



***Phytophthora*: A major threat to sustainability of horticultural crops**

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Dear members and colleagues of the Indian Society of Plantation Crops (ISPC)

I am extremely honoured to represent our society as the President and to follow and strengthen the philosophy and scientific contributions of leading researchers, who have served as the Society President in the past. This is a formidable task, yet I derive strength and inspiration from our Executive Committee. Please join me in welcoming our ISPC members. Moreover, I would like to extend many thanks for the contribution and work of the Executive Committee, who have been engaged in keeping our society running actively and smoothly. As President of the ISPC, it is my privilege to extend warm greetings from ISPC to ICAR-CPCRI for the year long centenary celebrations. The Society will continue to providing support and, in some cases, guidance, for the organization and development of courses, seminars, workshops and meetings in different parts of the India for the benefit of plantation sector. It is my wish that our partnership will promote the highest quality research and professional standards in plantation sector, that we will together disseminate useful information and knowledge, and foster professional growth and development among our society members.

Challenges pertaining to sustainable plantation sector, specifically pest and disease problems, market fluctuations, declining natural resources like water, labour force and climate change besiege us. Our problems require immediate solutions and strong collaborations among the various stakeholders. I encourage all members of plantation group to update ISPC web page and alert us about information that we could share, or that you would like to have included. This is the best forum for us

to stay connected and to enable mutual support. Let us continue working together toward strengthening plantation crops research and attaining sustainable plantation sector globally.

I am a plant pathologist and have been working on fungal diseases of horticultural crops, especially *Phytophthora* diseases for many years. I have organized the “International Conference on *Phytophthora*” during 2015 in Bengaluru, which was attended by more than 300 participants from different countries with great success and conducted winter school on “Molecular Identification of *Phytophthora*” in 2013 at Bengaluru for the benefit of young researchers. I have also conducted “Molecular Diagnosis of *Phytophthora* Associated with Horticultural Crops” in collaboration with Dr. Jean Ristiano, NC State University, USA and D.E.L. Cooke, James Hutton Institute, UK in Bengaluru in 2015. I have greatly appreciated and encouraged international interactions over all these years, and I hope to continue to do so in my current role in the Society. Science is nowadays a highly globally-connected and integrated activity, and our Society serves to bridge concepts, people and events from different parts of the world, fostering a cohesive scientific interactions.

During my presidential lecture, I will discuss the challenges and options in managing *Phytophthora* diseases of horticultural crops. First, I shall consider threat of *Phytophthora* to horticulture and natural vegetation. Next, I shall discuss the importance of genomics in identification of emerging populations and principals of disease management. Finally, I shall stress the importance of biosecurity measures to contain exotic threats to safe gourd Indian horticulture and forests.

1. Introduction

Stramenopile pathogens of the genus *Phytophthora* cause devastating diseases on a wide range of agricultural and horticultural crops, natural vegetation and forestry worldwide. There are over 120 species in the genus and many have wide host range. *Phytophthora infestans*, which caused the great Irish Potato Famine during late 1840s, still remains the most destructive pathogen of potatoes and tomatoes. The famine caused by *P. infestans* in 1845-57, changed the history of many countries. Other notable species that have emerged in more recent times are *P. ramorum* on oak, *P. alni* on alders, *P. kernoviae* on ornamentals, *P. cinnamomi* on forest crops, *P. agathis* on kauri, *P. cactorum* on hardwood trees, *P. capsici* on solanaceous and cucurbitaceous vegetables, *P. fragariae* on strawberries, *P. megakarya* on cocoa, *P. palmivora* on palms and *P. meadii* on arecanut, small cardamom and rubber from different parts of the world.

The genus *Phytophthora* has a long history. It is known to cause destructive plant diseases much before its discovery. Association of *Phytophthora* in destruction of citrus plants was recorded in 1836 in Azore island much before the potato famine of Ireland in 1845. However, the scientific discipline of plant pathology borne in the early 1860s when Anton de Bary recognized the *P. infestans* as the pathogen causing potato late blight (de Bary 1876). In India, E.J. Butler first described bud rot of coconut caused by *P. palmivora* in 1914.

The name *Phytophthora* was adopted from Greek that literally means *Phyto* (plant) and *phthora* (destroyer). Ever since the genus was erected, several plant pathogenic species have been described. Only 59 *Phytophthora* species were recorded till 1996 as per the famous book “*Phytophthora* worldwide” by Erwin and Rebeiro. Since then, several new *Phytophthora* species have been described from different parts of the world and now about 120 species have been recorded (Kroon *et al.*, 2012).

Most of the species of the genus have wide host range, for example about 2000 plant species are thought to be susceptible to infection by *P. cinnamomi* in Australia. There are also some species with narrow host range like *P. sojae* and *P. infestans*. In India, *P. palmivora* infects several

horticultural crops such as vegetables, fruits, ornamentals, spice crops, palms, cocoa, citrus, black pepper and cassava. Over 34 *Phytophthora* species have been recorded which cause diseases on different horticultural crops in India. Among them, the important ones are *P. infestans* on potato and tomato, *P. capsici* on black pepper and bell pepper, *P. meadii* on arecanut, cardamom and rubber, *P. parasitica* on tomato, *P. colacosiae* on taro, *P. cactorum* on apple, *P. citrophthora* on citrus and *P. palmivora* on cocoa and coconut. The recent emergence of 13_A2 clonal lineage of *P. infestans* on potato and tomato (Chowdappa *et al.*, 2013a, 2015) and *P. boehmeriae* on hot pepper (Chowdappa *et al.*, 2014) and host specific lineages of *P. nicotianae* on brinjal, tomato and cucurbits (Chowdappa *et al.*, 2016) are causing concern to Indian horticulture.

Phytophthora species have emerged as bio-security threats due to increases in international plant trade (Brasier, 2008). More than 90 members of the genus are considered as quarantine organisms. Accurate diagnosis and a proper risk assessment of *Phytophthora* species is required to implement control strategies. The emergence of new genotypes through sexual recombination and global migration, due to international agricultural trade, and concerns about bio-terrorism have necessitated use of sensitive and reliable diagnostic tools for rapid identification of the major *Phytophthora* species. Early detection, accurate identification and the ability to trace pathogens back to their source and eliminate them are the ultimate goals to reduce invasive species such as *Phytophthora*.

Phytophthora continues to be a major pathogen. One of the major reasons for the spread of pathogens is the difficulty in implementation of quarantine regulations in the open trade regime. The knowledge base on *Phytophthora* research and extension methodologies needs proper reorientation to meet the demands of farming community.

2. Crop loss

Late blight remains a major constraint to the production of potato, the world's third largest staple crop, and is a constant threat to food security (Fisher *et al.*, 2012). *P. ramorum*, the cause of sudden oak death, sudden larch death and ramorum blight, is the most destructive disease of oaks worldwide

(Brasier and Webber, 2010) *P. sojae* is one of the most damaging disease problems affecting soybean cultivation (Wrather and Koenning, 2006). *P. capsici* is a highly destructive pathogen that attacks solanaceous vegetables, black pepper, legume and cucurbit crops (Hausbeck and Lamour, 2004). *P. cinnamomi*, commonly called as the ‘biological bulldozer’, is known its capacity to destroy natural plant communities across the globe. *P. parasitica* (= *P. nicotianae*) is responsible for severe foliar and fruit diseases, as well as root and crown rots on herbaceous and perennial plant species in more than 250 genera, including solanaceous crops, and horticultural and fruit trees (Cline *et al.*, 2008). *P. palmivora* affects many tropical plants and cause severe damage to papaya, citrus, coconut, durian and cocoa and kills palms outrightly. *P. meadii* causes serious leaf loss of rubber and heavy nut shedding of arecanut and is also reported on cacao, cardamom, *Ficus*, *Piper* and pineapple. *P. megakarya*, causal agent of black pod disease of cocoa in West Africa, still remains as one of the most serious constraints on cocoa production.

3. Taxonomic position

Phytophthora species are diploid, algae-like oomycetes in the Kingdom Stramenopila (Gunderson *et al.*, 1987) and more closely related to brown algae than fungi. *Phytophthora* are “fungus-like” organisms. Fungal cell walls are made primarily of chitin and *Phytophthora* cell walls are mostly composed of cellulose.

4. Morphological identification

The genus *Phytophthora* has been widely recognized as taxonomically ‘difficult’ (Brasier, 1983) due to limited number of morphological characters. These characters are highly plastic, environmental dependant and show overlap between species. Leonian (1921) and Tucker (1931) developed dichotomous keys to the species. Waterhouse (1963) critically reviewed the genus and divided it into six major groups based on three sporangium types and two antheridium types. The revised taxonomic keys of Newhook (1978) and Stamps *et al.* (1990) were also based on morphological characters and they transformed Waterhouse’s dichotomous key into a more easily used tabular format. Ho (1981) developed a

regionally specialized dichotomous key to *Phytophthora* species in Taiwan. ‘Phytophthora Diseases Worldwide’ (Erwin and Ribeiro, 1996) is source book for techniques, morphological and bibliographic information. Gallegly and Hong (2008) developed a simplified dichotomous key for identifying *Phytophthora* species based on morphology in addition to single-strand conformational polymorphism (SSCP) analysis. Ristaino (2012) developed matrix-based computerized identification lucid key for identification of 55 common species of *Phytophthora* using morphological and molecular characters. The lucid key can be installed and run from a CD ROM drive on a laptop PC. Based upon sequences of ribosomal genes and their introns, 10 clades in the genus *Phytophthora* were described (Cooke *et al.*, 2000). Kang *et al.* (2008) developed the internet-based *Phytophthora* database using nuclear ITS sequences for identification and other genes for phylogenetic analysis. Robideau *et al.* (2011) highlighted the usefulness of the DNA barcoding region of the *cox1* gene for identification of *Phytophthora*. Sequence-based identification of *Phytophthora* species is now widely used, and identification using online tools such as GenBank, the *Phytophthora* database, or Phytophthora-ID (<http://www.phytophthoradb.org>) have been developed (Park *et al.*, 2008; Grünwald *et al.*, 2011). Based on multiloci phylogeny by Blair *et al.* (2008), 10 clades have been identified in the genus *Phytophthora*. Kroon *et al.* (2012) gave an overview of the 10 clades that are currently distinguished within the genus *Phytophthora*.

5. Molecular identification

A number of molecular techniques are available for identification of isolates to a species level. Various molecular techniques like DNA sequence analysis, gel based techniques, species-specific PCR markers that are specific for detection of a *Phytophthora* species in host tissues and micro and macroarrays have been developed.

5.1. Direct DNA Sequencing

DNA sequence analysis using nuclear encoded ITS region of the rDNA is the most novel method for identification of *Phytophthora* isolates to a species level. In addition, number of nuclear loci such as β -tubulin, translation elongation factor 1 α ,

elicitin, 60S ribosomal protein L10, enolase, heat shock protein 90, *TigA* gene fusion protein, and the large subunit of the rDNA can also be employed (Blair *et al.*, 2008). Schena *et al.* (2008) used additional nuclear loci, such as the *ras*-related protein *Ypt1*, which was found to be useful for some species. Mitochondrial genes such as *cox2* and *cox1* (Martin and Tooley 2003), *cox1* and *nad1* (Kroon *et al.*, 2004) have also been used. Target genes for DNA based detection of *Phytophthora* species is presented in the Table 1.

It is suggested to the users to begin the identification process using this ITS locus, which has been proven to establish its identity at or near the species level.

5.2. Gel based identification of species

There are several gel-based techniques that are useful for identification of isolates to a species level. RFLP techniques include digestion of the ITS region of the rDNA as well as the mitochondrially encoded

cox1 and *cox2* gene cluster. In addition, single stranded conformation polymorphism analysis has been found to be useful for differentiation of species.

5.3. RFLP analysis of the ITS region

Ristaino *et al.* (1998) reported *HaeIII*, *MspI* or *RsaI* generated restriction profiles of the amplified product of ITS region with primers ITS-4 and ITS-5 (White *et al.*, 1990) and found their utility for identification up to a species level. Cooke *et al.* (2000) replaced ITS-5 primer with ITS-6 and amplified ITS region and digested with *AluI*, *MspI* or *TaqI* and shown usefulness of these patterns for differentiating many species. They have suggested using 3% NuSieve 3:1 gels for accurate size estimations of digested fragments. PhytID, a website with restriction fragment sizes and tools has been created for identification of unknown isolates. Drenth *et al.* (2006) designed primer pair A2 and I2 for amplification of the ITS region from all

Table 1. Target genes for DNA-based detection of *Phytophthora* species

Target gene	Function	Available number of sequences
60S ribosomal protein L10	Conserved ribosomal protein	222
Beta-tubulin	Microtubule constituent protein	278
Enolase	Essential glycolytic enzyme	191
Heat shock protein 90	Cellular chaperone protein	250
Internal transcribed spacer 1 & 2	Spacer region between ribosomal RNA genes (18S and 28S)	2373
Large subunit rRNA	5' portion of 28S ribosomal RNA gene	229
Mitochondrial <i>cox1</i> locus	Mitochondrial cytochrome oxidase	320
Mitochondrial <i>cox2</i> locus	Mitochondrial cytochrome oxidase	889
Mitochondrial NADH dehydrogenase subunit 1	Mitochondrial NADH dehydrogenase subunit 1 and flanking regions	32
Mitochondrial NADH dehydrogenase subunit 9	Mitochondrial NADH dehydrogenase subunit 9 and flanking regions	445
Mitochondrial ribosomal protein S10	Mitochondrial ribosomal protein S10 and flanking regions	361
Mitochondrial sec-independent transporter protein	Mitochondrial sec-independent transporter protein (ymf16)	362
<i>TigA</i> gene fusion	Transcriptional fusion of genes encoding triose-phosphate isomerase and glyceraldehyde-3-phosphate dehydrogenase	142
Translation elongation factor 1 alpha	Translation elongation factor	247

Source: *Phytophthora* data base (<http://www.phytophthoradb.org>)

Phytophthora spp. and RFLP patterns generated with that amplicon using *MspI*, *RsaI* or *TaqI* could be employed for identification of isolates to a species level.

5.4. RFLP analysis of the mitochondrially encoded *cox1* and *cox2* gene cluster

Martin and Tooley (2004) identified primers that amplified the mitochondrially encoded *cox 1* and *2* genes from all 153 isolates of 31 species in the genus *Phytophthora*. Digestion of the amplicons with restriction enzymes generated species-specific RFLP banding profiles that were effective for isolate classification to a species level.

5.5. SSCP analysis

Kong *et al.* (2003) demonstrated the usefulness of single strand conformational polymorphism (SSCP) analysis of the ITS region of the rDNA for identification of isolates to a species level. Later, Gallegly and Hong (2008) published monograph with SSCP migration patterns along with morphological keys and photomicrographs of the different species for identification purposes.

5.6. Macro and micro arrays

Lee *et al.* (1993) developed dot blot hybridization technique using species specific oligonucleotides from ITS for identification of some *Phytophthora* species. Lievens *et al.* (2006) developed an array format capable of detection of SNPs.

6. Diagnosis

The globalization of world trade fuelled movement of a large volume of plant germplasm between countries and continents dramatically over the last two decades to meet the needs of consumer demand for new varieties of horticultural and forest plants (Cahill *et al.*, 2008; Surkov *et al.*, 2008). Preventing the spread of *Phytophthora* requires the development of detection techniques that are robust, highly specific, and sensitive.

6.1. Conventional

A number of conventional methods for detection of *Phytophthora* species are available (Erwin and Ribeiro, 1996). Among these, direct microscopic examination of diseased material,

baiting with plant materials and isolation of the pathogens from infected plant tissues, water and soil using general or selective agar media are important. Various reference baits such as leaf disks, cotyledons, and various fruits and seedlings were listed (Erwin and Ribeiro, 1996). Generic media can be used for isolation of *Phytophthora* from host and/or baiting tissues however the use of selective media can enhance rate of success.

6.2. Immunodetection

A series of diagnostic kits including immunofluorescence assays, ELISAs and a dipstick assay using monoclonal antibodies have been developed for identification of *Phytophthora* (Cahill and Hardham, 1994). Lateral flow devices that display a simple colour change in the presence of *Phytophthora* antigens have proved useful in the field.

6.3. DNA-based detection

In recent years, DNA based technique has emerged as the robust tool for detection of *Phytophthora* (Schena *et al.*, 2004, 2006, 2008). PCR tests have been developed for many species of *Phytophthora* ([http:// www.phytophthoradb.org](http://www.phytophthoradb.org)). The most commonly used gene targets for the development of species-specific primers are the ribosomal RNA internal transcribed sequences (ITS) (White *et al.*, 1990). Zhang *et al.* (2008) described the use of a macroarray for the detection of a range of pathogens that commonly infect solanaceous species. Preliminary studies have demonstrated that MALDI-TOF MS can also be used to differentiate species of *Phytophthora* in DNA extracted from soil (Siricord and O'Brien, 2008). The web-based database <http://phytophthora-id.org/> can be used for rapid identification of *Phytophthora* species based on sequencing of the ITS or Cox spacer regions, followed by BLAST searching the database.

6.4. Detection of *Phytophthora* in soil and water samples by PCR

Many species of *Phytophthora* are soil-borne pathogens and spread through the movement of infested soil, or by water flow through infested soil. A key element in the management of such diseases is the ability to detect the pathogen in soil and water. However, DNA extracted from soil contains

substances such as humic acids, lignins, carbohydrates, resins, and so on which are very inhibitory to PCR amplification (Robe *et al.*, 2003). The amounts of inhibitory substances will vary widely with soil type, vegetation type, and composition of the soil microflora. Strategies used to reduce the effects of PCR inhibitors in plant DNA (previous section) can also be used with soil DNA.

6.5. Detection by real-time PCR

A more recent innovation in PCR detection is the development of real-time PCR in which the amount of product is measured after each cycle of amplification. Real time PCR assays, using SYBR Green I or TaqMan probes, have been a powerful development with regard to early detection and quantification of fungal plant pathogens in plant and soil samples. Real-time PCR differs from conventional end-point PCR in the measurement of the amplified PCR product at each PCR cycle. Since the development of the exponential phase of the reaction is monitored, real-time PCR allows accurate template. An attractive feature of real-time PCR is the ability to include several pairs of primers in the same reaction (multiplexing). This allows identification of several species in one go. Real time PCR assays have been developed for identification of several *Phytophthora* species (<http://www.phytophthoradb.org>). Chowdappa *et al.* (2013b) developed a real-time PCR-based assay using SYBR Green I for detection of 13_A 2 clonal lineage of *P. infestans* in tomato, which has migrated from Europe.

6.6. Detection of *Phytophthora* in plant tissue samples by PCR

Most of the PCR detection assays are determined with DNA extracted from laboratory isolates. However, the efficiency of the PCR detection methods is less with DNA extracted from plant tissue or from soil due to the presence of PCR inhibitory substances that co-extract with the DNA. Martin and Tooley (2004) reported that plant DNA decreased the sensitivity of their *P. ramorum* detection assay by 100–1,000 fold depending on the plant species.

7. Genomics

The *Phytophthora* Functional Genomics Database (PFGD), developed by the National

Center for Genome Resources, in collaboration with Ohio State University-Ohio Agricultural Research and Development Center (OSU-OARDC) is an excellent source of information for *Phytophthora*–plant interaction research. The first *Phytophthora* genomes that became available were of *P. ramorum* and *P. sojae* in 2004 at the Joint Genome Institute (Tyler *et al.*, 2006). It was shortly followed the genome sequence of *P. infestans* in 2006 (Haas *et al.*, 2009). The draft genome sequence of *P. lateralis* has also become available (Quinn *et al.*, 2013). Later, the genomes of *P. capsici*, a devastating pathogen of vegetable crops and *P. parasitica*, a very broad host range pathogen that causes destructive diseases of a wide variety of crop plants were sequenced. Data mining of genome sequences and functional genomics of *P. sojae*, *P. ramorum* and *P. infestans* have revealed several hundred genes that potentially encode secreted effectors belong to the RXLR and CRN families proteins (Win *et al.*, 2007; Whisson *et al.*, 2007; Tyler *et al.*, 2006). Information on *Phytophthora* genome sequences are listed in the Table 2.

8. Principles of disease management

The management strategies for containing most of the *Phytophthora* species include: (1) cultural practices, (2) application of fungicides, (3) biological control and (4) resistance breeding.

8.1. Cultural practices

Removal and destruction of crop residue left from previous infected crops before replanting will reduce the inoculum. Introduction of pathogen to disease free areas can be avoided by adopting domestic plant quarantine principles. The field should be scouted regularly for *Phytophthora* symptoms, especially after major rainfalls, and particularly in low areas of the field. The wetness on aerial parts should be avoided to reduce the disease development. Avoid excess irrigation to reduce inoculum build up. In high rain fall areas, optimal horizontal and vertical drainage are required to prevent water-logging. Crop rotation with non-hosts, soil solarisation and organic amendments can reduce the propagules in soil, thereby reducing the disease intensity. An increase in the population of antagonistic bacteria, fungi and actinomycetes in the soil can cause suppression of *Phytophthora*.

Table 2. *Phytophthora* genomes sequenced

Species	Host	Genome size (Mb)	Number of genes predicted
<i>P. ramorum</i>	Oak trees, Japanese Larch trees	65	1,574
<i>P. sojae</i>	Soybean	95	19,027
<i>P. infestans</i>	Potato and tomato	240	17,887
<i>P. capsici</i>	Wide range of hosts in the Cucurbitaceae, Fabaceae and Solanaceae families	65	NA
<i>P. parasitica</i> INRA-310	Broad host range	82.39	20,501
<i>P. phaseoli</i> race F18	Lima bean	220	19,622
<i>P. ipomoeae</i> PIC 79916	Morning glory	230	20,545
<i>P. mirabilis</i> FIC 99114	Four O'clock plant	280	19,634

8.2. Fungicides

Bordeaux mixture or copper-based fungicides play a significant in management schedule of *Phytophthora* diseases and have found their utility as disease control agents in organic food production in vegetables and fruits. Development of mancozeb (dithiocarbamate) by Rohm and Haas in 1961 led to effective management of devastating disease such as potato late blight caused by *P. infestans*. The mancozeb is still the largest selling fungicide in the world due to its advantages of multi-site mode-of-action, low toxicity to mammals, plants and environment. The introduction of systemic phenylamide fungicide metalaxyl in 1977 by Ciba-Geigy helped the farmers for control of oomycete diseases. Rhone-Poulenc launched fosetyl-aluminum in 1977 for control of oomycete diseases, but has a more limited spectrum than metalaxyl. The natural products such as kresoxim-methyl, azoxystrobin and pyraclostrobin are broad spectrum strobilurins with a site-specific mode-of-action have been introduced in 1996 for control of oomycetes. Other important fungicides introduced for the control of *Phytophthora* and related pathogens in the last decade are oxazolidinediones (famoxadone), imidazoles (fenamidone), benzamides (fluopicolide, zoxamide), valinamides (iprovalicarb, benthiovalicarb), mandelamides (mandipropamid), cyanoimidazoles (cyazofamid), and thiocarbamates (ethaboxam) representing different chemistries and mode of action.

8.3. Biological control

A diverse group of bacterial and fungal antagonists like *Trichoderma*, *Bacillus*, *Pseudomonas*, *Streptomyces* and *Penicillium* etc. have been tested against *Phytophthora* diseases. The most commonly used species of *Trichoderma* are *T. virens*, *T. harzianum*, and *T. viride* and their biocontrol capabilities of have been extensively documented.

8.4. Effector triggered immunity

Phytophthora species secrete an arsenal of effector proteins that modulate plant innate immunity and enable parasitic infection (Kamoun, 2006). Based on data mining of genome sequences and functional assays, several genes that are likely to encode secreted effectors of both classes, apoplastic and cytoplasmic have been identified. Apoplastic effectors are often small cysteine-rich proteins. In contrast, cytoplasmic effectors belong to the RXLR and CRN families. Effector Triggered Immunity is one of the major components of host resistance in plants (Cui *et al.*, 2015). It is activated by direct or indirect interaction between one or more pathogen effectors and one or more plant R proteins, often resulting in host resistance. It has been reported that several RXLR effectors of *P. infestans* trigger HR in non-host pepper accessions, suggesting multiple interaction between effectors and putative target genes in non-host resistance (NHR) (Lee *et al.*, 2014).

9. Diseases caused by *Phytophthora* species

9.1. Vegetable crops

Diseases caused by *Phytophthora* spp. are emerging as a major concern in India due to emergence/migration of new clonal population with increased virulence and multiple fungicidal resistances. Although *Phytophthora* blight was a serious limiting factor in potato in India since 1952, these diseases never posed any threat to other vegetable crops. Since 2008, severe outbreaks of *Phytophthora* diseases such as late blight on tomato, fruit rot on brinjal, foliar blights and wilts in chili and capsicum, blights in cucurbits were noticed.

9.1.1. *Phytophthora* blight of bell Pepper and chillies: Diseases caused by *P. capsici* are normally called as *Phytophthora* blight. The *Phytophthora* blight first recorded on bell pepper in New Mexico in 1922 by Leonian. Now, it is a devastating disease on bell pepper and cucurbit crops like cucumber, water melon, squash and pumpkin worldwide (Babadoost, 2000, 2004). *P. capsici* also causes a root rot and crown rot, fruit rot, aerial blights of leaves and stem on bell pepper, tomato and many cucurbit crops.

Prior to 2011, foliar blight was not reported as a serious threat to hot pepper cultivation in India. During the June-to-January cropping season of 2011 and 2012, severe foliar blight epidemics were observed in Karnataka and Tamil Nadu states of India. Chowdappa *et al.* (2014) identified *P. boehmeriae* and *P. capsici* isolates as causal agents of foliar blight of hot pepper in India. The isolates of *P. boehmeriae* were metalaxyl sensitive while *P. capsici* isolates were intermediate in sensitivity. *P. boehmeriae* isolates were highly aggressive than those of *P. capsici* isolates. *P. boehmeriae* was emerged as dominant pathogen causing severe leaf blight epidemics on hot pepper in South India, although it is not serious pathogen on any crop in any part of the world. *P. boehmeriae* and *P. capsici* isolates exhibited characteristic protein as well as isozyme profiles but isolates within a species are identical (Madhura *et al.*, 2016).

9.1.2. Late blight of tomato: Prior to 2006, late blight was an annual threat in the states of northern India, but it was not considered a major problem on potato or tomato production in South India (Chowdappa *et al.*, 2011). Since 2008 growing

season, severe late blight epidemics have occurred on both tomato and potato crops in Karnataka, Tamil Nadu and Andhra Pradesh and have often caused 100 per cent crop loss (Chowdappa *et al.*, 2013a). The disease incidence was very severe even on Kufri Jyoti, a highly popular potato cultivar known to be partially resistant to late blight. Although late blight has been known on potato in the Karnataka state of India since 1953, serious epidemics have only been observed on tomato since 2008 (Chowdappa *et al.*, 2013a). On the basis of analysis of tomato isolates collected from certain localities in Bangalore, Chikkaballapur and Kolar districts of Karnataka from 2009 onwards, Chowdappa *et al.* (2013a) reported that the highly aggressive and metalaxyl tolerant 13_A2 clonal lineage migrated from Europe caused severe late blight epidemics on tomato in south west India. Further detailed studies conducted by Chowdappa *et al.* (2015) showed that the 13_A2 lineage was responsible for severe late blight outbreaks on potato and tomato in South India and has replaced the prior population represented by the US-1 and other genotypes. Revised management strategies will be required to combat this destructive 13_A2 clonal lineage and monitoring of the population across other potato- and tomato-growing regions of India is warranted. Multi locus phylogenetic analysis involving six genes of ITS, COX2+spacer region, beta-tubulin, elongation factor-1 alpha, enolase and Heat shock protein-90 of *P. infestans* populations from potato and tomato in India indicated single clonal lineage of *P. infestans*, 13_A2 genotype (Nirmal Kumar *et al.*, 2016).

9.1.3. Fruit rots in vegetable crops:

P. nicotianae has a broad host range, including over 255 plant genera in 90 families (Mammella *et al.*, 2013). *P. nicotianae* is also responsible for significant losses on a number of other economically important species including fruit, oilseed, vegetable crops ornamental plants and floricultural crops (Erwin and Ribiero, 1996). Severe outbreaks of *Phytophthora* fruit rot on brinjal, ridge gourd and tomato have been observed since 2011 in Andhra Pradesh, Karnataka, Telangana and Tamil Nadu states of India. Chowdappa *et al.* (2016) genotyped *P. nicotianae* isolates, recovered from brinjal, ridge gourd and tomato using three mitochondrial (ribosomal protein L5-small subunit ribosomal

RNA [*rpl5-rns*], small subunit ribosomal RNA-cytochrome c oxidase subunit 2 [*rns-cox2*], and *cox2+spacer*) and three nuclear loci (hypothetical protein [*hyp*], scp-like extracellular protein [*scp*], and beta-tubulin [β -*tub*]). The network analysis of genotypes using the combined dataset of three nuclear regions revealed a host-specific association. However, the network generated using mitochondrial regions limited such host-specific groupings only to brinjal isolates. *P. nicotianae* isolates were highly aggressive on their respective host of origin than on other hosts. This led to the identification of host-specific lineages responsible for severe outbreaks on brinjal, ridge gourd and tomato.

9.2. Fruit crops

Phytophthora spp. are the most important pathogens of fruit crops in India. It causes several types of diseases known as root rot, crown rot, collar rot, gummosis and brown rot in various economically important fruit crops such as citrus, guava (*P. nicotianae*), apple (*P. cactorum*), strawberry (*P. fragariae*, *P. cactorum*, *P. nicotianae*), papaya (*P. palmivora*), pear (*P. citricola*, *P. syringae*), pomegranate (*P. palmivora*, *P. nicotianae*), mango (*P. nicotianae*), pineapple (*P. nicotianae*, *P. cinnamomi*), avocado (*P. cinnamomi*), grapes (*P. nicotianae*) and jack fruit (*P. palmivora*). Of these, *Phytophthora* diseases on apple and citrus are economically important.

9.2.1. Citrus: It causes several types of diseases known as root rot, crown rot, collar rot, gummosis and brown rot in various economically important fruit crops. Tree and crop production losses occur from damping-off of seedlings in the seedbed, root and crown rot in nurseries, foot rot, gummosis, fibrous root rot and brown rot of fruits in citrus orchards. Three species, *P. nicotianae* (syn. *P. parasitica*), *P. palmivora* and *P. citrophthora* are the major and widely distributed species causing citrus diseases in India (Lele and Kapur, 1982; Naqvi, 2002). The best way to control a *Phytophthora* disease in citrus orchards is before it starts. Water regulation, clean stock, crop rotation, sanitation, host resistance, bio-agents and chemicals are among the controls that can be implemented.

The technology of soil solarization and fumigation is successfully adopted in eliminating

Phytophthora population from the potting mix on concrete floor and containerized nursery system is being used routinely in raising *Phytophthora*- free nursery stocks commercially at NRC for Citrus, Nagpur (Das, 2016). One of the most effective and widely recommended control measures is the practice of budding trees well above the soil line. In India, location specific root stock trials for last 50 years have given good indication for region wise use of root stocks such as Rangpur lime, rough lemon, macrophylla, Cleopatra, sour orange, swingle, trifoliata, troyer citrange, Carrizo, C-32 citrange and C-35 citrange. Biological agents like *Pseudomonas fluorescens*, *Pseudomonas putida*, *Trichoderma harzianum* and *Gliocladium viride* have been reported to reduce gummosis and root rot in citrus. In India, *Trichoderma harzianum* has been advocated to have a potent antagonistic action against *Phytophthora* root rot of Coorg mandarin when applied along with Coffee waste, poultry manure and FYM (Sawant *et al.*, 1995). Copper fungicides are effective in controlling collar, foot and root rot provided that they should be used at correct time. Bordeaux paste should always be applied before onset of monsoon on tree trunk as prophylactic measure. Two systemic chemicals, viz, metalaxyl/ mefenoxam and fosetyl-Al have been proved to be effective fungicides in the management of crown and root rot.

9.2.3. Apple: Collar rot or crown rot mainly caused by *P. cactorum* outrightly kills the apple plants both under nursery and field conditions and thus cause huge economic losses to the orchardists and nursery-men (Sharma *et al.*, 2016). Improved drainage around the tree base, removal of crop effuse including apple fruits from orchard floor after harvest and avoiding injury to the stem during field operation are helpful in restricting the disease spread. Use of mustard cakes (500g/ tree) and green manuring of plant basin with mustard plants help in keeping disease under control (Sharma and Negi, 2013). In India, rootstocks viz., M2, M4, M9, MM110, MM111, MM114, MM115 and Crab apple have been reported to possess high degree of resistance against *P. cactorum* and *P. ultimum* (Garg and Gupta, 1989; Sharma and Gupta, 1989; Sharma, 2004). Soil drenching with either of the fungicide namely metalaxyl+ mancozeb, copper hydroxide +mefenoxam,cymoxanil + mancozeb @ 300g in 100 liter of water, pyraclostrobin + metiram (250g in

100liters) and mancozeb (500g in 100liters of water), during the month of March (pink bud stage), June (second fortnight) and August -September (after harvest) is effective to control this disease (Sharma *et al.*, 2016). Bacterial antagonists like *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas fluorescens* (KB6) have been found to reduce the growth of *P. cactorum* *in vitro* as well as *in situ*.

9.3. Plantation Crops

Phytophthora causes several well-known diseases in plantation crops such as abnormal leaf fall in rubber, bud rot of coconut, black pod and stem canker of cocoa and fruit rot of arecanut. Proper identification or diagnosis of the disease is the most important and initial step in controlling any *Phytophthora* disease. It is often difficult to diagnose *Phytophthora* diseases of plantation crops especially in the initial stages of disease. Bud rot disease of coconut and arecanut and stem canker disease of cocoa are some of the classical examples for the difficulties faced not only by farmers but also by scientists in identifying the diseases at the very initial stage. Although bud rot of coconut is a well-recognized disease, it is often misdiagnosed as red palm weevil attack and *vice-versa*. Much attention should be given to disease diagnostic investigations so that quick diagnostic tools can be developed for accurate identification of *Phytophthora* diseases of plantation crops.

8.3.1. Cocoa: Presently, cocoa cultivation is facing threat of *Phytophthora* related diseases in the traditional high rainfall zones in seedlings, trees and pods, manifested through seedling dieback, stem canker and black pod rot (Chowdappa, 1995). Most *Phytophthora*-related losses can be attributed to black pod disease followed by stem canker disease. Among these, *P. palmivora* is the predominant pathogen worldwide and, *P. megakarya* and *P. citrophthora* in certain localities (Brasier and Griffin 1979; Brasier *et al.* 1981; Chowdappa and Chandra Mohanan, 1993). Chandra Mohanan (1979) first identified the species responsible for black pod disease as *P. palmivora* in Dakshina district of Karnataka state of India. The studies conducted in 1990s revealed that though *P. palmivora* was the predominant species causing black pod disease in India (Chowdappa *et al.*, 1993b). *P. capsici*

(Chowdappa *et al.*, 1993) and *P. citrophthora* (Chowdappa and Chandra Mohanan, 1996) also cause natural incidence of black pod disease in some of the localities in Kerala state. In India, both A1 and A2 mating types were found in *P. palmivora* and *P. capsici* and *P. citrophthora* isolates were sterile (Chowdappa and Chandra Mohanan, 1997 a, b).

All the three species of *P. palmivora*, *P. capsici* and *P. citrophthora* could be distinguished based on electrophoretic protein banding patterns (Chowdappa and Chandra Mohanan, 1995). Chowdappa *et al.* (2003b) showed that *P. palmivora* isolates of cocoa and coconut had identical restriction digestion patterns of ITS regions of rDNA and AFLP fingerprints. Pathogenicity studies indicated that *P. palmivora* isolates from cocoa and coconut are cross-inoculable. *P. palmivora* has been known as causal agent of bud rot on coconut in India since 1906 (Butler, 1906). When commercial cultivation of cocoa was started as mixed crop in coconut gardens in the sixties, *P. palmivora* isolates might have been moved from coconut to cocoa and resulted in the appearance of black pod for the first time in 1965 (Ramakrishnan and Thankappan, 1965). The lack of genetic diversity coupled with similar morphological and pathological features suggests the presence of clonal population of *P. palmivora* pathogenic to coconut and cocoa in India.

The isolates of *P. capsici* of cocoa exhibited ITS –RFLP patterns similar to Cap A sub group of *P. capsici* isolates on black pepper, betelvine and bell pepper (Chowdappa *et al.*, 2006). Inoculation tests suggest that Cap A isolates of *P. capsici* from cocoa infect black pepper, bell pepper, betel vine and vice versa while Cap B isolates of *P. capsici* caused infection only on black pepper. AFLP finger prints also showed cocoa genetic group was quite distinct from black pepper molecular group (Chowdappa *et al.*, 2003a). *P. capsici* has been known to be a pathogen of black pepper since 1966 (Samraj and Jose, 1960) and both Cap A and Cap B are prevalent on black pepper, which suggest that *P. capsici* has been first adapted as a pathogen on black pepper and later might have spread to cocoa (Chowdappa *et al.*, 2006). *Pythium vexans* de Bary has also been reported to cause black pod disease

in cocoa in certain localities of Kerala (Chowdappa and ChandraMohan, 1993a).

For effective control of black pod disease, phytosanitary measures like removal and destruction of all the disease affected plant parts (pods, flower cushion, stem and leaves) at frequent intervals is most important. Further extensive fungicidal trials conducted in Dakshina Kannada district of Karnataka indicated that spraying of pods with 1% Bordeaux mixture or 0.3% copper oxychloride at monthly intervals along with removal of infected pods during south-west monsoon were found to be highly effective in black pod disease management (ChandraMohan and Chowdappa, 1999). Adedeji *et al.* (2007) demonstrated the efficiency of *Trichoderma* strains as effective bio-control agents against *Phytophthora* pod rot. Guest (1994) used 10% potassium phosphonate as trunk injection two times in a year for the control of black pod and stem canker diseases. The disease can be controlled in the initial stage by the excision of diseased bark followed by wound dressing with Bordeaux paste. Treating the wound and soil around the base of the cocoa tree with *Trichoderma* coir pith cake (TCPC) is effective in reducing the incidence (Peter, 2012).

8.3.2. Arecanut: Fruit rot or koleroga or mahali is serious disease affecting arecanut. Bud rot or crown rot is another manifestation of fruit rot and this may occur independently or following severe fruit rot. *P. meadii* Mc Rae (Sastry and Hegde, 1987) has been reported to cause fruit rot. The identity was further confirmed by Chowdappa *et al.* (2003b) using molecular approaches. Based on ITS-RFLP of rDNA and AFLP patterns, Chowdappa *et al.* (2003b) have shown that isolates of *P. meadii* from arecanut, cardamom and rubber were synonymous. Chowdappa *et al.* (2002) recorded the occurrence of homothallic strain of *P. heveae* on fruit rot affected arecanut in addition to *P. meadii*. Continuous heavy rainfall with intermittent bright sunshine hours, low temperature (20-23°C) and high relative humidity (>90 %) are factors congenial for disease development. In a multi-locational trial on management of fruit rot using different fungicides revealed that Bordeaux mixture (1%) spray still holds good in controlling fruit rot compared to other systemic fungicides (Chowdappa *et al.*, 2000). They also demonstrated that covering the bunches with

polythene covers before initiation of southwest monsoon are equally effective in managing fruit rot.

9.3.3. Coconut: Bud rot and immature nut fall are the serious diseases affecting coconut. Bud rot caused by *P. palmivora* is fatal disease. Chowdappa *et al.* (2003a) reported that isolates of *P. palmivora* from coconut and cocoa were identical based on ITS-RFLP and AFLP patterns. The disease is generally noticed during both southwest and northeast monsoon periods when wet weather conditions prevail. The temperature range of 20 to 24°C and relative humidity of 98-100% are optimum for the development of the bud rot disease. Effective management of bud rot can be achieved only if the integrated plant protection measures are adopted at the right time. Spraying with Bordeaux mixture (1%) or pouring mancozeb (5g) dissolved in 300 ml of water or keeping 2 perforated mancozeb sachets are prophylactic measures to prevent the disease (Anonymous, 2011).

9.3.4. Rubber: In India, abnormal leaf fall (ALF) disease caused by *Phytophthora* spp. is one of the most destructive diseases of rubber. Six species of *Phytophthora*: *P. meadii*, *P. palmivora*, *P. botryosa*, *P. colocassiae*, *P. citrophthora* and *P. nicotianae* have been reported to cause abnormal leaf fall disease, however, the most common species encountered in the traditional rubber growing areas is *P. meadii* (Thankamma *et al.*, 1968; Roy *et al.*, 2005; 2009a). *P. meadii* isolates from arecanut, cardamom and rubber were identical based on ITS-RFLP and AFLP patterns (Chowdappa *et al.*, 2003). Earlier studies based on morphology also showed that *P. meadii* isolates from these crops were similar. *P. meadii* might originally have been a causal agent of fruit rot of arecanut in India since 1918 as arecanut is a traditional crop. Later, this pathogen might have moved from arecanut to rubber and cardamom.

Field sanitation and removal of all infected and dried up twigs, fruits and fruit stalks of the previous season from trees, to destroy the potential source of primary inoculum was reported to be essential for controlling the disease (McRae, 1918). All high yielding clones were found to be susceptible to abnormal leaf disease. However, clones RR II 105, PB 217 and GT 1 were observed to retain more leaves than the susceptible clones under similar prophylactic spraying. Copperoxychloride (COC)

dispersed in agricultural spray oil sprayed through low volume applicators proved effective for the control of this disease. As the maximum potency of copper fungicides lasts only for four to six weeks, spraying needs to be done as close to monsoon as possible. *Trichoderma viride*, *T. koningi* and *T. harzianum* were reported to inhibit the growth of *P. meadii* *in vitro* and cause lysis of oospores (Vanitha *et al.*, 1994). Some of the commercial preparations containing *Bacillus* sp. and *Pseudomonas* sp. were also ineffective under field conditions (Idicula and Joseph, 2011).

9.4. Spice crops

Phytophthora species that are predominant among spice crops include *P. capsici* which infects mostly black pepper, chillies and capsicum and *P. meadii* which infects mainly cardamom, vanilla and nutmeg (Bhai *et al.*, 2016). *P. cinnamomi*, *P. nicotianae* and *P. palmivora* have also been reported from spice crops occasionally. In black pepper *Phytophthora* infection occurs both in nurseries as well as main fields and on all the plant parts namely roots, leaves and spikes. Infection starts as water soaked lesions on capsules of cardamom and lead to rotting. Rotten capsules are shed from the panicle emitting a foul smell. The symptom of *Phytophthora* disease on vanilla mainly appears as rotting of beans.

In black pepper, cardamom and vanilla, diseases caused by species of *Phytophthora* occur at the same time during the onset of southwest monsoon and hence the management strategies are also almost common to all (Bhai *et al.*, 2016). In all the cases, phytosanitation is the most integral part of disease management followed by cultural and chemical or biological means. Prophylactic spraying of 1% Bordeaux mixture to all the black pepper vines with the onset of South West monsoon and drenching the plant basins at a radius of 45-50cm with 0.2% copper oxychloride @ 5-8 litres per vine and repeating this after about 45 days are suggested for black pepper. Three rounds of spraying with 1% Bordeaux mixture or 0.3% Fosetyl Aluminium could effectively control the spread of the capsule rot of the cardamom (Thomas *et al.* 1989, 1991). In vanilla, spraying 1% Bordeaux mixture or foliar spray with 0.4% potassium phosphonate is effective in preventing the incidence and spread of the disease.

9.5. Tuber crops

Leaf blight of taro caused by *P. colocasiae* and cassava tuber rot caused by *P. palmivora* are two serious diseases of tropical tuber crops.

9.5.1. Leaf Blight of Taro: Taro (*Colocasia esculenta* (L.) Schott.), a tropical aroid with nearly 1000 cultivars, is an important staple or subsistence crop for millions of people in developing countries. Leaf blight of taro, caused by *P. colocasiae* Raciborski, is the most destructive disease of colocasia. A1 and A2 mating types have been reported. The disease significantly reduces the number of functional leaves and can lead to yield reductions of the magnitude of 50% (Trujillo 1967). The pathogen can survive on self-grown taro plants and many collateral hosts (Thankappan 1985). Trujillo (1965) found that blight epidemics occur when night and day temperatures ranged between 20-22 and 25-28°C, respectively, with a relative humidity of 65% during the day and 100% at night and accompanied by overcast rainy weather.

A farmer-friendly IDM package for the management of the taro blight was developed by Misra *et al.* (2001). The package includes growing short-duration crop with early planting *i.e.*, in March, one protective spray with mancozeb (0.2%) at 45 days after planting followed by one spray with metalaxyl (0.05%) at 60 days after planting, intercropping with non-host crops like okra, use of disease free seed tubers and seed tuber treatment with *Trichoderma viride*. *Phytophthora* leaf blight of taro can be effectively managed by the use of tolerant cv. 'Muktakeshi' with mancozeb (0.25%) spray after the first appearance of the disease symptoms and an another metalaxyl + mancozeb (0.2%) spray 15 days after the spraying of mancozeb (Misra *et al.*, 2007).

9.5.2. Cassava tuber rot: Cassava tuber rot caused by *P. palmivora* accounts for up to 90 per cent yield loss in Tamil Nadu. The infected tubers emit a characteristic foul smell and rot within 5-7 days depending on the soil conditions.

10. *Phytophthora* biosecurity threats

New species or variants of existing species of *Phytophthora* posing biosecurity threats have been reported in many countries. International trade in plants has increased significantly and this increases the risk of these pathogens being brought into India.

Identifying future global *Phytophthora* threats to horticulture, natural vegetation and forests and potential routes of entry will be required in refining nursery practices and other national biosecurity measures.

Biosecurity threats include *P. ramorum* which is causing serious and widespread damage in nurseries and woodland systems of Europe and North America, *P. pinifolia* which attacks needles of *Pinus radiata* in South America resulting in tree death, *P. kernoviae* which produces bleeding cankers, leaf blight and dieback of rhododendron, magnolia and beech in a number of countries including New Zealand, *P. menzei* which infects the trunks of avocado trees in California and Mexico, *P. fragariae* var. *fragariae* which causes red stele root disease of strawberry, new strains of *P. infestans*, strains of *P. capsici* which attack capsicum, chilli and cucurbits, *P. cambivora*, which causes root rot and stem cankers on several forest tree species, *P. austrocedrae*, which infects *Austrocedrus chilensis* in Argentina. *P. cinnamomi*, which has a wide host range and causes mainly root diseases in eucalyptus, oaks, chestnuts, pines, and members of the Ericaceae as well as diverse agricultural crops, *P. katsurae*, which causes chestnut ink disease in Japan and Korea and immature nut fall in coconut in Hawaii, *P. kernoviae*, which causes a serious disease on European beech (*Fagus sylvatica*) in the UK, *P. lateralis*, which infects Port-Orford-cedar (*Chamaecyparis lawsoniana*) in France and the Netherlands, landscape plantings in France and Scotland and on *Chamaecyparis obtusa* var. *Formosana* in Taiwan, *P. megakarya* on cocoa in central and west Africa, *P. tentaculata* on greenhouse-grown nursery ornamentals in Germany, Italy, Spain, China and the U.S, *P. agathidicida* on kauri in Australia, *P. alni* on alders in many European countries and USA, *P. arenaria* on *Banksia* spp. in Western Australia, *P. austrocedrae* on *Austrocedrus chilensis* in southern Argentina and Chile, *P. frigida* on eucalyptus in South Africa and *P. pluvialis* on tan oak, pine and Douglas-fir in USA (www forest phytophthoras).

11. Future strategies

We need a strategy to “future-proof” to protect plantation and horticultural crops against *Phytophthora* diseases. Global initiative on

Phytophthora genome sequencing of tropical *Phytophthora* species, strengthening bio-security in agricultural trade; exploring the deployment of genetically modified crops wisely for disease management and use of new technologies such as CRISPR/Cas9 system gene editing; adequate human capacity building programmes to encourage young researchers; synergy between researchers and policy makers. There are a lot of good tools available (e.g. metabolomics, effector triggered immunity etc.) and these should be considered as part of the strategy. The “multi-pathed” approaches that include diagnostics capability to avoiding infection, improving soil health, use of disease-resistant crop plants, employing biological agents, fungicide resistance management strategies and utilization of new generation of molecules for combating *Phytophthora* diseases.

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