Chapter 1
INTRODUCTION

Introduction

Rubber plant, (*Hevea brasiliensis* (Willd.) Muell.-Arg., is an economically important crop in Thailand, the products of which are exported worldwide and produce significant revenue for the country. In 1998, 2.7 million tons of rubber were produced and valued at 55,010 million baht (Tachavuliporn, 1999) The planting area of *Hevea brasiliensis* has nowadays expanded to all over the country, mostly condensed in the South which has a suitable climate. It takes 5-6 years after planting before rubber latex can be obtained by tapping. However, the consequence of frequent tapping causes infection at the raw surface of tapping site. This problem is common in the southernmost part of Thailand where the humidity is high and suitable for pathogen growth, especially fungus.

Phytophthora palmivora is the causative agent of "leaf fall" and "black stripe" in rubber plants. It attacks the petioles, causing mature leaves to fall prematurely and attacks the tapping surface resulting in poor latex production. Therefore, the resistant cultivar of *H. brasiliensis* should be selected for planting. This is a more cost–effective way to prevent the loss from planting low-yield rubber tree. The commonly used strain of rubber for planting is RRIM600, it gives high-yield of latex but susceptible to *Phytophthora* spp. Currently, BPM-24 is considered to be more effective in this regard.

It is known that plants has been infected by many pathogens including bacteria, virus, fungi and nematodes and it can be infected with several pathogens at the same time. Each plant has its own defense mechanism to protect itself from the pathogens. The disease is expressed when a susceptible plant is infected with virulent pathogen (compatible reaction) and vice versa, it will show no symptom if it is infected with avirulent pathogen (incompatible reaction). In general, the incompatible reaction occurs in the resistant host and the compatible reaction causes disease in the susceptible host as well.

Structural defense mechanisms which prevent an infection are histological and cellular defense structures formed as a result of hypersensitive reaction in the plant, for example, cork layers formation, abscission regions formation, formation of tyloses and gum. Cork layers can prevent spreading of pathogen and its toxin by impeding water and nutrient flow to infected tissue resulting in localization of non-viable infected tissue. Abscission regions are formed by tissue disruption creating intercellular space surrounding infected area, therefore it prevents normal, non-infected tissue from further infection. Tyloses formation commonly develops during the invasion of pathogen into the xylem by the in-growth of the protoplasm of the adjacent parenchymal cells of the xylem making it obstructed. Tyloses can be enormously and rapidly formed in the resistant plant whereas it is formed more slowly in the susceptible plant usually after infection was spread. Furthermore, the accumulation of gum intra- or intercellularly surrounding the infected area is also helpful in this regard. The rate of gum accumulation differs between different kinds of plant.

Hypersensitive reaction is one of the most important defense mechanisms in plant. It causes the infected and its surrounding tissue of the resistant plant turn brown, having the characteristics of burn-like lesion and hypersensitive cell death. It makes the pathogen inside the dead tissue deprived of nutrient, therefore limits the pathogen growth and can inhibit further spread of infection. The rate of hypersensitive reaction is much slower or does not exist in the susceptible plant making it unable to defense itself (Jungpanich, 2525; Smitaman, 2534).

In addition to the numbers of pathogen, substances derived from pathogen or pathogen-host interaction, ultraviolet light as well as certain metal ions can also stimulate plant defense mechanism.

Review of Literature

1 Hevea brasiliensis

Hevea brasiliensis (Willd.) Muell.-Arg. or Para rubber is a number of in Family Euphorbiaceae. Formerly it was classified as *Siphonis brasiliensis* Willd. ex A. Juss. *H. brasiliensis* is a tropical tree and native to the Amazon Basin in Brazil and adjoining countries. *Hevea* was taken from the Amazon region to many other tropical regions of the world, such as South and South East Asia including Thailand, by the British Colonial Office (Reed, 1976).

Hevea plants grow best at temperatures of 20-28°C with a well-distributed annual rainfall of 1,800-2,000 mm. Mature *Hevea* trees on rubber plantations are 20-30 m high, with girth of 2.0-3.0 m; stems smooth and straight; bark grayish; taproot well-developed; leaves alternate, trifoliate, petioles 7.5-10 cm long; flowers numerous; female flowers apical; fruit a 3-lobed, 3-seeded ellipsoidal capsule, variable in size, 2.5-3 cm long, mottled brown, weighing 2-4 grams each (Fig.1) (Reed 1976). Such trees are flowering once a year, and after insect cross-pollination, produce large fruits containing several thimble-sized seeds with hard outer coats. Seeds are collected in July-September in India. If satisfactorily germinated and planted within 2-3 weeks, the seeds grow to produce seedling plants. Depending on conditions, the rubber trees take 5-10 years to reach 'maturity', which is defined as the stage when tapping can be started. In practice, this is the time when the trunk has about 500 mm in diameter at 0.75 meter above ground level. Tapping is conducted by removal of a thin cut of the bark about 1 mm deep at regular intervals, thus opening the latex vessels in the bark, which are arranged in concentric cylinders and run in counter-clockwise spirals up the

trunk. Usually, the cuts run half-way around the trunk, but may encircle the tree. Trees are tapped early in the morning when flow of latex is highest; latex flow decreases with temperature and usually ceases in about 3 hours. Major world supply of natural rubber at present is obtained from South East Asia. Major consumers are United States of America, United Kingdom, and France. Natural rubber accounts for one-third of the world's rubbers. Tires and tire accessories are nearly three-fourth of the U.S. natural rubber consumption in 1974 (Rogers, 1981).

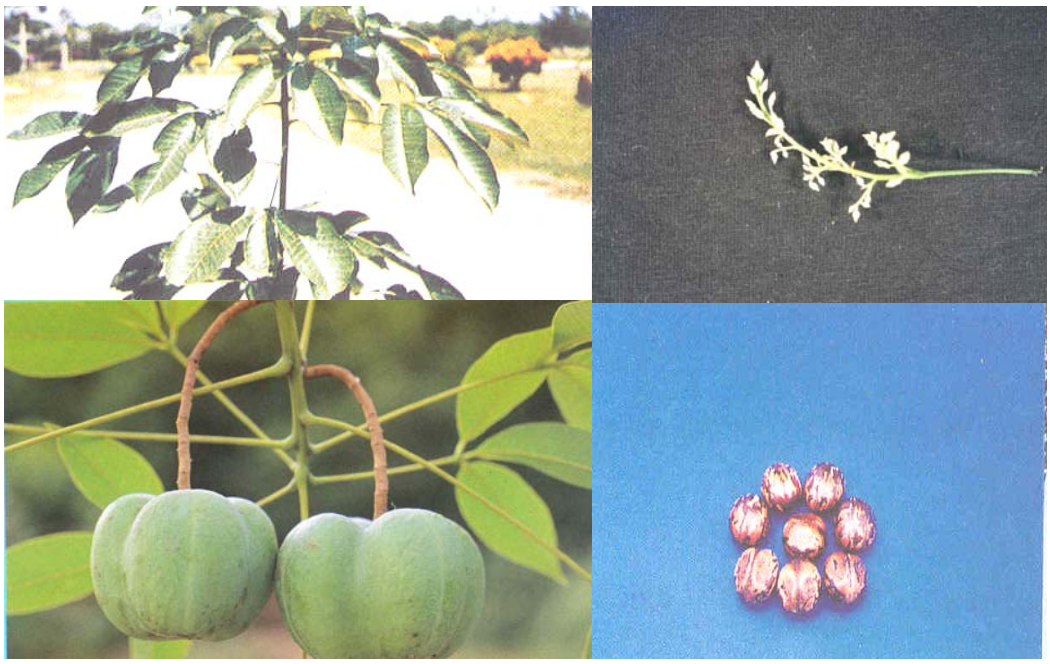

Fig. 1 Leaves (upper left), flowers (upper right), fruits (lower left) and seeds (lower right) of *Hevea brasiliensis* (Form Rubber Research Institute, 1999).

About 90 species of fungi are known to attack *Hevea* trees, the most prevalent ones being the following: *Botryodiplodia elactica* and *B. theobromae,*

Colletotrichum heveae (leaf spot), *Fomes lamaensis* (brown root rot), *Gloeosporium heveae* (die-back), *Oidium heveae* (powdery mildew), *Pellicularis salmonicolor* (pink disease), *Polystichus occidentalis* and *P. personii* (white spongy rot), *Sphaerella heveae* (rim bright), *Sphaerostilbe repens* (red rot), *Ustulina maxima* (charcoal rot) and *Phytophthora palmivora* (causing fruit rot, leaf-fall, black stripe (Fig. 2), and die-back). It is also attacked by bacteria, nematodes, insects, white ants, and snails (Golden, personal contact, 1984).

The leaf-fall and black stripe in *H. brasiliensis* are frequently found in Thailand and can decrease the quality and yield of rubber latex. Several species of *Phytophthora* have been reported to be responsible for leaf-fall and black stripe. The common species are: *Phytophthora palmivora* (Butl.) Butl., *P. meadii* Mc Rae, and *P. botryosa* Chee. In 1984, several other species of *Phytophthora* were identified as causes of black stripe infection in China: for example *P. citrophthora* (Smith & Smith) Leonian, *P. cactorum* (Lebert & Cohn) Schrodter, and *P. capsici* Leonian. The early symptoms of black stripe are not obvious. Series of sunken and slightly discolored areas just above the cut surface (tapping surface), followed by the appearance of vertical fissures in the renewing bark are observed. Dark vertical lines are visible when bark is removed. As the infection progresses, the stripes form broad lesions, and finally spread to the full width of the tapping panel. Occasionally, infection occurs on untapped bark resulting in a wound, called “canker”. This may arise on bark previously affected by black stripe or on wounds caused by spouts or wires. The early symptoms of canker are not obvious but in the advanced stage, the bark bursts and latex oozes out. Pads of coagulated latex are formed under the bark causing it to bulge and split

open. Black stripe incidence is associated with wet weather, being favoured by prolonged cool and rainy periods. The fungus is commonly present in the soil and its sporangia are spread by water droplets. In areas where abnormal leaf fall occurs, the sporangia are washed down from the canopy. Canker development is common in leaf fall areas via heavy inoculum from the tree canopy.

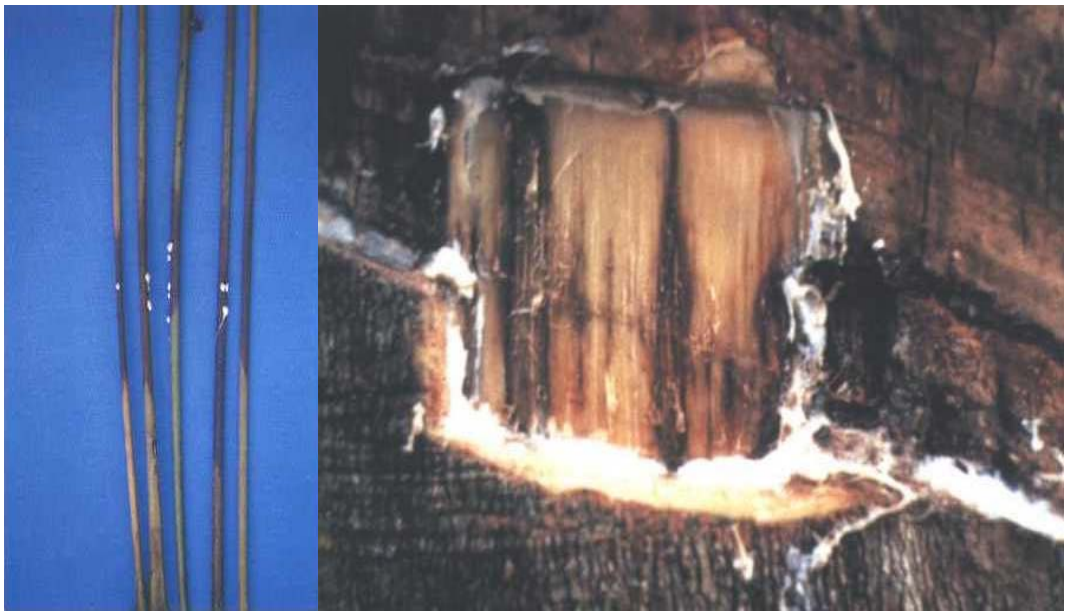

Fig. 2 The appearance of black stripe on leaf petiole and stem in *Hevea brasiliensis* (Form Rubber Research Institute, 1999)

2 *Phytophthora* spp.

The name of Phytophthora is derived from Greek, and literally means plant (phyto) destroyer (phthora). *Phytophthora* is a major genus of plant pathogens within the diploid, algae-like oomycete fungi. Currently, this genus is assigned to the order Pythiales, phylum Oomycota within the group of heterokont, biflagellate organisms that comprise the Kingdom Chromista (Cavalier-Smith, 1986; D.E.L.

Cooke, 2000). *Phytophthora* is fungus-like which is supported by a number of features including biflagellate zoospore, aseptate hyphae, diploid thallus and the cell wall is composed of cellulose and glycan rather than chitin.

There are 60 recognized species of *Phytophthora* (Erwin and Ribeiro, 1996). Most are primary plant pathogens with limited saprotrophic ability. Many species are responsible for serious diseases of economically important crops and some cause extensive damage to natural plant communities. In contrast to other soil-borne fungi, which are single-cycle pathogens, *Phytophthora* is multi-cyclic and can produce inoculum continuously after the initial infection as long as conditions remain favorable.

In the traditional taxonomy, *Phytophthora* was discriminated mainly on the structure of the sporangium (nonpapillate, semipapillate, or papillate), the form of sexual organ (amphigynous or paragynous), and on whether the taxon is inbreeding (homothallic) or outbreeding with A1 and A2 sexual incompatibility or mating types (heterothallic) (Tucker, 1931; Waterhouse, 1963). For asexual reproduction, the sporangium produces and releases zoospores which then encyst, germinate and elongate the germ tube and form appressorium. The penetrated hyphae is capable for host invasion. For sexual reproduction, hypha transforms to be antheridium (male) and oogonium (female). Mating of these organs are resulting in oospores, which can further grow to mycelia and then develop sporangia. Oospores and mycelia can survive in the host plants, later transform to sporangia and zoospores when exposed to appropriate conditions as shown in Fig. 3.

Phytophthora spp. causes root, stem and fruit rot in more than 100 plant species, including pineapple, papaya, orange, tomato, potato, tobacco, longan, and rubber tree (*H. brasiliensis*). *P. palmivora, P. botryosa, P. hevea, P. meadii* and *P. parasitica* have been described as pathogens of the rubber tree. *P. palmivora* and *P. meadii* are the most frequently isolated and are described as the causal agents of black stripe, green pod rot and abnormal leaf fall. In Malaysia and Thailand, these diseases are considered to be caused by *P. palmivora* and *P. botryos*a (Erwin and Ribeiro, 1996).

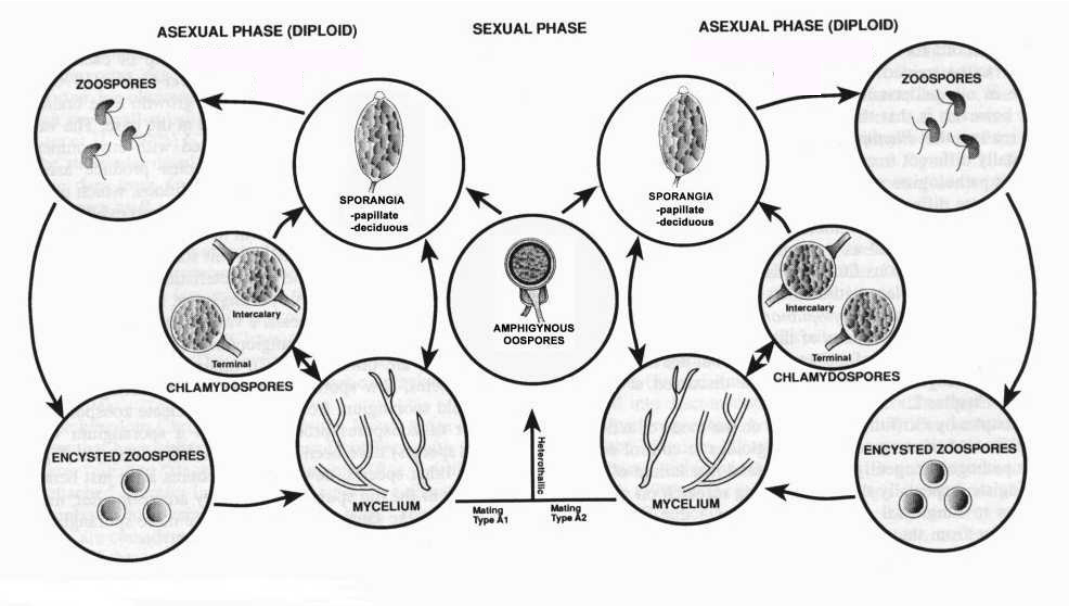

Fig. 3 The life-cycle of *Phytophthora* spp. (From D.C. Erwin and O.K. Ribeiro, 1996).

P. palmivora (Butler) is a ubiquitous pathogen with a wide host range (Erwin and Ribeiro,1996; Holiday, 1980). It causes many diseases such as black pot, stem canker and wilt of cocoa, root and fruit rot of papaya, fruit rot and premature nut fall in coconut, foot rot in black pepper; and black stripe, patch canker and

pod rot in rubber tree. *P. palmivora* is heterothallic and is distinguished from other species of *Phytophthora* mainly by the prominent papillate sporangia that are ellipsoidal to ovoid in shape, deciduous and have a short pedicel. However, the morphological and physiological characteristics of *P. palmivora* exhibit considerable variation, depending on the isolate and the host. *Phytophthora* is a soil-borne pathogen, it causes root rot and can spread easily from root to root contact, through the movement of infested soils, through irrigation system, rain splash, insects and pruning equipments (Holiday,1980).

Phytophthora spp. produce and secrete extracellular proteins, some of them are elicitors which can trigger plant defense reactions. Most *Phytophthora* spp. except *P. nicotianae*, secrete 10 kDa proteins which are generally called elicitins (Pernollet, *et al*., 1993 b). *P. nicotianae*, the causal agent of tobacco black shank disease, can invade tobacco stem whereas other *Phytophthora* species cause limited colonization, and leaf necrosis at a distance from the inoculation site (Bonnet, 1985). When elicitins of other *Phytophthora spp*. are applied to tobacco plants, they elicit leaf necrosis, cause the accumulation of pathogenesis-related proteins (Bonnet, *et al*., 1996), and induce protection against a subsequent inoculation with tobacco pathogen, *P. nicotianae* (Ricci, *et al*., 1989). In culture media of some *Phytophthora* spp, a 32 kDa glycoprotein of cell wall fragment is found. This protein can causes, similar necrosis of tobacco leaf was also reported (Baillieul, *et al*., 1996). In addition to a potent elicitor of phytoalexin accumulation in cultured parsley cells, a 42 kDa glycoprotein, was purified from the culture filtrate of *P. megasperma* f. sp*. glycinea* (Parker, *et al*., 1991).

3 The plant-pathogen interaction

Plants can respond to pathogens by two types of reactions, namely compatible and incompatible reactions. The compatible reaction is the interaction between a susceptible host and a pathogen, resulting in disease in the host whereas the incompatible reaction is the interaction between a resistant host and a pathogen that induces protective responses. Plant defense mechanism consists of passive and active defenses. The passive defense mechanisms are those that are already present prior to contact with the pathogen, while active defense mechanisms are activated only after pathogen recognition (Guest, 1997). The passive defenses include the natural physical and chemical barriers such as cuticle, stomatal aperture, phytoanticipins and plant defensins. The active defenses consist of two classes of responses which include the rapid active defenses such as the oxidative burst, cell wall reinforcement, phytoalexin accumulation, and hypersensitive cell death and the delayed active defenses such as pathogenesis-related proteins production and systemic acquired resistance (Table 1).

Table 1 Events involved in the coordination of defense responses in plants to challenge by pathogens (From Guest, D. and Brown, J., 1997).

Time	Event
Minutes	Membrane depolarisation and electrolyte leakage Reactive oxygen generation Expression of genes involved in phytoalexin biosynthesis
Hours	Oxidative burst Membrane lipid peroxidation Rise in salicylic acid levels Cytoplasmic aggregation, cell collapse and hypersensitive cell death Phytoalexin accumulation Cell wall reinforcements
Days	Accumulation of pathogenesis-related proteins Systemic acquired resistance

3.1 The rapid active defenses

Plant responses to infection are complex and there is no universal model or sequence of events that accurately describes the dynamics of resistance in the few interactions studied. Almost every host-parasite interaction is unique in the details of the activation, localization, timing and magnitude of each component of the defense responses. As previously stated, resistance is rarely absolute and whether a plant ends up being resistant or susceptible depending on the sum of many individual responses.

3.1.1 Changes in membrane function

Membrane permeability changes rapidly following the exposure of plant cell to fungal and bacterial elicitors, usually leading to a loss of cellular electrolytes such as K^+ and an uptake of H^+, and an influx of Ca^{2+}. The experimental blocking of Ca^{2+} transport across membranes in treated bean cells also inhibits gene activation and subsequent defense responses (Guest, 1997). Plasma membrane depolarization and Cl^- efflux are among the earliest signaling events detectable in elicitor-treated parsley and tobacco cells (Nürnberger, *et al*., 1994; Zimmermann, *et al.,* 1998). Moreover, anion channel antagonists have been shown to interfere with early and late elicitor- or pathogen-induced responses such as Ca^{2+} influx (Ebel, *et al*., 1995).

3.1.2 The oxidative burst

The rapid increase in respiration is known to be due to the generation of reactive oxygen species (ROS), especially hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and the superoxide anion (O_2^-) through the

addition of electrons to O_2 catalysed by the membrane-bound enzyme, NADPH oxidoreductase. Reactive oxygen species are also produced by errors in electron transport during respiratory and photosynthetic reactions in plant cells. Cells are normally protected from the damaging effects of reactive oxygen by superoxide dismutase, various peroxidases and catalase and by natural antioxidants such as carotene. The rapid oxidative burst generates levels of reactive oxygen species that initiate membrane lipid peroxidation and cell death. The signals trigger gene expression and the oxidative cross-linking of host cell wall component. Levels of reactive oxygen species that accumulate at the infection court are sufficient to kill microorganisms in vitro. The O_2^- -generating mixture of xanthine (X) and xanthine oxidase (XO) induced a hypersensitive response-like programmed cell death (HR-like PCD) in wild-type *Arabidopsis* plants in the presence of salicylic acid (SA) or of protein synthesis inhibitors (Mazel, 2000). In tobacco suspension cells, oxygen consumption rises sharply and superoxide is released in a burst that begins 6-8 hours after inoculation with zoospores of an incompatible race of the black shank pathogen caused by *Phytophthora nicotianae* (Guest, *et al*., 1989, Able, *et al*., 1998). However, the differential responses of tobacco cells depend on the ability of elicitors from *Phytophthora* spp. These elicitors could induce the common accumulation of some defence molecules such as proteinase inhibitors but they are different in their abilities to trigger the production of reactive oxygen species (Bottin, 1994).

3.1.3 Cell wall reinforcement

The first visible response to attempt penetration of plant cell walls by pathogens is often the intensification of cytoplasmic streaming followed by the

accumulation of host cytoplasm under the site of attempted penetration. Most pathogens must penetrate host cell walls at some stage, either as germ tubes, hyphae or haustoria. If the cell can respond quickly enough to repair or reinforce the cell wall, penetration efficiency may be reduced and pathogen development is retarded. A number of different types of cell wall fortifications are produced between the host cell wall and plasma membrane, directly under the penetration peg as the defense responses such as the formation of a papilla, accumulation of a branched β-1,3-glucan, callose along with silicon, lignin and hydroxyproline-rich glycoproteins. Hydrogen peroxide, released during the oxidative burst following pathogen challenge, causes extensive cross-linking between hydroxyproline-rich glycoproteins and other cell wall components making the walls even more resistant to microbial digestion. Cross-linked hydroxyproline-rich glycoproteins also provide a focus of lignin deposition on the plant cell wall. The rapid deposition of lignin and suberin following infection is associated with resistance to non-pathogens and avirulent pathogens in many plants. Lignin also binds to hyphal tips and bacterial cells, preventing further growth and movement and restricting the diffusion of pathogen enzymes and toxins and the uptake of water and nutrients by the pathogens.

Lignification of the mesophyll cell wall has been known to be caused by wounding or infection by pathogens. Because lignin is an extremely stable substance and plays a role as the barrier to fungal penetration, lignification is considered one of the defense reactions. Several examinations conducted on lignification were previously done by cytochemical staining with phloroglucinol-HCl, however Asada and Matsumoto (1972) were able to extract, isolate, and

chemically examine both lignins present in healthy and diseased Japanese radish roots inoculated with *Peronospora parasitica.* The healthy lignin is similar to the lignin of a broad-leaved tree, which contains syringyl-skeleton derived from sinapyl alcohol precursor. Diseased lignin contains less – OCH3 radicals and is similar to needle-leaved tree lignin derived from coumaryl alcohol and coniferyl alcohol as precursors. These lignins are produced by the oxidation and polymerization of precursors, which are induced by peroxidases (Fig. 4). The lignification of mesophyll cell walls occurs both in susceptible and resistant Japanese radish roots when they are infected. However, the lignification occurs very rapidly in resistant radish roots after the infection and serves as a barrier for further growth of the fungus. On the contrary, in the susceptible radish, the lignification occurs behind the extended fungal hyphae and thus does not serve as a barrier (Oku, 1994). Lignification of *H. brasiliensis* inoculated with spores of *Microcyclus ulei* are linked to the degree of resistance (Garcia, *et al*., 1995 a). Lignification appears to be more intense in marked partially resistant clones than that in weak partially resistant clones and sporulation of fungus is surrounded by lignin barriers. Thomas, *et al*. (1995) reported that lignin and suberin were deposited in all *Hevea* cell types surrounding the tapping wounds. Lignification of host tissues after microbial infection has been reported to be the cause of disease resistance in other host-parasite combinations. Thus, the rapid lignin deposition may provide a physical and/or chemical barrier to the invading pathogens.

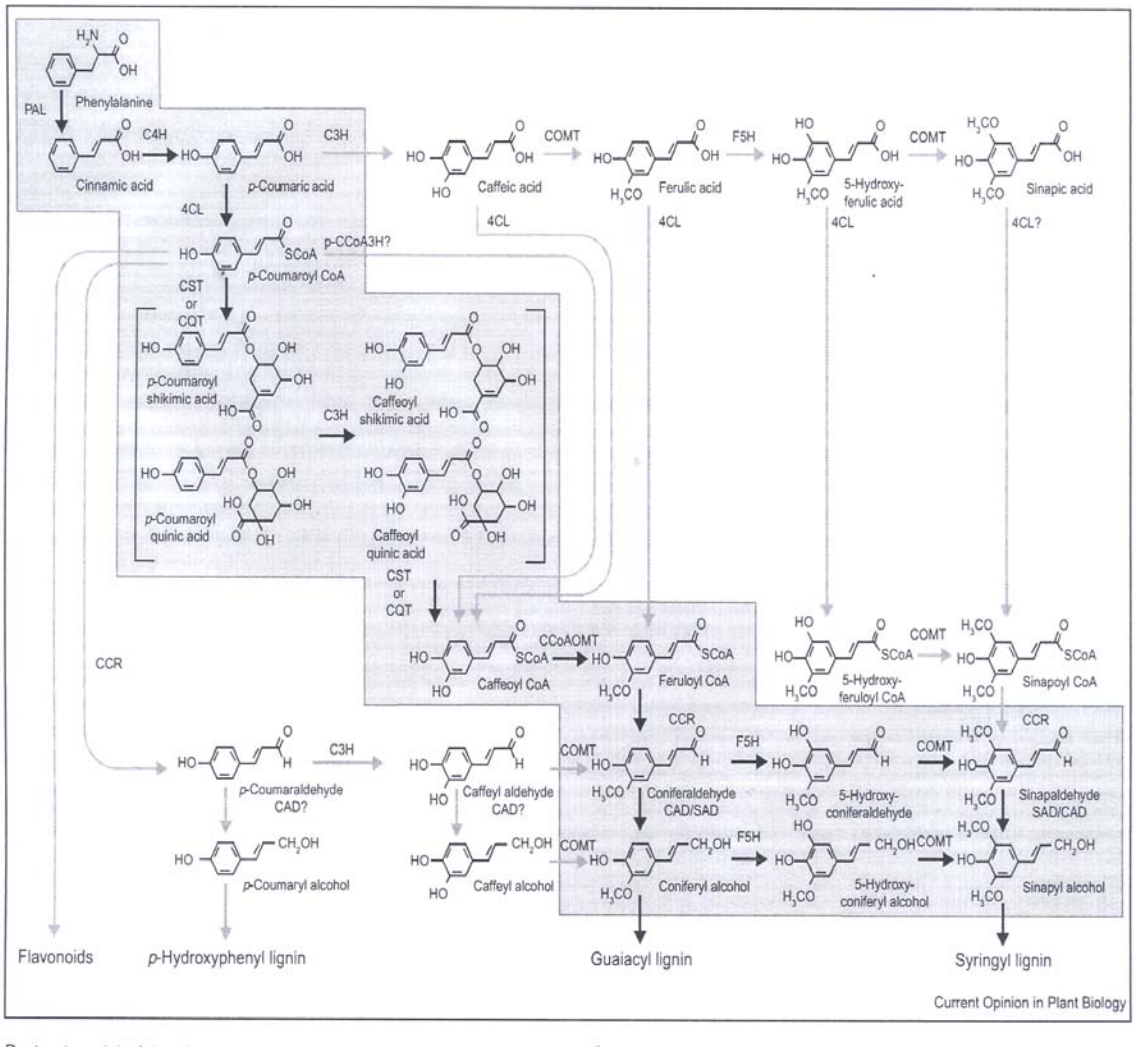

Fig. 4 Revise model of the phenylpropanoid pathway leading to lignin biosynthesis. Reactions thought to be key in lignin biosynthesis are indicated with black arrow. Intermidiate compounds and enzymes currently considered to form the prominent path to lignin are highlighted in grey. 4CL, 4-(hydroxy)cinnamoyl CoA ligase;C3H, p-coumarate 3-hydroxylase; C4H, cinnamate 4- hydroxylase; CAD, cinnamyl alcohol dehydrogenase; CCoAOMT, caffeoyl CoA *O*-methyltransferase; CCR, cinnamoyl CoA reductase; COMT, caffeic acid/5-hydroxyferulic acid *O*-methyl-transferase; CQT, hydroxycinnamoyl CoA:auinate hydroxycinnamoyltransferase; SAD, sinapyl alcohol dehydrogenase (from Humphreys and Chapple, 2002).

3.1.4 Hypersensitive cell death and necrosis

Necrosis is observed in plant at the infection site. It causes cells to turn brown and die. The size and progression of necrosis not only depend on a number and aggressiveness of pathogen but also the environment such as moisture and temperature, and the site of infection. Ward (1992) observed an association between necrotic mesophyll cells in *Bromus* spp. and attempted infection of resistant cultivars by the leaf rust fungus, *Puccinia recondita*. Stakman (1915) reported similar observations in resistant wheat cultivars infected with the stem rust pathogen, *P. graminis*, and introduced this type of necrosis as the term hypersensitivity. He contended that the more resistant the cultivar, the more rapid the collapse of host cells and the sooner the fungus was inactivated. Host cells died in the presence of the pathogen, preventing further spread or killing the invading pathogens. When the hypersensitive cell death (HR) occurs, the plant does not submit to infection and the damage is limited to the cells in the HR lesion. On the other hand, the necrosis expands to the neighboring cells in the susceptible plant. Typically, hypersensitive cell death is preceded by a rapid oxidative burst, and increases in cytoplasmic streaming, cytoplasmic aggregation followed by granulation, membrane disruption, cellular decompartmentalisation and browning, usually within 12-24 hours of attempted penetration (Fig 5). Hypersensitive cell death in plant cells shares many features in common with apoptosis, or programmed cell death (PCD) observed during development of defense against disease in animals. In animal cells undergoing genetically-determined cell death programs, a group of cysteine protease called caspases helps to dismantle the cells (Dangl, 1996). So far, a group of proteases equivalent to caspases has not yet been identified in plants and how the cells die

during the HR is not known with certainty. Solomon *et al*. (1999) provided evidence that cysteine proteases might be involved in regulating PCD in plants but unlike caspases, these proteases did not cleave after an aspartic acid residue and thus had different substrate specificity to the caspases involved in PCD in animals. Recent experiments have shown that in many host-parasite interactions, hypersensitive cell death precedes pathogen death regardless of whether biotrophic or necrotrophic pathogens are involved. In some interactions however, disease resistance does not depend on hypersensitive cell death. The success of hypersensitive cell death as a resistance mechanism in individual host-parasite interactions depends on the nutritional requirements of the pathogen and on the timing, location and magnitude of the host response in relation to pathogen development. In some interactions, the rapid suicidal effect of challenged host cells undoubtedly restricts pathogen development and contributes to the overall defense response.

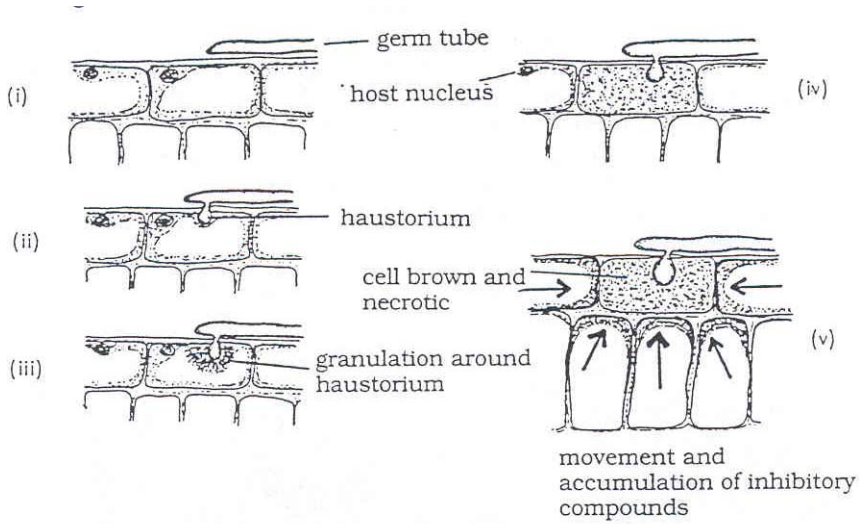

Fig. 5 Sequence of events leading to the hypersensitive reaction in plants infected by incompatible pathogens (From Guest, D. and Brown, J., 1997).

3.1.5 Phytoalexin production

Phytoalexins are low molecular weight antibiotics produced by plants under the stress from infection or elicitors stimulation. Phytoalexin molecule is composed of carbon, hydrogen and oxygen. Its toxicity is non-selective and the chemical affinity of most phytoalexins for lipids suggests that they accumulate in cell membranes. To play a role in disease resistance, phytoalexin must accumulate up to inhibitory levels at the infection court and restricts further development of the pathogen. The rate of phytoalexin synthesis is genetically determined and differs between the resistant and the susceptible cultivars. This responsive mechanism occurs only in the infected and surrounding areas (Darvill and Albersheim, 1984). Ernst Gäumann (1945) working in Switzerland identified these inhibitors as two phenolic compounds, orchinol and hircinol. At about the same time, Müller and Borger in Germany found that slices of potato tuber reacted hypersensitively to *Phytophthora infestans* and produced antibiotics that protected the tissue against subsequent infection by normally virulent strains of the pathogen. He also studied responses of the seed cavity of french bean pods to spores of the peach pathogen, *Monilinia fructicola*. While water droplets from uninoculated cavities stimulated fungal growth, inoculated cavities became necrotic and diffusates became inhibitory to fungal growth within 24 hours of inoculation. The unidentified inhibitor was extracted and termed a phytoalexin (from the Greek words meaning plant defender). This inhibitor was subsequently purified and found to be a phenylpropanoid and named phaseolin (Müller, 1940.

Since then, over 350 phytoalexins have been found in over 100 plant species from 30 families of dicotyledons and monocotyledons (Table 2), for

examples, phaseolin from bean pod; ipomeamarone, chlorogenic acid, umbelliferone and scopoletin from sweet potato; orchinol, hircinol and loroglosol from orchids; pisatin from green pea; glyceollins from soybean and 6-methoxymellein from carrot (KuĆ, 1995). It is possible that different part of plant can synthesize different phytoalexin. The closely-related plants in general synthesize similar structured phytoalexin, for instances, leguminous species produce phenylpropanoid whereas solanaceous species produce isoprenoid.

Table 2 Examples of phytoalexins produced by higher plants (From Guest, D. and Brown, J., 1997)

Structure	Name	Plants involved
Inorganic	sulphur	cocoa
Phenolic	chlorogenic acid	potato, tobacco, apple
	avenalumins	some cereals
Terpenoid	capsidiol	capsicum, tobacco
	rishitin	potato, tobacco, tomato
	ipomeamarone	sweet potato
	gossypol	cotton
Phenylpropanoid	pisatin	pea
	phaseollin	french bean, cowpea
	kievitone	french bean, cowpea
	glyceollins	soybean
	medicarpin	alfalfa, clover, broad bean, chickpea
	scoparone	citrus
Acetylenic	wyerone	broad bean
	safynol	safflower
Stilbene	resveratrol	grape, peanut
	batatasins	yam
Indole-sulphur	camalexin	*Arabidopsis*
	brassinins	cabbage, rape, turnip

Biosynthetic pathways of phytoalexin of all plants require shikimate, acetate-malonate and acetate-mevalonate pathways, which are the secondary metabolism pathways. For instances, chlorogenic acid is synthesized by shikimate pathways; 6-methoxymellein by acetate-malonate pathways and rishitin and ipomeamarone by acetate-mevalonate pathways. Some phytoalexins require more than one pathways, for instances, glycinol and pisatin are synthesized by acetate-malonate and shikimate pathways; xanthotoxin by shikimate and acetate-mevalonate pathways; kievitone, phaseollin and glyceollin by combination of shikimate, acetate-malonate and acetate-mevalonate pathways as shown in Fig. 6 (Darvill and Albersheim, 1984; Kuć, 1995). Some plants, such as soybean and chickpea, synthesize phytoalexins upon infection, but convert a proportion into inactive sugar conjugates and reserve in vacuoles. If the initial defense response fails to recognize pathogen growth, enzymes that cleave sugar molecule are activated and the phytoalexin reserves are rapidly released (Guest, 1997). Like other active defense responses, the success of phytoalexin accumulation depends on the speed, location and magnitude of the response. Phytoalexins accumulate faster and at higher concentrations in resistant cultivars. In resistant plants, gene transcription begins within one hour of recognition, phytoalexins appear within four hours and their concentration peak to fungitoxic levels about 18-24 hours after challenge. These events are delayed and more diffuse in susceptible plants (Bailey and Deverall, 1971).

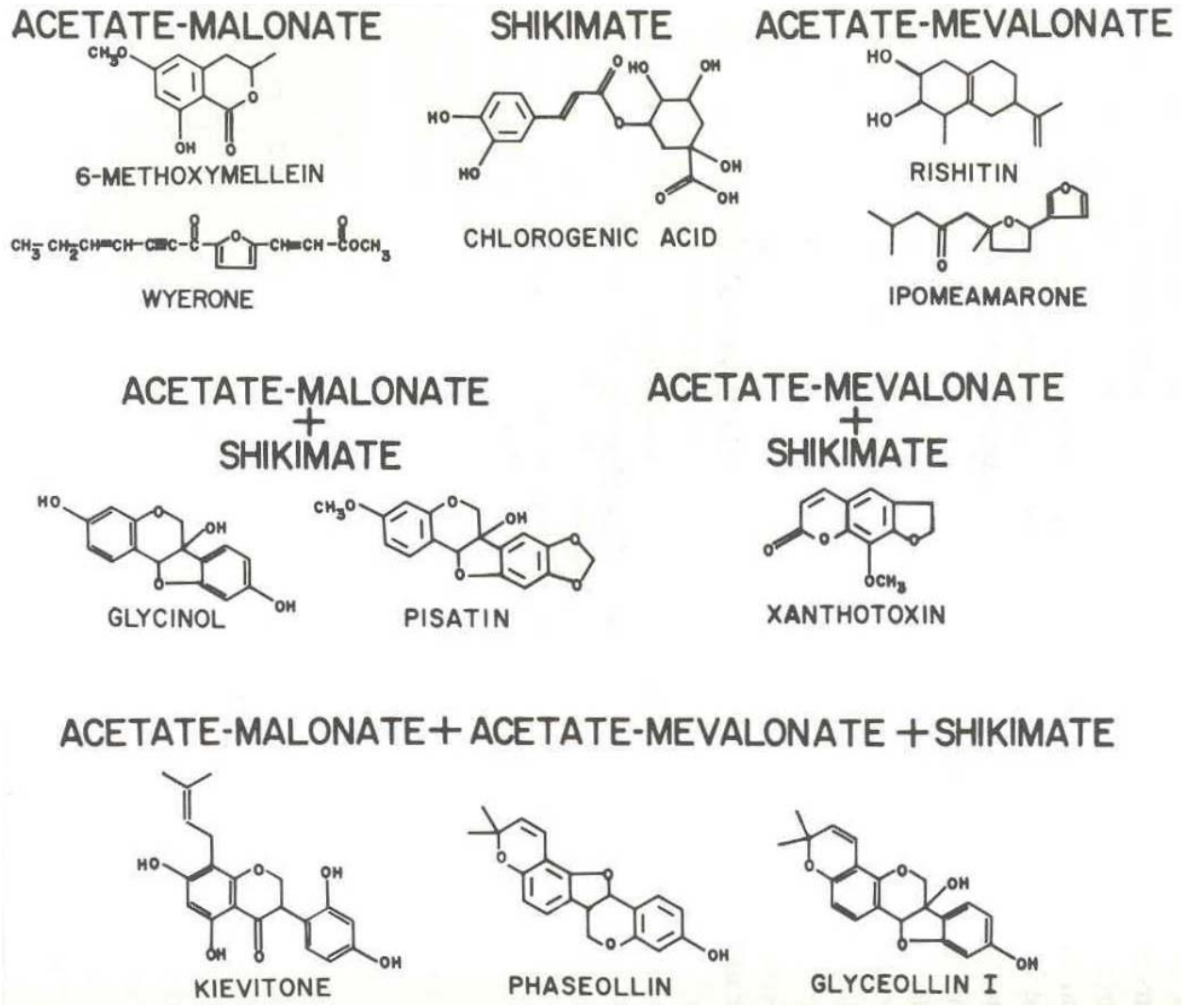

Fig. 6 Pathways for the biosynthesis of some phytoalexins. (Kuć,1995)

For rubber tree, Tan and Low (1975) demonstrated that, after the rubber leaf was incubated with the fungus, *Colletotrichum gloeosporioides*, it responded by producing phytoalexin at the infected site, the presence of which could be detected under ultraviolet light by blue-color illumination. Giesemann, *et*

al. (1986) analysed phytoalexin produced by rubber leaf against *Microcyclus ulei* and found to be scopoletin (Scp) which is hydroxycoumarin. The study of this responsive mechanism of different rubber clones against *C. gloeosporioides* and *M. ulei* demonstrated that Scp concentration was positively correlated with the resistance capability of rubber clones. Therefore, Scp concentration can be used to predict the degree of resistance; the higher Scp concentration, the greater the resistance capability (Garcia *et al*., 1995 b; Breton *et al*., 1994).

The rate of Scp production is also related with the resistance of rubber plant. The resistant clones produce Scp within 12-36 hours after infection whereas partially resistant clones produce Scp within 36-120 hours and susceptible clones produce Scp later than 120 hours (Garcia *et al*., 1995 b).

3.2 Delayed active defenses

3.2.1 Pathogenesis-related proteins (PR-proteins)

Plants synthesize many novel proteins following infection, which include phytoalexin biosynthesis and pathogenesis-related proteins, which have β-1,3 glucanase, chitinase or lysozyme activity. Some pathogenesis-related proteins are related to plant defensins while others are proteinase inhibitors that disrupt pathogen nutrition. PR-proteins are sometimes present in low levels before infection and are also induced by stress, wounding or flowering. These findings indicate that PR-proteins not only function in disease resistance but also serve in plant growth and development.

Chitinase and β-1,3-glucanase are PR-proteins found in infected monocotyledons and dicotyledons such as tobacco, barley and potatoes. Chitinase from barley can inhibit the growth of *Trichoderma reesei, Alternaria alternaria, Phycomyces blakesleesasus* and *Neurospora crassa* (Robert and

Selitrennikoff, 1986). Potato leaves infected with *P. infestans* accumulate two β-1,3 glucanases and six chitinases (Guest, 1997).

Martin, *et al.* (1991) prepared purified chitinase from rubber latex and found it to consist of 20% of all proteins compared with 1-2% in other plants. Churngchow, *et al.* (1995) also found β-1,3 glucanase amount to 15% of all proteins. The high amount of these enzymes may be the consequence of wounding, due to tapping, and as a result of the defense mechanisms of rubber plant against fungal infection through the tapping site.

Chitinase and β-1,3-glucanase accumulate in vacuoles, although some glucanases are secreted to the intercellular space (Fig. 7). These enzymes digest fungal cell walls and as a consequence, elicit hypersensitive cell death and phytoalexin biosynthesis. Cellular decompartmentalisation during hypersensitive cell death leads to an ambush of the pathogen by a flood of hydrolytic enzymes released from the vacuoles. Hydrolytic enzymes have antiviral, antibacterial and antifungal activities. PR-proteins accumulate over several days, and reach a maximum about seven to ten days after initial infection (Table 2). In contrast, gene-for-gene resistance is determined within hours of the initial attack. These results show that hydrolytic enzymes reduce disease susceptibility if they are present at the time of challenge, as observed in plants with systemic acquired resistance, a response that protects plants against re-infection. Chitinase and glucanase were purified from Carnauba wax and found that the N-terminal amino acid sequences showed a high degree of homology with the N-terminal region of an endochitinase, hevamine A, and with an internal sequences of glucanase precursor from *H. brasiliensis* (Cruz, *et al.,* 2002).

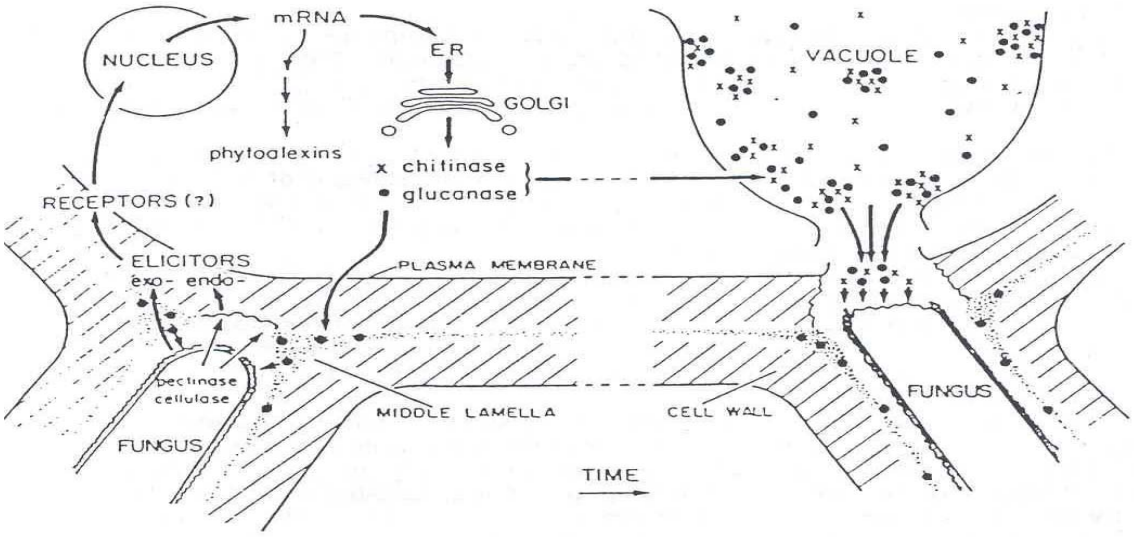

Fig. 7 Model outline the roles of chitinase and glucanase in a bean plant's defense against pathogen attacks. (from Mauch and Staehelin, 1989)

Several studies reported the infection of plants by plant pathogens, especially incompatible pathogens, by inducing many kinds of isozymes which are not present in uninfected plants and these isozymes arise from protein synthesis. As phytoalexin biosynthesis, activation of genes encoding enzymes involved in isoflavonoid phytoalexin has been detected by Northern blot hybridization with respective cDNAs as probes (Guest, 1993). The elicitors from *C. lindemuthianum* induced the accumulation of mRNA encoding cinnamyl-alcohol dehydrogenase, an enzyme for the synthesis of lignin monomers, in cultured bean cells (Walter, *et al*., 1998).

The mRNA levels of PR-proteins of the hypersensitive reaction in tobacco which are 1a, 1b and 1c have been shown to be strongly increased in tobacco which infected by tobacco mosaic virus (TMV) (Carr, *et al*., 1985). Two

glucanohydrolases, β-1,3-glucanase and chitinase, were induced as indicated by the increase in transcription (using Northern blot analysis) in *Nicotiana tabacum* infected by TMV. Furthermore, increasing of those enzymes was only observed in the tobacco which exhibited the hypersensitive reaction (Vögeli-Lange, *et al*., 1988). Induction of chitinase and β-1,3-glucanase in tobacco infected by *Pseudomonas tabaci* and *Phytophthora parasitica* were shown by the increasing of both proteins and mRNAs in tobacco leaves. Analysis of tobacco β-1,3-glucanase (pGL43) and chitinase (pCHN50) mRNA were performed using the probes which were the *PstI* inserts of cDNA clones (Meins, 1989). In addition, β-1,3-glucanase activity in leaves of the resistant melon cultivar increased more rapidly than in the susceptible cultivar in response to infection by the cucurbit powdery mildew fungus, *Sphaerotheca fusca*. Northern blot analysis using an homologous β-1,3-glucanase cDNA probe (Cmβgluc) isolated by RT-PCR from powdery mildew-infected leaves of the resistant cultivar showed an earlier induction of *βglu* transcripts in this cultivar than in the susceptible one (Rivera, 2002).

3.2.2 Systemic acquired resistance

In the plant-pathogen interaction, the infection of one part of a plant by viral, bacterial, and fungal pathogens induces resistance in the other parts of the same plant against a variety of pathogens and/or to subsequent infections, as well. This type of phenomenon is called systemic acquired resistance (SAR). This phenomenon has been used practically as a very effective control measure for some diseases although its mechanism remains unresolved. It should be noted that SAR fundamentally differs from the specific antigen-

antibody mediated immune response of mammals. The expression of SAR reduces disease severity rather than providing immunity. The development of SAR involves the slow expension expanding of necrotic lesion, the signals translocated in the phloem prime the rest of the plant against further pathogen challenge. SAR causes more rapid and more intense expression of defense responses in induced plant than in uninduced plants. There are several molecules that can induce features characteristic of SAR such as salicylic acid (SA), jasmonic acid (JA), and other elicitors including elicitins (Guest, 1997). Experimental, the inoculation of *Phythophthora nicotianae* after the necrosis induced by the elicitation on the stem of tobacco with elicitors, external symptoms remained very limited, whereas in the water-treatment controls, fungal invasion progressed downwards at a constant rate (Ricci, *et al.,* 1989). Application of either chitosan or oligandrin to the apex of decapitated tomato plants substantially reduced symptom severity of *Fusarium* wilt as compared with control. Five days after inoculation, the treated plants were free of visible symptoms such as wilting and exhibited a markedly reduced number of root lesions (Benhamou, *et al.,* 2001). Pathogen-associated cell death of tobacco, infected with *Thielaviopsis basicola* may be a necessary requirement for biological SAR activation (Hecht and Bateman, 1964). However, the induction of SAR by the exogenous elicitors such as SA or 2,6-dichloroisonicotinic acid (INA) does not involve cell death, this result suggests that these compounds stimulate the SAR pathway downstream from cell death (Hunt, *et a*l., 1996). Neuenschwander, *et al*., (1995) reported that no significant sustained increase in H_2O_2 was evident in the uninfected tobacco leaves of inoculated plant with tobacco mosaic virus (TMV) even though PR-1

mRNA accumulation was substantial by day 4 and SAR was established by day 9 as determined by a reduction in lesion size after tobacco TMV infection. According to these evidences, H_2O_2 plays role as a second messenger of SA in the SAR signal transduction. After the application of probenazole (PBZ) and 1,2,-benzisothiadiazole-1,1-dioxide (BIT) on the lower leaves of tobacco, the size of lesions on the upper leaves which were infected with TMV were smaller than those in the water-treated control plants after 7 days of PBZ and BIT applications (Nakashita, *et al*., 2002). The expression of *Triticum aestivum PR4* genes is also inducible upon treatments with SAR chemical inducers such as salicylic acid and methyl jasmonate (MeJA) indicating that activation of PR4 genes follows both SA- and JA-dependent defense response pathways. The expression of *PR4* gene in wheat is observed in response to *F. culmorum* infection as well as to chemical inducers of SAR. The induction of *PR4* transcripts in wheat coleoptile and roots is correlated with the expression of the corresponding proteins that are expressed only in the infected tissues. Wheat *PR1* and *PR5* genes used for comparison did not respond to either SAR activatiors or pathogen attack (Bertini, *et al*., 2003).

3.3 Gene-for-gene hypothesis

The plant-pathogen interaction causes plant resistance (R) proteins, which recognize pathogen-encoded effectors either directly or indirectly. In this role, pathogen-encoded effectors are called avirulence (Avr) proteins. *Avr* genes are structurally diverse and are theoretically maintained in their respective genomes by virtue of virulence roles advantageous to the pathogen. Evidence for virulence function has been demonstrated in several, but not for all, Avr proteins. Recognition is typically 'race specific', meaning that a given R protein recognizes the Avr proteins from one or very few pathogen isolates. This *R-avr* gene

interaction initiates host cell resistance which is referred to as gene-for-gene resistance (Holt, *et al*., 2003). It is shown in Fig. 8 that resistance is only expressed when a plant contains a specific *R* gene which recognizes a pathogen that has the corresponding avirulence gene (upper left panel). All other combinations lead to lack of recognition by the host, and the result is disease (Staskawicz, *et al.,* 1995). During the infection of tomato by the fungus *Cladosporium fulvum*, Avr proteins are secreted into the extracellular space. These Avr proteins are recognized by members of the Cf-9 which are R proteins and cause the cultivars resistant to pathogen (Piedras, *et al*., 2000, Van der Hoorn RAL, *et al.,* 2001). The pace of *R* gene discovery in crop plants such as barley, rice, maize and tomato has accelerated over the past five years owing to impressive developments in high-throughput molecular mapping, sequencing and gene isolation technologies. Striking similarities are found in the structures of R proteins from monocotyledonous and dicotyledonous species, implying that fundamental modes of recognition and defense signalling have been retained through plant evolution and diversification. The most prevalent class of functionally defined *R* genes encode intracellular nucleotide-binding/leucine-rich repeat (NB-LRR) proteins with variable N-terminal domains (Hammond-Kosack and Parker, 2003). The early signal transduction events in *Arabidopsis* leaf protoplast in response to the flagellin-derived bacterial elicitor was dependent on the LRR receptor kinase.

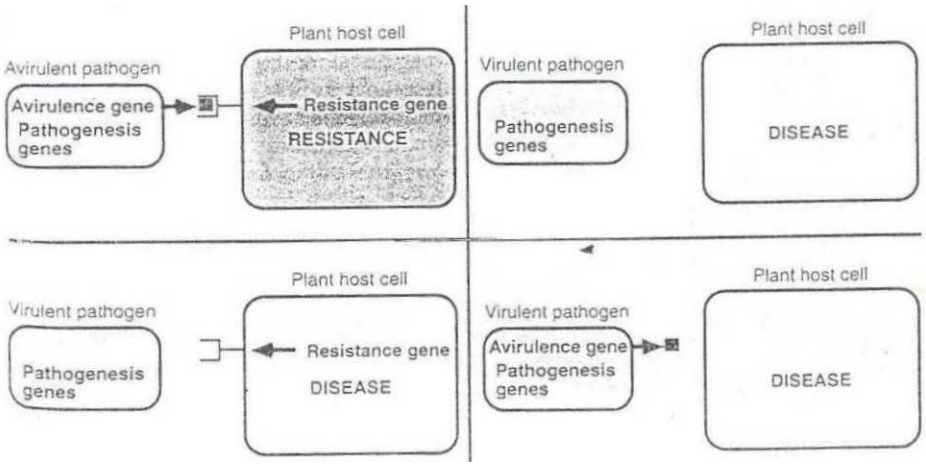

Fig. 8 Gene-for-Gene interaction specify plant disease resistance. (Staskawicz, *et al.,* 1995).

4 *Phytophthora* elicitin

All *Phytophthora* species except *P. parasitica* var. *nicotianae*, the virulent strain isolated from tobacco, produce and secrete protein called elicitins (Huet and Pernollet, 1993) when cultured in appropriate culture media. Elicitin is a non-glycosylated protein with molecular weight of 10 kDa. Its molecular structure consists of 98 amino acid residues with three disulfide bonds, 50% of which are in α-helix form and few or none in β-pleated sheet (Nespoulous, *et al*., 1992; Huet and Pernollet, 1993). Elicitins isolated from culture medium of each *Phytophthora* species have the same numbers of amino acids but differ only in amino acid sequences. The early study of amino acid compositions and sequences of cryptogein, capsicein and cinnamomin, the elicitins respectively isolated from *P. cryptogea, P. capsici* and *P. cinnamomi* (Huet and Pernollet, 1993b; Ricci, *et al*., 1989) demonstrated the 80% conservation of amino acid sequences particularly

the central core region. Subsequent studies of other *Phytophthora* elicitins, for examples, parasiticein from *P. parasitica* (Nespoulous, *et al*., 1992; Ricci, *et al*., 1992), Dreβ and Dreα which are β- and α-elicitins from *P. drechsleri (*Huet, *et al*., 1992). MgMβ and MgMα which are β- and α-elicitins from *P. megasperma* (Huet, *et al*., 1993), and cactorein from *P. cactorum* (Huet, *et al*., 1993; Dubery, *et al*., 1994) also demonstrated the identical of amino acid residues (98 aa) but different in amino acid compositions and amino acid sequences among these elicitins.

Elicitins are 10 kDa hydrophilic holoproteins displaying an α–helix fold stabilized by three disulfide bonds (Fig 9). Elicitins have sterol carrier activity which is probably the main function of these proteins since *Phytophthora* do not synthesize the sterols required for their reproductions (Hendrix, 1970). Elicitins have not exhibited any protease, β-glucanase or phospholipase activity and no other enzymatic activity has been reported so far (Tavernier, *et al*., 1995). The elicitin fold provides a hydrophobic cavity with a higher specificity for sterol to form a sterol-elicitin complexes and binds to the plant receptor at the outer membrane which causes the activation of plant defense mechanisms either a compatible (disease) or an incompatible interactions (Osman, *et al*., 2001). The elicitin and Lipid-Transfer Proteins (LTPs) are the small cysteine-rich lipid-binding proteins secreted by oomycetes and plant cells, respectively. Most recent works demonstrated that elicitins and LTPs share the same biological receptors and gives a new perspective to understand the role of LTPs in plant defense responses, mainly the early recognition of intruders. Unlike elicitins, the LTPs are small hydrophillic proteins probably involved in the formation and reinforcement of plant surface layers and defense against pathogens. Some of LTPs such as

nsLTP1 and nsLTP2 (non-specific Lipid-Transfer Proteins 1 and 2) are an elicitin antagonist. With unclear mechanism, they inhibit the cascade of signaling pathways of plant defense response. However, the low sensitivity to elicitins in some plants might be the result of the competition between endogenous nsLTPs and exogenous elicitin on the corresponding receptor, which has been observed on all plant cell membranes tested to date. This hypothesis is partly supported by a low level of soluble LTP content in tobacco, whereas plants that do not react to elicitins (e.g. tomato) contain ten times more of these proteins (Blein, *et al*., 2002).

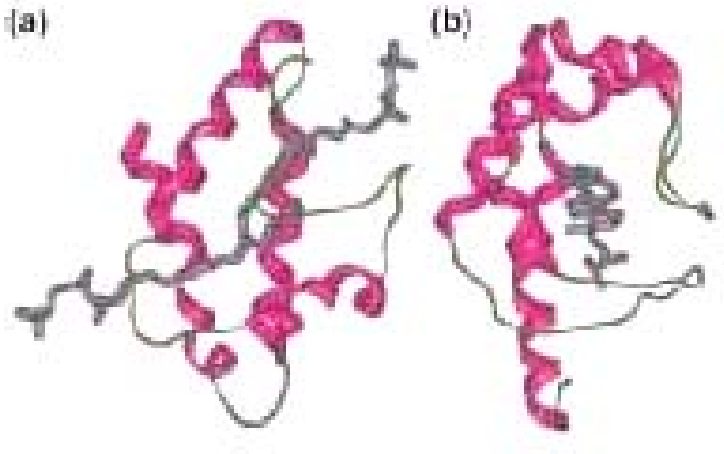

Fig. 9 Structures of Lipid-Transfer Proteins and elicitins. Three-dimensional structures of (a) wheat Lipid-Transfer Protein complexed with two molecules of lyso-myristoyl phosphatidylcholine in a head-to-tail orientation (Protein Data Bank 1BWO) and (b) cryptogein complexed by ergosterol (protein Data Bank BXM). Lipidic ligands are shown in grey (Blein, 2002).

Elicitin is a host-specific elicitor, although it can stimulate hypersensitive responses in some specific species of Solanaceae such as *Nicotiana* species or

certain species of *Cruciferae* such as turnip and radish, but it cannot stimulate such responses in Compositae, Leguminosae and Cucurbitaceae *(*Kamoun, *et al.,* 1993).

4.1 Classification of elicitins

Elicitins can be classified into two classes, α and β, depended on amino acid sequences and compositions, isoelectric point (pI), hydropathy index, as well as secondary and tertiary structures

4.1.1 α-elicitins

α-elicitin is acidic, having pI $\simeq$ 4.5 (Berre, *et al*., 1994). Elicitins in this class are capsicein, parasiticein, Dreα (α-elicitin from *P. drechsleri*), MgMα (α-elicitin from *P. megasperma*) and cactorein. The amino acid residue at position 13 is valine, the side chain of which is associated with its biological activity more than other sites. The study of three dimension structure of α-elicitin by using nuclear magnetic resonance (Bouaziz, *et al*., 1994) demonstrate that the amino acid at position 13 is located at the outer surface of the molecule. The amino acid at this site is important not only as a functional site (Huet, *et al*., 1992) but also affects the interaction between elicitin and its target receptor (Donohue, *et al*., 1995). The far UV CD spectra of the *P. drechsleri* elicitins indicate that Dreα is rich in α-helices (48±3%),devoid of β-sheets, and Dreβ exhibits less α–helices (36±2%), but contains some β–structure (14±9%) (Huet, *et al*., 1992).

4.1.2 β-elicitins

β-elicitin is basic protein ; its pI is $\simeq$ 8.5 (Berre, *et al*.,1994). Amino acid residue at position 13 is lysine (Donohue, *et al*., 1995). Elicitins in this class are cryptogein, Dreβ (β-elicitin from *P. drechsleri*), MgMβ (β-elicitin from *P. megasperma)* and cinnamomin.

On the basis of petiole dip assay with Dreα and β, β–elicitins are more necrogenic than α-elicitin (Huet, *et al*., 1992). This result was supported by Kamoun that the petiole dip assay on *N. tabacum* and radish cultivars with cryptogein showed more severe necrosis than parasiticein did at the same concentration. On the other hand, infiltration of leaves with the two elicitins induced necrotic lesion of similar intensity. Also, no difference in the intensity of CHS8::GUS induction between α– and β-elicitins were observed in a series of side-by-side infiltration, confirming that the two isoforms induce a similar HR response by the infiltration method. This is in contrast with the petiole dip assay which showed a stronger distal necrosis induction by β-elicitin than by α–elicitin (Table 3). Furthermore, by using the low amount of both elicitin isoforms result that, β–elicitin induced a more effective resistance of tobacco than α–elicitin (Kamoun, *et al*., 1993). Donohue, *et al.,* (1995) suggested that this effect of elicitins was probably the receptor–mediated defense response mechanism stimulated by elicitins. Zanetti, *et al*., (1992) demonstrated that both cryptogein (β) and capsicein (α) were equally able to migrate in the tobacco vascular system without undergoing any detectable molecular alteration. This indicates different biological activities between these two classes, the cause of which is likely due to the difference in amino acid sequences rather than the difference in intracellular movement of β- and α-elicitins. Ricci, *et al*. (1989) also suggested that changes in amino acid sequences appeared to affect biological activities. Dreβ differs from Dreα which accounts for the difference in isoelectric point and the behavior on Sephadex G-50. Like other elicitin isoforms, Dreβ elicites more distal necrosis on detached leaves than Dreα does which correlates with the difference of amino

acid at position 13 (Huet, *et a*l., 1992). Donohue, *et al*., (1995) reported significant decrease in biological activity of cryptogein after changing amino acid at position 13 from lysine to valine. This is because valine at position 13 of α-elicitin is aliphatic and its side chain is nonpolar and hydrophobic whereas lysine at position 13 of β-elicitin has polar side chain and is hydrophilic; therefore β-elicitin is more toxic than α-elicitin. Not only amino acid at position 13 but also amino acids at position 2, 14, 72 and 94 of elicitins can affect biological activities but are less important (Donohue, *et al*., 1995).

With regards to amino acid compositions, about 50% of both β- and α-elicitins polypeptides consisted of leucine, serine, threonine and alanine, whereas none is detected for tryptophan, histidine and arginine (Yu , 1995).

Some *Phytophthora* species can produce only acidic elicitins, for examples, *P. parasitica* produces only parasiticein (Nespoulous, *et al*., 1992; Ricci, *et al*., 1992). *P. cactorum* produces only cactorein (Huet, *et al*., 1993 Dubery, *et al*., 1994). Some can produce both acidic and basic elicitins, for example, *P. drechsleri* can produce Dreα (acidic) and Dreβ (basic) which have 92% similarity in amino acid sequences. Huet, *et al*., (1993) reported that *P. megasperma* could also produce both acidic (MgMα) and basic (MgMβ) elicitins which had 86.7% similarity in amino acid sequences and found that MgMβ was less toxic when compared with other β-

elicitins but more toxic than α–elicitins including MgMα. Among this group of α–elicitins, there are some variations; Para secreted by *P. parasitica* and Cap

Table 3 Comparative properties of elicitin isoforms. Isoforms are listed in increasing order of their toxicities to tobacco. (Pernollet, *et al*., 1993)

Phytophthora	Elicitin	Relative distal necrotic index	13th residue
P. cactorum	Cacto	0.75	Val
P. capsici	Cap	1.00	Val
P. cryptogea	Cryα	1.03	Val
P. citrophthora	Citro	1.08	Val
P. megasperma	MgMα	1.13	Val
P. drechsleri	Dreα	1.19	Val
P. cinnamomi	Cinα	1.63	Val
P. megasperma	MgMβ	1.66	Lys
P. drechsleri	Dreβ	2.00	Thr
P. cryptogea	Cryβ	2.00	Lys
P. cinnamomi	Cinβ	2.03	Lys

secreated by *P. capsici,* are less toxic than Dreα secreted by *P. drechsleri* and MgMα secreted by *P. megasperma*. The study of primary structure of MgMβ demonstrates the difference of amino acids at position 5, 57 and 61 of MgMβ from MgMα indicating that biological activities of elicitins depend on amino acid not only at position 13 but also at positions 5 and 57-65 (Huet, *et al*., 1993). *P. cryptogea* produces two acidic and one basic elicitins, 95% of which is basic elicitin called cryptogein β (pI = 8.5) and the other 2 and 3% are acidic elicitins respectively called cryptogein A_1 and cryptogein A_2 (pI = 4.5 and 4.6). Similar to

MgMβ and MgMα, cryptogein β is more toxic than cryptogein A_1, and A_2. It should be noted that none of *Phytophthora* species produces only basic elicitin (Berre, *et al*., 1994).

4.2 Elicitin properties in plant-pathogen interaction

Elicitins have elicitor properties; i.e. it can stimulate plant responsive activities such as phytoalexin accumulation, synthesis of pathogenesis-related proteins and apoptosis or cell necrosis.

There are two types of elicitor, namely biotic and abiotic. Substances from pathogens or from the reactions of plant and pathogen are called biotic elicitors whereas light, ultraviolet radiation, heavy metal ions are called abiotic elicitors. Biotic elicitor can be polypeptide, polysaccharides, glycoprotein, chitosan or fatty acid (Darvill and Albersheim, 1984).

Elicitin is classified as biotic elicitor when tested with tobacco cell suspension, it stimulates hypersensitive response by increasing pH and conductivity of the extracellular medium. It also increases the synthesis of ethylene and capsidiol which is phytoalexin. The necrosis and the accumulation of pathogenesis-related proteins, PR1a, are also observed when elicitin is tested with tobacco leaves and trees (Huet, *et al*., 1991). The biological activities of elicitins can be inhibited by pronase enzyme. In addition to stimulating cellular response in tobacco plant, elicitins can induce systemic acquired resistance to *P. parasitica var. nicotianae* which causes black shank disease (Ricci, *et al*., 1989). Furthermore, it can induce acquired resistance to *Xanthomonas,* the causative pathogen of radish (Kamoun, *et al*., 1993).

P. parasitica isolated from tobacco belongs to different genotypes and demonstrates various virulence levels towards tobacco with similar elicitin

patterns *in vitro* and in planta. Although elicitins are encoded by a multigene family, *parA1* is the main elicitin gene expressed. This gene is highly conserved among isolates, regardless of the elicitin production and virulence levels toward tobacco. The elicitin-producing *P. parasitica* isolates that are virulent on tobacco down- regulate *parA1* expression during compatible interaction. Conversely, one elicitin-producing *P. parasitica* isolate that is pathogenic on tomato but avirulent on tobacco still expresses *parA1* in the compatible interaction with tomato. Therefore, some *P. parasitica* isolates may escape tobacco recognition by down regulating *parA1* in the plant. The in-planta down regulation of *parA1* may constitute a suitable mechanism for *P. parasitica* to infect tobacco without deleterious consequences of the pathogen (Colas, *et al*., 2001).

Pp-elicitor from *P. parasitica* induces effluxes of K^+ and Cl^-, phytoalexin production and expression of defense-related genes encoding phenylalanine ammonia lyase, 4-coumarate:coenzyme A ligase and *eli 12,* an elicitor-responsive gene of unknown function in cultured cells of *Petroselinum crispum* (Fellbrich, *et al*., 2000).

Infiltration of tobacco leaves with Cry and Para did not induce significant activation of *tcI 7,* a gene encoding a β–subunit of proteasome, in tobacco leaves and a slightly accumulation of *PR-1b,* a SAR gene, corresponding mRNAs were detected after the treatments with elicitins as well (Etienne, *et al*., 2000). Decapitated treatment of Cry and Para in tobacco resulted in HR, and SAR; the expression of 20S proteasome subunits, β1din, α3din and α6din, were strongly induced by Cry greater than by Para (Suty, *et al.,* 2003). These observation, (Kamoun, *et al.,* 1993, Etienne, *et al.,* 2000 and Suty, *et al., 2003)*, suggest that

the difference in biological activities noted between the two elicitin isoforms may reside in a greater ability of β-elicitins to induce distal HR rather than in a difference in the interaction between elicitins and target leaf cells.

Objectives

1. To identify the main defense mechanisms involved in resistance of rubber tree (*H. brasiliensis*) to *Phytophthora palmivora*.
2. To study the distinction of necrosis induced by zoospores or elicitins in the resistant and the susceptible *H. brasiliensis* clones.
3. To study the extent of each defense mechanism of *H. brasiliensis* including phytoalexin production, lignification and pathogenesis-related proteins (PR-proteins) induced by zoospores or by elicitin of *P. palmivora*.
4. To examine the toxicity of elicitin to the resistant and the susceptible *H. brasiliensis* clones.
5. To study the transcription of PR-proteins of *H. brasiliensis* in response to stimulation by elicitin.