Chapter 6

General discussion

Screening methods

The mechanisms of *A. flavus* invasion and the groundnut responses to invasion are desired to identify the type of host resistance. Interestingly, it has been possible to visualize growth of the fungus in tissue by encoding glucoronidase (GUS) reporter gene into the fungus (Xu *et al.,* 2000). Nevertheless, visualization of fungal growth within tissue requires incubation of the sample with GUS substrate that is time consuming and prevents following real time colonization of tissue (Brown *et al.,* 1995). Future research is required other the method that can be distinguished by accelerated, provided rapid and effective documentation of plant-fungal interaction. This thesis presented the novel screening method for identifies the resistant genotypes that showing the plant-fungal interaction. In accordance with the beneficial fluorescent light microscope was brought into play aniline blue fluorescence and hematoxylin staining (AFHS) method as Chapter 3. Of considerable is the observation that the infected tissue fluorescence in groundnut varied with genotypes. *A. flavus* grew and spread faster in a susceptible genotypes than in a resistance one. One possibility is that the retarded colonization of the host tissue by the pathogen was probably conditioned by a physiological factor. Factors that could influence this include the morphological alterations in the growing of groundnut pod after infection at the ultrastructural level and chemical components of resistant and susceptible groundnut genotypes. Presumably, the fluorescence in the seedcoat tissues was the

fragment of the fungus because of seedcoat structure was completed growth or alterable chemical production. It was the mechanism of resistance of seedcoat barrier, which act as some instances such structural and biochemical characters are already present in plant prior to pathogen invasion as preformed (passive resistance) resistance mechanisms (Isaac, 1992; Lucus, 1998). In corn (*Zea mays* L.), phenolic compounds in kernel wax were associated with the inhibition of *A. flavus* infection and aflatoxins production (Gembeh *et al.,* 2001). The large molecule weight of protein, 14 kDa trypsin inhibitor content and amount of kernel cuticle were potential sources of *A. flavus* resistance in drought-tolerant maize genotypes (Tubajika and Damann, 2001). Effects of volatile aldehydes from corn inhibited *A. parasiticus* radial growth (Wright *et al.,* 2000). Further, calcium nutrition was influence to groundnut seedcoat thickness under liming application that decreased seed infection by *Aspergillus* spp. and *Penicillium* spp. (Fernandez *et al.,* 1997). However, the fluorescence in groundnut peg was not only caused of the fungal fragment, but also aflatoxins accumulation and/or the alterable plant responsiveness. The previously researches found that aflatoxins-producing strains were associated with a higher frequency of bright greenish fluorescence of corn kernel sample (Wicklow, 1999). The fluorescence is produced by the oxidative action of heat-labile enzymes (peroxidase) in living plant on kojic acid, which is formed with aflatoxins by *A. flavus* (Marsh *et al.,* 1969). For defensive 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). A previous study on pearl millet (*Pennisetum glaucum*) found that seedlings responding to invasion by the downy

mildew pathogen *Sclerospora graminicola* strongly suggested that lignin and callose deposition were host structural responses for cultivar resistance. A time-course study showed that the accumulation of the polymers in cell walls of resistant seedlings was rapid and localized around the pathogen, apparently to restrict pathogen entry (Kumudini and Shetty, 2002). 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. Thus, the screening for resistance to infection by the way of mechanism of plant-fungal interaction was required the pre-harvesting determination in the field environment when the plant is growing.

Variation of resistance to *Aspergillus flavus*

Although researchers have not been able to locate germplasms lines which show complete resistance to aflatoxins-producing fungi. It was expected that the levels of resistance could be improved further by pyramiding resistance genes from different and diverse sources. The current study found that these groundnut population genotypes variably resisted to infection by *A. flavus* along with genotypes in peg screening, seed screening and AFHS methods. Percent-infected peg was positive correlation with percent-infected seed but not with AFHS method (Table 6.1). It was indicated that almost the low-infected peg genotypes were less seed infection. Thus, the pre-harvest resistance supported to post-harvest resistance.

Four genotypes were less seed and peg infection, percent infection 0.0 to 30.0, identified as resistant group. However, other remaining genotypes were separated as susceptible group, percent infection from 30.0 to 100.0 (Figure 6.1). These peg and seed screening method were evaluated the amount of infected peg or seed while AFHS method was determined the area of infection of each peg. Hence, the characteristics of resistances are likely to be very different between these two methods to screening. Although, in this study AFHS method was not associated the many chemicals concentration and the quantitative screening methods, peg and seed screening methods (Table 6.1). However, only AFHS method was effective to show the mechanisms of resistance to the fungus. Further work is needed to screen the resistant genotypes to *A. flavus* by this AFHS method.

Table 6.1 Correlation coefficient of percent infection with calcium and tannins in seedcoat and peg of groundnut.

	% Seed Infection	Seedcoat Ca	Aerial Peg Ca	Soil Peg Ca	Seedcoat Tannins	% Area Fluorescence
% Peg Infection	0.3654*	-0.1304	-0.5188**	-0.5998**	-0.3185	0.1777
% Seed Infection		-0.5261**	-0.1795	-0.1645	-0.4451**	0.1848
Seed Ca			0.1742	0.1586	0.0719	-0.0448
Aerial Peg Ca				0.7420**	-0.0589	-0.0027
Soil Peg Ca					0.1838	-0.1815
Seed Tannins						-0.2309

*,** Correlation significant level at p = 0.05 and 0.01 respectively.

Compared with J_{11}, standard genotype, which is a widely grown cultivar in western and center India and improved for resistance to *A. flavus* seed infection and seed colonization by *A. flavus* by ICRISAT (Mehan and McDonald, 1980). Most groundnut genotypes in this research evaluated were susceptible to *A. flavus* infection both pre-harvest and post-harvest periods, including ICGV91066 and KK4, susceptible under pre-harvest inoculation, post-harvest uninoculation and inoculation condition. Thus, in this work, calcium and tannins contents in both genotypes less than J_{11} genotype. Calcium concentration in seedcoat and peg and tannins in seedcoat were negative correlated to *A. flavus* infect groundnut (Table 6.1). Factors that could influence this include the chemical concentration in seedcoat and peg structures. Presumably, concentration of calcium in seedcoat and peg and tannins in seedcoat suppressed *A. flavus* infection to groundnut. However, there were genotypes resistances to infection by the fungus, consistently those from ICRISAT derived resistant genotypes e.g. ICGX990093 and ICGX990094. The two lines resulted from two single cross, involving ICGV91278 × ICGV86590 for ICGX990093 and ICGV91279 × ICGV86590 for ICGX990094. Thus, ICGV91278 and ICGV91279 have shown superior performance for yield and resistant to *A. flavus* seed infection in Thailand and Vietnam (Mehan and Gowda, 1997; Upadhyaya *et al.*, 2001). Interestingly, J_{11} genotypes also have been reported to resistant to seed infection in Thailand (Thailand Coordinated Groundnut Improvement Program, 1985). Additionally, in this screening, US120 genotype illustrated better resistance to the infection than those standard genotypes, J_{11}. In contrast to ICGV91066 and KK4 susceptible genotypes, US120 contained high calcium and tannins content in seedcoat and peg structures. Interestingly, it has been possible to increase the Ca concentration

in the groundnut seedcoat threefold by lime application to soil (Fernandez *et al.*, 1997). This suggests that lime application influenced to seedcoat thickness, leading to decrease in seed infection by *A. flavus*. Further research is needed on Ca transporter and other genes to improve the Ca concentration of the peg, seedcoat or groundnut structures. Since genetically modified groundnut may not be acceptable to all con summers, exploration of alternative strategies, such as those explored in this thesis, are warranted. Of considerable interest is the observation that the accumulation of Ca in groundnut peg and seedcoat structure varied with genotype.

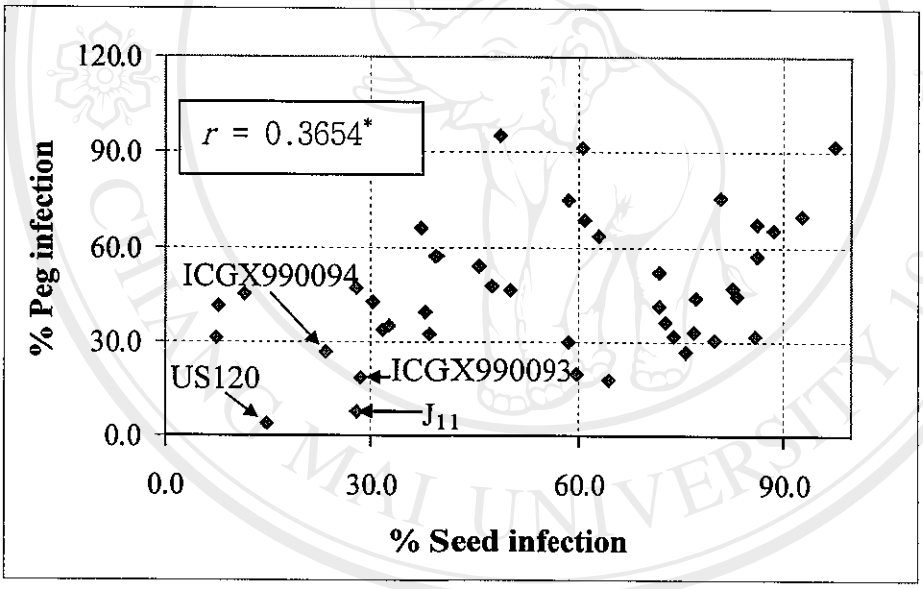

Figure 6.1 Correlation of percent seed infection and percent peg infection.

Several drought-tolerant groundnut genotypes were found among the resistant to *A. flavus* infection and aflatoxins production. This study found resistance to *A. flavus* infection in drought-tolerant groundnut genotype such as 511CC and 419CC was not tolerant. However, several drought-tolerant genotypes were susceptible to colonization and subsequent contamination by aflatoxins (Mehan, 1989; Mehan *et al.*,

1987). Thus, water stress alone was not responsible to *A. flavus* invasion or aflatoxins production since stressed groundnuts were not always contaminated (Sanders *et al.*, 1985). Further, Dicken *et al.*, (1973) demonstrated that the lesser cornstalk borer, commonly associated with water and often contributing to the aflatoxins problem, was not always involved since kernels from pods without damage often contained aflatoxins. In addition, the water deficit stress influenced to the epicuticular wax load on leaves reduces surface transpiration and thus improves crop water use efficiency (Samdur *et al.*, 2003). In similarity, the accumulation wax of kernel may be associated with the drought stress that inhibited to the invasion by *A. flavus*. Therefore, these high accumulation wax or drought-tolerance genotypes should be useful in future research to improved by breeding programs.

In addition to the genotype, groundnut resistant to *A. flavus* infection was affected differently by the fungus density (Chapter 4). The virulence of the strain, together with the size of the inoculum received by plant, will affect the rate of disease development (Isaac, 1992). Thus, under inoculated condition, high density or large size of the inoculum, groundnut in this research represented the higher infection by *A. flavus* than in uninoculated condition. The stages of growth, location, and type of tissue infected and the degree of inherent resistance of the plant to that pathogen will also affect the outcome of infection.

Inheritance of resistance to *Aspergillus flavus*

Inheritance of resistance to pre-harvest *A. flavus* infection, combining ability was investigated in groundnut populations obtained from half-diallel crossing among 5 parental lines. The selection of the parents for breeding programs was one of the

aims of this study. Thus, the estimate of the general combining ability (g_i) of a parent in the diallel is an importance indicator of its potential for generating superior breeding populations. A low g_i estimate, positive or negative, indicates that the mean of a parent in crossing with the other does not differ greatly from the general mean of the crosses. On the contrary, a high g_i estimate indicates that the parental mean is superior or inferior to that general mean. This represents a strong evidence of favorable gene flow from parent to offspring at high frequency and gives information about the concentration of predominantly additive genes (Mather and Jinks, 1971; Srivastava *et al.,* 1976). Thus, crosses involving with greater estimates of general combining ability should be potentially superior for the selection of lines in advances generations.

In present results, the general combining ability (GCA) mean square were significant for all traits, which shows the variability of GCA of the parents. The specific combining ability (SCA) mean squares were also significant, except for percent-infected peg. When the SCA mean squares were not significant, the performance of a single-cross progeny could be adequately predicted on the basis of GCA (Baker, 1978). There was indicated that the importance of additive gene effects was the main cause of the observed genetic variation for percent-infected peg. However, the highly significant of GCA as well as SCA in percent-infected peg area fluorescence indicates the importance of both the additive and the non-additive gene effects. The significant effect of SCA and high magnitude of its variance component is a clear evidence of the elevated diversity of genetic material under study. These materials should have complementation and could show transgressive individuals in generation under selection. The existence of

significant effect for GCA indicates the possibility of genetic gain to be obtained through the selection practice over the segregant population due to interracial crosses.

Large positive GCA values with high percent-infected peg of KK4 (70.0) was effected to its progeny had high percent infection, J_{11} × KK4 (42.2), ICGX990093 × KK4 (46.4), ICGX990094 × KK4 (62.4) and ICGV91066 × KK4 (43.3). Large negative GCA with low percent-infected peg of J_{11} (7.4) was also inherited to its progeny, J_{11} × ICGV91066 (8.4) and J_{11} × ICGX990094 (21.8). It was indicated effective transmission of genes for these traits from parent to their offspring (Bdliya and Burris, 1988). Predominance of positive GCA effects indicated that selection based on the performance of individual lines should be effective in improving resistant groundnut peg to infection by *A. flavus* using peg screening method. While, for percent-infected area fluorescence, based on these considerations and estimates of the SCA effects, it can be inferred that the hybrids from J_{11} × KK4, ICGV91066 × ICGX990093 and KK4 × ICGX990094 performed outstandingly for resistance to *A. flavus* infection using AFHS screening method. Thus, hybrid combinations with low percent-infected area fluorescence, with the favorable SCA estimates and involving at least one of the parents with high negative GCA, would tend to increase concentration of favorable alleles, a situation of great interest to the breeder. The resistant genotypes could be improved through hybridization and selection using the good combiner, negative GCA of parents and less percent infection with identified in this study. However, to elimination aflatoxins in groundnut not only a matter of resistance genotypes but also need to fond solution via the implementation of the optimizing agriculture

practical managements and/or crop modeling strategies that can minimizing its impact for grower in both pre and post-harvesting.