanism underlying *A. alternata* resistance (Zhang et al., 2015). Likewise, 2-DE and MS were used in identifying different proteins expressed during *Liberibacter asiaticus* (*Las*) infection on *Citrus*. The management of *L. asiaticus* disease commonly known as *Citrus* Huanglongbing (HBL) has been successful using heat treatment. Through comparative proteomics study, 107 Las/heat-induced proteins such as HSP70-like proteins, ribulose-1, 5-bisulphate and carboxylase were identified. They were up-regulated due to heat treatment, which gives an insight on the underlying heat-induced host defense mechanism (Nwugo et al., 2016). Proteomics as an approach in controlling plant disease is more efficient than conventional methods; however, it has some limitations in dynamic resolution for large-scale proteomes analysis as well as in quantifying proteomes (using Mass Spectrometry). Furthermore, separation, visualization and identification of hydrophobic proteins can be a challenge (Van Wijk, 2001).

Metabolomics

Plant-pathogen interaction could be better understood based on the identification and quantification of small molecules called metabolites (Rojas et al., 2014). A number of techniques have been used in the past in metabolomics analysis. To-date the commonly used are high performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS) as well as nuclear magnetic resonance spectroscopy (NMRS) (Kasture et al., 2012). Using GC-MS approach, Warth et al. (2015) showed that wheat metabolome is modified by deoxynivalenol (DOIN) secreted by *Fusarium graminearum* that causes Fusarium Head Blight (FBH) disease. Earlier, Levenfors et al. (2008) investigated biological management of snow pink mould (*Micrcodochium nivale*) on wheat and rye using *Pseudomonas brassicacearum* MA250. The study found a significant biocontrol effect of *P. brassicacearum* on *M. nivale.* Later, Anderson (2012) discovered that the biocontrol activity of *P. brassicacearum* on *M. nivale* was associated with the secondary metabolites Piliferolide A and SB0253514. Parker et al. (2009) had hinted on the possibility that metabolites control pathogenesis, when *Magnaporthe grisea* was observed to counteract rice, barley and *Brachypodium distachyon* responses by reprogramming its hosts through secretion of different patterns of metabolites.

Known for their devastating effect, *Botrytis cinerea* (*Bot*) and *Pseudomonas syringae* pv *tomato* (*Psd*) are two major pathogens affecting tomato production. Analysis of altered metabolites isolated from both *B. cinerea* and *P. syringae* infected tomatoes revealed that the host resistance is associated with metabolomics reprograming in the host allowing biochemical changes in tomatoes (Camañes et al., 2015). Melatonin-mediated innate immunity against host specific bacteria in *Arabidopsis* has been determined to be reinforced by sugars and glycerol increases.

Link between quorum sensing (bacterial communication system) and plant disease resistance

Quorum sensing (QS) is a cell-to-cell communication mechanism in bacteria allowing them to control their local population density and virulence factors (Bouayed et al., 2016). Through this mechanism small signaling molecules are secreted and detected by bacteria enabling them to assess their population. A number of signaling molecules are involved in QS. These include oligopeptides in Gram-positive bacteria and N-acylhomoserine lactones (AHL) in Gram-negative bacteria and some auto inducers (AI-2) in both Gram-positive and Gram-negative bacteria. AHL is the best characterized amongst QS molecules. Studies have shown the positive effect of AHL in priming induced resistance in plants against phytopathogens. It has been demonstrated that AHL-derived from *Serratia plymuthica* can induce systemic resistance in bean and tomato against *Pythium aphanidermatum* (Pang et al., 2009). Oxo-C14-HSL has been reported to induce resistance

against *P. syringae* in *Arabidopsis thaliana*. The induced resistance is due to accumulation of callose, phenolic compounds and lignification in plant cell wall (Schenk et al., 2014). Similarly, Oxo-C14-HSL derived from *Ensifer melioloti*, a rhizobium of root nodulation in legume, which has been found to fortify host response mechanism in plants. This has been reported for *Phytopthora infestans*, *Blumeria graminis* and *Puccinia graminis* resistance in tomato, barley and wheat, respectively. This Oxo-C14-HSL induced systemic resistance was later confirmed in *Arabidopsis* and barley against *B. graminis* and *Golovinomyces orontii* (Schikora et al., 2011).

TISSUE CULTURE AS AN APPROACH TO MANAGING PLANT DISEASES

Haberlandt (1969) published a paper which envisioned the idea of tissue culture procedures and provided a paradigm for many scientists to delve deeper into the aseptic production of plant cells, tissues and organs in culture (Akin-Idowu et al., 2009). In plant tissue culture, plant cells, tissues, and organs are propagated *in vitro* under aseptic conditions on artificial medium (Hussain et al., 2012). Plant tissue culture has gained popularity in the recent past, and it has been of great importance in plant disease elimination, large scale plant multiplication and plant improvement (Ogero et al., 2012) as well as in the production of secondary metabolites. Subsequently, the application of tissue culture in managing plant diseases is elucidated.

Meristem-tip culture and meristem heat therapy

Organized apex of the shoot from a selected donor plant can be subsequently cultured *in vitro* (Grout, 1990). The cultures are established from axillary buds or from shoot tips, after excision the explants are inoculated into a culture medium that allows the explant to propagate into shoot. The explant of meristem culture may either be the apical dome (apical meristem) or the apical dome plus a few leaf primordia. Studies have shown that larger explants are desirable as they are easier to dissect and have much higher survival growth rate than the smaller ones. The excised apical meristem tip often measures 0.1 mm in diameter and 0.25 to 3.0 mm in length and is done under sterile condition. The significant importance of using meristem tip cultures is that small explants are paramount for excluding devastating pathogens present in the donor plant (Grout, 1990). Besides, axillary shoot proliferation offers lower risks of genetic instability and is easily achievable in most plant species. This technique has been used to eliminate virus infection in sweet potato (Frison and Ng, 1981). Smith (2013) reported that meristem culture technique had made it possible to save many vegetative propagated plants from viruses. Earlier, Ogero et al. (2012) demonstrated an optimized tissue culture approach for disease-free sweet potato seedlings production in Kenya. Furthermore, this technique can be optimized by combining with heat treatment prior to meristem culture, hence contributing vastly to production of healthy plants. Mwangangi et al. (2014) combined thermotherapy at 38°C with meristem tip culture to eliminate brown streak virus from infected cassava. Meristems excised from plants subjected to thermo-therapy had enhanced CBSV eradication as compared to the control resulting in 68.8% plant survival with 84% of the plants surviving being virus-free. These findings confirm previous reports (Acedo, 2006) and elimination of sweet potato fatherly mottle virus (Mashilo et al., 2013). Application of meristem culture combined with thermotherapy at 35°C is reported to increase the survival rate of *in vitro* explants (Manganaris et al., 2003; Mashilo et al., 2013). This is because larger tips can be obtained from heat-treated plants while ensuring virus-free plant production.

In some cases, it is problematic to eliminate viruses from meristem tip culture; hence, thermotherapy coupled with meristem culture has been proposed. Thermotherapy is applied before *in vitro* meristem-tip culture and has been shown to be effective against potato virus S (PVS) and PVX (López-Delgado et al., 2004). Chatenet et al. (2001) and Fitch et al. (2001) proved apical meristem culture to be effective in eliminating sugarcane yellow leaf virus (SCYLV). What is more, a combination of meristem culture, heat therapy, and cryotherapy has been used successfully in the elimination of various plant diseases. Thermotherapy coupled with meristem-tip culture has enabled the elimination of bean yellow mosaic virus (BYMV) from infected corms, hence leading to production of BYMV free plants (Sharifi Nezamabad et al., 2015).

In vitro shoot grafting and callus culture

In vitro shoot-tip grafting has also been applied successfully in the elimination of viruses in some woody plants. It entails grafting of apical meristem on young root stock seedling. According to Navarro (1992), this technique has been applied successfully in the elimination of approximately 16 diseases in citrus plants, including Psorosis (Navarro et al., 1980).

Calli is a group of unorganized proliferative cells produced by subjecting explants to suspension culture. During culturing, some cells may escape from viral infections due to the high rate of cell proliferation and attain viral resistance due to mutation. In callus culture derived from infected cells, it is evident that not all calli uniformly contain the viral infections. Studies showed that approximately 40% of calli derived from tobacco infected with tobacco mosaic virus (TMV) contained the virus (Hansen and Hildebrandt, 1966). The main reason for the escape from this devastating virus is the high rate of cell

proliferation hence the virus is unable to keep pace with the high rate of cell multiplication, and acquisition of resistance by some cells through mutagenesis (Warren et al., 1992).

Through somaclonal variation, an array of disease resistant plants has been developed. Out of 370 *Solanum lycopersicum* plants propagated from callus cultures, six showed enhanced resistance to TMV. Similarly, late blight *(P. infestans)* resistant potato plants and calli resistant to bacterial blight of rice have been developed. Different pathogens produce different secondary metabolites which can be used to screen different calli for disease resistance. Resistant calli can survive in the presence of toxins, hence generation of disease resistant plants. Through this technique, different disease resistant plants have been developed such as rice resistant to the brown spot pathogen *Helminthosporium oryzae* (Mwendo et al., 2017). Similarly, TMV resistant plants, *Helminthosporium maydlis* toxin resistant *Zea mays* plants and *Helminthosporium sacchari* resistant sugarcane have been generated. Besides, meristem callus culture has been used effectively in the eradication of PVX. Experiments on potato used culture media made up with culture filtrates of different *P. infestans* pathotypes to successfully isolate resistant lines. Embryo culture is the other technique used in tissue culture in embryo rescue in wide crosses, monoploid production and overcoming seed dormancy. It proved to be a very effective tool for transfer of *Alternaria* blight tolerance in oilseed brassicas (Yadav et al., 1991; Aneja and Agnihotri, 2016).

Somatic embryogenesis

Somatic embryogenesis refers to the *in vitro* development of embryo like structures from somatic cells rather than from combination of male and female gametes. According to D'onghia et al. (2001), somatic embryogenesis has been applied successfully in the management of devastating citrus psorosis virus (CPsV) from three different *Citrus* species, namely, Dweet tangor, Common Mandarin and sweet orange. Psorosis virus-free citrus can be propagated via heat therapy, and shoot grafting or combination of both techniques (Calavan et al., 1972; Navarro et al., 1980; D'onghia et al., 2001). However, enhanced competence during the sanitation procedure is necessary because virus eradication differs between isolates and hardly exceeded 70 to 80% (Roistacher, 1993). Somatic embryogenesis obtained by culture of style and stigma has been used successfully in the management of CPsV, hence a promising technique in the propagation of healthy citrus plants (D'onghia et al., 2001).

Protoplast fusion and somaclonal variation

The variability generated from *in vitro* cultured somatic cells, may be due to genetic, epigenetic or physiological causes. Somaclonal variation has been observed in economically important crop species such as wheat, rice, sugarcane, oats, potato, tobacco, among many other plant species with numerous traits, for example resistance to viruses, bacterial and fungal infections. This technique has been used for developing disease resistance in wheat and production of dihaploids through wheat × maize hybrids (Mehta and Angra, 2000) and also for resistance to *Verticillium dahliae* in potato (*S. tuberosum* var L.) plants regenerated from callus (Sebastiani et al., 1994). Protoplast fusion is a vital technique for the generation of hybrid plants among different incompatible species and incorporation of an alien genetic factor for pathogen resistance (Larkin and Scowcroft, 1981). In various cases, vital gene variability in the cultivated germplasm may be missing, and use of protoplast fusion can curb this. Hassan (2014) showed that protoplast fusion of two fungicide tolerant mutants of *Trichoderma harzianum* and *Trichoderma viride* enhances β-glucanase, chitinase and protease enzyme activity in fusant strains (fusion of two different species of fungus) as compared to the parental strains and they had a powerful antagonistic activity against grapevine pathogens *Macrophomina phaseolina, Pythium ultimum* and *Sclerotium rolfsii*. Plant fusion can also cause hypovirulence in other phytopathogenic fungi (Lee et al., 2011).

Haploid and polyploid plants

Haploid plants have been generated from anther and ovule culture. The production of homozygous lines within a span of a short period has paved the way for many types of research. Also, haploid plants are highly useful for research related to plant breeding and genetics. Furthermore, they provide convenient systems for induction of mutations and selection of plants with desired traits. Through these techniques, mutants that are resistant to various pathogens have been developed. For example haploids were used to produce melon with resistance to multiple virus diseases (Lotfi et al., 2003). Polyploidy usually occurs from one generation to another and it results from variation that alters the number of chromosomes in the cells. Different methods describe the origin of polyploidy but they mainly occur due to doubling of somatic cells in mitosis, non-reduction in meiosis yielding gametes that are unreduced, polyspermy and endo-replication (Bharadwaj, 2015). Due to increase in number of chromosomes of related gene dose in polyploids, the gene expression and some secondary metabolites production can be enhanced, thus, boosting host plant resistance mechanisms. According to Van (1975), *Lolium*, an autotetraploid has extra structural carbohydrate and good resistance to diseases than the diploids due to relationship changes in dose of genes, silencing of gene and secondary metabolites expression.

By applying polyploidy, one can produce allopolyploids from parent plants having multiple endogenic chemicals of protection and secondary metabolites which usually provide all metabolites and biocatalysts of the two fused parent plants, thus, successfully promoting the resistance to pest characteristic (resistance thus is more in horizontal form). This strategy can also strengthen tolerance to specific stresses of the environment (Bharadwaj, 2015).

FUTURE PROSPECTS OF APPLYING MOLECULAR TECHNIQUES IN PLANT DISEASE MANAGEMENT

Pathogens have the potential to develop resistance against the mechanisms employed to manage them. The use of only one type of R genes is quite a temporary disease solution. For example, the *Brassica* resistance mediated in Rim1 collapsed in 5 years of use (Sprague et al., 2006). As done in *S. tuberosum* (Kim et al., 2012; Vossen et al., 2014), integrating several R genes may be the way out to make sure that whenever mutations occur in the pathogen to surpass one of the R genes, additional sources of resistance will persist. R genes have to be prolonged by studying the complex of resistance of the evolution of R gene. Grzeskowiak et al. (2014) studied the mechanism of resistance in tomato focusing on Pto/Fen/Prf resistance complex. Some studies have shown that studying non-host resistance (NHR) may lead to and devise mechanisms of resistance that are long lasting and independent of recognition of R protein. According to Singh et al. (2013), non-host resistance emerges when the whole species of a plant is pathogen resistance and compared to resistance mediated by R gene, it is more persistent, thus being a new method for enhancement of crops.

There are two promising tools to exploit for genome editing; the first one is the system of nuclease (Christian et al., 2010; Bogdanove and Voytas, 2011; Schornack et al., 2013) which uses effectors of TAL from *Xanthomonas* species a pathogenic plant to bind DNA regions that are short in a way that is specific to sequence a method described by Boch et al. (2009). The second method is referred to as modulate gene expression and the system of CRISPR which is a technique that enables creation points of gene mutations in absence of placing additional unwanted DNA that is foreign (Belhaj et al., 2013).

Various techniques have emerged that permit hasty cloning and gathering of standard constructs. For instance, fusion of USER that uses cloning based on excision of uracil (Geu-Flores et al., 2007) and golden gate with its associated organization like golden braid (Engler et al., 2008; Sarrion-Perdigones et al., 2013). Tool kits of molecular studies are also available and permit cloning to be prompt and adjustable as discussed by Engler et al. (2014) and Binder et al. (2014). Such techniques may be utilized both in favor of the prevailing immunity mediated by R gene through assembling of R genes in cassettes of resistance and in recent constructed biology advances. Constructed biology methods have thrilling possibility of forming inductive resistance of diseases in plants. According to Hou et al. (2012), one can direct constructed promoters that are sensible to stress in *Arabidopsis*. This upcoming technology may aid in dealing with larger problems related to crops inclusive of genome of polyploids (Galletta and Maas, 1990). As described by Wang et al. (2014), three homoalleles of the susceptible gene of powdery mildew Mlo was fruitfully mutated from wheat that was hexaploid for prevention of growth of pathogen.

CONCLUSION

In this work, how plant diseases are being managed through application of molecular techniques was discussed. However, plants are still being affected by diseases thus, there is need for sustained innovation in this area of science to identify more effective strategies. The use of ecologically safe and environmental friendly methods of protecting crops from diseases is gaining importance. Among the various methods of control of plant diseases, resistance of host is still the method of preference despite the fact that shortfall of persistence has been a repeated limitation. Advancement in transfer of genes systems in crops is perhaps the most difficult form of research in plants. Presently, the preferred two methods are the biolistic-mediated and *Agrobacterium*-mediated DNA delivery systems. The evolution of nanoparticles for delivery of DNA cells of plants is coming up and the probabilities of incorporating the success of *Agrobacterium* and biolistic mediated systems is looming. The use of *Agrobacterium* in transforming plants will continue to have consideration since one avoids the step of tissue culture during regeneration of plants. Genomics has proven to be a potential tool in plant disease management either by targeting virulence of pathogen or by genetic manipulation of the host plant. The most important advantage of utilizing transcriptomics techniques is the capability of being able to carry out detailed studies at transcriptional level of interactions between host plant-pathogens interplay. Some of the other molecular methods are associated with limitations for example. Polymerase chain reaction (PCR) can only be used to find sequences that are known in some details due to primer requirement, but is still a useful tool in crop disease management. Tissue culture on the other hand can be associated with problems in maintaining uniformity and stability of clonally propagated plants and can lead to loss of morphogenetic capacity. Luckily, new advancements will arrive through combination of the available techniques that will enable gene transfer that is accessible and easy to apply plant species in large numbers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Acedo VZ (2006). Improvement of *in-vitro* techniques for rapid meristem development and mass propagation of Philippine cassava (*Manihot esculenta* Crantz). *Journal of Food, Agriculture and Environment.* 4(1):220-224.

Adams IP, Harju VA, Hodges T, Hany U, Skelton A, Rai S, Deka MK, Smith J, Fox A, Uzayisenga B, Ngaboyisonga C, Uwumukiza B, Rutikanga A, Rutherford M, Ricthis B, Phiri N, Boonham N (2014). First report of maize lethal necrosis disease in Rwanda. New Dis Rep, 29: 22.

Akin-Idowu PE, Ibitoye DO, Ademoyegun OT (2009).Tissue culture as a plant production technique for horticultural crops. African. Journal. Biotechnology 8(16):3782-3788.

Anderson PF (2012). Secondary metabolites associated with plant disease, plant defense and biocontrol. *diss.* (sammanfattning/ summary) Uppsala: Sveriges lantbruksuniv. Acta Universitatis agriculturae Sueciae, 1652-6880; 2012: 52 ISBN 978-91-576-7699-3 (Doctoral thesis).

Andolfo G, Iovieno P, Frusciante L, Ercolano MR (2016). Genome-editing technologies for enhancing plant disease resistance. Frontiers in Plant Science 7: 1813.

Andrade CM, Tinoco MLP, Rieth AF, Maia FCO, Aragão FJL (2016). Host-induced gene silencing in the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*. Plant Pathology 65(4):626-632.

Aneja JK, Agnihotri A (2016). *Alternaria* blight of oilseed brassicas: epidemiology and disease control strategies with special reference to use of biotechnological approaches for attaining host resistance. Journal of Oilseed Brassica 1(1):1-10.

Anjanappa RB, Mehta D, Okoniewski MJ, Szabelska A, Gruissem W, Vanderschuren H (2017). Molecular insights into *cassava brown streak virus* susceptibility and resistance by profiling of the early host response. Molecular Plant Pathology 19(2):476-489.

Balogh B, Jones JB, Momol MT, Olson SM, Obradovic A, King P, Jackson LE (2003). Improved efficacy of newly formulated bacteriophages for management of bacterial spot on tomato. Plant Disease 87(8):949-954.

Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V (2013). Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods 9(1):39.

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001). Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409(6818):363.

Bharadwaj DN (2015). Polyploidy in crop improvement and evolution. In: Bahadur B, Rajam MV, Sahijram L, Krishnamurthy KV (eds) Plant biology and biotechnology, vol I. Plant diversity, organization, Function And Improvement. Springer, India pp 619–638. https://doi.org/10.1007/978-81-322-2286-6_24.

Binder A, Lambert J, Morbitzer R, Popp C, Ott T, Lahaye T, Parniske M (2014). A modular plasmid assembly kit for multigene expression, gene silencing and silencing rescue in plants. PLoS One 9(2):88218.

Biruma M, Pillay M, Tripathi L, Blomme G, Abele S, Mwangi M (2007). *Banana* Xanthomonas wilt: a review of the disease, management strategies and future research directions. African Journal of Biotechnology 6(8):953-962.

Boch J, Scholze H, Schornack S, Landgraf A, Hahn S, Kay S (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. Science 326(5959):1509-151512.

Bogdanove AJ, Voytas DF (2011). TAL effectors: customizable proteins for DNA targeting. Science 333(6051):1843-1846.

Bouayed N, Dietrich N, Lafforgue C, Lee CH, Guigui C (2016). Process-oriented review of bacterial quorum quenching for membrane biofouling mitigation in membrane bioreactors. Membranes 6(4):52.

Bregar O, Mandelc S, Celar F, Javornik B (2012). Proteome analysis of the plant pathogenic fungus *Monilinia laxa* showing host specificity. Food Technology and Biotechnology 50(3):326.

Brooks C, Nekrasov V, Lippman ZB, Van Eck J (2014). Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiology 166(3):1292-1297.

Bruez E, Larignon P, Compant S, Rey P (2017). Investigating the durable effect of the hot water treatment used in nurseries on pathogenic fungi inhabiting grapevine wood and involved in Grapevine trunk diseases. Crop Protection 100:203-210.

Calavan EC, Roistacher CN, Nauer EM (1972). Thermotherapy of citrus for inactivation of certain viruses. Plant Disease Reporter 56:976-980.

Camañes G, Scalschi L, Vicedo B, González-Bosch C, García-Agustín P (2015). An untargeted global metabolomic analysis reveals the biochemical changes underlying basal resistance and priming in *Solanum lycopersicum*, and identifies 1-methyltryptophan as a metabolite involved in plant responses to *Botrytis cinerea* and *Pseudomonas syringae*. The Plant Journal 84(1):125-139.

Castel SE, Martienssen RA (2013). RNA interference in the nucleus : roles for small RNAs in transcription, epigenetics and beyond. Nature Reviews Genetics 14(2):100-112. https://doi.org/10.1038/nrg3355.

Chandramouli K, Qian, PY (2009). Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Human Genomics and Proteomics 2009:239204.

Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M, Gal-On A (2016). Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Molecular Plant Pathology 17(7):1140-1153.

Chatenet M, Delage C, Ripolles M, Irey M, Lockhart BEL, Rott P (2001). Detection of Sugarcane yellow leaf virus in quarantine and production of virus-free sugarcane by apical meristem culture. Plant Disease 85(11):1177-1180.

Cheng XJ, He B, Chen L, Xiao SQ, Fu, J, Chen Y (2016). Transcriptome analysis confers a complex disease resistance network in wild rice *Oryza meyeriana* against *Xanthomonas oryzae* pv. *oryzae*. Scientific Reports 6:38215.

Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A (2010). Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 186 (2):757–761.

De Werra P, Bussereau F, Keiser A, Ziegler D (2015). First report of potato blackleg caused by *Pectobacterium carotovorum subsp. brasiliense* in Switzerland. Plant Disease 99(551):10-1094.

Del Pozo O, Lam E (2003). Expression of the baculovirus p35 protein in tobacco affects cell death progression and compromises N gene-mediated disease resistance response to *Tobacco mosaic virus.* Molecular plant-microbe interactions 16(6):485-494.

D'onghia AM, Carimi F, De Pasquale F, Djelouah KMGP, Martelli GP (2001). Elimination *of Citrus psorosis virus* by somatic embryogenesis from stigma and style cultures. Plant Pathology 50(2):266-269.

Engler C, Kandzia R, Marillonnet S (2008). A one pot, one step, precision cloning method with high throughput capability. PloS one 3(11):3647.

Engler C, Youles M, Gruetzner R, Ehnert TM, Werner S, Jones, JD (2014). A golden gate modular cloning toolbox for plants. ACS synthetic biology 3(11): 839-843.

Food and Agriculture Organization (FAO) (2017). The future of food and agriculture – Trends and challenges. Rome. ISBN 978-92-5-109551-5.

Fitch MMM, Lehrer AT, Komor E, Moore PH (2001). Elimination of Sugarcane yellow leaf virus from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. Plant Pathology 50(6):676-680.

Flor H (1955). Host-parasite interaction in flax rust–its genetics and other implications. Phytopathology 45:680-685.

Frison EA, Ng SY (1981). Elimination of sweet potato virus disease agents by meristem tip culture. International Journal of Pest Management 27(4):452-454.61-73.

Fry WE (1982). Principles of Plant Disease Management. New York Academic Press P 378.

Galletta GJ, Maas JL (1990). Strawberry genetics. HortScience 25(8):871-879.

Gao J, Wang G, Ma S, Xie X, Wu X, Zhang X, Xia Q (2015). CRISPR/Cas9-mediated targeted mutagenesis in *Nicotiana tabacum*. Plant molecular biology 87(1-2):99-110.

Gao S, Sosnoskie LM, Cabrera JA, Qin R, Hanson BD, Gerik JS, Thomas JE (2016). Fumigation efficacy and emission reduction using low permeability film in orchard soil fumigation. Pest Management Science 72(2):306-314.
Geu-Flores F, Nour-Eldin HH, Nielsen MT, Halkier BA (2007). USER fusion: A rapid and efficient method for simultaneous fusion and cloning of multiple PCR products. Nucleic Acids Research 35(7):55.
Grout BWW (1990). Meristem tip culture. In: Pollard JW, Walker JM (eds) Methods in molecular biology: Plants cell and tissue culture. New Jersey Humana Press 597 p.
Grzeskowiak L, Stephan W, Rose LE (2014). Epistatic selection and coadaptation in the Prf resistance complex of wild tomato. *Infection,* Genetics and Evolution 27:456-471.
Haberlandt G (1969). Experiments on the culture of isolated plant cells. The Botanical Review 35(1):68-88.
Hansen AJ, Hildebrandt AC (1966). The distribution of *Tobacco mosaic virus* in plant callus cultures. Virology 28(1):15-21.
Hassan M (2014). Influence of protoplast fusion between two *Trichoderma spp*. on extracellular enzymes production and antagonistic activity. Biotechnology and Biotechnological Equipment 28(6):1014-1023.
Hayes RJ, Trent MA, Mou B, Simko I, Gebben SJ, Bull CT (2014). Baby leaf lettuce germplasm enhancement: developing diverse populations with resistance to bacterial leaf spot caused by *Xanthomonas campestris* pv. *vitians*. HortScience 49(1):18-24.
Horgan RP, Kenny LC (2011). 'Omic'technologies: genomics, transcriptomics, proteomics and metabolomics. The Obstetrician & Gynaecologist 13(3):189-195.
Hou L, Chen L, Wang J, Xu D, Dai L, Zhang H, Zhao Y (2012). Construction of stress responsive synthetic promoters and analysis of their activity in transgenic *Arabidopsis thaliana*. Plant Molecular Biology Reporter 30(6):1496-1506.
Hu Z, Parekh U, Maruta N, Trusov Y, Botella JR (2015). Down-regulation of Fusarium oxysporum endogenous genes by host-delivered RNA interference enhances disease resistance. Frontiers of Chemical 3:1-10.
Hussain A, Qarshi IA, Nazir H, Ullah I (2012). Plant Tissue Culture: Current Status and Opportunities, Recent Advances in Plant *in vitro* Culture, Annarita Leva (Ed.), ISBN: 978-953-51-0787-3, InTech. doi: 10.5772/50568.
Iqbal MM, Nazir F, Ali S, Asif MA, Zafar Y, Iqbal J, Ali GM (2012). Over expression of rice chitinase gene in transgenic peanut (*Arachis hypogaea L*.) improves resistance against leaf spot. Molecular Biotechnology 50(2): 129-136.
Iriarte FB, Balogh B, Momol MT, Smith LM, Wilson M, Jones JB (2007). Factors affecting survival of bacteriophage on tomato leaf surfaces. Applied and Environmental Microbiology 73(6):704-1711.
Jabeen N, Chaudhary Z, Gulfraz M, Rashid H, Mirza B (2015). Expression of rice chitinase gene in genetically engineered tomato confers enhanced resistance to Fusarium wilt and early blight. The Plant Pathology Journal 31(3):252.
Jones JB, Jackson LE, Balogh B, Obradovic A, Iriarte FB, Momol MT (2007). Bacteriophages for plant disease control. Annual Review of Phytopathology 45:245-262.
Jones JD, Dangl JL (2006). The plant immune system. Nature 444(7117):323.
Kasture VS, Musmade DS, Vakte MB, Sonawane SB, Patil PP (2012). Current technologies and future trends. Int J. Res Dev. Pharm. Life Science 2:206-217.
Katan J (2000). Physical and cultural methods for the management of soil-borne pathogens. Crop Protection 19(8):725-731.
Katan J (2010). "Cultural approaches for disease management: Present status and future prospects," Journal of Plant Pathology 92(4):S7-S9.
Kebede T, Gemmeda L (2017). Evaluation of banana genotype resistant to Xanthomonas wilt disease (*Xanthomonas campestris* pv. *musacearum*) in south east of Ethiopia. African Journal of Microbiology Research 11(15):603-612.
Kim G, LeBlanc ML, Wafula EK, de Pamphilis CW, Westwood JH (2014). Genomic-scale exchange of mRNA between a parasitic plant and its hosts. Science 345:808-811.
Kim HJ, Lee HR, Jo KR, Mortazavian M, Huigen J, Evenhuis B (2012). Broad spectrum late blight resistance in potato differential set plants MaR8 and MaR9 is conferred by multiple stacked R genes. Journal of Theoretical Applied Genetics 124:923-935. doi: 10.1007/s00122-011-1757-7.
Lamichhane JR, Venturi V (2015). Synergisms between microbial pathogens in plant disease complexes: a growing trend. Frontiers in plant science 6:385.
Larkin PJ, Scowcroft WR (1981). Somaclonal variation—a novel source of variability from cell cultures for plant improvement. Theoretical Applied Genetics 60(4):197-214.
Lee KM, Yu J, Son M, Lee YW, Kim KH (2011). Transmission of *Fusarium boothii* Mycovirus via Protoplast Fusion Causes Hypovirulence in Other Phytopathogenic Fungi. PLos One 6(6):e21629.
Legg JP, Kumar PL, Makeshkumar T, Tripathi L, Ferguson M, Kanju E, Cuellar W (2015). Cassava Virus Diseases: Biology, Epidemiology, and Management. Advances in Virus Research 91:85-142.
Levenfors JP, Eberhard TH, Levenfors JJ, Gerhardson B, Hökeberg M (2008). Biological control of snow mould (*Mcirodochium nivale*) in winter cereals by *Pseudomonas brassicacearum,* MA250. BioContro 53(4):651-665.
Li D, Wang L, Teng S, Zhang G, Guo L, Mao Q (2012). Proteomics analysis of rice proteins up-regulated in response to bacterial leaf streak disease. Journal of Plant Biology 55(4):316-324.
Li R, Shen Z, Sun L, Zhang R, Fu L, Deng X, Shen Q (2016). Novel soil fumigation method for suppressing cucumber Fusarium wilt disease associated with soil microflora alterations. Applied Soil Ecology 101:28-36.
Lincoln JE, Richael C, Overduin B, Smith K, Bostock R, Gilchrist DG (2002). Expression of the antiapoptotic baculovirus p35 gene in tomato blocks programmed cell death and provides broad-spectrum resistance to disease. Proceedings of the National Academy of Sciences 99(23):15217-15221.
López-Delgado H, Mora-Herrera ME, Zavaleta-Mancera HA, Cadena-Hinojosa M, Scott IM (2004). Salicylic acid enhances heat tolerance and *potato virus X* (*PVX*) elimination during thermotherapy of potato microplants. American Journal of Potato Research 81(3):171-176. doi:10.1007/bf02871746.
Lotfi M, Alan AR, Henning MJ, Jahn MM, Earle ED (2003). Production of haploid and doubled haploid plants of melon (*Cucumis melo* L.) for use in breeding for multiple virus resistance. Plant Cell Reports 21(11):1121-1128. doi:10.1007/s00299-003-0636-3.
Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T (2017). Transcriptomics technologies. PLoS Computational Biology 13(5):1005457.
Lu Y, Ye X, Guo R, Huang J, Wang W, Tang J (2017). Genome-wide targeted mutagenesis in rice using the CRISPR/Cas9 system. Molecular Plant 10(9): 242-1245.
Mach J (2016). The Viroid, the Polymerase, and the Transcription Factor: Replication of a Naked, Noncoding RNA Pathogen by Host Proteins. The Plant Cell 28(5):999-1000.
Mahlein AK (2016). Plant disease detection by imaging sensors–parallels and specific demands for precision agriculture and plant phenotyping. Plant Disease 100(2):241-251.
Mahuku G, Lockhart BE, Wanjala B, Jones MW, Kimunye JN, Stewart LR, Kumar PL (2015). Maize lethal necrosis (MLN), an emerging threat to maize-based food security in sub-Saharan Africa. Phytopathology 105(7):956-965.
Manganaris GA, Economou AS, Boubourakas IN, Katis NI (2003). Elimination of PPV and PNRSV through thermotherapy and meristem-tip culture in nectarine. Plant Cell Reports 22(3):195-200.
Mashilo J, Van Niekerk R, Shanahan P (2013). Combined thermotherapy and meristem tip culture for efficient elimination of feathery mottle virus in sweet potato (*Ipomoea batatas*). Acta Horticulturae, 1007:719-725. doi:10.17660/actahortic.2013.1007.83
Mehta CM, Palni U, Franke-Whittle IH, Sharma AK (2014). Compost: its role, mechanism and impact on reducing soil-borne plant diseases. Waste Management 34(3):607-622.
Mehta YR, Angra DC (2000). Somaclonal variation for disease resistance in wheat and production of dihaploids through wheat x maize hybrids. Genetics and Molecular Biology 23(3):617-622.
Meyers BC (2003). Genome-Wide Analysis of NBS-LRR-Encoding Genes in *Arabidopsis*. The plant cell online 15(4):809-834.

doi:10.1105/tpc.009308.
Miklas PN, Kelly JD, Beebe SE, Blair MW (2006). Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147(1-2):105-131.
Munir I, Hussan W, Kazi MSK, Farhatullah AAM, Iqbal A, Munir R (2016). Production of transgenic brassica juncea with the synthetic chitinase gene (NIC) Conferring resistance to *Alternaria brassicicola*. Pakistan Journal of Botany 48(5): 2063-2070.
Mwangangi M, Ateka E, Nyende A, Kagundu A (2014). Elimination of cassava brown streak virus from infected cassava. Journal of Biology, Agriculture and Healthcare 4(13):32-40.
Mwendo M, Ochwo-ssemakula M., Lamo J, Gibson P, Edema R (2017). Reaction of upland rice genotypes to the brown spot disease pathogen *Bipolaris oryzae*. African Journal of Rural Development 2(1):127-133.
Navarro L (1992). Citrus shoot tip grafting *in vitro*. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry. High-tech and micropropagation. Berlin: Springer-Verlag pp. 327-338.
Navarro L, Juarez J, Ballester JF, Pina JA (1980). Elimination of some citrus pathogens producing psorosis-like leaf symptoms, by shoot-tip grafting *in vitro*. In International Organization of Citrus Virologists Conference Proceedings (1957-2010) 8:8.
Nwugo CC, Doud MS, Duan YP, Lin H (2016). Proteomics analysis reveals novel host molecular mechanisms associated with thermotherapy of 'Ca. Liberibacter asiaticus'-infected citrus plants. BMC Plant Biology 16(1):253.
Ocsoy I, Paret ML, Ocsoy MA, Kunwar S, Chen T, You M, Tan W (2013). Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against *Xanthomonas perforans*. Acs Nano 7(10):8972-8980.
Ogero K, Gitonga NM, Mwangi M, Ngugi M, Ombori O (2012). Low cost tissue culture technology in the regeneration of sweet potato (*Ipomoea batatas* (L) Lam). Research Journal of Biological 2(2):71-78.
Onkendi EM, Kariuki GM, Marais M, Moleleki LN (2014). The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: A review. Plant Pathology 63(4):727-737.
Pang Y, Liu X, Ma Y, Chernin L, Berg G, Gao K (2009). Induction of systemic resistance, root colonisation and biocontrol activities of the rhizospheric strain of *Serratia plymuthica* are dependent on N-acyl homoserine lactones. European Journal of Plant Pathology 124(2):261-268. doi:10.1007/s10658-008-9411-1.
Parker D, Beckmann M, Zubair H, Enot DP, Caracuel-rios Z, Overy DP, Draper J (2009). Metabolomics analysis reveals a common pattern of metabolic re-programming during invasion of three host plant species by *Magnaporthe grisea*. The Plant Journal 59(5):723-737.
Patil BL, Legg JP, Kanju E, Fauquet CM (2015). Cassava brown streak disease: a threat to food security in Africa. Journal of General Virology 96(5):956-968.
Prabha K, Baranwal VK, Jainm RK (2013). Application of Next Generation High Throughput Sequencing Technology in Characterization, Discovery and Molecular Interaction of Plant Viruses. Indian Journal of Virology 24(2):157-165. doi.org/10.1007/s13337-013-0133-4.
Praveen S, Ramesh SV, Mishra AK, Koundal V, Palukaitis P (2010). Silencing potential of viral derived RNAi constructs in Tomato leaf curl virus-AC4 gene suppression in tomato. Transgenic Research 19(1):45-55.
Prince G, Clarkson JP, Rajpoot NM (2015). Automatic detection of diseased tomato plants using thermal and stereo visible light images. Plos one 10(4):0123262.
Pyott DE, Sheehan E, Molnar A (2016). Engineering of CRISPR/Cas9-mediated potyvirus resistance in transgene-free *Arabidopsis* plants. Molecular Plant Pathology 17(8):1276-1288.
Richa K, Tiwari IM, Devanna BN, Botella JR, Sharma V,Sharma TR (2017). Novel chitinase gene LOC_Os11g47510 from indica rice Tetep provides enhanced resistance against sheath blight pathogen *Rhizoctonia solani* in rice. Frontiers in Plant Science 8:596.
Richa K, Tiwari IM, Kumari M, Devanna BN, Sonah H, Kumari A, Sharma TR (2016). Functional characterization of novel chitinase genes present in the sheath blight resistance QTL: qSBR11-1 in rice line tetep. Frontiers in Plant Science 7:244.
Rodrigues SP, Lindsey GG, Machado P, Fernandes B (2009). Biotechnological Approaches for Plant Viruses Resistance : From General to the Modern RNA Silencing Pathway. Brazilian Archive of Biology and Technology 52(4):795-808.
Roistacher CN (1993). Psorosis - a review. Proceedings of the 12th Conference of the International Organization of Citrus Virologists, India (12):139-1.
Rojas CM, Senthil-Kumar M, Tzin V, Mysore KS (2014). Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Frontiers in Plant Science 5:17.
Sankaran S, Mishra A, Ehsani R, Davis C (2010). A review of advanced techniques for detecting plant diseases. Computers and Electronics in Agriculture 72(1):1-13.
Sarkar S, Chaudhuri S (2016). Bacterial wilt and its management. Current Science 110(8):1439-1445.
Sarrion-Perdigones A, Vazquez-Vilar M, Palací J, Castelijns B, Forment J, Ziarsolo P, Blanca J, Granell A, Orzaez D (2013). GoldenBraid 2.0: a comprehensive DNA assembly framework for plant synthetic biology. Journal of Plant Physiology 162:1618-1631. doi: 10.1104/pp.113.217661.
Schenk ST, Hernandez-Reyes C, Samans B, Stein E, Neumann C, Schikora M, Schikora A (2014). N-Acyl-Homoserine Lactone Primes Plants for Cell Wall Reinforcement and Induces Resistance to Bacterial Pathogens via the Salicylic Acid/Oxylipin Pathway. The Plant Cell 26(6):2708-2723.
Schikora A, Schenk ST, Stein E, Molitor A, Zuccaro A, Kogel K (2011). N-Acyl-Homoserine Lactone Confers Resistance toward Biotrophic and Hemibiotrophic Pathogens via Altered Activation of AtMPK6. Plant Physiology 157(3):1407-1418. doi:10.1104/pp.111.180604.
Schornack S, Moscou MJ, Ward ER, Horvath DM (2013). Engineering plant disease resistance based on TAL effectors. Annual Review of Phytopathology 51:383-406. doi: 10.1146/annurev-phyto-082712-102255.
Sebastiani P, Lenzi A, Pugliesi C, Fambrini M (1994). Somaclonal variation for resistance to *Verticillium dahliae* in potato (*Solanum tuberosum* L.) plants regenerated from callus. Journal of Euphytica 80:5-11.
Sharifi NP, Koohi HM, Dizadji A, Kalantari S (2015). Elimination of Bean yellow mosaic virus through thermotherapy combined with meristem-tip culture in gladiolus corms. Journal of Crop Protection 4(4):533-543.
Singh AK, Sharma VISHAL, Pal AK, Acharya VISHAL, Ahuja PS (2013). Genome-wide organization and expression profiling of the NAC transcription factor family in potato (*Solanum tuberosum L.*). DNA Research 20(4):403-423.
Smith RH (2013). Plant Tissue Culture. Techniques and Experiments. Experimental Agriculture 49(2):317. doi:10.1017/s0014479712001433.
Soler N, Fagoaga C, Chiibi S, López C, Moreno P, Navarro L (2011). RNAi-Mediated Protection Against Citrus Tristeza Virus in Transgenic Citrus Plants. Non Coding RNAs in Plants, 447-460. doi:10.1007/978-3-642-19454-2_27.
Soliman A, Barsoum B, Mohamed G, Rezk A, Aboul-Ata A, Mazyad H (2008). SiRNA Silencing of PVX Coat Protein Gene Affects Accumulation of Viral RNA in Potato and Tobacco Plants. International Journal of Virology 4(1):14-25. doi:10.3923/ijv.2008.14.25.
Sprague SJ, Balesdent MH, Brun H, Hayden HL, Marcroft SJ, Pinochet X (2006). Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. European Journal of Plant Pathology 114:33-40.
Stark-Lorenzen P, Nelke B, Hänßler G, Mühlbach HP, Thomzik JE (1997). Transfer of a grapevine stilbene synthase gene to rice (*Oryza sativa L.*). Plant Cell Reports 16(10):668-673.
Thorpe P, Mantelin S, Cock PJ, Blok VC, Coke MC, Eves-van den Akker S, Wright KM (2014). Genomic characterisation of the effector complement of the potato cyst nematode Globodera pallida. BMC Genomics 15(1):923.
Tjamos EC, Papavizas GC, Cook RJ (1992). Biological Control of Plant Diseases Progress and Challenges for the Future. Boston, MA:

Springer US: Imprint: Springer P 462. https://trove.nla.gov.au/work/14848438?q&versionId=229758745
Van Bogaert G (1975). A comparison between colchicineinduced tetraploid and diploid cultivars of *Lolium* species. In: Nuesch B (ed) Ploidy in fodder plants. Eucarpia Rep Meet Fodder Crops Sect, Zürich pp. 101-110.
Van Wijk KJ (2001). Challenges and Prospects of Plant Proteomics. Plant Physiology 126(2):501-508.
Vleeshouwers VG, Oliver RP (2014). Effectors as tools in disease resistance breeding against biotrophic, hemibiotrophic, and necrotrophic plant pathogens. Molecular Plant-Microbe Interactions 27(3):196-206.
Vossen JH, Jo KR, Vosman B (2014) Mining the genus *Solanum* for increasing disease resistance. Genomics of Plant Genetic Resources. Springer, Netherlands pp. 27-46
Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Zhao K (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PloS one 11(4):0154027.
Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL (2014). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology 32:947-951. doi: 10.1038/nbt.2969.
Warren GS, Thomas P, Herrera MT, Hill SL, Terry RF (1992). The use of plant cell cultures for studying virus resistance, and enhancing the production of virus-resistant and virus-free plants. Journal of biotechnology 22(3):171-200.
Warth B, Parich A, Bueschl C, Schoefbeck D, Katharina N, Neumann N, Schuhmacher R (2015). GC – MS based targeted metabolic profiling identifies changes in the wheat metabolome following deoxynivalenol Treatment. Metabolomics 11(3):722-738.
Wisniewski M, Droby S , Norelli J, Liu J, Schena L (2016). Alternative management technologies for postharvest disease control: The journey from simplicity to complexity. Postharvest Biology and Technology 122:3-10.
Yadav RC, Sareen PK, Chowdhury JB (1991). Interspecific hybridization in *Brassica juncea* x *Brassica tournefortii* using ovary culture. Cruciferae Newsletter 84:14-15.
Yang JK, Tong ZJ, Fang DH, Chen XJ, Zhang KQ, Xiao BG (2017). Transcriptomic profile of tobacco in response to *Phytophthora nicotianae* infection. Scientific Reports 7(1):401.
Zhang CX, Tian Y, Cong PH (2015). Proteome analysis of pathogen-responsive proteins from apple leaves induced by the *alternaria* blotch *Alternaria alternata*. PloS One 10(6):0122233.
Zhou H, Liu B, Weeks DP, Spalding MH, Yang B (2014). Large chromosomal deletions and heritable small genetic changes induced by CRISPR/Cas9 in rice. Nucleic Acids Research 42(17):10903-10914.
Zulkarnain Z, Tapingkae T, Taji A (2015). Applications of *In Vitro* Techniques in Plant Breeding. Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools pp. 293-{"IsDistinguishedFolder":true,"FolderId":{"__type":"FolderId:#Exchange","Id":"AQMkADJkMmMzZTRiLWY4YmEtNDkwNC04Nzk4LTEyYjdkMTU0YmNiYwAuAAADC86hcQn+E0i0ivtqFK/3HwEA9NwpG98wlU+eaqXMujbJIwAAAdYH8QAAAA==","ChangeKey":"AQAAABYAAADAtPysI3R8RrOUhTWWMWYJAAEpSlHI"},"DragItemType":3}328. doi:10.1007/978-3-319-22521-0_10.