Chapter 2

Literature Review

2.1 Groundnut or Peanut

Groundnut or peanut (*Arachis hypogaea* L.) is an ancient crop, which originated in South America (Bolivia and adjoining countries) and is now grown throughout the tropical, sub-tropical and warm temperate regions of the world. This crop was grown widely by native peoples of the New World at the time of European expansion in the sixteenth century and was subsequently taken to Europe, Africa, Asia, and the Pacific Islands. Today groundnut is widely distributed and has adapted in various countries of the World. The most important countries for production are India, China, USA, West and Southern Africa, and Brazil. The diffusion of crop can be traced along the varietal lines (Figure 2.1).

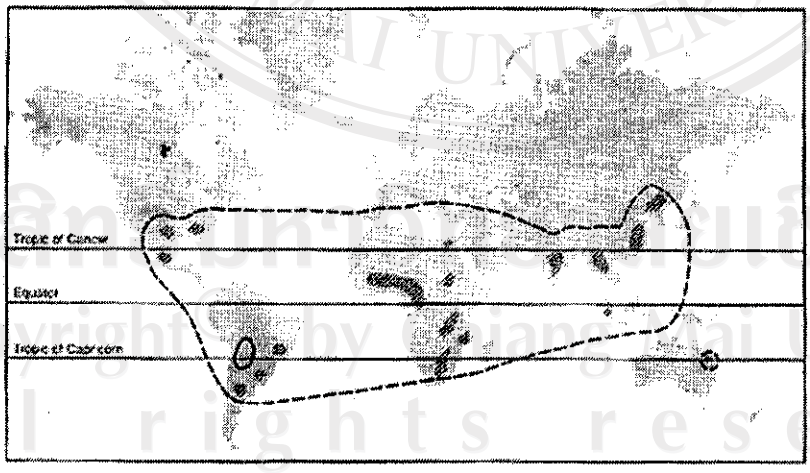


Figure 2.1 Groundnut center of origin (solid line), area of intensive cultivation (dotted line) and areas of maximum cultivation (shaded) (Weiss, 2000)

Groundnut, an important oil and food crop, is currently produced on approximately 33 million metric ton worldwide. It is the third major oilseed of the world next to soybean and cotton (FAO, 2003). India, China, and the United States have been the leading producers for over 25 years and grow about 70 % of the world crop (Weiss, 2000). Figure 2.2 shows the groundnut production trend in the world since 1961. Figure 2.3 and 2.4 shows the percentage of groundnut production and area under cultivation in major groundnut producing countries in 2003 respectively (FAO, 2003).

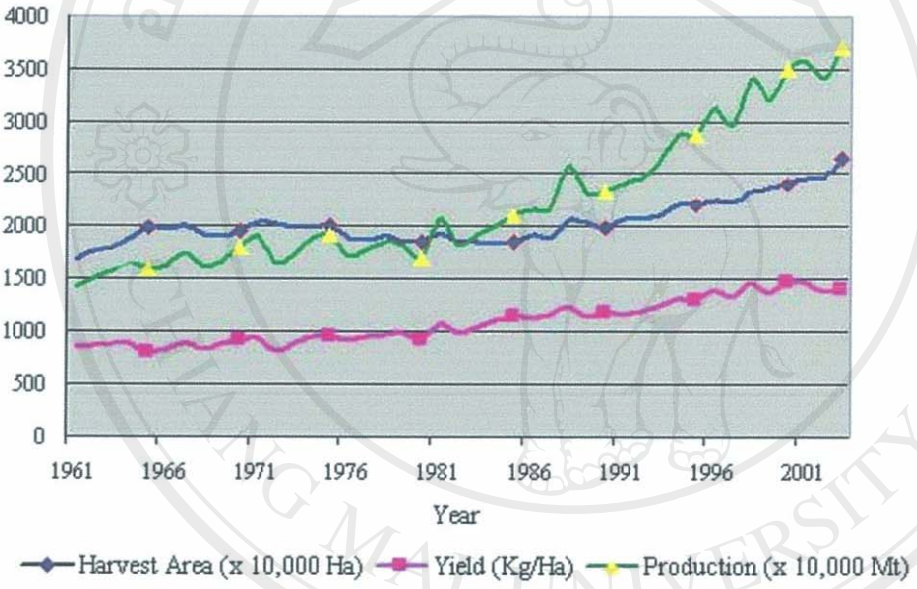


Figure 2.2 Groundnut harvest area, yield and production trend in the world since 1961 to 2001 (FAO, 2003)

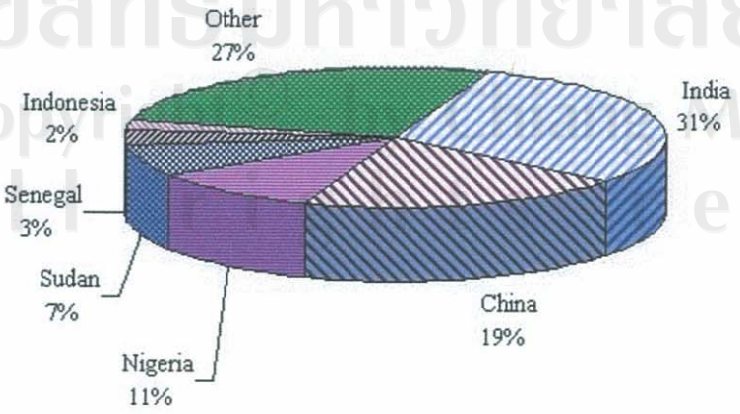


Figure 2.3 World area of groundnut production in year 2003 (FAO, 2003)

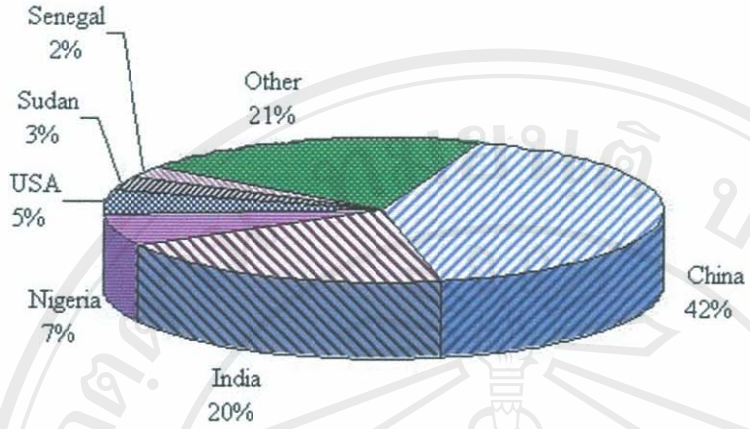


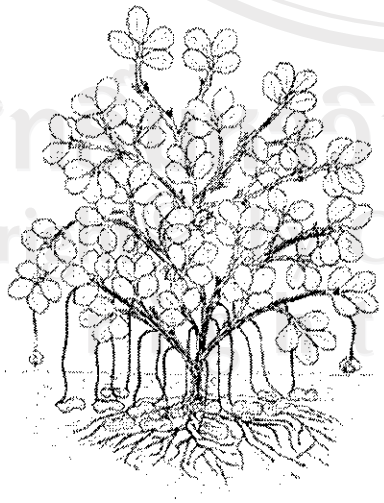
Figure 2.4 World production of groundnut in year 2003(FAO, 2003)

Although, India and China are the large producers groundnut of the world, but production is insufficient to supply domestic demand for oil and meal. A high proportion is used as edible nuts or in processed food of all kinds. Groundnut, indeed all oilseed production in most Africa countries has suffered from the wars that have raged almost continually since 1970, and the continent now has an overall edible oil shortfall and this trend is unlikely to be reversed. World groundnut production for crushing is expected to decline as competition from rapeseed, sunflower and oil palm rises and their oils fall in price. Edible nut production will probably increase as food products containing groundnuts increase in popularity, especially peanut butter and huge range of snacks and confectionery (Weiss, 2000).

2.1.1 Botany

Groundnut (*Arachis hypogaea* L.) belongs to the family Leguminous and subfamily Papilioceae, which is an annual herb of indeterminate growth habit. *Arachis hypogaea* is an allotrtraploid ($2n = 4x = 40$) species, which likely evolved from two diploids in section *Arachis* (Kochert *et al.,* 1996; Hommons, 1973).

Subspecific and varietal classifications are mostly based on location of flower on the plant, patterns of reproductive nodes on branches, numbers of trichomes and pod morphology (Krapovickas and Gregory, 1994). Cultivated groundnut plant is an elect or prostrate, usually 15 to 60 cm tall (Figure 2.5). The leaves are compound with leaflets varying in size and shape, either narrow or slender. Flowers are born on inflorescence located in the axils of the leaves (Figure 2.6A). Each flower is perfect and highly self-pollinated, commonly yellow but can vary from nearly white to deep orange (Figure 2.6B). About 7-10 days after fertilization the receptacle thickens, elongates, turns downward and forces the ovary into the ground (Cattan and Fleury, 1998; Umen, 1976). The long carpophore bearing the fertilized ovule is commonly known as a peg (Figure 2.6C), the action of burying the immature pod as pegging, and groundnut growers describe a crop as pegging well. Mature fruits (pods) cylindrical, 1-8 cm x 0.5-2 cm, containing one to five seeds (Figure 2.6D). Seed may be round or elliptical vary in seedcoat color from off-white to deep purple (Figur 2.6E). Seed size ranges from about 0.15 to more than 1.3 g / seed in *A. hypogaea* (Singh and Simpson, 1994), but seeds as small as 0.047 g / seed are produced by *Arachis* wild species.



Fugure 2.5 Groundnut plant

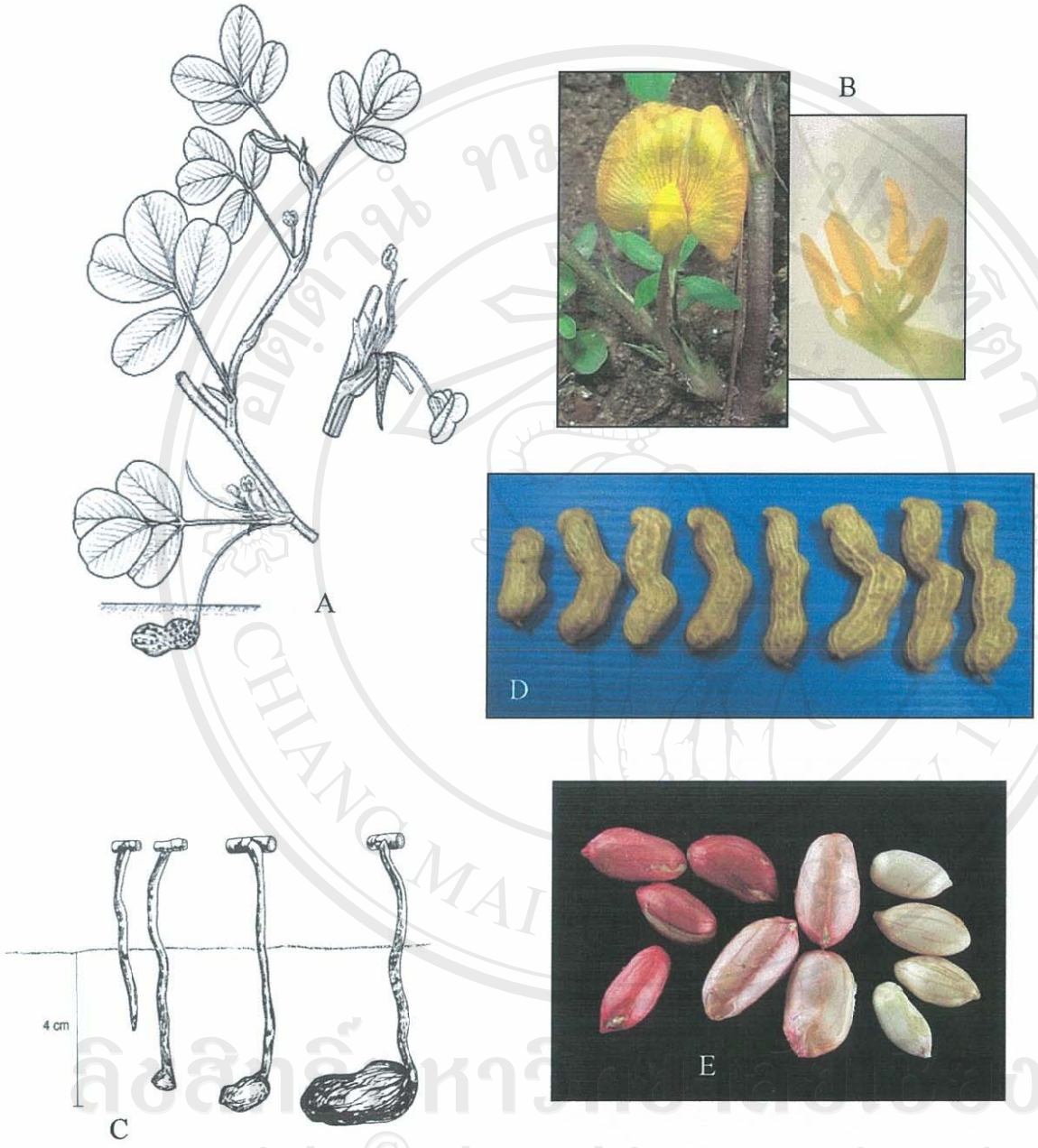


Figure 2.6 Compound leaves (A), flower (B), and pegs structure(C), pods (D) and seed color (E) of groundnut

Groundnut is usually divided into 4 varieties (Stalker, 1997): Virginia, Peruvian runner, Valencia, and Spanish as follows:

Arachis hypogaea subsp. *hypogaea*	No floral axes on main axis: alternating pair of vegetative and floral axes along lateral branches.
subsp. *hypogaea* cv Virginia	Less hairy, branches short.
subsp. *hirsute* cv Peruvian runner	More hairy, branches long.
subsp. *fastigiata*	Floral axes on the main axes of muti-floral axes along lateral branches.
subsp. *fastigiata* cv Valencia	Little branched.
subsp. *hypogaea* cv Spanish	More branched.

2.1.2 Ecology

Suitable regions for groundnut growth are around 35°S to 45°N latitude (Figure 2.1). Like other annual oilseeds with a short growing season, groundnut can adapt to a wide range of environments. It is normally grown commercially below 1250 m, although many varieties could be found at much higher elevations. Temperature between 25 and 30 °C appear to be optimum, below 20 °C retards development. Grows on light, friable, well-drained sandy loams, well supplied with calcium and moderate amount of organic matter, but will grow in heavier soils (Weiss, 2000). Spanish-type cultivars usually mature within 90 to 120 days after planting, whereas most Virginia-types cultivars take 130 or more days, and some groundnut grown in the highlands of south America may take up to 6 month to mature

(Stalker, 1997). Groundnut is drought resistant once established and to some extent it also tolerates flooding. A rainfall of 500- 1000 mm will allow commercial groundnut production (Weiss, 2000).

2.1.3 Cultivation and management

Commercial groundnut crops are grown from seed and usually planted manually either by hill or drill method. Optimum plant population depending on soil type, season, plant typical, expected rainfall, etc., but recommended plant densities for 100 000-120 000 plant/ha for bunch types and half this for runner is acceptable in areas of adequate rainfall, 750-1000 mm. Population should be reduced as the expected rainfall decreases; for instance at 600 mm, a population of 50 000-60 000 plants/ha would be appropriate. Irrigated bunch types can be sown up to 150 000 plants/ha (Weiss, 2000). In most countries, cultivation is in row with plant spacing from 40 cm × 20 cm to 30 cm × 20 cm (Shorter and Patanothai, 1997).

Smallholder production is mainly manual growth either as a rain-fed crop during the wet season or on residual moisture. They are grown as a sole high-value cash crop and also intercropped with maize, soya beans and cassava. In some areas, they are grown under perennial tree crops such as coconut, oil palm. Transitions from mixed cropping to monoculture have increased disease pressures in many regions. Production is mechanized in developed countries, but a large proportion of the global crop is grown with manual labor and with minimal on-farm inputs. Bunch types are commonly grown in small production systems where hand labor is used because they have higher yields than runner and because harvesting is easier (Stalker, 1997).

To achieve maximum economic yield, competing weed must be eliminated. The peculiar nutrition requirement is for Ca in the pod zone that resulted in empty pods. The most serious fungal diseases of foliage are leaf spot and rust that can cause significant losses, particularly during the wet season. While, the important soil borne fungus *Aspergillus flavus* and related species are widespread in the region and infected groundnuts can be contaminated with carcinogen aflatoxins (Weiss, 2000; Stalker, 1997). However, future efforts will be needed to solve several production problems, especially those related to diseases, aflatoxin, and seed quality (Stalker, 1997).

2.2 *Aspergillus flavus* associated with aflatoxin production

Aspergillus flavus is a cosmopolitan, filamentous fungus that is known to occur mostly in soils, but it is also found in plant products, particularly oil-rich seeds and in living plant. Concerns about its ability to produce aflatoxins association with plant products, particularly corn, groundnut, cotton and tree nuts.

2.2.1 *Aspergillus flavus*

A. flavus is a widely distributed saprophytic fungus that like approximately one third of ascomycete fungi. Colonies of *A. flavus* are yellow to green colors on Czapek's agar medium (Figure 2.7). The hyphae are well developed, profusely branched, septate and hyaline; their cells are, as a rule, multinucleate. While still young and vigorous, the mycelium produces and abundance of conidiophores. The conidiophores are long, erect hyphae, each terminating in bulbous head, the vesicle. Sterigmata on vesicle have either uiseriate or biseriate sterigmata that begin to form spore called conidia at their tips (Figure 2.8). These asexual spores are an efficient

form of dissemination and serve as the primary inoculation of members of this genus (Raper *et al.*, 1965).

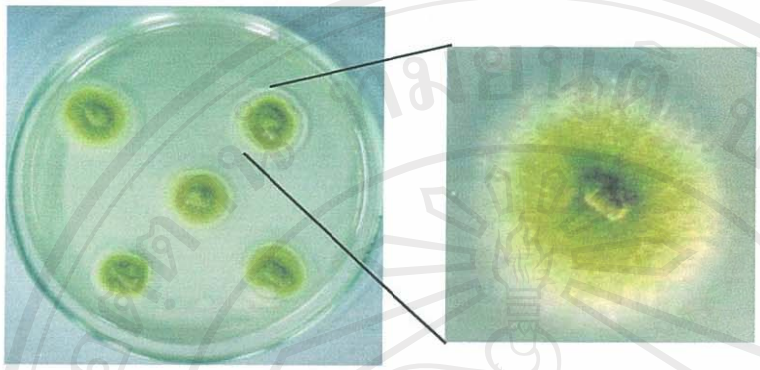


Figure 2.7 Colonies of *Aspergillus flavus* on M3S1B selective medium

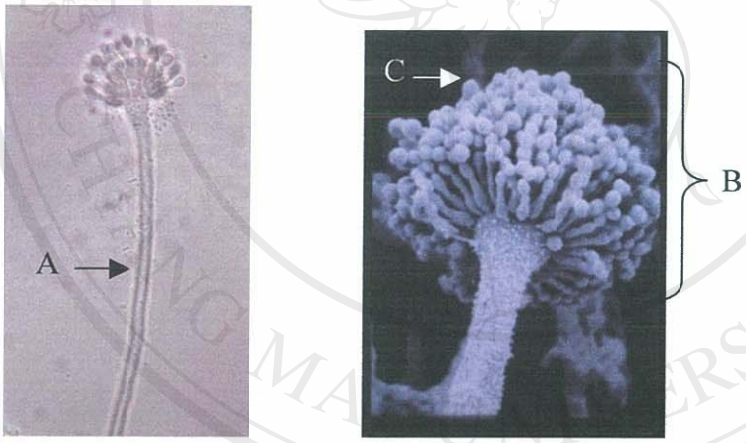


Figure 2.8 Conidiophores (A), conidial head (B) and conidia (C) of *Aspergillus flavus*

Strains of the *A. flavus* group may not only differ in host or nutrient use, but also in host/nutrient location and strategy to exploit resources. It may occur on the same crop or the same field (Cotty and Cardwell, 1999). All developing groundnut crops were contaminated with *A. flavus* even at flowering and pod development stage (Yingthongchai, 1994). Pod can be infected from the soil, by airborne spore or

systemically, and often through insect or mechanical damage and split pod (Hill *et al.*, 1983). Under favorable conditions of high temperature and humidity, these fungi grow on certain foods and feeds, resulting in the production of aflatoxins. Water and temperature stress are important in stimulating amounts of *A. flavus* infection developing pod of groundnut. Infection and heavy aflatoxin contamination is favored at 25-31 °C with 0.85-0.95 a_w in pod during drought periods (Sautour *et al.*, 2002; Molina and Giannuzzi, 2002; Sander *et al.*, 1985). Drought stress could decrease metabolic activity in the kernels, temperature stress could affect the development of microbial competitors in the geocarpophere and both could affect phytoalexin production (Mehan *et al.*, 1991). Other environmental factors influence the growth of *A. flavus* and production of aflatoxin are linoleic acid, light and pH (Calvo *et al.*, 1999; Molina and Giannuzzi, 2002). However, severity of *A. flavus* contamination also occurs in post harvest period, storage environmental and it is influenced by factors such as temperature, humidity, insect populations, etc. (Asevedo *et al.*, 1993; Dange and Patel, 1984).

These toxins are usually found together in various foods and feeds in various proportions. Variability in production of aflatoxins, *A. flavus* isolates may produce anywhere from no detectable aflatoxins (<1 μgkg^{-1}) to over 1000 000 μgkg^{-1}. Diversity of *A. flavus* to produce aflatoxins has reported in consistent differences among vegetative compatibility groups in several characters, including enzyme production, plant virulence, sclerotial morphology, and other physiological traits (Cotty *et al.*, 1990; Bayman and Cotty, 1993).

Phylogenetic studies of *A. flavus* have shown that it consists of two subgroups, called groups I and II. Most group I strains produced B aflatoxins to some degree, and none produced G aflatoxins. Four of six group II strains produced both B and G aflatoxins (Geiser *et al.,* 2000). On the basis of physiological and morphological criteria, *A. flavus* can be divided on sclerotium size into two strains, S and L. Isolates in S strain of *A. flavus* produce numerous small sclerotia and fewer conidia than other *A. flavus* isolates. The L strain produce larger and fewer sclerotia (Bayman and Cotty, 1993; Geiser *et al.,* 2000). Typical isolates (L strains) may produce only aflatoxin B or no aflatoxins at all (Egel *et al.,* 1994), while all *A. flavus* S strains produce relatively large quantities of aflatoxins, some producing only AFB and others producing both B and G aflatoxins (Egel *et al.,* 1994; Cotty and Cardwell, 1999). Vaamonde *et al.,* (2003) found the *A. flavus* able to produce simultaneously aflatoxins type B in Argentina groundnut also produced numerous small sclerotia like S strains of *A. flavus* detected in cottonseed in Arizona and in soils of Thailand and West Africa.

2.2.2 Aflatoxins

Afltoxins are one of the most potent toxic substances that occur naturally and have been found in a wide range of commodities used for animal and human consumption. They are secondary fungal metabolites with high highly mutagenic and carcinogenic properties that are products of polyketide pathway (Figure 2. 9) (Atkins and Norman, 1998; Moss, 2002; Varga *et al.,* 2003; Yu *et al.,* 2002). The four major aflatoxins of concern are designated B_1, B_2, G_1, and G_2 (Yu *et al.,* 1998). These four compounds are separated by the color of their fluorescence under long-wave ultraviolet illumination (B = blue; G = green). Other significant members of the

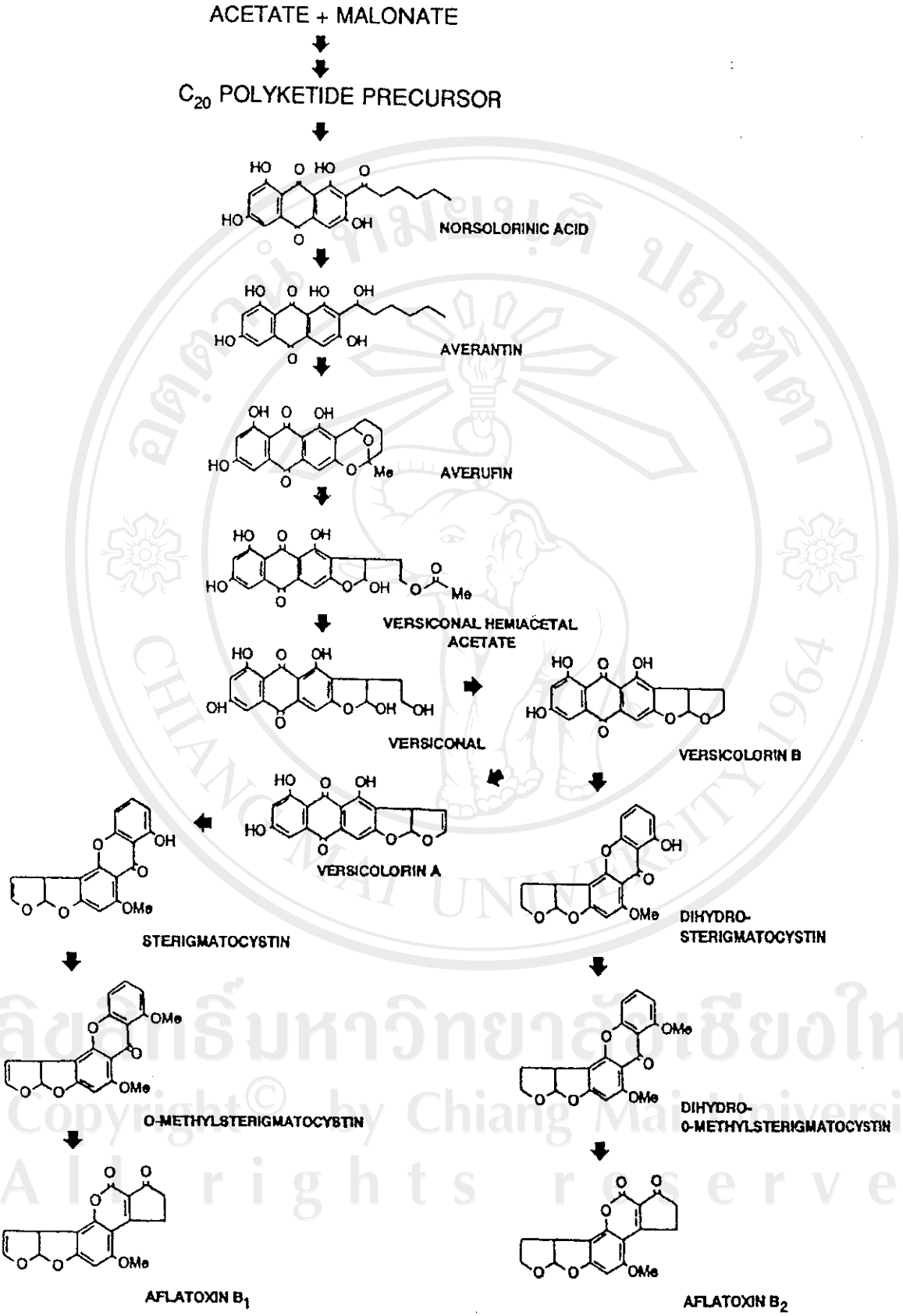


Figure 2.9 The aflatoxins biosynthesis pathway (Bennett *et al.*, 1994)

aflatoxin family, M_1 and M_2, are oxidative forms aflatoxin B_1 modified in the digestive tract of some animals and isolated from milk, urine and feces (Squir, 1989). Aflatoxins were discovered back in 1960 after the outbreak of the turkey "X" disease, in England. This resulted in more than 100,000 deaths of turkeys as well as other farm animals in a few months. The cause was found to be a feed containing Brazilian Peanuts, which was infested heavily with *A. flavus*. After much analysis of this feed, thin-layer chromatography revealed that a series of fluorescent compounds, later called aflatoxins were responsible for this outbreak (Rustom 1997). Epidemiology, clinical and experimental studies reveal that exposure to large doses (> 6000 mg) or LD_{50} as Table 2.1 of aflatoxin may cause acute toxicity with lethal effect. Evidence of acute aflatoxicosis in humans has been reported from many parts of the world like Taiwan, Uganda, India, and many others. The syndrome is characterized by vomiting, abdominal pain, pulmonary edema, convulsions, coma, and death with cerebral edema and fatty involvement of the liver, kidneys and heart. Whereas effects of long-term exposure to low levels of these important mycotoxins on animals and humans for prolonged periods is chronic toxicity. Because of aflatoxins, especially aflatoxin B1 is the most potent hepatocarcinogenic and carcinogenic naturally occurring substance known, causing liver damage to most experimental animal species tested and to humans (Atkins and Norman, 1998; Moss, 2002; Wogan, 2002; Yu *et al.*, 2002; Yu *et al.*, 2004). Statistical estimate of potency (TD_{50} values) allow quantitative comparisons of carcinogenic potency of aflatoxin B_1 among species. Wogan (2000) calculated TD_{50} values for several relatively susceptible and resistant species (Table 2.2).

Table 2.1 Oral acute toxicity LD_{50} value of aflatoxin B_1 for a number of animal species

Animal species	LD_{50} (mg kg^{-1})
Rabbit	0.3
Cat	0.6
Dog	0.5–1.0
Pig	0.6
Baboon	2.0
Rat (male)	5.5
Rat (female)	17.9
Macaque monkey	7.8
Mouse	9.0
Hamster	10.2
Humans	ca 5.0 (?)

Extracted from Moss (2002).

Table 2.2 Interspecies differences in liver cancer induction by aflatoxin B_1 ingestion (Wogan, 2000)

Species	Sex	Duration of exposure	TD_{50} ($\mu g\ kg^{-1}$ bwt day^{-1})
Rat			
Fischer	M	104 weeks	1.3
	F	104 weeks	7.5
Porton	M	104 weeks	3.1
	F	104 weeks	12.5
Mouse			
Swiss	M	80 weeks	>5300
C3H	M	80 weeks	>70
Primates			
Rhesus monkey	M&F	3.3 y	156
Cynomolgus monkey	M&F	14.0 y	848

Although the aflatoxin contamination does not affect crop productivity but it makes produce unfit for consumption, as toxins are injurious to health. Especially, the economic impact of aflatoxins derive directly from crop and livestock losses as well as indirectly from the cost of regulatory programs designed to reduce risks to animal and human health. The Food and Agriculture Organization (FAO) estimates that 25%

of the world's food crops are affected by mycotoxins, of which the most notorious are aflatoxins. Aflatoxin losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and the more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency. The marketability of contaminated produce, particularly in international trade is diminished to nil due stringent standards of permissible limits on aflatoxin contamination set by the importing countries. Several countries stipulated the maximum tolerated levels of aflatoxin B_1 in food shown in Table 2.3 (Moss, 2002).

Table 2.3 A selection of maximum tolerated levels of aflatoxin B_1 in food (Moss, 2002)

Country	Max. level ($\mu g\ kg^{-1}$)	Products
Argentina	0	Groundnuts, maize and products
Brazil	15	All foodstuffs
China	10	Rice and edible oils
Czech Republic	5	All foods
Hungary	5	All foods
India	30	All foods
Japan	10	All foods
Nigeria	20	All foods
Poland	0	All foods
South Africa	5	All foods
Zimbabwe	5	Foods

The ability of aflatoxins to cause cancer and related diseases in humans given their seemingly unavoidable occurrence in foods and feeds make the prevention and detoxification of these mycotoxins one of the most challenging toxicology issues of present time (Dorner *et al.*, 1998; Ellis *et al.*, 1994; Lo´pez-Malo, 2002 and Tamil Selvi, 2003). Minimized Aflatoxins could be managed by intensive cultural, produce handling and storage practices. However, these practices were not widely adopted particularly by small farmers in developing countries, which contribute about 60 % of the world groundnut production (Upadhyaya *et al.*, 2004). While, one of the possible

means of reducing aflatxin contamination of groundnut is the use of plant resistant to invasion by aflatoxin-producing fungi or aflatoxin production. This plant will be of great value to the farmers in both developed and developing countries as there are no cost input and consequently associated economic losses and health hazards (Upadhyaya *et al.*, 2004).

2.3 Mechanism of plant resistance to pathogens

Plants are continually exposed to insects, nematodes and other potentially damaging pests, as well as to a wide variety of parasitic microorganisms. The majority of plants remain healthy most of the time. This observation suggests that plant must possess highly effective mechanisms for preventing invaders (Lucus, 1998).

Mechanism barriers, which act as physical inhibitors to pathogen invasion, and biochemical resistance mechanisms, including toxic or inhibitory compounds undoubtedly function in combination. In some instances such structural and biochemical characters are already present in plant prior to pathogen invasion as preformed (passive resistance) resistance mechanisms. However, in other cases resistance reactions are induced (active resistance) by pathogen invasion (Figure 2.10) (Isaac, 1992; Lucus, 1998).

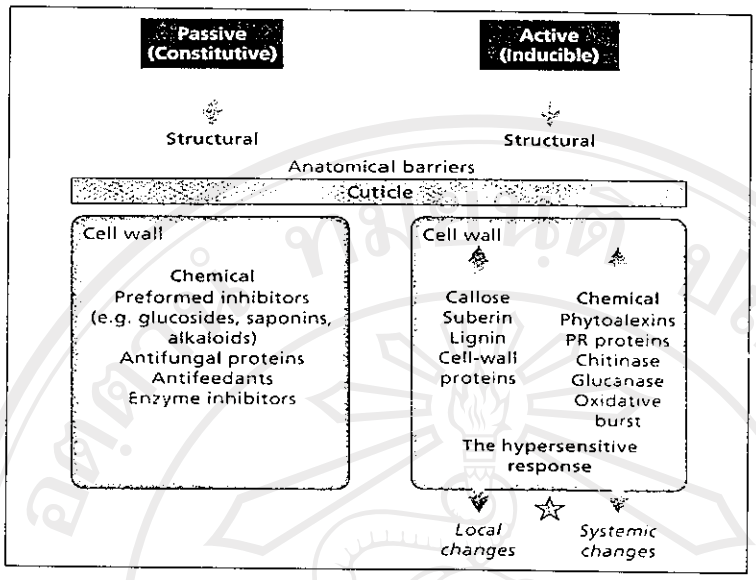


Figure. 2.10 Classification of types of plant defence, based on existing anatomical or biochemical features, or active changes induced after challenge by pathogens (Lucus, 1998)

2.3.1 Passive defense mechanisms

A passive anatomical feature is the structural and biochemical characters that are already present in the plant prior to pathogen invasion. Plant structure such as the cuticle, bark and cell wall represent highly effective obstacles to penetration by most microorganisms. Where this is impregnated with chemicals such as waxs, cutin, pectins, cellulose, hemicellulose, suberin and lignin it forms an even more effective barrier against pathogens. Plants synthesize a vast array of secondary metabolites, many of which are toxic to pathogens. Compounds such as phenols, alkaloids, glycosides, saponins, tannins and resins all possess antibiotic properties and may therefore contribute to resistance (Grayer *et al,* 1992; Nicholson and Hammerschmidt, 1992; Adegoke and Odesola, 1996).

2.3.1.1 Preformed structure barriers

Although some pathogen can force entry through closed stomata or directly puncture pass plant cell structure, many fungal pathogens are able to enter plant through stomata but the structure characteristics of these opening do influence penetration. In particular for example, stomata with very narrow or elevated guard cells and small apertures may restrict the entry of hyphae. In addition, the frequency of epidermal hair or papillae and the differently rough surface of plant structures support to obstruct the pathogen penetration (Huang, 2001; Commenil *et al.,* 1997). The initial contact between a fungus and plant most usually occurs at the cuticle, which overlies the epidermal cell wall (Figure. 2.11). The role of the cuticle as barrier to fungal invasion is supported by a direct correlation between disease resistance and cuticle thickness in several host-parasite interactions. Next to inner, the cell wall functions as a skeleton and a skin in higher plant. As the skeleton, it provides physical coherence, strength and morphology to plant cells (Huang, 2001). This is the primary site for polymerization of cell wall polysaccharides (cellulose, hemicellulose and pectin polysaccharides), lignin and amorphous silica gel in plant that accumulate this polymer (Kaufman *et al.,* 1999). In more deeply seated tissues subepidermal sclerenchyma can act as a structure barrier. Such thickened, lignified, secondary walls are highly resistant to penetration, which varies depending on species, organ, developing stage and environmental condition (Isaac, 1992; Huang, 2001).

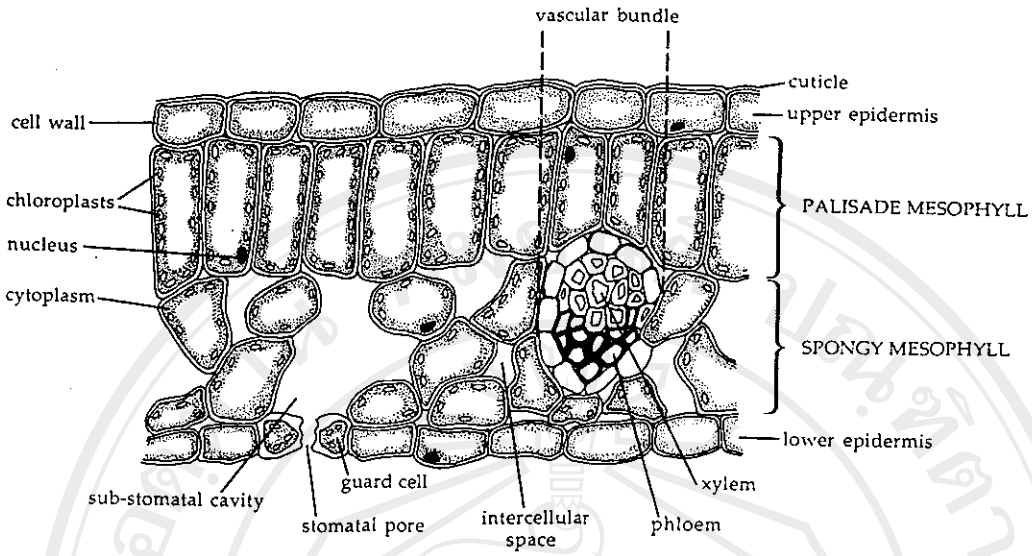


Fig. 2. 11 Vertical sections through leaf tissue to show the arrangement of cell and intercellular spaces (Isaac, 1992)

2.3.1.2 Preformed biochemical compounds

Resistance to invasion may be the direct result of the appearance of substances in or on plant cell before infection that are poisonous to attacker. The first line of defense and possibly the single important is the excretion and polymerization of the cuticle at the outside of plant cell walls. There are three major compound constituents of cuticle, wax, cellulose and cutin (Huang, 2001). The waxes provide the plant with a highly water-repellent surface. According to Laprade *et al.* (1973) use of a scanning electron microscope (SEM) revealed that the accumulation of surface wax on the testa of dry groundnut seeds in tolerant lines greater than in highly susceptible lines to colonization by *A. flavus*. Gembeh *et al.,* (2001) also found phenolic compounds may contribute to corn kernel wax inhibition of *A. flavus* infection/aflatoxin production. Russin *et al.,* (1997) reported that kernels of corn resistant genotype to *A. flavus* appeared rough and showed abundant wax deposits on kernel surface. The susceptible

kernels appeared much more smooth and lacked the abundant surface deposits observed in resistant genotypes. The phenolic compounds have also been entangled in resistance, either present in surface waxes or within host cell. Tannins are very diverse family of polyphenolic compounds complexing with proteins, polysaccharides and other macromolecules (Nicholson and Hammerschmidt, 1992). Grayer *et al.*, (1992) proposed that high procyanidin or condensed tannins in groundnut leaf bud petioles are related to groundnut resistance against *Aphis craccivora*. In reinforcement, the fibers in cuticle are cellulose wall like a rigid basket of plant cell. However, the amount of cellulose in the cuticle is small. Cutin is the main structure component of plant cuticles (Huang, 2001). The underlying cutin polymer, as a polyester and polyether, is obviously difficult to degrade enzymatically of fungi (Slusarenko *et al.*, 2000). In more profoundly, three structures of cell wall, middle lamella, primary wall and secondary wall serve as signal molecules that regulate plant growth, development and defense responses (Isaac, 1992). A diagram illustrating the distribution and relative concentrations of the chemical component in the mature cell wall is given in Figure 2.12. Cellulose fibers take up the tensile forces while hemicellulose and pectin make up the pressure-bearing matrix. Some cell wall in order to provide specialized function; lignin to give strength to dead, turgor-less cell, e.g., in cork cell and in the endodermis; cutin and epicuticular waxes to protect the above ground part of land plants from desiccation. Tannins are common to vascular plants existing primarity within woody tissue. Tannins consist of various phenolic compounds that react with protein to form water insoluble copolymers. Plant tissues threat are high in tannin content have a highly bitter taste and are avoided by most feeder (Kaufman *et al.*, 1999). In addition, it is becoming increasingly apparent that

cell wall also contain many different proteins and glycoproteins which fulfill both structure and enzymatic functions (Slusarenko *et al.*, 2000; Huang, 2001).

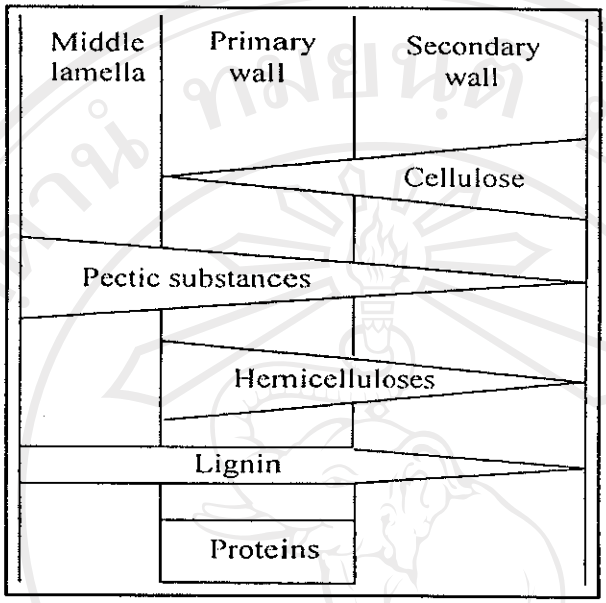


Figure 2.12 Distribution and relative concentration of each cell-wall component in the mature cell wall (Huang, 2001)

Application of mineral nutrient fertilizer in different amounts and forms not only affects the growth and composition of plants directly but also has profound effects on microbial activities in soil and plant resistance and tolerance to pathogens. Plants with an optimal nutritional status have the highest resistance to disease and that susceptibility increases as nutritional status deviates from this optimum. Most parasitic fungi invade the apoplasm by releasing pectolytic enzymes, which dissolve the middle lamella. The activity of these enzymes is strongly inhibited by Ca^{2+}, with explains the close correlation between the calcium content of tissue and their resistance to fungal diseases (Huang, 2001; Marschner, 1986). Fernandez *et al.* (1997) also found that the fungus incidences on groundnut grains were affected by Ca

nutrition. The development of *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and potential aflatoxin production fungi was decreased and even suppressed when the Ca content of the seedcoat was increased from 2.2 to 5.5 g. kg^{-1}. Pectic enzymes of the parasite not only dissolve the middle lamella but these enzymes, or the products of pectin breakdown, also increase passive permeability of the plasma membrane and enhance K^+ efflux and H^+ influx which probably trigger hypersensitive reaction such as localize necrosis (Atkinson *et al.,* 1986)). In other pathogenic fungi such as leaf spot (*Helminthosporium cynodontis* Marig.), severity of disease symptoms strongly decreases with increase in potassium content of the leaves (Richardson and Croughan, 1989).

The accumulation and deposition of silicon in the epidermal cell layers may form an effective physical barrier to hyphal penetration. As the silicon supply increases the silicon content of leaves also rises, inducing a corresponding decline in susceptibility to fungal diseases such as rice blast. The increase in resistance appears to be related directly to the silicon concentration in the external solution and in leaves (Osuna-Canizalez *et al.,* 1991). Of the various defense mechanisms available to plants the phenolic and lignin are the most understood well, and of the micronutrients, at least boron, manganese and copper play a key role in phenol metabolism and lignin biosynthesis (Marschner, 1995). Micronutrients can also affect resistance indirectly. In boron-deficient wheat plants the rate of infection with powdery mildew is several fold higher than that in boron sufficient plants, and the fungus also spreads more rapidly over the plant (Schütte, 1967).

2.3.2 Active defense mechanisms

The various structural and chemical compounds described in the previous section serve as a first line of defense against microbial attack. When a microorganism has breached the entire preformed barrier, which a plant has put up a passive, plant cells usually exhibit typical morphological reactions as a second line of active structure defense (Slusarenko *et al.*, 2000). If combination is compatible, further hyphal growth and invasion of host tissue continues unrestricted. In resistant host, though, a number of changes take place in penetrated cells and adjacent tissues that ultimately halt the advance of the pathogen (Lucus, 1998). These infection induced active resistant mechanisms involve the formation new structures and biochemicals or a change in composition or interaction of existing plant cell materials. During penetration, normal plant wall may induce phenolic, suberin, lignin, proteins, calcium and silicon. In addition, walls may contain inhibitors of enzymes or toxins that deter a possible parasite. Numerous reviews have covered cell wall alteration such as modification the structural barrier or changes in composition after fungal invasion (Agrios, 1997; Huang, 2001; Jones, 1987).

2.3.2.1 Induced structural barrier

Although the accumulation of reaction material in cells is one of the earliest at most regular plant responses to challenge by a pathogen, there is debate about the importance of these changes in host resistance. The most obvious suggestion is that wall appositions impede penetration by the pathogen, either by increasing the strength and thickness of the cell and cell wall or by enhancing its resistance to enzymes attack. The deposition of a plug of materials known as a papilla spread

over epidermal cell wall may be changed to leave a disc-shaped zone or halo. Plant cells penetrated by fungi may form characteristic protrusions and layers of the wall known as cork layer, tyloses and lignituber (Isaac, 1992; Lucus, 1998; Moerschbacher and Mendgen, 2000).

Cork layer

Cork layers are laid down in response to mechanical injury to cells activity in the cork cambium results in the information of cork cell in areas surrounding penetration points. Nutrient supplies to the attacker are thereby removed and attempt to protect themselves from any toxins released by the pathogen. The speed of cork layer formation has also been related to resistance levels in plants (Moerschbacher and Mendgen, 2000).

Tyloses

Tyloses are formed in xylem vessels as a response to abiotic stress, invasion an aging. The cellulosic wall of the tyloses extends to a degree and number that the xylem vessels may become completely blocked. In fact plant that are resistant to vascular wilts have the capacity to form many tyloses, whereas more susceptible plants form relatively few. In similar way hemicellulose gels and gums may be produced, in cells adjacent to infected tissue, blocking pathogen growth and spread through vascular tissue but also restricting solute movement through plants (Mansfield, 2000).

Lignitubers

The progress of penetration may be progressively impeded by the deposit of extra deposits of cellulose, callose and lignin, often with additional insoluble phenolic compounds. A sheath or lignituber may form around the invading hyphae increasing the mechanical strength of cell walls involved and preventing water loss. Lignifications and suberinisation are limit the proliferation of an infection fungus and are therefore important factors in disease resistance. It is interesting that the exact composition of the materials laid down after the damage has occurred differs from those accumulated during natural plant development. It has also been suggested that superior resistance is afforded by these depositions as wounds age. Lignin deposits may increase the mechanical strength of cell walls preventing further penetration and may also alter the susceptibility to lytic enzyme attack by invading fungal pathogens. Additionally, it is likely that phenolic precursors of lignin may act as antifungal compounds (Cseke and Kaufman, 1999).

Moreover, deposition of callose, lignin-like materials and suberin can also occur upon wounding, but during defense reactions to pathogenic attack, they are often highly stimulated in a zone surrounding the site of tissue penetration. Callose can block plasmodesmata and thereby inhibit cell-to-cell transportation. Callose papillae may be deposited on the inside of cell wall as a response to fungi invasion, e.g. the response of barley plant to powdery mildew (Bayles *et al.*, 1990).

2.3.2.2 Induced biochemical responses

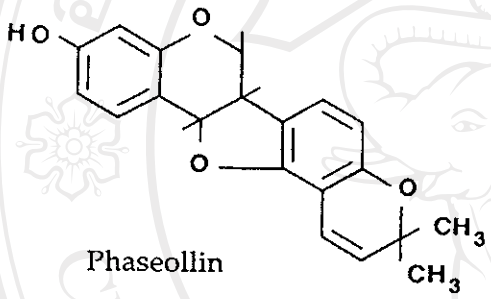
Biochemical inhibitors

Phenols have long been associated with the passive and active defense responses of plant, because of their universal presence in vascular plants and their

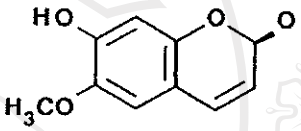
accumulation in both compatible and incompatible interactions. The production and accumulation of phenolic compounds are often associated with injury responses and the wound healing of plant, but these substances also have fungitoxic activity (Nicholson and Hammerschmidt, 1992). Rapid accumulation of the compounds may occur in resistant plants after infection, e.g. scopoletin, umbelliferone, caffeic acid and orchinol (Figure 2.13). The concentration of phenolic may not sufficient to inhibit the development of infection but in combination the compounds present may well be effective antifungals (Issac, 1992).

When plant tissues are damaged, for instance by mechanical wounding or attack by pathogen, defense genes are switched on in cell adjacent to the wound site. These wound-inducible genes encode products such as proteinase inhibitors that are active against digestive enzymes. Similar inhibitor proteins are also synthesized in tissue remote from the initial injury. Huang *et al.* (1997) estimated corn seed protein inhibitory mass of *A. flavus* growth is approximately 28-kDa and of aflatoxin production appears to be greater than 100-kDa. Subsequently, several researcher found the specific 14-kDa corn trypsin inhibitor reduced the production of extracellular fungal α-amylose and its activity, thereby limiting the availability of simple sugars of fungal growth (Chen *et al.,* 1999; Tubajika and Damann, 2001). One active class of chemical inducers is cell wall fragments, known as oligosacaharins, which may be released as a result of cell damage (Van Loon, 2000). Others are the hormone abscisic acid, lipid-derived compounds such as jasmonic acid (Feys and Parker, 2000; Huang *et al.,* 1997; Juergen *et al.,* 2003; Zeringue Jr., 2002) and a small peptide molecule comprising 18 amino acid and christened systemin shown in Figure 2.14. Systemin is released from the precursor protein, prosystemin and translocated

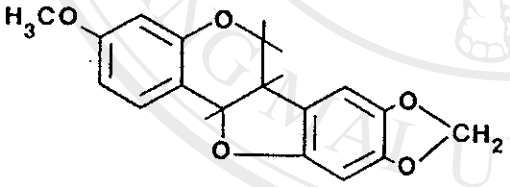
throughout the plant inducing defence function in remote tissues. (Lucus, 1998; Pieterse and van Loon, 1999). Binding of oligosacaharins or systemin to receptors in the plasma membrane generates intracellular signals derived from membran lipids. The enzyme lipoxygenase is involved in the conversion of lipid precursors to jasmonic acid, which switches on the genes encoding defence proteins, such as the proteinase inhibitors (Lucus, 1998; Jabs and Slusarenko, 2000).



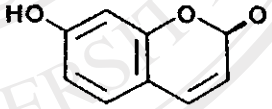
Phaseollin



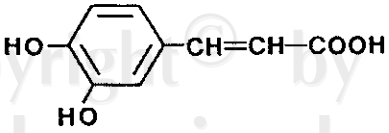
Scopoletin



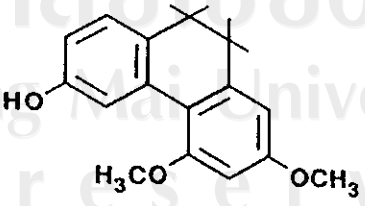
Pisatin



Umbelliferone



Caffeic acid



Orchinol

Figure 2.13 Chemical structures of some phenolic compounds that are induced in plants as a response to fungal attraction

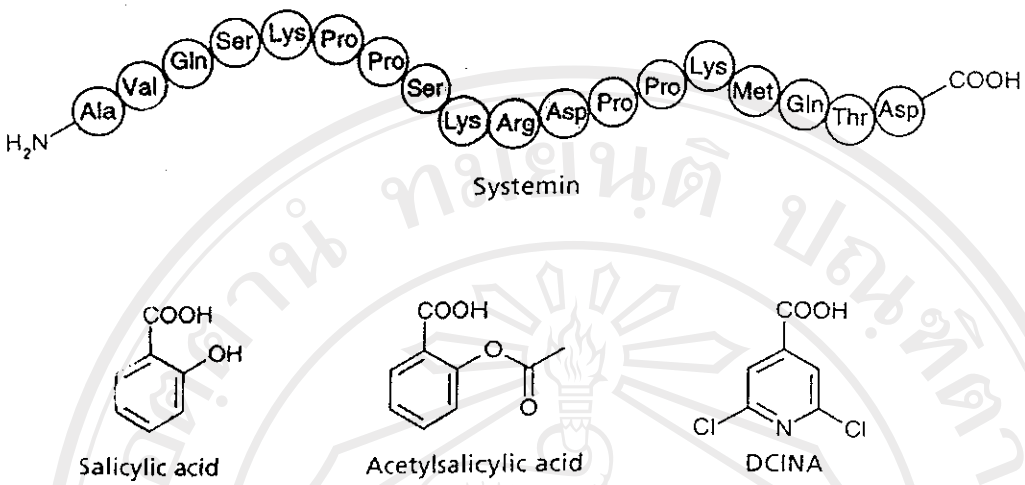


Fig. 2.14 Signal molecules implicated in the induction of plant defense, systemin, salicylic acid, acetylsalicylic acid and dichloroisonicotinic (DCINA)

Hypersensitivity

Nicholson and Hammerschmidt (1992) have argued effectively for the differentiation of the responses of plants to pathogens based on host and non-host interactions. While the responses are characterized by the early accumulation of phenolic compounds at the infection site that limited development of pathogen occurs as a result of rapid cell death. Although it is often suggested that lignin accumulates early in response to infection, little direct chemical evidence supports its formation as an initial host response. One responses observed consistently to attempted infection, in both compatible and incompatible interactions, is the necrosis of cells. The breakdown of membranes and the cellular disorganization that accompanies necrosis may in fact trigger metabolic changes in adjacent living cells. The sum total of these changes is the creation of an inhibitory environment which restricts further growth of

pathogen, either by starving it or poisoning it or physically walling it in, or a combination of all three (Lucus, 1998).

Hypersensitive reaction occurs as the result of the recognition of infection by the host plant and as a consequence of incompatibility between host and pathogen. The term response relates to the visual symptoms of dynamic host cell necrosis associated with penetration in resistant host cultivars. Infected cells rapidly and suddenly loose membrane permeability and turgor, are subject to dynamic incresses in respiratory activity, accumulate phenolic compounds and phytoalexins (Figure 2.15). An increase in oxidation reactions and a decrease in reduction reactions result in necrosis; limited numbers of cells die and turn brown. The pathogen is therefore isolated in localized necrotic tissue and does not spread to healthy regions. It is interesting that the biochemical changes, which are associated with hypersensitivity, are similar to those that occur after wounding, at senescence or as stress responses (Isaac, 1992).

Phytoalexins

Phytoalexins are low molecular weight antimicrobial compounds, which are synthesized by and accumulate in plant cells after microbial infection (Paxton, 1981). The phytoalexin synthesis in plant occurs in cell adjacent to those which have been damaged, either by physical wounding or biochemical injury, as the result of penetration or toxin effect (Isaac, 1992). However, probably in low concentrations may be present prior to infection as a result of plant response to abiotic stresses (Van Etten *et al.*, 1989). Both resistant and susceptible necrotic tissues produce phytoalexins although resistance appears to be related to the concentration that accumulate.

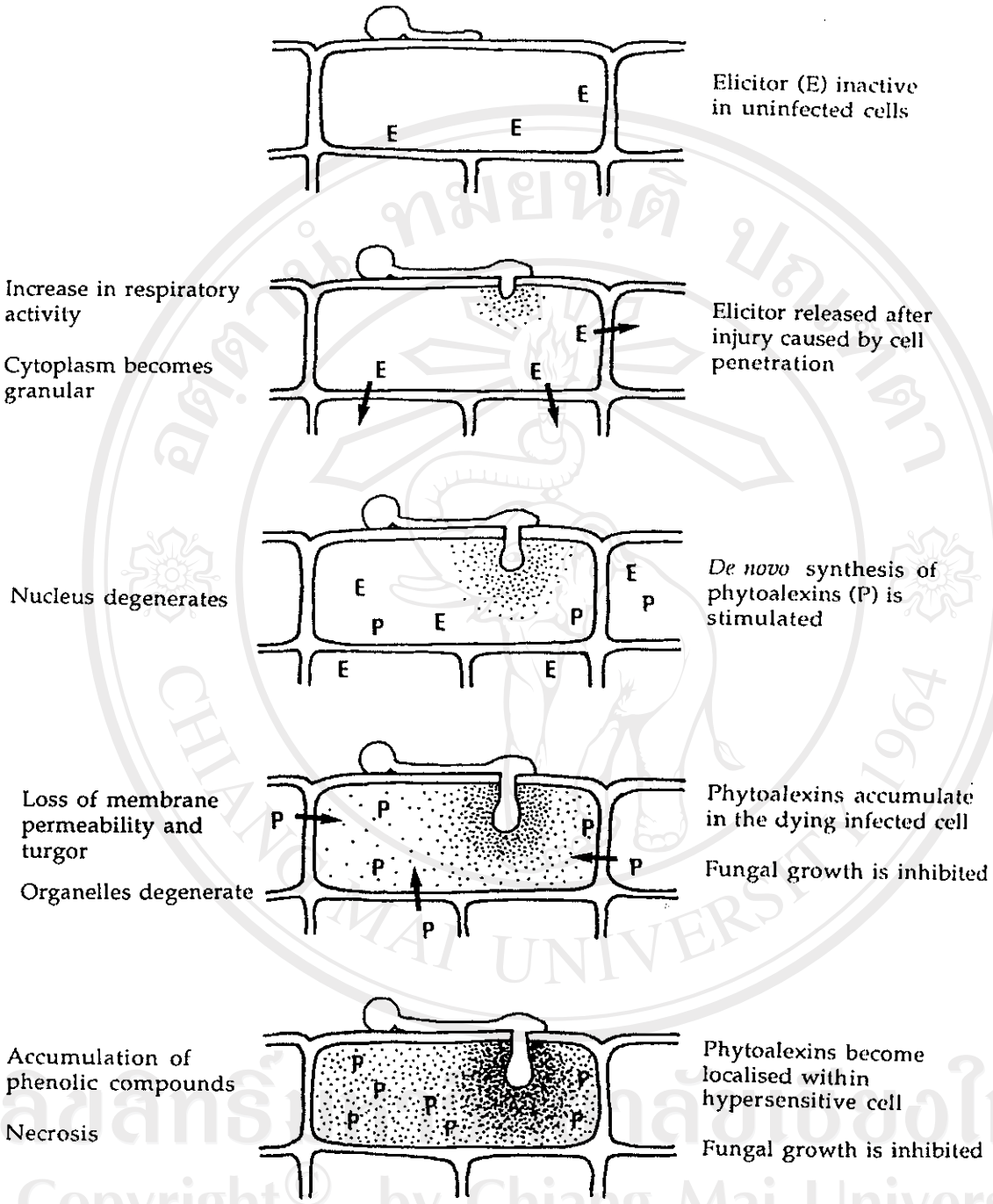


Figure 2.15 The stages of morphological and physiological change occurring during development of the hypersensitive response of plant cells and the possible role of constitutive elicitors in phytoalexin accumulation (Issac, 1992)

The actual amounts of phytoalexins, which have been challenged, are very variable and may depend on a number of factors including the physiological status of the plant and its genetic composition. Phytoalexin concentrations were influenced by cultivars, length in storage and viability. It may be significant that the resistant cultivars accumulated more than three times as much arachidin IV as the susceptible cultivars. Storage for 9 months drastically reduced the ability of cotyledons to synthesize phytoalexins. Seed with low viability had also lost its ability to accumulate phytoalexins although it was not determined whether the low viability was attributable to dormancy or mortality. Maturity also influenced the capacity of groundnuts to accumulate phytoalexins (Arora and Strange, 1991). Pegs were able to synthesize phytoalexins and this may be one reason why infection of this part of the plant by airborne conidia of *Aspergillus* spp. has been reported to be low (Griffin and Garren, 1976). All part of the developing pods was able to synthesize phytoalexins but as the testa and pod matured they lost this capacity. Mature pods are lignified and they as well as mature testas also contain preformed antifungal compounds. Thus, these tissues, despite losing their capacity to produce phytoalexins, afford the seed some protection from microorganisms of the surrounding soil. In contrast to pod and testa tissue, the more mature cotyledons accumulated greater concentration of phytoalexins (Arora and Strange, 1991). The involvement of phytoalexins in disease resistance is not restricted to a particular interaction between genotypes, for examples race-specific or non-host resistance, but is more closely associated with morphologically similar types of response. Most data supporting a role for phytoalexins accumulation as the cause of the inhibition of microbial growth come

from interactions in which resistance is expressed following penetration into the plant and is associated with the necrosis of plant cells (Hammerschmidt, 1999).

Phytoalexins affect the growth of fungi, inhibiting germ tube elongation, colony growth and dry weight accumulation. The main effect of phytoalexins on fungi is via the membrane. The plasma membrane is rapidly disrupted and structural integrity is affected, resulting in an often dramatic and massive loss of electrolytes. There is also evidence that phytoalexins disrupt respiratory pathways (Zeringue, 2002). Cytoplasmic streaming is prevented and cell contents become granular. Membrane disruption and distortion has been observed in electron microscope sections of affected hyphae. Newly synthesis regions of hyphae tips are particularly vulnerable and sensitive and swell to bursting. There is also some evidence that phytoalexins inhibit the action of fungal cell wall synthesizing enzymes but this may be primarily a disruption of the highly integrated synthesis system of wall growth at hyphal trips (Isaac, 1992).

Arora and Strange (1991) found that groundnut accumulated the phytoalexins resveratrol, 4-(3-methyl-but-1-enyl)-3,5,4′ trihydroxystilbene (arachidin III) and 3-isopentadienyl-4,3′ ,5′- trihydroxystilbene (arachidin IV) in response to wounding. They suggested that these three phytoalexins seem to have the potential for playing a role in resistance of groundnut to invasion by *Aspergillus* spp. fungi. Compounds that induce phytoalexins synthesis in plants are termed elicitors and may be microbial in origin (exogenous elicitors) or plant-derived (endogenous elicitors). Such biotic elicitors are often complex molecules such as carbohydrates, glycoproteins, polypeptides, enzymes or lipids. Additionally phytoalexins also accumulate in response to abiotic elicitors. These are usually factors, which cause plant stress,

such as cold, ultraviolet light, or the presence of heavy metals in the immediate environment. Additionally, it has been suggested that the receptor-ion channels may be ion-specific (e.g. for K^+, Ca^{2+}, Cl^- and H^+) so that different fluxes would occur through the membrane. In this way alternations in Cytoplasmic ion concentrations may affect exchanges with the vacuole and generate different signals within the plant. Calcium ions are implicated particularly in the functioning of this model, since these are so important in plant cells and very small increases in calcium ion concentrations cause rapid response in cells. Different types of cells, at different locations in the plant, may respond to signals in different ways and cells surrounding one which has been invaded may respond to secondary signals from their damaged neighbor (Isaac, 1992).

Enzyme and protein activities

Enzymes with the ability to attack the structure of microorganisms are potential defense arsenals against pathogenic invasion. Most of fungal pathogens contain β-1,3-glucan, chitin and chitosan as cell wall components (Broekaert *et al.*, 2000; Sharathchandra *et al.*, 2004; Van Loon, 2000). It is conceivable that enzymes hydrolyzing these fungal components are able to inhibit fungal growth and cause death of mycelia. Increase in activity of these hydrolases in plant tissues has been considered an attribute of resistance.

2.4 Breeding for resistance

There are many ways in which the occurrence of fungal infection of plants can be minimized in a crop. Good hygiene and careful practice, such as removing dead and infected material from the site, the use of clean seed and crop rotation, may avoid disease at the site. The use of fungicides as a preventative or direct control measure, and some biological control systems, also prove to be economically viable. However, in many developing countries poor farmers do not have the financial resources and education for a safe application of pesticide whereas natural resistance is a potentially cheap and efficient way to fight diseases. As a result many genetically different lines and cultivars have gradually arisen. More recently the value of plant breeding has been recognized and more intensive methods, accelerating this process, have been employed, combining useful genetic characters and giving rise to varieties with greatly increased yields, improved quality, harvest ability, storage potential and appearance. Breeding for lines which are more tolerant to stress environmental conditions and resistant to pathogen invasion has improved a valuable contribution. It is indisputable that an inherent level of resistance to pathogen invasion or establishment in the crop plant is the most useful and cost-effective means for controlling disease (Agrios, 1997; Isaac, 1992; Vanderplank, 1968).

2.4.1 The gene-for-gene hypothesis

The basis of the plant resistance reaction is therefore a specific recognition between the two components. Genetic factors of both the plant and the pathogen are required for a successful defense reaction of plant. The specificity of plant-pathogen interaction is determined by the interaction of an avirulence gene product encode by a

dominant gene in the pathogen and a product of the resistance gene from plant. This recognition triggers further physiological defence reaction resulting in hypersensitive cell death and the accumulation of molecules, which are toxic for pathogen as above. This is also called an incompatible interaction between the plant ant the pathogen. In the absence of either the resistance gene product or the avirulence gene product, there is no recognition of the pathogen by the plant. This allows the further growth of the pathogen, resulting in a compatible interaction and susceptibility. Thus, a mutation in either the avirulence or the resistance gene that results in a loss of function will result in a change from an incompatible to a compatible interaction. The presence of a resistance gene in the host plant therefore exerts a strong selective pressure for a mutation in the avirulence gene if the product of the avirulence gene is not essential for the survival of the pathogen. This selection pressure has important epidemiological consequences for the development of new pathogen races and the losses in crop production and quality. Thus, resistance in the gene-for-gene interaction is race-specific whereas susceptibility is not specific. The genetic basis of specific resistance is best understood by quadratic (Figure 2.16) that can be used to describe the gene-for-gene interaction. In this graphical description, resistance occurs only when both a dominant *R* gene from the plant and the dominant avirulence gene *A* from the pathogen are present in the upper left guardant. In all the other quadratic the interaction is compatible, resulting in susceptibility. To prove a gene-for-gene interaction, the quadratic check must be reciprocal, i.e. it must be true for at least two resistance genes in the host and two matching avirulence genes in the pathogen (Figure 2.16B) (Vanderplank, 1982). If this condition is fulfilled, a gene-for-gene interaction occurs in this particular disease. A more molecular model derived from the

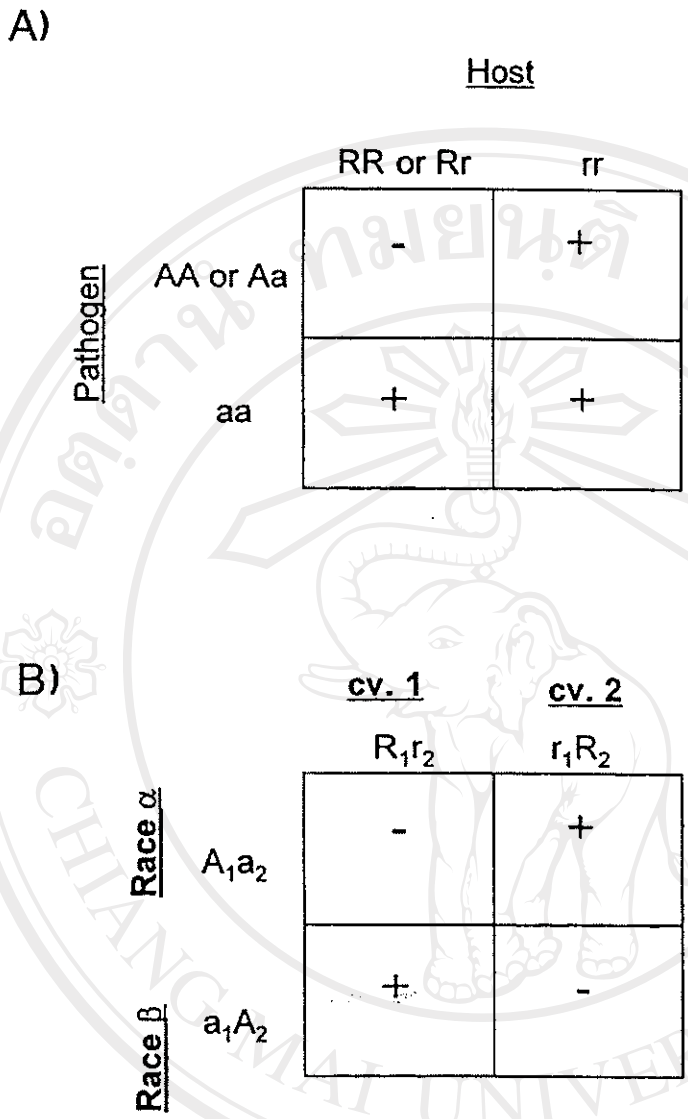


Figure 2.16 Quadratic check of gene combinations and the resulting different interaction types in gene-for-gene interaction (Keller *et al.*, 2000). The pathogen can grown in the compatible (+), but not in the incompatible (-) interaction. A indicates a dominant avirulence gene in the pathogen, R a dominant resistance gene in plant. (A): The quadratic check for a single locus in the host and in the pathogen. (B): Reciprocal check for two genetic loci of resistance (R_1 and R_2) in the two plant cultivars (cv.1and cv.2) and the corresponding two avirulence loci in two pathogen races (A_1 and A_2).observation of the dominant character of both the avirulence gene and the

resistance gene is shown in Figure 2.17 . The product of the resistance gene in this model would be a receptor that actively recognizes a direct product of the avirulence gene. Only the receptor-ligand interaction (Figure 2.17A) results in specific recognition indicated by the hypersensitive response and disease resistance.

2.4.2 Variable system of plant –pathogen interaction

Race-specific resistance gene and the corresponding avirulence genes in the pathogens belong to the genetically most intensively studied systems. These monogenic resistances are also of great importance for resistance breeding in many crop plants However, it has to be emphasized that this type of resistance represents only one form of resistance present in the gene pool of plants. Race-specific resistance is also called vertical resistance. It has been shown that the resistance may be controlled by a single or very few (2-3) genes (oligogenic resistance). Vanderplank (1968) introduced the term vertical resistance to describe the situation in which plants showed very high levels of resistance to a particular physiological race of pathogen but very little resistance to other races. Such hosts often show the hypersensitive response, usually very early in infection, and the pathogen is not able to establish or multiply within the plant tissues. In contrast to the genetically different type of non-specific resistance, which is often referred to as horizontal or polygenic resistance as there are several gene involved (Figure 2.18). Other terms used for this type of resistance are quantitative or partial resistance. The genes involved in this type of resistance are often called minor genes in contrast to the major genes in vertical resistance.

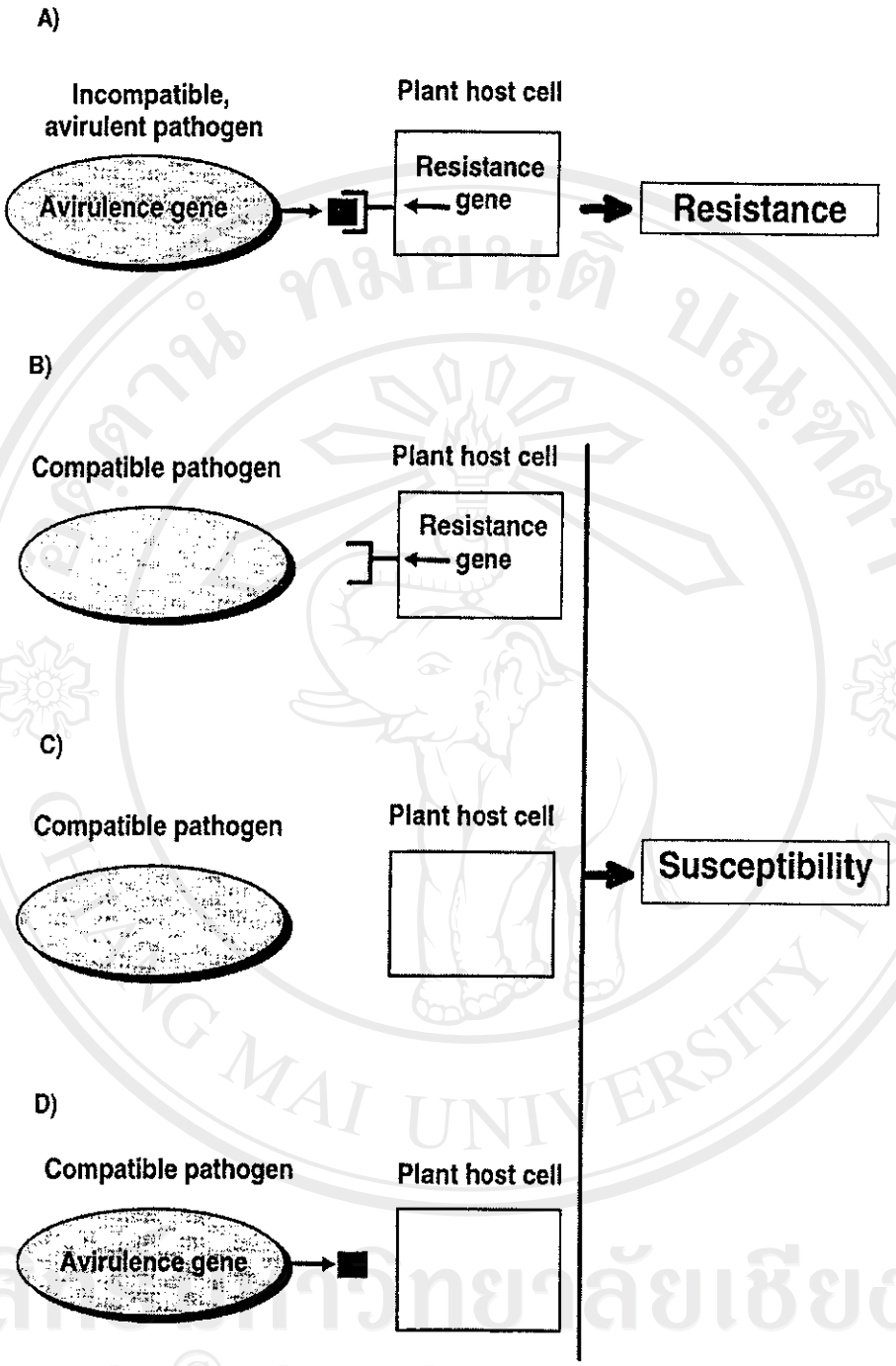


Figure 2.17 Molecular model of the gene-for-gene interaction (Keller *et al.*, 2000). Resistance occurs only if there is a specific recognition between the resistance gene product and the product of the matching avirulence gene (A). In the absence of any recognition (B, C, D), no resistance reaction occurs and the pathogen can colonize the plant, which results in a susceptible phenotype.

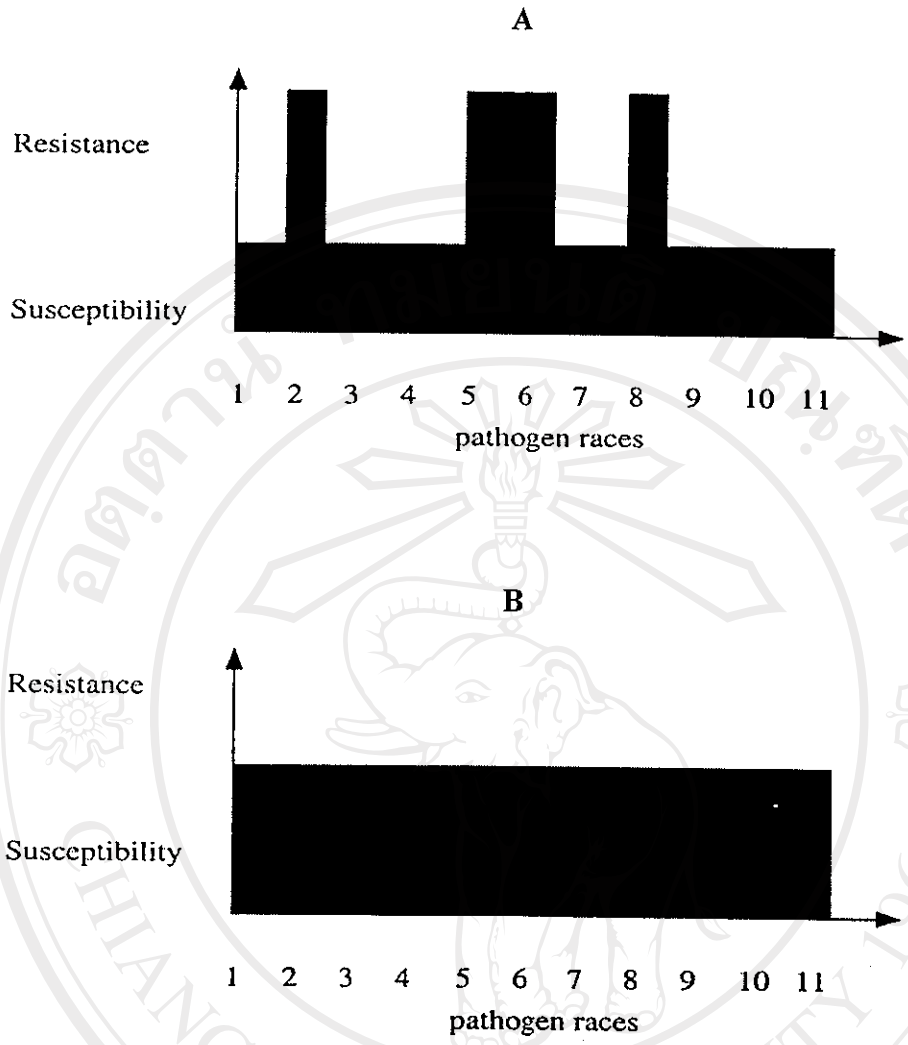


Figure 2.18 Vertical (A) and horizontal (B) resistance

Horizontal resistance is characterized by the absence of genetic interaction between the host genotype and pathogen genotype in contrast to the race-specific genetic interaction in vertical resistance in gene-fore-gene relationship. Horizontal resistant plants have a level of general resistance to pathogens, which is controlled by a large number of genes. Equal resistance is shown to all pathogen races. While, it may not totally protect plant from infection, and the actual level of resistance is mediated by environment, but it does reduce susceptibility and slows down the establishment of the pathogen. A high horizontal resistance is useful in crop cultivars to slow down the

spread of disease. It has been argued that horizontal resistance is an artifact and in fact corresponds to vertical resistance. Additionally, it is likely that the molecular mechanism of resistance is the same (Isaac, 1992; Vanderplank, 1968).

2.4.3 Genetic resources for resistance

Due to the increasing demands on agricultural resources through an increasing world population the putative sources of resistance have become even more valuable material for plant breeding. One of the first important for breeding for *A. flavus* resistance is must to have the resources of genetic resistant or the resistant genotypes of groundnut (Allard, 1966). In conventional resistant breeding, the major source of resistant germplasms comes from the gene pool of the crop plant itself. Resistances can be found in lines from other breeding programs in the same or different geographic areas and can be crossed into lines with the desired genetic background. Gene banks containing many different plant accessions, collection from various geographic regions, can also be very valuable resource for resistant germplasms. The International Crops Research Institute for the Semiarid Tropics (ICRISAT) in India maintains the largest world groundnut germplasms collection of over 12,000 accessions (Stalker, 1997). While, landraces in general, native plant or wild varieties are very important sources of genetic variability for resistance breeding and usually consist of mixtures of various genotypes. The conservation of the genetic diversity present in this genetic material is one of the most important tasks not only for breeder but also for society in general.

Most sources of resistance to soil-borne fungi in groundnut show low levels of resistance or tolerance. Such partial resistance is presumably governed by polygenic and is assumed to be similar to horizontal resistance (Fry, 1982). Upadhyaya *et al.* (2004) identified resistant cultivars at ICRISAT to aflatoxin-producing fungi in three types; resistance to pod infection (pod wall); resistance to seed invasion and colonization (seedcoat); and resistance to aflatoxin production (cotyledons). Sources of all the three types of resistance have been reported. These included Shulamit and Darou IV for resistance to pod infection, PI337394, PI337394F, PI337409, GFA1, GFA2, UF71513, Ah7223, J11, Var27, U 4-47-7, ICGV88145, ICGV89104, ICGV91283, ICGV91278, ICGV91284, ICG239, B95, B88, B99-1 and ICG2946 for resistance to seed colonization and U 4-7-5, VRR245, B99-1 and B95 for resistance to aflatoxin production (Ghewande *et al.,* 1993; Mehan, 1989; Mixon, 1980; Mixon and Roger, 1973; Rao *et al.,* 1995; Upashyaya *et al.,* 2001; Zambettakis *et al.,* 1981). These reported only the post harvest resistant that resistance to pod wall infection, seed colonization and aflatoxin production by cotyledons properties. However, the pre-harvest resistant expressions are also the one important criterion to develop the resistance genotypes. Some of seed resistant genotypes, PI337394F, PI337409, GFA1, GFA2, UF71513, Ah7223 and J11, were reported to have considerably lower natural seed infection by *A. flavus* than various seed susceptible genotypes (Mehan, 1989).

2.4.4 Methods used in breeding for resistance (Isaac, 1992)

Breeding methods require that levels of disease resistance be screened to allow comparison between the levels of resistance achieved. Assessments must be carefully carried out and can often be labor intensive and time consuming. Viable,

standardized inoculum is required and trials must be conducted under controlled, uniform conditions in order to avoid interaction with the environment that might interfere with the interpretation of the results. It is therefore necessary to understand the mechanism of host invasion and disease establishment to reliably test for disease (the percentage plants infected), the severity of the disease (the proportion of the total plant affected) and may also allow the identification of differences between lesion size and type, which may reflect the reaction of the plant. There were concluded that the main methods used in the breeding for disease resistance are considered below.

Mass selection

Mass selection is the simplest method used for breeding for resistance. Seed from the most highly resistant plants is used to grow future generations. Plants with high levels of resistance, and other characters where these are important, are selected from a self-pollinating population and the progeny are then bulked together for a further growth cycle and subsequent selection. The individuals are heterozygous and therefore there is always likely to be some degree of variation between them. This method is straightforward to carry out but the level of resistance increases only slowly through each consecutive generation. In cross-pollinated plants like groundnut there is no control over the pollen source so that recurrent selection for resistant and advantageous plants operates slowly over successive generation.

Pure line selection

Pure lines (on pedigrees) are selected from the progeny of highly resistant homozygous plants. These are tested for resistance and other characters and those individuals with desirable features are then multiplied. In this way the genetic properties of new lines can be well defined. For self-pollinating species off-types that carry useful characters arise occasionally, probably as a result of mutation or hybridization within the crop. Such plants can also be selected and multiplied, to great effect. This is a widely used method of breeding for resistance.

Back-crossing

Desirable characters from parental varieties can be combined by crossing plants. Enabling useful traits to be transferred to the progeny. Those resultant individuals are tested for resistance and then backcrossed to standard cultivars. Eventually, over successive generations of backcrosses the level of resistance becomes stabilized and is expressed within the useful cultivars. This system is more easily controlled with cross-pollinated crops and has the advantage that several forms of resistance may be transferred simultaneously. Back-crossing is used to introduce resistance into a susceptible cultivars but it is often a lengthy process taking many years. If the resistance to be bred in is a dominant character then backcrossing will retain all traits of the parents plus that resistance. If the resistance is a recessive character then the first generation of backcross progeny (F1) must be selfed to enable the selection of resistant plants. This method is not used in cases where resistance is a multigene character.

F_1 hybrids

Inbred lines with high levels of resistance can be produced from cross-pollinating species by selfing or sib mating (brother × sister crosses) which have been selected for resistance. First generation (F_1) hybrids of homozygous lines carrying different genes for resistance are then used. Two separate lines are selfed (inbred parental lines) and are then crossed. The F_1 hybrid progeny are heterozygous for most alleles, which may enhance the level of disease resistance. A new F_1 hybrid must be produced each year for new crop planting since the seed from any progeny cannot be guaranteed to retain the resistance.

Mutations

Mutations in the chromosome complement of plants do occur naturally and can give rise to useful variants. However, the process is random and very slow (1 in 10^4 to 10^6 alleles per locus). It is possible to induce mutations in selected material by the use of chemical agent. e.g. colchicines, or ultraviolet radiation. A range of chromosome deletions and additions may occur. Colchicine treatment disrupts the movement of chromosomes at cell division and results in changes in the chromosome number of progeny. Diploids (2n) and triploids (3n) contain two or three complete sets of chromosomes, respectively. Plants with three or more sets of chromosomes are termed polyploids. Autopolyploids contain multiples of haploid or diploid chromosome numbers. Aneuploid plants contain multiples of the normal chromosome number with either extra or fewer chromosomes, as in trisomics (2n+1) and tetrasomics (3n+2) which contain the diploid chromosome number plus one or two chromosomes respectively and monosomics (2n-1) which have the diploid number less one chromosome from a pair.

Many different changes are possible and although large changes are likely to be lethal alterations in chromosome number may enhance levels of resistance.

2.4.5 The use of resistant cultivars

Resistant varieties are very useful for disease control but must be carefully planted in order to avoid problems, which arise from the so-called durability of that resistance (Issac, 1992). The durability of resistance in a particular variety is modified by interactions between the host and the pathogen. It is important to understand that the breakdown of resistance in a crop is not as the result of changes in the host or in host resistance but is a reflection of adaptations in the pathogen. The presence of resistant plants increases the selection pressure on the potential fungal pathogens in that area and eventually new strains, or races, appear (by mutation) which are able to overcome the host resistance presented. The speed at which this occurs depends on the level of variability, and mutation rate in the pathogen but may occur relatively rapidly for some species and in some conditions.

Lines carrying vertical resistance are useful if they are planted in controlled, confined areas. However, one such variety planted over a large acreage may unduly raise the selection pressure on the pathogen population at that site. If a new pathogen race develops which can overcome those particular resistance characters expressed then the crop will succumb to the pathogen and infections of severe epidemic proportions may occur. The vertical resistance can be very effective in annual crops, which have a relatively short life span. It is also effective when directed towards races of the pathogen which do not spread very easily or which are relatively genetically stable, i.e. in which there is little genetic change.

In general terms immunity to disease is not normally required. Horizontal resistance provides plants with incomplete protection but such resistance is more durable in agricultural situations. This non-specific resistance operates against all races of the pathogen and will be maintained where the pathogen is present since there is always positive selection pressure on the potential host plants to retain it (Allard, 1966a).

The widespread planting of a genetically uniform cultivar has advantages in terms of the uniformity of ripening, harvesting and quality of a crop and may be required for commercial purposes. However, the generation of a new race of pathogen will eventually result in the infection of resistant plants within such a stand. Infected individuals then act as an inoculum source and in a genetically uniform crop the pathogen may subsequently spread in epidemic proportions (Lucus, 1998). The same effect may occur with the influx of an imported or air-born pathogen. More genetically heterogeneous crops are less likely to suffer in this way. Although some plant may be immediately susceptible it is not likely that all will become infected. A lower inoculum potential is therefore generated. Interspersed plants, which are more resistant, also act as barriers to the rapid spread of the disease.

It can be seen therefore that the planting of mixtures of cultivars, which are similar in their characteristics but which are derived from a number of lines containing many resistance genes, is sound agricultural practice. Commonly used varieties must usually be replaced after 3-5 years after widespread planting, to guard against the development of epidemics. Multilines are also used. These are isogonics lines, derived by backcrossing individuals to a common parent and differing in the vertical resistance, which is carried.