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An insight into *Hevea* - *Phytophthora* interaction: The story of *Hevea* defense and *Phytophthora* counter defense mediated through molecular signalling

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ABSTRACT

Hevea brasiliensis is the major commercial source of natural rubber in the world accounting for 99% of the world's total rubber production. Abnormal leaf fall disease caused by *Phytophthora* spp. is the most destructive, annually recurring disease of rubber tree in India causing a loss of 38–56% in latex yield as most of the major cultivated clones are susceptible to this disease. Conventional breeding programmes aimed at incorporating disease resistance involves complex and time consuming steps especially in perennial tree crops like rubber tree, which warrants the need to identify molecular solutions for developing immunity in plant towards the pathogen. Currently, adopting efficient disease control measures against *Phytophthora* is a difficult task due to the peculiarities in the pathogen physiological characteristics, broad host range of clonal susceptibility and the unpredictable prevailing environmental conditions. To develop fruitful disease management strategies, it is essential to focus our research on the complex molecular interaction involved in *Phytophthora* pathogenicity and corresponding *Hevea* tolerance. In this review an attempt is made to consolidate the available data on host pathogen interaction between *Hevea brasiliensis* and various *Phytophthora* species that infect *H. brasiliensis*.

1. Introduction

The Para rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Muell., a perennial tree in tropical countries, is the major source of natural rubber in the world with an annual world production of more than 13.5 million metric tons [1–3]. It provides employment for several million people across the world and serves as the primary source of income for people in south and south-east Asia alone [2,4]. The future of this crop is hopefully bright and the total production is increasing every year. Kerala, Tamil Nadu and Karnataka are the major rubber tree growing states in India as the availability of high rainfall (2000–4000 mm) in these regions favour luxurious growth of rubber trees. However, this high humidity supports growth of harmful fungal pathogens [2–4]. The adverse climatic conditions serve as predisposing factors for various diseases on rubber tree [5]. Of the various diseases affecting different parts of the rubber tree namely leaves, stem and root, leaf diseases are considered most important as they cause significant economic loss. In addition, root and stem diseases also create considerable damage if not managed properly. Furthermore, some of these pathogens damage rubber trees in young plantations, thereby affecting growth of these plants [1–12] and extending the immaturity period.

Of all the diseases described in rubber tree, those caused by *Phytophthora* infection causes the major threat to rubber tree cultivation. Black stripe of tapping panel was the first report of a *Phytophthora* disease, made in Sri Lanka in the early 1900s [1–4]. Later on, leaf fall, stem canker and green pod rot were reported from across the world. *Phytophthora* cause infection in most parts of the rubber tree except in root. Abnormal leaf fall (ALF) disease caused by *Phytophthora* spp. is one of the most damaging diseases of rubber tree [2,12]. In India, the disease occurs annually during southwest monsoon months of June, July and August. On young rubber trees up to three years and in nursery plants, leaf fall and shoot rot occur causing extensive die back. In such cases, growth is retarded leading to extended period of immaturity. In mature rubber trees, extensive defoliation leads to considerable loss of crop. Crop loss due to ALF disease in clones RRII 118, GT 1 and RRIM 600 over 14 years was reported to be 7.15, 8.21 and 31.66% respectively [2–4]. The mean leaf retention in clone RRIM 600 was only 14.32% as against 54.82 in RRII 105. *Phytophthora* infection on tapping panel and ALF disease cause reduced growth of trees leading to considerable decrease in latex yield. Apart from this, pod rot affects production of seeds which in turn interferes with the development of good quality root stock for propagation [12].

Considering the damages inflicted upon by these pathogens on the rubber plant and the resulting significant economic loss, efficient

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disease management is a matter of prime importance. Well-organized disease control can be achieved by developing plant immunity towards the pathogen attack or with the use of active fungicides. These, to a greater extent, depend upon a detailed knowledge of the host, the pathogen and the plant-pathogen interaction. Currently, scarce data is available on the molecular mechanism behind *Phytophthora* infection on rubber tree. The present review is an attempt to understand the *Phytophthora* - *Hevea* interaction.

2. Phytophthora

Phytophthora species possesses aseptate, coenocytic hyphae with a slight constriction at the base of its branches which is initially right-angled; sporangiophore is sympodially branched and is of indeterminate growth; sporangia is ovoid, obpyriform to limoniform in shape and contains upto 50 laterally biflagellate zoospores; globose oogonium contains a single spherical oospore and thin or no periplasm; amphigynous and or perigynous antheridial configuration [13]. Due to the variability and overlapping of morphological characteristics, species-level identification of *Phytophthora* has always been difficult but is possible.

3. Life cycle

Phytophthora, known as water mould prefers a tropical climate with prolonged wet conditions as aquatic environment is very conducive for the pathogen. Many of the species are hemibiotrophic. During the initial stages of infection hemibiotrophic pathogens acquire nutrients from living cells, but later they become necrotrophic causing cell death to facilitate colonisation as depicted in Fig. 1 [14]. Diseases develop and spread during the wet rainy season when there is plenty of water for reproduction. They reproduce by means of asexual sporangia which are dispersed by wind or water. Germination of sporangia may be direct or indirect. Sporangia germinate directly by forming a germtube or invasive hyphae, or it differentiates into biflagellate zoospores. The zoospores move or swim actively in water for short distances with the aid of their flagella and the motility plays an essential role in the local spread and development of epidemics by *Phytophthora* species [15–17]. Zoospores come in contact with the suitable host surface chemotactically or electrotactically [18] before they encyst or settle down and adhere to the host surface to initiate infection. The settled zoospores germinate to give rise to a germ tube which spreads across the plant surface until it develops an appresorium for penetrating into the plant epidermis [19]. Vegetative hyphae grow and rapidly occupy the intercellular spaces of plant tissues. Then bulb like structures called haustoria [20,21] develops as lateral branches from intercellular hyphae for translocating nutrients to fungal hyphae. Extrahaustorial matrix surrounds the haustoria and is the primary battlefield where all potential effectors are secreted [22,23].

4. Various species of *Phytophthora* reported in *Hevea brasiliensis*

Phytophthora diseases have been reported from all countries where *H. brasiliensis* is grown including India, Sri Lanka, Myanmar, Malaysia, Thailand, Cambodia, Vietnam, Liberia, Costa Rica and Venezuela [3–13,24–26], as indicated in Table 1. Six species of *Phytophthora* namely *P. meadii*, *P. palmivora*, *P. botryosa*, *P. colocassiae*, *P. citrophthora* and *P. nicotianae* have been reported to cause ALF disease. The most common species encountered in the traditional rubber tree growing areas is *P. meadii*. *P. faberi, P. heveae and P. capsici* are other species associated with the disease in other countries [8,4–13,24–28]. In Thailand, *P. palmivora* and *P. botryosa* have been reported to be the most destructive *Phytophthora* species leading to ALF disease in rubber tree [29].

5. *Phytophthora* infection on rubber tree

The diseases caused by *Phytophthora* on rubber plant becomes prominent in the monsoon season during the months of June to September (Fig. 2). In leaf fall, the individual leaves remain healthy and green at first while the lesions in petiole turn in colour from dark brown to black with a white spot of coagulated latex in the centre. The fallen leaf lamina shows green to yellow shades. In severe case of infection, the fruit may be covered with mycelium, turns dark in colour and rot, but remain attached to the tree. Seeds inside the rotten pod are not viable. At the final stage of disease, affected terminal twigs dieback leading to

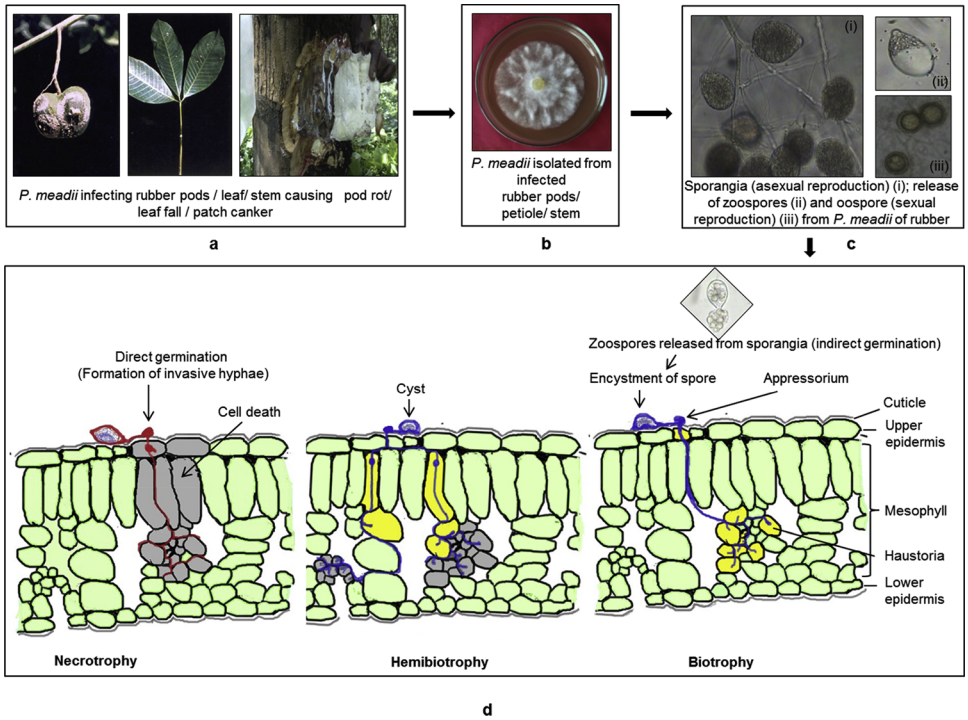

Fig. 1. The mode of infection and life cycle of Oomycetes. (a) *P. meadii* on its host *H. brasiliensis*;(b) *P. meadii* growing on potato dextrose agar media; (c) Sporangia which are the asexual reproductive structures of *Phytophthora* releasing zoospores; (d) Penetration and colonization on leaf surface. Oomycetes may be biotrophic/ necrotrophic/ hemibiotrophic or a combination of these three.

Table 1
Various species of *Phytophthora* reported in rubber tree.

Species	Country	Disease Caused	Reference
P. meadii	India, Sri Lanka, Malaysia, Thailand, Myanmar, China, Nigeria	Abnormal leaf fall, Pod rot, Stripe canker, Black stripe	[30,31,32,33,34,35,36,37]
P. palmivora	Malaysia, Indonesia, Brazil, China, Sri Lanka, Thailand, Vietnam, Philippines, Nigeria, Liberia, Costa Rica	Abnormal leaf fall, Stripe canker	[4,31,38,39,40,41,42,43,44]
P. botryosa	Thailand, Vietnam, Malaysia, Nigeria	Abnormal leaf fall, Stripe canker	[4,45,31,46,47,43,44]
P. citrophthora	Ivory Coast, Brazil, Indonesia, China, Nigeria, Thailand	Abnormal leaf fall	[4,15,16,38]
P. capsici	Brazil, China, Nigeria	Black stripe, Stem canker	[36,39]
P. nicotianae	China, Nigeria	Abnormal leaf fall, Pod rot, Stripe canker, Black stripe	[16,36]
P. phaseoli	Philippines	Seedling blight	[17]
P. hevea	Malaysia	Pod rot, Black stripe	[31]

complete abscission of leaves. Leaf fall, apart from causing a reduction in crop yield, adversely affects timber output too. Tapping panel diseases prevent tapping and hinder regeneration of bark.

6. Disease symptoms

Inoculum development starts with the germination of previous season's oospores, which are resting spores, present in infected dried pods, leaves and twigs deposited on the soil as well as remaining on the trees [4,48]. Green pods that remain on the tree or fallen on the ground show the first signs of disease where water soaked rotting lesions of dull grey colour, oozing of latex and cheesy coating on the surface occur. The fungal mycelia penetrate inside the endosperm of the seed and numerous sporangia produced on mycelium gives a cheesy coating to the pods. The infected fruits do not produce viable seeds. On the leaves, infection is more common on the petioles with a drop of latex oozing out of it. The petiole shows water soaked lesions which turns dark brown or black and a drop of coagulated latex ooze out from the lesion. The affected leaves are green even when they fall off. Water-soaked lesions are also observed on the leaf lamina with a dull green colour which later turns to black. The fungi also infect growing shoots and young twigs leading to rotting and dieback back of shoots. *Phytophthora* also infects pods, petiole, leaves and tender shoots causing heavy defoliation and crop loss [1–12,48]. Under favourable climatic conditions, leaf fall is severe especially in susceptible clones that the fallen leaves cover the entire ground forming a carpet (Fig. 2).

7. The molecular basis of pathogenesis

Plants respond to pathogen infection by means of a two-branched

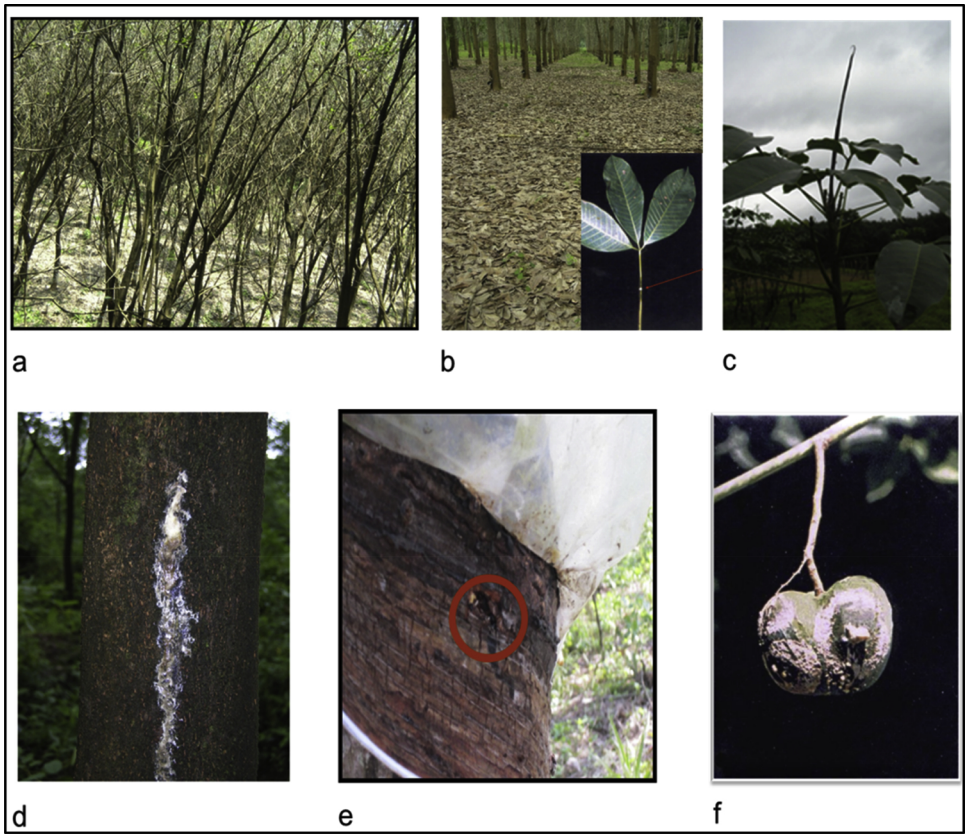

Fig. 2. Diseases caused by *Phytophthora* spp. on rubber trees. a) Extensive leaf fall disease from the canopy of the trees; b) Carpet of leaves formed on ground resulting from heavy leaf fall; a petiole showing typical symptom of black lesion with a central drop of coagulated latex; c) Shoot rot in terminal twigs of young rubber plant; d) Latex exudation from a stem canker; e) Black stripe disease on the tapping panel; e) Green pod rot in which white cottony mass of mycelium cover the surface of pod. Source: Photographs taken by scientists of Plant Pathology division of RRII.

immune system which includes preformed and inducible defense response. The first branch recognizes and responds to molecules common to many classes of microbes, including non-pathogens. These preformed barriers of defense like cell wall, waxy epidermal layer, bark etc., form a part of constitutive defense of the plant. The second branch responds to pathogen virulence factors or elicitors, either directly or through their effects on host targets and hence forms inducible defense system [49,50]. The inducible defense responses may operate at two levels. One is basal resistance or innate immunity, which is considered to be the first line of active plant defense that involves the recognition of pathogen which trigger general plant defense responses referred to as PAMP-triggered immunity (PTI). This recognition could be based on an essential molecule displayed on the surface of the microbe like flg22 (a part of bacterial flagellar protein flagellin), chitin, fungal xylanase, oomycete heptoglucans and bacterial lipopolysaccharides [50–52]. The recognition of a microorganism at the cell surface may prevent infection before the microbe gains a hold in the plant.

The species of *Phytophthora* and all true fungi, may withstand immune response of plant by disabling PTI or alter cellular functions and structures of the host cells in a way conducive for the pathogen, by using an array of variable effectors that promote effector-triggered susceptibility (ETS) that facilitates infection [49,53]. The pathogen has evolved this counter strategy of secreting effector proteins into the plant cytosol to suppress the different components of PTI that apparently alter resistance signalling or expression of resistance responses [54]. Once the pathogens evolve the mechanisms to suppress PTI, plants develop a more dedicated mechanism, known as effector triggered immunity (ETI). ETI involves recognition of specific microbial effectors (avirulence/ Avr proteins) that challenge PTI, by plant resistance (R) genes which is termed as 'gene-for-gene resistance' [55]. Activation of R protein-mediated resistance also affect microbial growth, but not before the pathogen has had an opportunity for limited proliferation. R effector association resulting in the commencement of defense signalling and host resistance leads to localized hypersensitive cell death response (HR) at the site of penetration and inhibition of pathogen growth [49,56,57]. On the other hand, in the absence of this R/ Avr interaction, the pathogen escapes detection by the host plant, resulting in the explosion of pathogen growth within the plant cell and the onset of disease.

HR response is a complex and early defense response that limits the access of pathogen from water and nutrients in the plant tissue. The HR is a suicidal/ sacrificial event of the plant cell at the site of pathogen invasion to save the rest of the plant [58]. Wide range of pathogens belonging to different category *viz*. bacteria, fungi, viruses, nematodes etc., can cause hypersensitive response in plants. Recognition of effector molecule by the host plant elicits HR and plants may become highly resistant to wide range of pathogens for extended period of time after the HR [46,49]. This represents an elevated state of preparedness where the plant resources will be directed against further pathogen encounter. Activation of defense-related genes and production of HR, precedes "whole plant" resistance known as systemic acquired resistance (SAR) [59,60]. SAR is one of the best-studied signal transduction pathways involved in complex resistance response, of which salicylic acid is a key signalling component [61]. Even though molecular mechanism behind defense responses is studied in detail in many plant species, little information is available about the molecular dialogues involved in the interaction between *Hevea* and its major pathogen *Phytophthora*.

8. Plant barriers against pathogen entry

Immediately following infection, plants activate various constitutive and induced basal defense mechanisms in order to restrict the progression of pathogen to healthy uninfected tissues. The initial reactions of plant defense include structural changes in cell wall by deposition of lignin, suberin and callose and oxidative bursts while active expression of *pathogenesis-related* (*PR*) genes, production and accumulation of phytoalexins and antimicrobial proteins occur at a later phase of pathogenesis [62]. All plant genes that favor pathogen compatibility can be considered *susceptibility* (*S*) genes. Mutation or loss of an S gene reduces the ability of the pathogen to cause disease. S genes that act as susceptibility factors facilitating infection by important oomycetes have been identified. These genes may be involved in early pathogen establishment, regulating host defenses or in the pathogen sustenance [41,43,59–62].

To restrict spread of the fungal pathogen to healthy unaffected tissues, *H. brasiliensis* synthesize a variety of anti-fungal compounds [63]. Cell wall acts as a natural physical barrier in preventing pathogen entry into the plant tissue. A study conducted on resistant and susceptible clones (with respect to *Phytophthora*) of rubber tree revealed that the formation of structural component lignin from phenolic aldehydes around the infected area may be critical in disease resistance [63]. Elmer et al. [64] demonstrated the importance of anti-fungal phenolic compounds in inhibiting various fungal pathogens. It was observed that petioles of healthy and resistant clones (RRIC 100 and BPM 24) of rubber tree had higher concentrations of phenolics than susceptible clones (RRIC 121, RRIM 600 and PB 86). The infected petioles always had a lower level of inhibitory phenolics than healthy petioles while challenge inoculated leaves contained higher concentration of phenolics [63]. Those phenolics were identified either as hydroxycoumarins, phenanthrenes or stilbenes.

Enzymes involved in the synthesis of many natural phenolic products show higher activity upon infection. For example, phenylalanine ammonia-lyase (PAL) enzyme, which catalyses the synthesis of precursors for lignin biosynthesis [65] shows higher activity in petiolar tissues of the resistant clones. All these observations highlight the role of lignin and other phenolics in restricting the spread of fungal hyphae into healthy tissues of affected plants. In addition to these, small amount of highly fungitoxic compounds such as vanillin were extracted from infected tissues of the petioles of RRIC 100 by mass extraction methods. Two of the phenolic compounds vanillin and umbelliferone were found to be toxic to zoospore germination *in-vitro,* of which vanillin is more active than umbelliferone. But the major phenolic compound involved in defense was found to be lignin, which deposits around the infected area. A difference in the rate of production and accumulation of lignin around the infection site in resistant and susceptible clones was also observed in this study; which is earlier and faster in resistant clones [63].

The oxidative burst is the rapid and transient process in which high levels of reactive oxygen species (ROS) is produced at the earlier onset of cellular defense responses [62,66,67]. ROS produced as a by-product of oxidative cellular metabolism is one of the first observable feature of plant's defense strategy. In plants, ROS are regularly synthesized during vital processes like photosynthesis, photorespiration, breaking down of fatty acids and electron transport system [68]. Among the various ROS such as superoxide radical, hydroxyl radical, singlet oxygen and hydrogen peroxide, H_2O_2 is the rather steady non-radical ROS which is able to diffuse through aquaporins of plant cell membranes. H_2O_2 can protect plants directly from pathogen infection and also it works as an intercellular and intracellular signaling molecule for the stimulation of systemic acquired resistance [69]. To control the ROS toxicity in a way that maintain ROS homeostasis and to utilise it as an effective signal molecule, plants depend on various ROS-detoxifying systems or antioxidant components [70] such as catalase, ascorbate peroxidase and guaiacol peroxidase (POD) that supress the toxic levels of H_2O_2 [71].

POD is considered to be involved in cell wall modification and biosynthesis of phenolics in the defense against pathogens [71,72]. Phenylalanine ammonia lyase, the enzyme that catalyzes the conversion of phenylalanine to trans-cinnamic acid [73], which serves as the precursor for biosynthesis of polyphenyl compounds including lignins, flavonoids, plant hormones such as salicylic acid (SA) and phytoalexins is induced in response to pathogenic attack [74]. Studies conducted in different plants which were artificially inoculated with pathogen

revealed that all these enzymes get activated as a response to various elicitors secreted by the pathogen [75,76]. SA is an important defense hormone which is mainly associated with resistance against biotrophic and hemibiotrophic pathogen such as *Phytophthora palmivora* [77]. This hormone acts as an endogenous elicitor and it activates both localized and systemic acquired resistance [59]. Exogenous application of SA and Jasmonic acid trigger various physiological, biochemical and molecular processes in plants including antioxidative reactions. The rubber trees pre-treated with salicylic acid show more tolerance to *Phytophthora palmivora* disease as SA induces increase in H_2O_2 production and accumulation, catalase (CAT), peroxidase (POD) and phenylalanine ammonia lyase (PAL) activities. The elevated levels of lignin, endogenous SA, scopoletin and phytoalexins and increased expression of PR defense genes such as *HbCAT1*, *HbPAL* and *HbPR1* triggered by the SA could contribute to the resistance [77,78].

9. Plant elicitors

The changes immediately following infection are detected by the host using a group of pathogen associated molecules called elicitors which some plants perceive as a microbial signature [53,59,69,79,80]. Plants are highly specific in recognising the elicitors. Even a single amino acid change in its sequence, might aid the pathogen to evade defense responses developed by the host [81]. These molecules that trigger immune responses in plants are components or products derived from pathogen during its life cycle or infection process and are structurally conserved pathogen associated molecular patterns (PAMPs) [17,22]. These molecules derived from pathogens are recognised by the plants and elicit defense responses even in the absence of actual pathogen. The elicitors may be non-specific molecules, like fungal cell wall components, the outer membrane or flagella of bacteria or specific *avr* proteins produced by particular strains of pathogens [81–84]. *Nicotiana tabacum* leaves infiltrated with OPEL proteins, secreted by culture filtrates of *Phytophthora parasitica* show increased callose deposition, oxidative bursts and induction of PAMP-triggered immunity (PTI) and salicylic acid-responsive defense. From the improved plant immune response and tolerance to *P. parasitica,* exhibited by plants upon infiltration of OPEL, we can conclude that it is a potential elicitor of this pathogen [85]. The cellulose-binding elicitor lectin (CBEL) isolated from *P. parasitica* is an apoplastic elicitor that trigger immune responses upon infiltration in tobacco and *Arabidopsis* leaves [86–88]. It induces defense reactions in tobacco cells having cell wall, as CBEL-induced defense reactions require binding to plant cell wall. A study on the role of *HbASI* gene in the defense response of *Hevea* against *P. palmivora* proved that *HbASI* gene expression was induced by a biotic elicitor in leaves inoculated with zoospores of *Phytophthora*. Also the result suggested a negative correlation between cutin content and *HbASI* gene expression. The young rubber tree leaves contain less cutins so the *HbASI* gene expression was observed to be higher. The level of *HbASI* gene expression decreases with time as the plants accumulate more cutin [89].

Elicitors trigger immune responses in the plant and the effect of elicitor vary with the tolerance rate of plants with respect to the particular pathogen. In order to prevent pathogen ingress and spread, plants develop two types of immune responses namely PAMP triggered immunity and effector-triggered immunity. Plant cell membrane hosts certain pattern recognition receptors (PRRs) with specific PAMP recognition domains to perceive conserved oomycete elicitors or PAMPs that results in PAMP triggered immunity. The pathogenic fungi secrete a set of molecules called effectors in order to evade or suppress PTI for initiating disease in the plant. These effectors were targeted and get destroyed by various intracellular disease resistance proteins in plants and activate effector-triggered immunity (ETI) [49,50,54,81,83,84].

Zoospore exudates by *Phytophthora* play important role in the disease development and it promotes zoospore communication, homing and germination during infection. Exudates from swimming zoospores of *Phytophthora* can function as virulence factors capable of invoking plant immune reactions like elicitors [90].

10. Effector suppresses host immunity

When the elicitors work in a way to inhibit pathogen establishment, oomycete pathogens counteract to suppress those immune responses in order to sustain a close link with the plant. That is, the interaction between oomycetes and plants are characterized by molecular co-evolution. To alter the plants vital processes in a way conducive for the pathogen, they secrete an array of effector proteins to alter host cell structure and function facilitating infection and colonization [53,91]. The species of *Phytophthora* and all true fungi use variable effectors that work in many different cellular compartments to promote effector-triggered susceptibility for parasitic infection [49,53,91]. Effectors are proteinaceous key virulence factors encoded by the oomycete pathogens that can act in many different ways inside the host such as suppressing PTI responses, inhibiting protease and peroxidase enzymes, inactivating ubiquitination system, salicylic acid signaling and separating plant plasma membrane from cell wall. [54,92],

Effectors are classified into two groups based on their subcellular localization namely as apoplastic effectors and cytoplasmic effectors. Apoplastic effectors are released into the plant apoplast while cytoplasmic effectors enter the plant cell. The apoplastic effectors of *Phytophthora* include cell wall-degrading enzymes, toxins, elicitins [93–97] and enzyme inhibitors. The cytoplasmic effectors contain additional sequences required for its translocation across the plant plasma membrane [14]. Crinkler effectors (CRN) are cytoplasmic effectors present in oomycete species and these effector with RxLRs motif is characteristic to *Phytophthora* [98,99].

Elicitins are a group of small (10 kDa) protein elicitors, secreted by different species of *Phytophthora* [97]. It was reported that zoospores and elicitin produce similar defense responses such as wilting, necrosis, lignification and synthesis of scopoletin and PR proteins. But elicitins are found to be more effective than zoospores in inducing different immune responses in rubber tree. These responses occur earlier and faster in resistant varieties and also prolong for a long time [100]. Capsicein, cinnamomin, cryptogein and parasiticein are various elicitins isolated from the culture filtrates of *P. capsici, P. cinnamomi, P. cryptogea,* and *P. parasitica* respectively [101–103]. The complete sequences of these holoproteins containing 98 amino acids are known [104–107]. Elicitins are categorised into two groups based on their molecular characteristics. The α-class consists of acidic elicitins and the β-class is characterized by a hydrophillic residue at the thirteenth position and basic isoelectric point (pI). β-elicitins are considered to be more toxic than α based on their necrotic effect on tobacco leaves [104,105].

Recently, another effector namely palmivorein was extracted from the culture filtrate of *P. palmivora* infecting *Hevea*. The molecular and biochemical characteristics of palmivorein were studied in detail and the SDS-PAGE results revealed that it is a small protein and an α-elicitin based on its acidic pI and valine at thirteenth position. It results in severe wilting and necrosis in the leaves of tobacco and *Hevea.* The leaves of susceptible rubber clone (with respect to *P. palmivora*) are more prone to wilting and necrosis than the leaves of resistant clone. High concentration of this elicitin leads to severe wilting and thereby leads to secondary leaf fall in *Hevea* [107]. The leaves from resistant clone BPM 24 (with respect to *P. palmivora*) inoculated with palmivorein shows higher amount of scopoletin production than in the susceptible clone, RRIM600 [108]. The role of this elicitin as a pathogenicity factor in *Hevea*, which leads to invasion, establishment and colonisation is yet to be investigated in detail. The RXLR effector of *P. parasitica* PSE1, identified in a cDNA library was reported to favour disease development by regulating the accumulation of auxin during the penetration process [109].

Phytopathogenic fungi secretes a large number of effector proteins

that can interfere with host innate immune systems to alter cell physiology leading to successful infection, colonisation and reproduction [51,53] The effector proteins such as EPI 1 and EPI 10 (extracellular protease inhibitors secreted by *P. infestans*), possess biochemical activities that suppress primary defense responses in host [110]. The inhibition of plant defensive proteases is an intelligent counter measure adopted by many plant pathogens to shut down host defense mechanisms for successful colonization of plant tissue [100]. The antagonistic interactions taking place between various protease inhibitors secreted by *Phytophthora* spp. and the corresponding host plant proteases are one such strategy. The subtilisin-like serine proteases/ subtilases are ubiquitously found serine endopeptidase enzymes in plants that play essential roles in vital processes including response to environmental stress. The role of plant subtilases in defense mechanisms was first elucidated in tomato. *Hevea brasiliensis* serine protease –HbSPA is one such protease enzyme involved in immune response against *Phytophthora*. But the exact molecular dialogue by which this protein contributes to plant resistance is still under study. It is assumed that this enzyme has some participation in recognising the pathogen, destructing various pathogenic proteins and thereby activating immune responses. An increase in the expression of *HbSPA* gene found during the infection of *P. palmivora* on *Hevea* again point towards its role as an antifungal component. Various effectors produced by pathogens target these protective proteases in plants and degrade them. This again suggests the role of protease enzymes in controlling pathogenicity like the extracellular serine protease inhibitor PpEPI 10 which is released into the plant apoplast by *P. palmivora* during infection. The domains Kazal 1 and Kazal 2 of PpEPI 10 interact and inhibit a 95 kDa protease extracted from the leaves of *Hevea* [110]. But this protease was not recognised. Also, the role of PpEPI 10 as a potent virulence factor in *Hevea* needs to be explored further.

11. Delayed active defences

Plants develop various effective defense strategies to prevent invasion and establishment of pathogen. Pathogenesis-related (PR) proteins, which are synthesized and accumulated both in local and systemic tissues with time are one of the strongest plant defensive proteins [111–113]. Some PR proteins are directly involved in plant defense while others are inhibitors that prevent pathogen from getting nutrients for survival. Based on their properties and functions at least 17 families of PR proteins have been identified till date [112–114]. Eleven PR proteins were detected in *H. brasiliensis* infected with *Phytophthora* [115] and a new anionic PR 9 peroxidase protein was detected in resistant interactions [115]. They might be directly antimicrobial such as PR 2 (β-1,3-glucanase), PR 3 (chitinase) and PR 7 (endoprotease) or they might be hydrolytic enzymes catalyzing degradation of structural components in the cell walls of pathogen. These enzymes are released to plant intercellular space (extracellular matrix) where the initial processes of disease cycle such as host-pathogen interaction, recognition and signalling takes place [50,76]. The PR 2 protein β-1,3 glucanase was found to play a major role in the defense mechanism of many crops against pathogenic oomycetes. The gene for this protein show increased activity in tolerant clone RRII 105 upon challenge inoculation with *Phytophthora.* Recombinant PR 2 protein exhibits antifungal activity against *P. meadii* under *in vitro* conditions [115].

Phytoalexins, low molecular weight plant secondary metabolites, are one of the important early discoveries about the molecular basis of plant-pathogen defense [116,117]. These antimicrobial metabolites produced *de novo* as a response to biotic and abiotic stresses control the invading microorganisms. Elicitors produced during the initial phase of pathogenesis, induce the production of phytoalexins [78]. Cavalcanti et al. (2005) [118] reported that phytoalexins work by different strategies such as cytoplasmic granulation, disorganization of the cellular contents, rupture of the plasma membrane, inhibition of fungal enzymes, retarding elongation of the germ tube and reduction or inhibition of mycelial growth.

Tan and Low [119], the pioneers in the field of plant defense mechanism of *Hevea*, discovered two fungal toxic substances in leaf tissues after infection with *Colletotrichum gloeosporioides*. They detected a blue fluorescent compound which was later identified as scopoletin, a hydroxycoumarin by its chromatographic and spectrometric properties [120]. Scopoletin is described as a phytoalexin of *Hevea* due to the induction, the localization or restriction of the substance around the fungal hyphae, to its positive correlation to resistance of *Hevea* to *M. ulei*. Scopoletin is found to be toxic to a large range of fungi, as published by Jurd et al. [121]. The speed and extent of scopoletin production and accumulation were very important in the resistance of *Hevea* against *M. ulei* [122,123] and *C. gloeosporioides* [124]. The lesion size and the amount of Scopoletin after infection were directly proportional to the concentration of spores applied to *Hevea* leaves. In addition, scopoletin showed fungitoxic effect on mycelial growth in bioassays [108].

12. Response of rubber tree to *Phytophthora*

Molecular studies to understand resistance mechanism identified a few genes responsible in imparting *Phytophthora* resistance in tolerant clones [115]. Expression of pathogenesis related proteins were reported in *Hevea* during pathogen infection [116,125–127]. Expression of major PR proteins like β-1,3 glucanase and peroxidases were observed in tolerant clone RRII 105 subsequent to challenge inoculation with *Phytophthora.* Anionic peroxidase was detected in tolerant clone RRII 105 during *Phytophthora* infection. Two anionic peroxidase proteins corresponding to molecular weights 29 and 33 kDa were prominent in the extracts from challenged clone RRII 105. Vacuum filtration studies revealed presence of similar protein in the intercellular fluids obtained from the leaves of RRII 105 [115]. Thanseem et al. [116,125] reported the role of β-1,3 glucanase gene in *Phytophthora* infection in rubber tree. β-1,3 glucanase gene expression was analysed upon artificial inoculation of *Phytophthora* in susceptible clone, RRIM 600 and tolerant clone RRII 105 by northern blot analysis and higher level of expression was observed in RRII 105. Full length β-1,3 glucanase cDNA was amplified from the clone RRII 105 and expressed in a bacterial system. It was shown that recombinant β-1,3 glucanase protein possessed antifungal property inhibiting growth of *Phytophthora in vitro.* Although induction of β-1,3 glucanase gene occurred in both tolerant and susceptible clones, the predominant difference between the clones was in the intensity and duration of response. The tolerance of clone RRII 105 may be associated with the prolonged expression of the gene following infection.

Isoforms and major sequence variation within the intronic sequences of β-1,3 glucanase gene with “CT” repeats was reported by Supriya et al. [128]. They also characterized a novel form of β-1,3 glucanase gene from the genomic DNA of rubber clone RRII 105. The full length genomic sequence possessed a single intronic region with 1233 nucleotides. This form coded for a class II acidic glucanase with 373 amino acids and designated as glucanase 4 which varied with the other reported forms of *Hevea* in having a different promoter and possessing a different stop codon ‘ochre’ instead of ‘opal’ in the other form of β-1,3 glucanase.

Saha et al. [129] studied allelic diversity based on simple sequence repeat (SSR) polymorphisms at the locus encoding β-1,3 glucanase, a PR 2 protein having antifungal property in rubber tree. SSR marker (*hglu*), existing in the intronic region of β-1,3 glucanase gene was developed in rubber tree, which appeared to be highly polymorphic. Seven alleles and consequently 15 allelic combinations were identified in cultivated clones of rubber. Allele mining for β-1,3 glucanase gene in wild accessions, revealed the existence of 12 alleles forming several genotypes. A putative RAPD marker ($OPF10_{600}$) linked to the loci conferring resistance to ALF disease in rubber tree was also identified and characterized at the nucleotide level. Association of this marker

was noticed in wild accessions showing tolerance to *Phytophthora*.

13. Conclusion

Considering the sustained deleterious impact of *Phytophthora* pathogens on rubber tree cultivation and the resulting economic loss incurred, unravelling the complex molecular cross talk between the host and pathogen is a matter of prime importance. However, most part of this interaction story remains unexplored and need to be studied to completely interpret the mechanism of their interactions. For this, all the components involved in the interaction between *Phytophthora and Hevea* such as elicitors, effectors, pathogen associated molecular patterns, membrane receptors, defensive chemicals and proteins have to be identified. The multitudinous processes involved in the interaction between pathogen and the host have to be well understood to know about plant defense signals and to develop novel methods for improving host tolerance against pathogen. Research on this phytopathogenic oomycete is gaining new dimensions and novel approaches using high-throughput strategies in the field of genetics, -omics and cell biology for their efficient management. Identification of genetic determinants involved in host defense and pathogen counter defense is inevitable for incorporating the resistance genes to create disease-resistant clones. Combining a detailed knowledge of the molecular processes required for disease, with improved cell biology resources and tools will open up new arena to develop control measures against *Phytophthora* infection in *Hevea brasiliensis*.

Conflicts of interest

The authors declare no conflict of interest.

References

[1] C.K. Jacob, History of *Phytophthora* disease control of natural rubber in India, Phytophthora Diseases of Plantations Crops, (2015), pp. 1–5.
[2] C.K. Jacob, Disease control in rubber: latest trends, Planters' Chronicle 99 (2003) 38–47.
[3] C.K. Jacob, T.T. Edathil, S.P. Idicula, K. Jayarathnam, M.R. Sethuraj, Effect of abnormal leaf fall disease caused by *Phytophthora* sp. On the yield of rubber tree, Indian J. Nat. Rubber Res. 2 (1989) 77–80.
[4] A. Drenth, D.I. Guest, Diversity and Management of *Phytophthora* in Southeast Asia, Australian Centre for International Agricultural Research (ACIAR), 2004, pp. 136–143.
[5] P.M. Priyadarshan, Contributions of weather variables for specific adaptation of rubber tree *(Hevea brasiliensis* Muell. Arg.) clones, Genet. Mol. Biol. 26 (4) (2003) 435–440, https://doi.org/10.1590/S1415-47572003000400006.
[6] D.V.T. Thanh, N.V. Vien, A. Drenth, *Phytophthora* diseases in Vietnam, Diversity and Management of Phytophthora in Southeast Asia, (2004), p. 83.
[7] L. Thankamma, M.K. George, K.V. George, Occurrence of two species of *Phytophthora* on *Hevea brasiliensis* in India, Rubber Board Bull. 10 (1968) 43.
[8] T.T. Edathil, M.K. George, *Phytophthora nicotianae* var parasitica (Dastur) Waterhouse on *Hevea brasiliensis*in South India, Rubber Board Bull. 13 (1–2) (1976) 3–4.
[9] T.T. Edathil, M.K. George, *Phytophthora botryosa* causing abnormal leaf fall disease of *Hevea brasiliensis* in South Andaman Island in India, International Rubber Conference (1980).
[10] C.B. Roy, C.A. Zachariah, S. Philip, S.P. Idicula, C.K. Jacob, T. Saha, Molecular characterization of *Phytophthora* infecting rubber plantations in South Western India, International Natural Rubber Conference (2005) 489–498.
[11] J. Mathew, C.B. Roy, C.K. Jacob, N. Ramachandran, *Phytophthora* Diseases of Plantation Crops, Westville Publishing House, New Delhi, 2015 pp 176.
[12] C.K. Jacob, E.E. Prem, M.J. Manju, S.P. Idicula, T. Edathil, Crop loss due to abnormal leaf fall disease of rubber (*Hevea brasiliensis*) caused by *Phytophthora* spp, Nat. Rubber Res. 19 (1–2) (2006) 1–8.
[13] H. Hon-Hing, Taiwan *Phytophthora*, Bot. Bull. Acad. Sinica 31 (1990) 89–106.
[14] S. Fawke, M. Doumane, S. Schornack, Oomycete interactions with plants: infection strategies and resistance principles, Microbiol. Mol. Biol. Rev. 79 (3) (2015) 263–280, https://doi.org/10.1128/MMBR.00010-15.
[15] N.I.S. Liyanage, B.E.J. Wheeler, Comparative morphology of *Phytophthora* species on rubber, Plant Pathol. 38 (4) (1989) 592–597, https://doi.org/10.1111/j.1365-3059.1989.tb01456.x.
[16] N.I.S. Liyanage, Phytophthoras on Rubber *Hevea brasiliensis*: Pathogen Taxonomy, Survival and Disease Control, (1986).
[17] Cline, E.T., Farr, D.F., & Rossman, A.Y. (2008). A synopsis of Phytophthora with accurate scientific names, host range, and geographic distribution. Plant Health Progress, (March), 0318-01. doi: https://doi.org/10.1094/PHP-2008-0318-01-RS.
[18] B.M. Tyler, Molecular basis of recognition between *Phytophthora* pathogens and their hosts, Annu. Rev. Phytopathol. 40 (1) (2002) 137–167, https://doi.org/10.1146/annurev.phyto.40.120601.125310.
[19] M. Latijnhouwers, P.J. de Wit, F. Govers, Oomycetes and fungi: similar weaponry to attack plants, Trends Mcrobiol. 11 (10) (2003) 462–469, https://doi.org/10.1016/j.tim.2003.08.002.
[20] R.J. O'Connell, R. Panstruga, Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes, New Phytol. 171 (4) (2006) 699–718, https://doi.org/10.1111/j.1469-8137 .2006.01829.x.
[21] S.C. Whisson, P.C. Boevink, L. Moleleki, A.O. Avrova, J.G. Morales, E.M. Gilroy, M.R. Armstrong, S. Grouffaud, P. Van West, S. Chapman, I. Hein, A translocation signal for delivery of oomycete effector proteins into host plant cells, Nature 450 (7166) (2007) 115, https://doi.org/10.1038/nature06203.
[22] P.N. Dodds, J.P. Rathjen, Plant immunity: towards an integrated view of plant–pathogen interactions, Nat. Rev. Genet. 11 (8) (2010) 539, https://doi.org/10.1038/nrg2812.
[23] N. Leborgne-Castel, T. Adam, K. Bouhidel, Endocytosis in plant–microbe interactions, Protoplasma 247 (3–4) (2010) 177–193.
[24] T. Petch, The Diseases and Pests of the Rubber Tree, Macmillan and Co. Limited, London, 1921, p. 278.
[25] T.S. Ramakrishnan, P.N.R. Pillay, Abnormal leaf fall of rubber (*Hevea brasiliensis*) caused by *Phytophthora palmivora* Butl. In South India, Rubber Board Bull. 5 (1) (1961) 11–20.
[26] T.S. Ramakrishnan, P.N.R. Pillay, Abnormal leaf fall of rubber caused by *Phytophthora palmivora*, Rubber Board Bull. 5 (2) (1961) 76–84.
[27] K.H. Chee, *Phytophthora* leaf disease in Malaya, J. Rubber Res. Inst. Malaya 21 (1) (1969) 79–87.
[28] C.B. Roy, G. Douhan, M.D. Coffey, C.K. Jacob, T. Saha, Existence of two mating types causing extensive genetic diversity in the population of *Phytophthora* sp. Associated with abnormal leaf fall disease of rubber (*Hevea brasiliensis*), Indian Phytopathol. 62 (3) (2009) 390–391.
[29] E.J. Butler, *Phytophthora palmivora* (butler) var. *palmivora*, *Phytophthora Disease Worldwide*, The American Phytopathological Society St. Paul Minnesota, 1996, pp. 408–422.
[30] W. McRae, *Phytophthora meadii* on *Hevea brasiliensis*, Memoirs of the Department of Agriculture in India (Botanical Series) vol. 9, (1918), pp. 219–273.
[31] A. Thompson, *Phytophthora* species in Malaya, Malayan Agric. J. 17 (3–4) (1929).
[32] K.H. Chee, J.M. Greenwood, *Phytophthora* leaf fall and pod rot of *Hevea brasiliensis* in Thailand, FAO Plant Prot. Bull. 16 (1) (1968) 1–5.
[33] S. Liyanage, C.K. Jacob, Diseases of economic importance in rubber, Dev. Crop Sci. 23 (1992) 324–359, https://doi.org/10.1016/B978-0-444-88329-2.50021-0.
[34] J. Kochuthresiamma, R. Kothandaraman, M. Jacob, Actinomycetes population of rubber growing soil and its antagonistic activity against *Phytophthora meadii* (McRae), Indian J. Nat. Rubber Res. 1 (1988) 27–30.
[35] A. Johnston, Diseases and pests, Rubber (1989) 415–458.
[36] F.C. Zeng, E. Ward, Variation within and between *Phytophthora* species from rubber and citrus in China, determined by polymerase chain reaction using RAPDs, J. Phytopathol. 146 (2–3) (1998) 103–109 1111/j.1439-0434.1998.tb04665.x.
[37] H.G. Jayatissa, N.I.S. Liyanage, R.L.C. Wijesundera, Fungicides in the control of *Phytophthora* diseases of rubber in Sri Lanka, J. Nat. Sci. Counc. Sri Lanka 22 (1994) 7–13.
[38] A.F. Dos Santos, K. Matsuoka, A.C. Alfenas, L.A. Maffia, Identification of *Phytophthora* species that infect *Hevea* sp, Fitopatologia Brasileira 20 (1995) 151–159.
[39] J.C.R. Pereira, A.F. dos Santos, A.F. Dos-Santos,). Stem diseases of rubber tree caused by *Phytophthora* spp. And their control, Agrotropica 7 (3) (1995) 63–69.
[40] Y. Parnata, The role of *Phytophthora palmivora* in cacao cultivation in North Sumatra, Bulletin Balai Penelitian Perkebuan Medan 14 (2) (1983) 53–57.
[41] K.H. Chee, Hosts of *Phytophthora palmivora*, Rev. Appl. Mycol. 48 (1969) 337–344.
[42] D.M. Dantanarayana, O.S. Peries, A.D.S. Liyanage, Taxonomy of *Phytophthora* species isolated from rubber in Sri Lanka, Trans. Br. Mycol. Soc. 82 (1) (1984) 113–126, https://doi.org/10.1016/S0007-1536(84)80218-1.
[43] P.H. Tsao, N. Chew-Chin, R. Syamananda, Occurrence of *Phytophthora palmivora* on *Hevea* rubber in Thailand, Plant Dis. Rep. 59 (12) (1975) 955–958.
[44] N. Duong, H.V. Thanh, T. Doan, N. Yen, T.T.M. Tam, T. Dung-Phan, L.T.T. Phuong, N.H. Duong, H.N. Thanh, N.T. Yen, P.T. Dung, Diseases and pests of *Hevea brasilliensis* in Vietnam, Symposium on Natural Rubber (*Hevea brasilliensis*) 2 (1998) 80–91.
[45] K.H. Chee, Variability of *Phytophthora* species from *Hevea brasiliensis*, Trans. Br. Mycol. Soc. 52 (3) (1969), https://doi.org/10.1016/S0007-1536(69)80126-9 425-IN9.
[46] T. Suzui, Y. Kueprakone, T. Kamphangridthrong, *Phytophthora* spp. Isolated from some economic plants in Thailand, Tech. Bull. TARC (Japan) 12 (1979) 32–41.
[47] V.T.T. Dang, M.T. Ha, List of Diseases on Fruit Crops. Survey Results of Insect Pests and Diseases on Fruit Trees, 1997–1998, Agricultural Publishing House, Hanoi, 1999 158p..
[48] M.K. George, T.T. Edathil, Over Summering of *Phytophthora* causing Abnormal Leaf Fall Disease of Rubber (India), Rubber Board Bulletin, 1975.
[49] J.D. Jones, J.L. Dangl, The plant immune system, Nature 444 (7117) (2006) 323, https://doi.org/10.1038/nature05286.
[50] L.C. van Loon, M. Rep, C.M. Pieterse, Significance of inducible defense-related proteins in infected plants, Annu. Rev. Phytopathol. 44 (2006) 135–162, https://doi.org/10.1146/annurev.phyto.44.070505.143425.
[51] P. Ahmad, C.A. Jaleel, M.A. Salem, G. Nabi, S. Sharma, Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress, Crit. Rev. Biotechnol.

30 (3) (2010) 161–175, https://doi.org/10.3109/07388550903524243.
[52] C.M. Pieterse, A. Leon-Reyes, S. Van der Ent, S.C. Van Wees, Networking by small-molecule hormones in plant immunity, Nat. Chem. Biol. 5 (5) (2009) 308, https://doi.org/10.1038/nchembio.164.
[53] S. Kamoun, A catalogue of the effector secretome of plant pathogenic oomycetes, Annu. Rev. Phytopathol. 44 (2006) 41–60, https://doi.org/10.1146/annurev.phyto.44.070505.143436.
[54] T. Boller, S.Y. He, Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens, Science 324 (5928) (2009) 742–744, https://doi.org/10.1126/science.1171647.
[55] N.T. Keen, Gene-for-gene complementarity in plant-pathogen interactions, Annu. Rev. Genet. 24 (1990) 447–463, https://doi.org/10.1146/annurev.ge.24.120190.002311.
[56] N.S. Coll, P. Epple, J.L. Dangl, Programmed cell death in the plant immune system, Cell Death Differ. 18 (8) (2011) 1247, https://doi.org/10.1038/cdd.2011.37.
[57] K. Tsuda, F. Katagiri, Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity, Curr. Opin. Plant Biol. 13 (4) (2010) 459–465, https://doi.org/10.1016/j.pbi.2010.04.006.
[58] M.C. Heath, Involvement of reactive oxygen species in the response of resistant (hypersensitive) or susceptible cowpeas to the cowpea rust fungus, New Phytol. 138 (2) (1998) 251–263.
[59] W.E. Durrant, X. Dong, Systemic acquired resistance, Annu. Rev. Phytopathol. 42 (2004) 185–209, https://doi.org/10.1146/annurev.phyto.42.040803.140421.
[60] J.A. Ryals, U.H. Neuenschwander, M.G. Willits, A. Molina, H.Y. Steiner, M.D. Hunt, Systemic acquired resistance, Plant Cell 8 (10) (1996) 1809, https://doi.org/10.1105/tpc.8.10.1809.
[61] T. Gaffney, L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, J. Ryals, Requirement of salicylic acid for the induction of systemic acquired resistance, Science 261 (5122) (1993) 754–756, https://doi.org/10.1126/science.261.5122.754.
[62] P. Wojtaszek, Oxidative burst: an early plant response to pathogen infection, Biochem. J. 322 (3) (1997) 681–692, https://doi.org/10.1042/bj3220681.
[63] K.E. Jayasuriya, R.L.C. Wijesundera, S.A. Deraniyagala, Isolation of anti-fungal phenolic compounds from petioles of two *Hevea brasiliensis* (rubber) genotypes and their effect on *Phytophthora meadii*, Ann. Appl. Biol. 142 (1) (2003) 63–69, https://doi.org/10.1111/j.1744-7348.2003.tb00230.x.
[64] W.H. Elmer, M.J.I. Mattina, G.J. MacEachern, Sensitivity of plant pathogenic fungi to taxane extracts from ornamental yews, Phytopathology 84 (10) (1994) 1179–1185, https://doi.org/10.1094/Phyto-84-1179.
[65] C.J. Lamb, M.A. Lawton, M. Dron, R.A. Dixon, Signals and transduction mechanisms for activation of plant defenses against microbial attack, Cell 56 (2) (1989) 215–224, https://doi.org/10.1016/0092-8674(89)90894-5.
[66] T. Kawano, Roles of the reactive oxygen species-generating peroxidase reactions in plant defense and growth induction, Plant Cell Rep. 21 (9) (2003) 829–837, https://doi.org/10.1007/s00299-003-0591-z.
[67] M.A. Torres, J.D. Jones, J.L. Dangl, Reactive oxygen species signaling in response to pathogens, Plant Physiol. 141 (2) (2006) 373–378, https://doi.org/10.1104/pp.106.079467.
[68] J. Dat, S. Vandenabeele, E. Vranová, M. Van Montagu, D. Inzé, F. Van Breusegem, Dual action of the active oxygen species during plant stress responses, Cell. Mol. Life Sci. 57 (5) (2000) 779–795, https://doi.org/10.1007/s000180050.
[69] Z. Chen, H. Silva, D.F. Klessig, Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid, Science 262 (5141) (1993) 1883–1886, https://doi.org/10.1126/science.8266079.
[70] R. Mittler, S. Vanderauwera, M. Gollery, F. Van Breusegem, Reactive oxygen gene network of plants, Trends Plant Sci. 9 (10) (2004) 490–498, https://doi.org/10.1016/j.tplants.2004.08.009.
[71] P.L. Gratao, A. Polle, P.J. Lea, R.A. Azevedo, Making the life of heavy metal-stressed plants a little easier, Funct. Plant Biol. 32 (6) (2005) 481–494, https://doi.org/10.1071/FP05016.
[72] P.M. Seevers, J.M. Daly, F.F. Catedral, The role of peroxidase isozymes in resistance to wheat stem rust disease, Plant Physiol. 48 (3) (1971) 353–360, https://doi.org/10.1104/pp.48.3.353.
[73] D.'Maris Amick, A.C. Dempsey, M.C.W. Vlot, F.K. Daniel, Salicylic Acid Biosynthesis and Metabolism, The Arabidopsis book/ American Society of Plant Biologists, 2011, p. 9, https://doi.org/10.1199/tab.0156.
[74] J. Huang, M. Gu, Z. Lai, B. Fan, K. Shi, Y.H. Zhou, Z. Chen, Functional analysis of the *Arabidopsis* PAL gene family in plant growth, development and response to environmental stress, Plant Physiol. (2010), https://doi.org/10.1104/pp.110.157370 pp-110.
[75] Y.C. Chen, B.N. Kidd, L.C. Carvalhais, P.M. Schenk, Molecular defense responses in roots and the rhizosphere against *Fusarium oxysporum*, Plant Signalling Behav. 9 (12) (2014) e977710, https://doi.org/10.4161/15592324.2014.977710.
[76] R.A. Dixon, C.J. Lamb, Molecular communication in interactions between plants and microbial pathogens, Annu. Rev. Plant Biol. 41 (1) (1990) 339–367, https://doi.org/10.1146/annurev.pp.41.060190.002011.
[77] N. Deenamo, A. Kuyyogsuy, K. Khompatara, T. Chanwun, K. Ekchaweng, N. Churngchow, Salicylic acid induces resistance in rubber tree against *Phytophthora palmivora*, Int. J. Mol. Sci. 19 (7) (2018) 1883, https://doi.org/10.3390/ijms19071883.
[78] U. Khunjan, K. Ekchaweng, T. Panrat, M. Tian, N. Churngchow, Molecular cloning of *HbPR-1* gene from rubber tree, expression of *HbPR-1* gene in *Nicotiana benthamiana* and its inhibition of *Phytophthora palmivora*, PLoS One 11 (6) (2016) e0157591, https://doi.org/10.1371/journal.pone.0157591.
[79] A. Heese, D.R. Hann, S. Gimenez-Ibanez, A.M. Jones, K. He, J. Li, J.I. Schroeder, S.C. Peck, J.P. Rathjen, The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants, Proc. Natl. Acad. Sci. 104 (29) (2007) 12217–12222, https://doi.org/10.1073/pnas.0705306104.
[80] A. Chaparro-Garcia, R.C. Wilkinson, S. Gimenez-Ibanez, K. Findlay, M.D. Coffey, C. Zipfel, S. Schornack, The receptor-like kinase SERK3/BAK1 is required for basal resistance against the late blight pathogen *Phytophthora infestans* in *Nicotiana benthamiana*, PLoS One 6 (1) (2011) e16608, https://doi.org/10.1371/journal.pone.0016608.
[81] G. Henry, P. Thonart, M. Ongena, PAMPs, MAMPs, DAMPs and others: an update on the diversity of plant immunity elicitors, Biotechnol. Agron. Soc. Environ. 16 (2) (2012) 257–268.
[82] D.J. Bradley, P. Kjellbom, C.J. Lamb, Elicitor-and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response, Cell 70 (1) (1992) 21–30, https://doi.org/10.1016/0092-8674(92)90530-PGet.
[83] A. Garcia-Brugger, O. Lamotte, E. Vandelle, S. Bourque, D. Lecourieux, B. Poinssot, A. Pugin, Early signaling events induced by elicitors of plant defences, Mol. Plant-microbe Interact. 19 (7) (2006) 711–724, https://doi.org/10.1094/MPMI-19-0711.
[84] J. Eder, E.G. Cosio, Elicitors of plant defense responses, Int. Rev. Cytol. 148 (1994) 1–36, https://doi.org/10.1016/S0074-7696(08)62404-3.
[85] Y.H. Chang, H.Z. Yan, R.F. Liou, A novel elicitor protein from *Phytophthora parasitica* induces plant basal immunity and systemic acquired resistance, Mol. Plant Pathol. 16 (2) (2015) 123–136, https://doi.org/10.1111/mpp.12166.
[86] F.V. Mateos, M. Rickauer, M.T. Esquerré-Tugayé, Cloning and characterization of a cDNA encoding an elicitor of *Phytophthora parasitica* var. *Nicotianae* that shows cellulose-binding and lectin-like activities, Mol. Plant-microbe Interact. 10 (9) (1997) 1045–1053, https://doi.org/10.1094/MPMI.1997.10.9.1045.
[87] N. Séjalon-Delmas, F.V. Mateos, A. Bottin, M. Rickauer, R. Dargent, M.T. Esquerré-Tugayé, Purification, elicitor activity, and cell wall localization of a glycoprotein from *Phytophthora parasitica var. nicotianae*, a fungal pathogen of tobacco, Phytopathology 87 (9) (1997) 899–909, https://doi.org/10.1094/PHYTO.1997.87.9.899.
[88] M. Khatib, C. Lafitte, M.T. Esquerré-Tugayé, A. Bottin, M. Rickauer, The CBEL elicitor of *Phytophthora parasitica var. Nicotianae* activates defence in *Arabidopsis thaliana* via three different signalling pathways, New Phytol. 162 (2) (2004) 501–510, https://doi.org/10.1111/j.1469-8137.2004.01043.x.
[89] O. Bunyatang, N. Chirapongsatonkul, P. Bangrak, R. Henry, N. Churngchow, Molecular cloning and characterization of a novel bi-functional α-amylase/subtilisin inhibitor from *Hevea brasiliensis*, Plant Physiol. Biochem. 101 (2016) 76–87, https://doi.org/10.1016/j.plaphy.2016.01.014.
[90] P. Kong, J.M. McDowell, C. Hong, Zoospore exudates from *Phytophthora nicotianae* affect immune responses in *Arabidopsis*, PLoS One 12 (6) (2017) e0180523, https://doi.org/10.1371/journal.pone.0180523.
[91] S. Kamoun, Groovy times: filamentous pathogen effectors revealed, Curr. Opin. Plant Biol. 10 (4) (2007) 358–365, https://doi.org/10.1016/j.pbi.2007.04.017 PMID: 17611143 41.
[92] M.C. Giraldo, B. Valent, Filamentous plant pathogen effectors in action, Nat. Rev. Microbiol. 11 (11) (2013) 800.
[93] P.R. Birch, A.P. Rehmany, L. Pritchard, S. Kamoun, J.L. Beynon, Trafficking arms: oomycete effectors enter host plant cells, Trends Microbiol. 14 (1) (2006) 8–11, https://doi.org/10.1016/j.tim.2005.11.007.
[94] A.R. Hardham, D.M. Cahill, The role of oomycete effectors in plant–pathogen interactions, Funct. Plant Biol. 37 (10) (2010) 919–925, https://doi.org/10.1071/FP10073.
[95] P.H. Gan, M. Rafiqi, A.R. Hardham, P.N. Dodds, Effectors of biotrophic fungal plant pathogens, Funct. Plant Biol. 37 (10) (2010) 913–918, https://doi.org/10.1071/FP10072.
[96] W. Oswald, F. Fleischmann, D. Rigling, A.C. Coelho, A. Cravador, J. Diez, G. Sipos, Strategies of attack and defence in woody plant–*Phytophthora* interactions, For. Pathol. 44 (3) (2014) 169–190, https://doi.org/10.1111/efp.12096.
[97] A.R. Hardham, L.M. Blackman, Molecular cytology of *Phytophthora*-plant interactions, Australas. Plant Pathol. 39 (1) (2010) 29–35.
[98] S. Schornack, M. van Damme, T.O. Bozkurt, L.M. Cano, M. Smoker, M. Thines, E. Huitema, Ancient class of translocated oomycete effectors targets the host nucleus, Proc. Natl. Acad. Sci. 107 (40) (2010) 17421–17426 1073/pnas.1008491107.
[99] T.O. Bozkurt, S. Schornack, M.J. Banfield, S. Kamoun, Oomycetes, effectors, and all that jazz, Curr. Opin. Plant Biol. 15 (4) (2012) 483–492, https://doi.org/10.1016/j.pbi.2012.03.008.
[100] N. Chirapongsatonkul, P. Nak-udom, N. Churngchow, Defence responses of calli and seeds of *Hevea brasiliensis* to zoospores and the elicitin of *Phytophthora palmivora*, J. Phytopathol. 156 (11–12) (2008) 732–741, https://doi.org/10.1111/j.1439-0434.2008.01437.x.
[101] P. Ricci, P. Bonnet, J.C. Huet, M. Sallantin, F. Beauvais-Cante, M. Bruneteau, J.C. Pernollet, Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco, Eur. J. Biochem. 183 (3) (1989) 555–563.
[102] P. Ricci, F. Trentin, P. Bonnet, P. Venard, F. Mouton-Perronnet, M. Bruneteau, Differential production of parasiticein, an elicitor of necrosis and resistance in tobacco, by isolates of *Phytophthora parasitica*, Plant Pathol. 41 (3) (1992) 298–307, https://doi.org/10.1111/j.1365-3059.1992.tb02351.x.
[103] J.C. Huet, C. Nespoulous, J.C. Pernollet, Structures of elicitin isoforms secreted by *Phytophthora drechsleri*, Phytochemistry 31 (5) (1992) 1471–1476.
[104] C. Nespoulous, J.C. Huet, J.C. Pernollet, Structure-function relationships of α and β elicitins, signal proteins involved in the plant-*Phytophthora* interaction, Planta 186 (4) (1992) 551–557.
[105] J.C. Huet, J.C. Pernollet, Sequences of acidic and basic elicitin isoforms secreted

by *Phytophthora megasperma megasperma*, Phytochemistry 33 (4) (1993) 797–805, https://doi.org/10.1016/0031-9422(93)85277-X.
[106] F. Mouton-Perronnet, M. Bruneteau, L. Denoroy, P. Bouliteau, P. Ricci, P.H. Bonnet, G. Michel, Elicitin produced by an isolate of *Phytophthora parasitica* pathogenic to tobacco, Phytochemistry 38 (1) (1995) 41–44, https://doi.org/10.1016/0031-9422(94)0058X-A.
[107] N. Churngchow, M. Rattarasarn, The elicitin secreted by *Phytophthora palmivora*, a rubber tree pathogen, Phytochemistry 54 (1) (2000) 33–38, https://doi.org/10.1016/S0031-9422(99)00530-0.
[108] N. Churngchow, M. Rattarasarn, Biosynthesis of scopoletin in *Hevea brasiliensis* leaves inoculated with *Phytophthora palmivora*, J. Plant Physiol. 158 (7) (2001) 875–882, https://doi.org/10.1078/0176-1617-00230.
[109] M. Tian, B. Benedetti, S. Kamoun, A second Kazal-like protease inhibitor from *Phytophthora infestans* inhibits and interacts with the apoplastic *pathogenesis*-related protease P69B of tomato, Plant Physiol. 138 (3) (2005) 1785–1793, https://doi.org/10.1104/pp.105.061226 PMID: 15980196.
[110] K. Ekchaweng, E. Evangelisti, S. Schornack, M. Tian, N. Churngchow, The plant defense and pathogen counter defense mediated by *Hevea brasiliensis* serine protease HbSPA and *Phytophthora palmivora* extracellular protease inhibitor PpEPI10, PLoS One 12 (5) (2017) e0175795, https://doi.org/10.1371/journal.pone.0175795.
[111] L.C. Van Loon, W.S. Pierpoint, T.H. Boller, V. Conejero, Recommendations for naming plant pathogenesis-related proteins, Plant Mol. Biol. Rep. 12 (3) (1994) 245–264.
[112] L.C. Van Loon, M. Rep, C.M. Pieterse, Significance of inducible defense-related proteins in infected plants, Annu. Rev. Phytopathol. 44 (2006) 135–162, https://doi.org/10.1146/annurev.phyto.44.070505.
[113] S. Breen, S.J. Williams, M. Outram, B. Kobe, P.S. Solomon, Emerging insights into the functions of pathogenesis-related protein 1, Trends Plant Sci. 22 (10) (2017) 871–879, https://doi.org/10.1016/j.tplants.2017.06.013.
[114] K. Narasimhan, A. Thulaseedharan, R. Kothandaraman, Detection of pathogenesis related protein in *Hevea brasiliensis* by *Phytophthora meadii*, Indian J. Nat. Rubber Res. 13 (1–2) (2000) 30–37.
[115] I. Thanseem, A. Joseph, A. Thulaseedharan, Induction and differential expression of β-1, 3-glucanase mRNAs in tolerant and susceptible *Hevea* clones in response to infection by *Phytophthora meadii*, Tree Physiol. 25 (11) (2005) 1361–1368, https://doi.org/10.1093/treephys/25.11.1361.
[116] R. Hammerschmidt, Phytoalexins: what have we learned after 60 years? Annu. Rev. Phytopathol. 37 (1) (1999) 285–306, https://doi.org/10.1146/annurev.phyto.37.1.285.
[117] M.A. Naoumkina, Q. Zhao, L.I.N.A. Gallego-Giraldo, X. Dai, P.X. Zhao, R.A. Dixon, Genome-wide analysis of phenylpropanoid defence pathways, Mol. Plant Pathol. 11 (6) (2010) 829–846, https://doi.org/10.1111/j.1364-3703.2010.00648.x.
[118] F.R. Cavalcanti, M.L.V. Resende, C.P.S. Carvalho, J.A.G. Silveira, J.T.A. Oliveira, Induced defence responses and protective effects on tomato against *Xanthomonas vesicatoria* by an aqueous extract from *Solanum lycocarpum* infected with *Crinipellis perniciosa*, Biol. Control. 39 (3) (2006) 408–417, https://doi.org/10.1016/j.biocontrol.2006.05.009.
[119] A.M. Tan, F.C. Low, Phytoalexin production by *Hevea brasiliensis* in response to infection by *Collectotrichum gloesporioides* and its effect on other fungi, International Rubber Conference (1975).
[120] A. Giesemann, B. Biehl, R. Lieberei, Identification of scopoletin as a phytoalexin of the rubber tree *Hevea brasiliensis*, J. Phytopathol. 117 (4) (1986) 373–376, https://doi.org/10.1111/j.1439-0434.1986.tb04377.x.
[121] L. Jurd, J. Corse, A.D. King Jr., H. Bayne, K. Mihara, Antimicrobial properties of 6, 7-dihydroxy-, 7, 8-dihydroxy-, 6-hydroxy-and 8-hydroxycoumarins, Phytochemistry 10 (12) (1971) 2971–2974, https://doi.org/10.1016/S0031-9422(00)97334-5.
[122] D. Garcia, E. Cazaux, F. Rivano, J. D'Auzac, Chemical and structural barriers to *Microcyclus ulei,* the agent of South American leaf blight, in *Hevea* spp, Eur. J. For. Pathol. 25 (5) (1995) 282–292, https://doi.org/10.1111/j.1439-0329.1995.tb01013.x.
[123] D. Garcia, C. Sanier, J.J. Macheix, J. D'Auzac, Accumulation of scopoletin in *Hevea brasiliensis* infected by *Microcyclus ulei* (P. Henn.) V. ARX and evaluation of its fungitoxicity for three leaf pathogens of rubber tree, Physiol. Mol. Plant Pathol. 47 (4) (1995) 213–223, https://doi.org/10.1006/pmpp.1995.1053.
[124] F. Breton, C. Sanier, J. d'Auzac, Scopoletin production and degradation in relation to resistance of *Hevea brasiliensis* to *Corynespora cassiicola*, J. Plant Physiol. 151 (5) (1997) 595–602, https://doi.org/10.1016/S0176-1617(97)80236-2.
[125] I. Thanseem, P. Venkatachalam, A. Thulaseedharan, Sequence characterization of β-1,3 glucanase gene from *Hevea brasiliensis* through genomic and cDNA cloning, Indian J. Nat. Rubber Res. 16 (1–2) (2003) 106–114.
[126] S. Philip, A. Joseph, K.A. Kumar, C.K. Jacob, R. Kothandaraman, Detection of β-1, 3-glucanase isoforms against *Corynespora* leaf disease of rubber (*Hevea brasiliensis*), Indian J. Nat. Rubber Res. 14 (1) (2001) 1–6.
[127] S. Philip, The host factors in *Phytophthora – Hevea* interaction, Phytophthora Diseases of Plantations Crops, (2015), pp. 28–32.
[128] R. Supriya, A. Saleena, A. Thulaseedharan, Cloning and characterization of β-1,3 glucanase mRNAs in tolerant and susceptible *Hevea* clones in response to infection by *Phytophthora meadii*, Tree Physiol. 25 (2006) 1361–1368.
[129] T. Saha, C.B. Roy, M. Ravindran, Towards development of molecular markers for *Phytophthora* tolerance in rubber, Phytophthora Diseases of Plantations Crops, (2015), pp. 144–151.