Journal of Agricultural Science and Food Technology
Vol. 3 (3), pp. 39-51, May, 2017
ISSN: 2465-7522
Review
http://pearlresearchjournals.org/journals/jasft/index.html

# Genomics Based Approaches towards Management of Plant Diseases with Emphasis on *in silico* Methods as a Prudent Approach

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Accepted 24 March, 2017

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## ABSTRACT

**Feeding the growing population of the world in next thirty years or so requires raising overall food production on an average by fifty percent of the current food production. In view of the fact that globally on an average fifty percent of food production is lost due to various kinds of diseases, the plant disease management strategies become quite significant in fulfilling the future global food supply. Thus, the present review illustrates the various conventional as well as contemporary plant disease management strategies with emphasis on the genomics and *in silico* based approaches. Early detection of diseases is a crucial component of plant disease management, accordingly, a number of direct and indirect methods, which have been developed to detect various kinds of plant diseases, have been discussed. Furthermore, the various types of defence responses exhibited by plants to protect themselves against these pathogens, such as production of toxic chemicals, pathogen degrading enzymes, secondary metabolites, volatile organic compounds, etc., have been described. The present review describes both the traditional as well as contemporary disease management strategies covering the basic concepts involving the process of infection and progression of the pathogen as well as defence response exhibited by plants and their interaction with the environment. The present day biotechnology and bioinformatics have immense potential in management of plant diseases in globally shifting agricultural and environmental priorities. They have provided newer methodologies in genomics based approaches for management of plant disease resistance. Thus, the present review emphasises the recent methodologies and developments in the area of agrigenomics which have led to the development of highly promising disease management strategies along with an insight into the understanding of the impact of plant pathogen interactions at genome level.**

**Key words: Plant disease management, Agrigenomics, *in silico* approach, Single nucleotide polymorphism and detection methods.**

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## INTRODUCTION

World population is continuously increasing and is expected to increase from the current figure of 6.9 billion to 9.1 billion by 2050. Therefore, feeding the growing population of the world require raising overall food production by more than 50% of the current production (Alexandratos and Bruinsma, 2012). At the same time, global food production is hampered by various kinds of diseases leading to huge losses in terms of both the quality and quantity. Thus, according to one estimate, the worldwide losses due to plant diseases range between 9 to 16% in rice, wheat, barley, maize, potato, soybean, cotton and coffee amounting to an average loss of 14% of global food production. Therefore, methods to prevent or reduce the loss of crop productivity due to various kinds of diseases are crucial to meet the supply of food to the growing population of the world. Also monitoring of health and detection of diseases in plants is critical for sustainable agriculture which is an integrated system of plant production practices having a site specific application to satisfy the human food, enhance environmental quality and natural resources (Hagaag, 2002; Sankarana et al., 2010). To ensure agricultural sustainability, disease management programs that reflect the dynamics of pathogen population structure as well as their interaction with target plants are essential and are frequently determined by disease forecasting or modelling (Zhan et al., 2014; Trivedi et al., 2016). Thus, 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 negative environmental impacts of diseases and their management (Pinstrup-Anderson, 2001). Successful implementation of these methods at both local as well as global levels of changing agricultural environments demands new approaches for their durable control (Gilligan, 2008). The key role of bioinformatics is acquiring striking importance in the era of outstanding advances in omics technologies for its fundamental support in describing the multifaceted aspects of biological functionalities.

The manifold omics efforts flourishing worldwide are also contributing fundamental novelties in many aspects of agricultural sciences and, as a consequence, bioinformatics is acquiring a crucial role in these research fields (Esposito et al., 2016). *In silico* plant disease management leads to encompassing a systematic development and application of information technology solutions to handle biological information by addressing biological data collection and warehousing, data mining, database searches, analyses and interpretation, modeling and product design using bioinformatics tools and techniques (Alemu, 2015). Rapid and huge whole genome sequence data generated as a result of next-generation sequencing are of immense importance in understanding of plant pathogen interactions at molecular level (Trivedi et al., 2016). The potential of modern biology to identify new resources for genetic, chemical and biological control of plant disease is remarkably high. A major motivation for genome sequencing has been the identification of biologically significant differences in gene repertoire of plant and pathogen through comparative genomics as well as insight into the evolutionary mechanisms by which variations have been achieved. As more genome sequences become available, so does the potential for deeper understanding of the nature and evolution of genetic factors that account for specific types of interactions with host plants (Lindeberg, 2012). Studies of model plants have significantly enhanced our understanding of plant innate immune perception and signalling. The identification of classical plant resistant genes and other model dicots facilitated the successful cloning of multiple disease resistant genes (Ellis et al., 2014; Wulff and Moscou, 2014).Thus, with the advancements in genome sequencing and analyses, we are now at a stage to exploit the basic knowledge gained in plant model species at a full genome scale in crops (Piquerez et al., 2014). The present review describes various plant disease management strategies with emphasis on genomics and *in silico* based methods as a prudent approach.

## PLANT PATHOGEN TYPES AND DISEASES

There are a number of pathogens causing a variety of diseases in different plants, in different environment with effects ranging from mild symptoms to calamities (Strange and Scott, 2005). These pathogens display striking differences in the nature of their interactions with host plants with regard to host range, tissue specificity, and optimal environmental conditions. Thus, it is necessary to understand the biology of the each pathogen and their effective diagnosis and management strategies. In recent times, functional analyses of genes as well as availability of plethora of sequenced genomes of both pathogens as well as respective host plants, have greatly aided studies aimed at identifying disease-related genes and approaches towards developing resistant plants. These newer approaches have great potential towards maintenance as well as increasing the plant productivity. Major plant pathogens causing various diseases can be categorized in to bacteria, fungi, virus, nematodes and parasitic plants. From the available literature, depending on variety of the crop and/or pathogen and environmental conditions the losses in crop yield due to diseases caused by major pathogens exhibit wide variation ranging, on an average, between 10 to 40%. Among these, the majority of the losses have been projected due to fungal pathogens (~40%) (Godfrey et al., 2016; Moore et al., 2011) followed by bacteria (~20%) (Kumar et al., 2012) nematodes (~15%) (Nicol et al., 2011), viruses (~15%) (Coutts et al., 2009) and parasitic plant pathogens (~10%) (Rodenburg et al., 2016; Parker, 2008). Relative contribution of losses in plant productivity due to various pathogens is depicted in Figure 1. A brief description of each category of the pathogens along with the diseases they cause is provided in following sections.

### Bacteria

Because of the extreme diversity exhibited by bacteria, they are found almost everywhere on Earth and affect a number of plants growing in diverse conditions. Symptoms caused by plant pathogenic bacteria include lesions and overgrowths, wilts, leaf spots, specks and blights, soft rots, as well as scabs and cankers (Brian et al., 2009; Ellis et al., 2008). Bacteria are dependent on

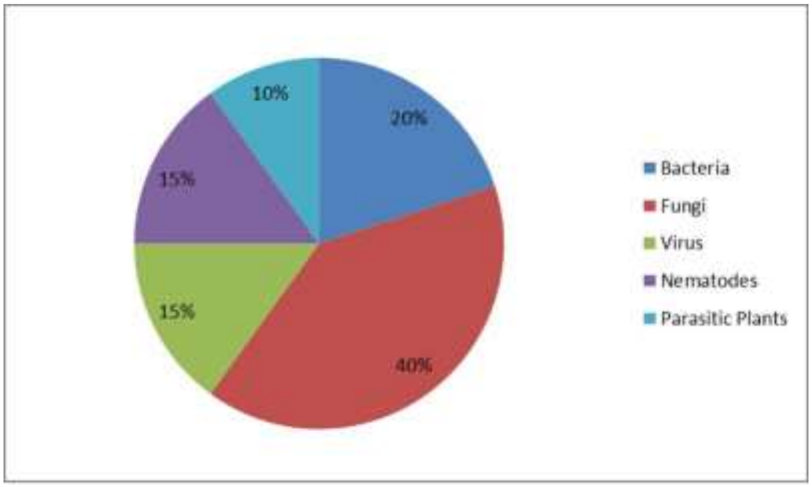

**Figure 1.** Contribution of approximated losses in plant productivity due to various kinds of pathogens.

the major outside agents for dispersal of bacterial infection from plant to plant includes splashing of water through irrigation, human contact or open wounds (Pernezny et al., 2014). More than 80 species of bacteria consisting of numerous subspecies belonging to the following genera: *Erwinia, Pectobacterium, Pantoea, Agrobacterium, Pseudomonas, Ralstonia, Burkholderia, Acidovorax, Xanthomonas, Clavibacter, Streptomyces, Xylella, Spiroplasma,* and *Phytoplasma* have been found to cause diseases in plants. Thus, *Ralstonia (Pseudomonas) solanacearum*, a plant pathogen with a worldwide distribution, causes diseases in more than 250 host species belonging to 50 families including potato (Schell, 2000). *Clavibacter sp* causes bacterial canker of pepper causing around 20% yield losses at different locations in Himanchal Pradesh, India (Kumar et al., 2012). Among a number of endophytic bacterial parasites, the one which are exclusively restricted to xylem cells (known as xylem-limited bacteria, XLB) are important. For example, various strains of *Xylella fastidiosa,* which are responsible for most known XLB plant diseases (Purcell and Hopkins 1996) have emerged as worldwide threats. This bacteria is an important plant pathogen affecting many economically important crops, such as Pierce's disease (PD) in grapevine, citrus variegated chlorosis in citrus, almond leaf scorch disease in almond (Almedia and Nunny, 2015, Bhattacharyya et al., 2002).

## Fungi

It is estimated that fungi share various kinds of diseases caused by around 15,000 different species, majority of which belong to the Ascomycetes and Basidiomycetes (Aiyere, 2004). All fungi that colonize plants are recognized by the plant immune system and elicit host defences. Looking to the magnitude of losses as well as diversity of pathogenic fungi, fungal plant pathogens are of great economic importance (Presti et al., 2015). Thus, *Puccinia graminis tritici* strain Race Ug99, causing black stem rust disease in wheat is reported to be a major threat to wheat production in Africa, Middle East and South Asia (Pretorius et al., 2000). *Pyricularia oryzae,* an ascomycete fungus, and attacks rice and maize, severely affects their global production (Talbot, 2003). (*Magnaporthe oryzae* (causing rice blast disease), *Blumeria graminis* (causing powdery mildew of wheat and barley), *Rhizoctonia solani* (causing sheath blight of rice), *Fusarium xylarioides* (causing coffee wilt disease) are a few examples of fungi effecting productivity drastically at global level. *Leptosphaeri amaculans*, a pathogen of *Brassica napus* (canola), has been studied using functional genomics approach in order to avert yield losses due to blackleg disease in Australia (Howlett et al., 2015).

## Virus

Viruses also constitute one of the important and diverse groups of pathogens which infect a variety of plants (Prendeville et al., 2014). Vectors are responsible for the spread of viruses which include insects, mites, nematodes, fungi and even humans. Symptoms associated with viral infections include reduced growth, mosaic pattern of light and dark patches on leaves, cup shaped leaves, curling of leaves etc. Viruses exhibit a great extent of diversity with regards to host range. Thus, for example, CMV has the widest host range for any plant virus, including more than 1200 species in over 100 families of dicotyledonous and monocotyledonous angiosperms (Edwardson and Christie, 1991). Barley yellow dwarf viruses (BYDV), are distributed worldwide and infect over 150 species of the Poaceae, including most of the staple cereals such as wheat, barley, oats, rye, rice, and maize (Edwards et al., 2001). Tobacco mosaic virus (TMV) occur in all tobacco growing countries in the world affecting more than 150 genera of herbaceous dicotyledonous plants including many vegetables, flowers and weeds. Yellow vein mosaic virus (YVMV) of *Abelomoschus esculents* (lady finger) is the most devastating disease in all lady finger growing regions of India. In case the plants get infected at early stages of development it causes 80% of crop loss. Lettuce infectious yellows crinivirus (LIYC), Cucurbit yellow stunting disorder crinivirus (CYSDC), are some other economically important viral diseases in plants.

### Nematodes

More than 4,100 species have been identified as plant parasitic nematodes. These plant-parasitic nematodes vary in shapes and sizes. Thus, the typical nematode has a long and slender worm-like shape, but often the adult animals are so swollen that they no longer even resemble worms. The size of plant-parasitic nematodes range between 250 µm to 12 mm in length to about 15 to 35 µm in width. Though nematodes feed on all parts of the plant, including roots, stems, leaves, flowers and seeds but as most plant parasitic nematodes are soil borne, they primarily feed on root tissue with only few species feeding on shoot tissues (Lambert and Bekal, 2002). One of the most devastating nematode species, namely *Ditylenchus dipsaci,* attacks over 450 different plant species, including weeds. Species of *Meloidogyne* such as *Meloidogyne hapla* which attack many important crop plants, including groundnut, potato, carrot, sugar beet, strawberry, and onion, and on occasions, may cause total crop loss (Strange and Scott, 2005, Evans et al., 1993). Among some of the highly damaging species of nematodes, common in India, *Anguina tritici* is worth mentioning which causes Ear Cockle Disease of wheat, resulting into losses of about 10000 tonnes of wheat costing more than 70 million rupees annually. Similarly, the annual loss to Indian Coffee due to root lesion nematode *Pratylenchus coffeae* is estimated to be about 40 million rupees. The golden cyst nematode of potato *Globodera rostochinensis* is also a serious problem in southern hill area of India.

### Parasitic Plants

Parasitic plants comprise of about 3000 species distributed among 16 families (Musselman and Press, 1995). These parasitic plants are classified into two classes namely hemiparasite and holoparasite. Hemiparasites are partially dependent on their host for nutrition due to presence of chlorophyll. Holoparasites, such as mistletoe, have chlorophyll but no roots and depend on their host for minerals and water (George, 1992; Deeks et al., 1994). Some other parasitic seed plants, such as dodder (*Cuscuta*), having neither chlorophyll nor true roots, depend entirely on their host for their existence. It belongs to family *Cuscutaceae* and consists of about 200 species which live as stem holoparasites on other plants (Kaiser et al., 2015). Dodder, Broomrapes and Witchweed are some of the important examples of parasitic plants. Thus, dodder plants, which appear as conspicuous tangles of intertwined yellow threads on the aerial parts of host plants, parasitize a large number of host plants causing incredible destruction to a number of economically important plants (Lucas et al., 1992). The most frequently affected host plants belong to legumes, citrus and edible oil plants (Orr et al., 1996). Another important parasitic plant, namely Broomrapes (*Orobanche* species), widely spread in many countries of Central Asia and the Mediterranean region, affect productivity of a number of economically important plants such as potato, sunflower, beans, eggplant, tomato etc. Witchweed (*Striga* species), is an obligate root parasite of grain grasses and legumes. It has bright red flowers, which grows in clumps to about 50 cm in height. Plants attacked by witchweed look like they are suffering from drought. They are stunted, wilted, and yellowish. Heavily parasitized plants eventually die and produce little or no yield. A single witch weed plant may produce half a million tiny brown seeds, which are easily spread by water and wind (Joel, 2000).

## MECHANISM OF PLANT DEFENSES AGAINST PATHOGENS

In order to protect against pathogen, plants develop a wide variety of defence responses. Some may be categorized as constitutive ones which include a variety of barriers to pathogens such as cell wall, epidermal cuticle, bark etc. while other type of defense response, categorized as the inducible defence responses, include production of toxic chemicals, pathogen degrading enzymes, secondary metabolites, volatile organic compounds etc. Inducible defence responses are classified into two major types of responses, namely hypersensitive response (HR) and systemic acquired resistance (SAR) response. HR is characterized by plant cell suicide at the site of infection. The diversity observed in plant cell death morphologies suggests that there may be multiple pathways through which the HR can be triggered. Signals from pathogens appear to interfere with these pathways (Morel and Dangal, 1997; Kombrink and Schemelzer, 2001). However, the two major HR responses include hyper-lignification and extensive oxidative damages, in localised fashion, resulting into restriction of pathogen entry to the cell and subsequent cell death. Lignin, a highly branched heterogeneous phenolic polymer found principally in the secondary cell walls of plants, provides a strong physical barrier against pathogen attack due to its insoluble, rigid, and virtually indigestible nature (Freeman and Beattie, 2008).

Furthermore, the interference between host and pathogen affects a number of genes belonging to a broad range of cellular processes, such as hormonal regulation, cell cycle control and endogenous transport of macromolecules. Expression of these genes is in tune with the severity of the infection (Pallas and Antonio, 2011). HR responses are typically more pathogen specific and are frequently triggered by pathogen specific effectors and their interaction with a plant cell gene product. Though majority of pathogens such as bacteria, fungi viruses and nematodes are capable of inducing HR in plants, HR was first studied in relation to TMV infection. It was observed that the zone surrounding TMV-induced local lesions on some tobacco species was completely resistant to subsequent TMV infection (Mandadi and Scholthof, 2011). Plant tissues may become resistant to a broad variety of pathogens for an extended period of time, known as SAR (Freeman and Beattie, 2008; Fu and Dong, 2013).

In the SAR, plants are primed (sensitized) to more quickly and more effectively activate defense responses the second time they encounter pathogen attack (Conrath, 2006). Commonly associated with the HR and SAR is the systemic synthesis of several families of serologically distinct, low molecular weight pathogenesis related (PR) proteins. These proteins are induced during SAR and develop resistance to pathogen attack. Many secondary metabolites in plants have role in defence against pathogens. Secondary metabolites are involved in deterrence activity, toxicity or acting as precursors to physical defence system (Bennet and Wallsgrov, 1994). Pyrethrins are monoterpenoid esters produced by chrysanthemum plants that act as insect neurotoxins. Many commercially available insecticides are synthetic analogues of pyrethrins, called pyrethroids. Gossypol, a terpenoid produced by cotton (*Gossypium hirsutum*) that has strong antifungal and antibacterial properties. Citronella is an essential oil isolated from lemon grass (*Cymbopogon citratus*) it contains high limonoid levels and has become a popular insect repellent.

## COMMON METHODS FOR PLANT DISEASE DETECTION

An early and accurate diagnosis of plant disease is a crucial component of plant management system. Monitoring plant health and detecting pathogen early are essential to reduce disease spread and facilitate effective management practices (Miller and Martin, 1988). Thus, a number of methods to detect a variety of plant diseases have been developed which are categorized as direct and indirect methods. Direct methods include, laboratory-based techniques such as polymerase chain reaction (PCR), immunofluorescence (IF), fluorescence in-situ hybridization (FISH), enzyme-linked immunosorbent assay (ELISA), flow cytometry (FCM) and gas chromatography-mass spectrometry (GC-MS). On the other hand, methods such as infrared thermography, fluorescence imaging and hyperspectral techniques are included under indirect methods. In addition to this a number of relatively newer methods have been developed which include biosensors. Some of these methods are briefly described in the following sections.

### Direct Methods

#### *Immunochemical Methods / Serological Methods*

Methods such as, enzyme-linked immunosorbent assay (ELISA), IF and FCM, have been developed for diagnosis of bacterial, fungal as well as viral pathogens (Martinelli et al., 2014). ELISA is by far the most widely used immunodiagnostic technique, because of its high throughput potential. In this method, the target epitopes (antigens) from the viruses, bacteria and fungi are made to specifically bind with antibodies conjugated to an enzyme. The detection can be visualized based on color changes resulting from the interaction between the substrate and the coupled enzyme. The sensitivity of ELISA varies depending on the organism and sample freshness (Schaad et al., 2001). IF is a microscopic method which harnesses both the power of antibodies to bind to their cognate antigens along with the use of the fluorescence microscope to visualise the structures to which they bind. The technique is of particular use in pathology where the location and morphology of the bacterial cells can be viewed due to the fluorescently labelled antibodies. FCM is a technique which enables the determination of physical and/or biochemical characteristics of biological particles, such as bacterial cells, in a suspension. This technique has capacity for simultaneously measuring several parameters, based on light scattering and fluorescence, on thousands of individual cells within a few minutes (Chittara and van den Bulk, 2003). FCM has been proven to be efficient for detection of soil borne bacteria such as *Bacillus subtilis* in mushroom composts (Diaper and Edwards, 1994).

#### *Nucleic Acid Based Methods*

Among the tools available for pathogen detection, nucleic acid (NA) based methods, the PCR based methods are currently very common due to thier rapid, specificity, sensitivity as well as speed (Vincelli 2016). Many PCR variants such as nested PCR (nPCR), multiplex PCR (m-PCR), inverse PCR (Inv-PCR) etc. have also been employed. In addition to this, the real-time PCR (RT-PCR) also finds great application with regards to accurate detection and quantification of pathogens (López et al., 2009). PCR offers several advantages by having the capability to detect a single target in complex mixtures, rapid and specific detection of multiple targets, and the

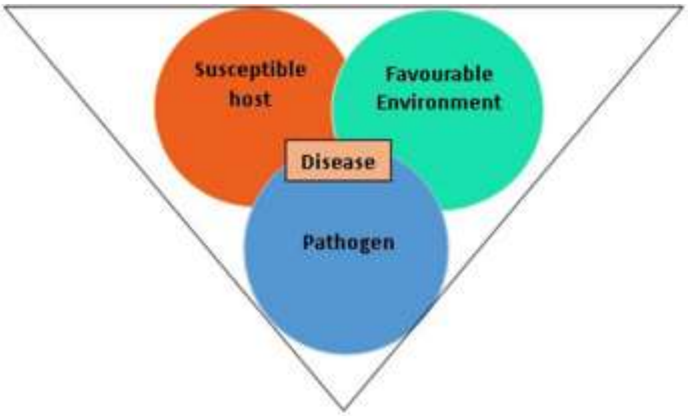

**Figure 2.** Disease triangle involving interaction between host, pathogen and environment.

potential to detect unculturable pathogens such as viruses or some bacteria and phytoplasma. Many other nucleic acid based methods like fluorescence in situ hybridization (FISH), which is applied to bacterial pathogens in combination with microscopy and hybridization of DNA probes and target gene from diseased plant samples. Due to the presence of pathogen-specific ribosomal RNA (rRNA) sequences in plants, recognizing this specific information by FISH can help in identifying 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 (Kliot et al., 2014).

### Indirect Methods

#### *Infrared Thermography*

Infrared thermography assesses plant temperature and is correlated with plant water status. It allows imaging the differences in surface temperature of leaves and canopies of infected and healthy plants. The emitted infrared radiation can be captured by thermographic cameras and color difference can be analyzed (Mahlein, 2016).

#### *Fluorescence Imaging*

In this technique various chlorophyll fluorescence parameters are used to estimate photosynthetic activity of plants (photosynthetic electron transfer) using LED or laser light sources (Bourigel and Herppich, 2014). This technique has been shown to be useful for quantification as well as discrimination of fungal infections.

#### *Hyperspectral Techniques*

This technique provides spectral spatial information for the imaged object. It can be used to obtain information about plant health over a wide range of spectrum between 350 to 2500 nm. The spatial resolution has strong influence on detection of plant diseases or plant pathogen interaction (Mahlein et al., 2012).

#### *Biosensors*

These are analytical devices that convert a biological response into an electrical signal. These sensors are also based on chemical, electrochemical, optical, magnetic or vibrational signals. In the recent developments, several types of biosensors have been developed to increase their specificity and overall performance. Recently nanoparticles have been used to increase their performance because they provide a friendly platform for the assembly of bio-recognition element, the high surface area and high electronic conductivity that enhance the limit of detection (sensitivity). The nanomaterials used for biosensor construction include metal and metal oxide nanoparticles, quantum dots, carbon nanomaterials such as carbon nanotubes and graphene as well as polymeric nanomaterials. Other than this several other biosensors such as affinity biosensors, antibody-based biosensors, enzymatic electrochemical biosensors and bacteriophage based biosensors are some commonly used sensors for plant disease detection (Fang and Ramasamy, 2015).

## DISEASE MANAGEMENT PRACTICES AND STRATEGIES

Management of any plant disease, traditionally, is based on two important concepts. The first is known as the concept of disease triangle while the second one is known as the concept of disease cycle. Thus, all kinds of the plant disease development and progression are manifestations of the of the three way interaction between the host, the pathogen and the environment that is, the concept of disease triangle (Figure 2).

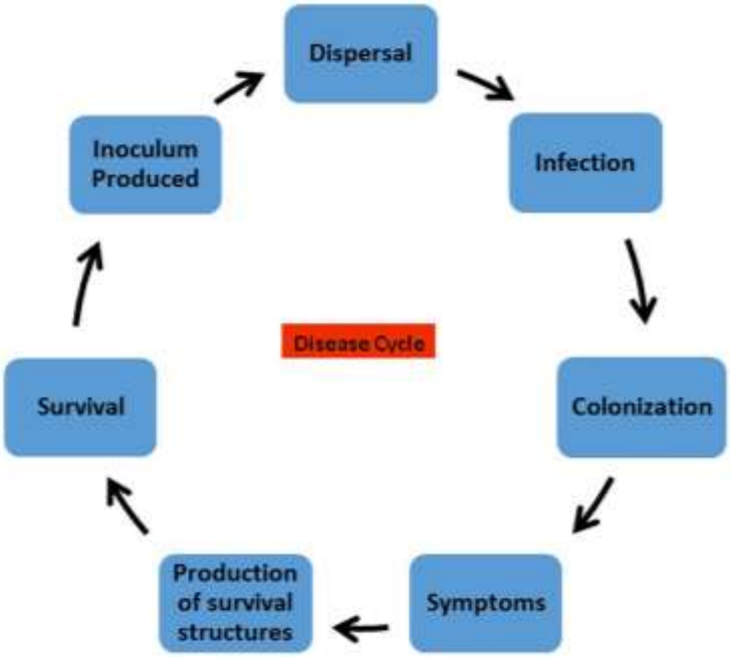

**Figure 3.** Disease cycle depicting seven components.

Conceptually, in order to reduce the level of a disease, according to the concept of disease triangle, the interaction should be such that the host is less susceptible, the pathogen is less virulent, and the environment (physical, chemical and biological) is less favourable (Nelson, 1994). Similarly, development and progression of disease also depends on seven major components covered under the concept of disease cycle (Figure 3). The concept of the host pathogen interaction and disease cycle are important in understanding what makes diseases develop and how to control them. The stages of the disease cycle form the basis of many plant disease prediction as well disease management practice models (De Wolf and Esard, 2007). Plant disease management practices rely on anticipating occurrence of disease and attacking the weak links in the infection chain in the disease cycle. A thorough understanding of the disease cycle, including climatic and other environmental factors that influence the cycle, and cultural requirements of the host plant, are essential to effective management of any disease (Maloy, 2005). In general, plant disease management involves six basic principles, namely avoidance, exclusion, eradication, protection, resistance and therapy. All the six principles have a common objective of targeting the interrelation between the host, pathogen and environment in such a way that disease development is hampered. Accordingly, based on of these six principles, several strategies have been developed for successful disease management. Thus, the disease management strategies may broadly be categorized under two heads, namely traditional/ conventional and contemporary as briefly described in the following sections.

## Traditional/ Conventional Plant Disease Management Practices

Since the beginning of agriculture, generations of farmers have been evolving practices for fighting the various infections suffered by crops. Most farmers in developing nations use traditional agricultural practices for effective and sustainable means of disease control. Traditional disease management included altering plant and crop architecture, biological control, fresh burning, adjusting crop density or depth or time of planting, planting diverse crops, fallowing, flooding, mulching, multiple cropping, planting under zero tillage, using organic amendments, planting in raised beds and sanitation. Among these, crop rotation is one of the very commonly used method help which breaks the cycle by reducing pathogen level. In most cases crop rotation effectively controls those pathogens that survive in soil or on crop residues (Thurston, 1990). Thus, few examples of conventional methods for the control of pathogens include mulching to control the web blight disease of common beans; rotation and fallow to control potato cyst nematode; multistory cropping to control pathogens in household gardens (Maloy, 2005).

## Contemporary Plant Disease Management Practices

### *Pesticides and Biopesticide*

**Table 1.** Commonly used plants (extracts) as bio pesticides in plant disease management.

| S/No. | Plant / Plant Part (Extracts) | Used against Plant Disease | References |
|---|---|---|---|
| 1 | Garlic and onion bulb (aqueous extract) | Alternaria blight, White rust, Downy mildew and powdery mildew | Meena et al. (2004), (2013) Yadav et al. (2012) and Meena et al. (2013) |
| 2 | Neem and seed kernel (oil) | Rice tungro virus | Muthamilan and Revathy (2007). |
|  | Neem leaf and/or seed (aqueous extract) | Bacterial blight of rice | Sunder et al. (2005) |
| 3 | *Lantana camara* /*Pongamia pinnata* (leaf extract) | Leaf blight of onion | Bhosale et al. (2008) |
| 4 | Burma dhania (*Eryngium foetidum*) (Aqueous extract) | Soil borne pathogens of tomato and black pepper | Bhagat (2010) |

In case of an imminent attack, use of pesticides is a popular alternative. Many crops do benefit from routine or managed application of pesticides and this remains one of the principal control methods available for pathogens, especially fungi and nematodes, and their vectors, especially insects and nematodes (Thurston, 1990). Though tremendous benefits have been derived from the use of pesticides in agriculture sector, upon which the Indian economy is largely dependent, it has also led to drawbacks which have resulted in serious health implications to human as well as the environment. Biological control is, thus, being considered as an alternative and eco-friendly way to control plant diseases and reduce the use of chemicals in agriculture (Aktar et al., 2009; Singh, 2014). The indiscriminate use of chemical pesticides have led to several disadvantages such as the problem of pesticide residues in food products, risk of development of new pathotypes and pollution of soil and water ecosystem resulting into several ill effects on flora and fauna including human beings. To overcome these disadvantages of chemical pesticides, attention had been paid to explore into use of non harmful microbes and their products (fungi and bacteria, microbial pesticides), insects, biopesticides (extracts of plants) etc. towards plant disease management as eco-friendly and sustainable approaches.

Furthermore, these approaches can suitably fit in any integrated pest management framework as well as in organic farming system which is a necessity in the present day situation (Bhagat et al., 2014). Biopesticides include organisms and naturally occurring substances which control pests. Thus, *Bacillus thuringiensis* (Bt) (Rosas-García, 2009) which is an insecticide with unusual properties that make it useful for pest control in certain situations. Bt is a naturally occurring bacterium common in soils throughout the world. Several strains can infect and kill insects. Because of this property, Bt has been developed for insect control. Bio pesticides also exist for the management of weeds, insects, and nematodes. Keeping in view the ever-increasing demand gained a pivotal role in the management of plant diseases in comparison to the conventional chemical pesticides. Several plants have been identified for antimicrobial properties which can suppress the growth and multiplication of plant pathogens. List of some plants (extracts) used as bio pesticides in plant disease management has been given in Table 1.

## GENETIC DIVERSITY AND DEVELOPING DISEASE RESISTANT PLANTS

Genetic resistance to disease is one of the most effective approaches used in plant disease management. Resistant genes are often clustered or occur in tandem repeats, suggesting that resistance genes with different specificities arise by gene duplication followed by intragenic and intergenic recombination, gene conversion, and diversifying selection. Maintaining the genetic diversity of crop plants is most important approach of plant disease management because it acts as barrier in built-up of new virulence race of pathogen population. It has been strongly argued that genetic mixtures possess greater stability of performance and that their inherent resistance to disease is rendered more effective and more durable by their diversity (Strange and Scott, 2005). Non–availability of resistance to plant pathogens can be overcome by introducing resistance genes from other sexually incompatible species (Khoury and Makkouk, 2010). Genetic engineering technologies play a significant role in this and permit the expeditious introduction into crops of targeted, diverse resistance mechanisms. Genetic engineering are possible, ranging from very modest, targeted mutagenesis, through cisgenics and transgenics, to insertion of transgenes from other crops, from other (non-crop) plants, and from evolutionarily distant organisms (Vincelli, 2016). The most common example to this category is the trait of developing insect resistant plants by incorporating the insecticidal toxin (Crystalline protein, Cry) gene from the bacterium *Bacillus* thuringiensis (as mentioned in previous section) to a number of plants of economic importance such as for safe food, biopesticides coming from plant have cotton, brinjal, maize, lady finger etc.

**Table 2.** Bioinformatics tools and databases for plant genome analyses.

| **Plant Genome Database** | |
|---|---|
| Genome online database | www.genomesonline.org |
| NCBI genomes | www.ncbi.nlm.nih.gov/genome |
| CoGePedia | genomevolution.org/wiki/index.php/Sequenced_plant_genomes |
| Citrus Genome Database | www.citrusgenomedb.org |
| Ensembl Plants | Plants.ensmbl.org |
| Phytozome9.1 | www.phytozome.net |
| Gramene | www.gramene.org |
| EST database | www.ncbi.nlm.nih.gov/dbEST |
| Uniprot | www.uniprot.org |
| DEG- Database of Essential genes | tubic.tju.edu.cn/deg |
| **Computational tools** | |
| DNASTAR Lasergene 9 | SNP analyses |
| HaploSNPer | SNP analyses |
| PHRAP | Sequence alignment |
| Clustal W | Sequence alignment |
| Discovery Studio 4.5 | Molecular modelling |

## INTEGRATED PLANT DISEASE MANAGEMENT

Integrated plant disease management approach is an ideal combination of various methods of plant disease management against a group of pests, diseases and weeds in a specified farming ecosystem, with the objective of bringing down their infestation to economically insignificant levels with minimum interference on the activity of natural beneficial organisms. Thus, the essence of integrated plant protection concept lays in the harmonious integration of compatible multiple methods use singly or in combination against insect pests, pathogens and weeds (Khoury and Makkouk, 2010). Integrated plant disease management is considered as a sustainable disease management strategy. Plants and their pathogens are engaged in continuous evolutionary battles and sustainable disease management requires novel systems to create environments conducive for short-term and long-term disease control. Sustainable disease management requires changes in agriculture and plant protection goals that also focus on sustainable environmental and economic issues without compromising yields. Such evolutionary principles can be used to guide the formulation of sustainable disease management strategies which can minimize disease epidemics while simultaneously reducing pressure on pathogens to evolve increased infectivity and aggressiveness (Zhan et al., 2014).

## GENOMICS BASED PLANT DISEASES MANAGEMENT

The revolution in genomics has emerged as a very powerful strategy towards disease management in the area of plant pathology. With a wealth of new crop genome data, discovery of new resistant and defense related genes are possible (Klosterman, 2016). In recent years, rapid developments in genomics and proteomics have generated a large amount of biological data. Bioinformatics has already played a great role and will continue to play enormous role in future in generating and integrating large quantities of genomic, proteomic, and other data (Anamitra et al., 2002). Comparative genomics analyses of plant-associated pathogens and respective hosts have facilitated prediction of their interactions (Van Sulys et al., 2003). The increased application of genomics in agriculture is inevitable in meeting out the dual challenge of unparalleled population growth and climate change (Batley and Edwards, 2016).

The combination of all available approaches like comparative genomic and proteomics, pathogenesis (PR proteins) induced proteins, resistance/tolerance proteins when combine with other advanced techniques,functional genomics, biochemical and metabolomics profiling and host- pathogen interaction studies, provides better idea on account of host-pathogen interactions (Nagrale et al., 2016).

Thus, in recent past a number of new plant-specific comparative genomic databases have been developed. Using these databases and the implementation of novel methods can prove beneficial to integrate published functional data into comparative genomic databases (Martinez, 2013). Some important plant genome databases and computational tools have been listed in Table 2. Genomics and associated high-throughput technologies provide opportunities for better understanding of infectious disease mechanism, as well as their prevention and treatment. Subtractive genomics approaches are useful for screening pathogen specific targets which are non- homologous genes/proteins regulating pathogen specific metabolic pathways or biological reactions. These *in silico* approaches are quite efficient with regards to time as well as the cost of target the area of plant pathogen interaction (Barh et al., 2011).

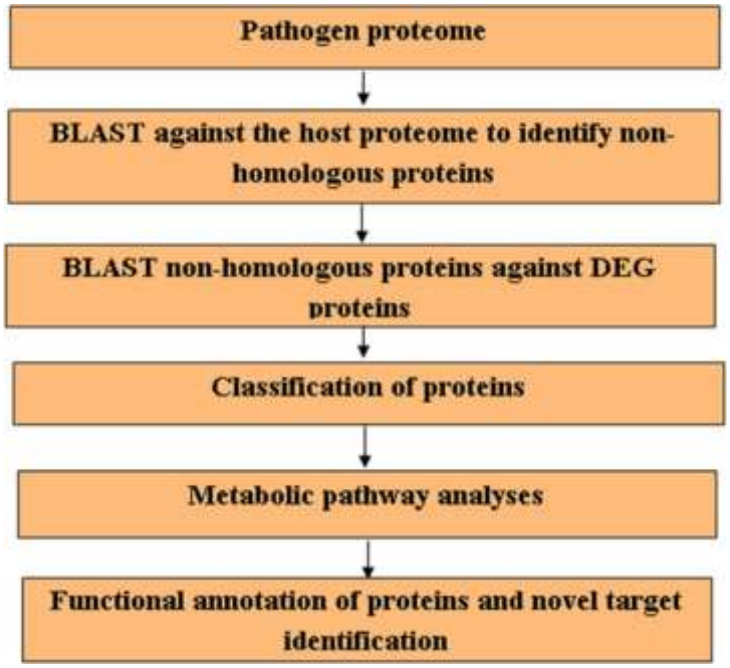

**Figure 4.** Flow chart of systematic identification of novel targets using subtractive genomics approach.

Steps involved identification of such novel targets using subtractive genomics approach are presented in the Figure 4. Subtractive genomics approach for identification of novel antimicrobial targets in *Xanthomonas oryzae pv.* PXO99A, the causative agent of bacterial blight in rice, has successfully been used. Thus, comparative analyses of the bacterial genome led to subsequent analyses of 27 essential proteins which were involved in different metabolic activities essential for its survival and pathogenicity. Further analyses revealed three essential non-homologous proteins as novel antimicrobial targets (Keshri et al., 2014). Single nucleotide polymorphisms (SNPs) are another valuable resource for investigating the genetic basis of disease.

The availability of SNPs within coding sequences is a powerful tool to detect mutation and utilize them to screen for susceptibility to diseases and improve the efficiency of selecting desirable genotypes through plant breeding (Fang et al., 2012). SNPs are also valuable markers for the construction of genetic and physical maps, genome sequencing, marker-assisted selection, and for other genetic and genomic applications. Thus, computational strategies for SNP discovery make use of a large number of sequences present in public databases (as expressed sequence tags (ESTs)) and are considered to be faster and more cost-effective than experimental procedures. A major challenge in computational SNP discovery is distinguishing allelic variation from sequence variation between paralogous sequences, in addition to recognizing sequencing errors. These tools include both computational procedures for data analyses as well as methods to efficiently store and retrieve information. Thus, in a study on *Sorghum bicolor* genome, using online SNP and allele detection tool HaploSNPer (based on Quality SNP pipeline), the mining of SNPs have been reported to be useful for producing high yield producing varieties of sorghum. Potential of discovered SNPs were also proposed to be useful for identifying disease causing genes in sorghum (Singhal et al., 2011). Similarly, analyses and functional annotation of SNPs in the genomes of infected (by bacteria *Xylella fastidiosa)* and uninfected *C. sinensis* plants using computational tools have led to identification of eight candidate genes. It was revealed that these SNPs trigger a defense mechanism in the infected plant and might be responsible for the modulation of gene and/or enzyme expression leading to resistance/susceptibility towards the Citrus Varigiated Chlorosis disease. These findings offered novel agrigenomics insights in developing future molecular targets and strategies for citrus fruit cultivation in ways that are resistant to *X. fastidiosa* infection (Dwivedi et al., 2016).

## Conclusion

With increasing world population, concomitant with deteriorating and depleting natural resources in the form of land, soil and water, feeding the growing population of the world, which would require raising overall food production by more than 50% of the current production, is a major challenge (Alexandratos and Bruinsma, 2012). In this direction, in view of the fact that global food production is hampered on an average of 50% by various kinds of diseases emphasis must now be given on developing disease control strategies. Furthermore, emphasis must also be given on to evaluate the efficacy of current physical, chemical and biological control strategies including disease-resistant cultivars, and future climate scenarios in all research aimed at developing new tools and strategies for managing diseases (Chakraborty and Newton, 2011). In the past 20 years, molecular biology and plant pathology has added new perspectives in the field of plant disease management. The continuously increasing amount of data has resulted in a greater understanding of the function of proteins and their targets in specific plant hosts and has protracted the potential of what applications can be considered as new possibilities for the management of disease with host resistance. Genomics offers tools to address the challenge of increasing food yield, quality and stability of production through advanced breeding techniques (Kole et al., 2015; Muthamilarasan et al., 2013). Advances in plant genomics provide further means to improve the understandings of crop diversity at species and gene levels, and other DNA markers to accelerate the speed of genetic improvement. Novel genome editing technologies provide plant breeders with tools for the generation of new sources of resistance, at a much bigger level. The increased precision of these new methodologies offer new set up in the field of plant disease management, with a reduced effort and time framework compared with traditional methods. Advancement in these techniques will provide consistent food supply as well as sustainable plant disease management in near future (Sundin et al., 2016). Recent advances in genome sequencing and genotyping assays allow for many strategies at the genomics level, which can be developed to understand the impact of climate change on plant diseases. The newly available genome sequences for plants, pathogens and pests provide the resources to study their co-evolution in response to climate change. An understanding of the co-evolution of genes responsible for virulence and resistance will lead to improved plant protection strategies and provide a model to understand plant-pathogen and plant-insect interactions in diverse species (Kole et al., 2015). The potential for modern biology to identify new sources for genetical, chemical and biological control of plant disease is remarkably high. Successful application of these methods within globally and locally changing agricultural environments demands new approaches to durable control.

## ACKNOWLEDGEMENT

Financial supports from the Department of Biotechnology (DBT), New Delhi, under Bioinformatics Infrastructure Facility, Department of Higher Education, Government of UP, Lucknow, under the Centre of Excellence Grant and Promotion of University Research and Scientific Excellence (DST-PURSE) program are gratefully acknowledged.

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