Table of Contents

	Page
Acknowledgements	**iv**
Abstract (English)	**vi**
Abstract (Thai)	**x**
Table of Contents	**xiv**
List of Tables	**xvii**
List of Illustrations	**xix**
Abbreviations and Symbols	**xxiii**
Chapter 1 General Introduction	1
Chapter 2 Literature Review	**6**
2.1 Groundnut or Peanut	**6**
2.1.1 Botany	8
2.1.2 Ecology	11
2.1.3 Cultivation and management	12
2.2 *Aspergillus flavus* associated with aflatoxin production	**13**
2.2.1 *Aspergillus flavus*	13
2.2.2 Aflatoxins	16
2.3 Mechanism of plant resistance to pathogens	**21**
2.3.1 Passive defense mechanisms	22
2.3.1.1 Preformed structure barriers	23
2.3.1.2 Preformed biochemical compounds	24
2.3.2 Active defense mechanisms	28
2.3.2.1 Induced structural barrier	28
2.3.2.2 Induced biochemical responses	30
2.4 Breeding for resistance	**39**
2.4.1 The gene-for-gene hypothesis	39
2.4.2 Variable system of plant–pathogen interaction	42
2.4.3 Genetic resources for resistance	45
2.4.4 Methods used in breeding for resistance	46
2.4.5 The use of resistant cultivars	50

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Chapter 3 Screening Procedures **52**

3.1 Introduction **52**

3.2 Materials and methods **53**

3.2.1 Aniline blue fluorescence and hematoxylin staining (AFHS) method 53

3.2.1.1 Preliminary finding 53

3.2.1.2 Preparation of suspension of *Aspergillus flavus* 56

3.2.1.3 Source of seeds 56

3.2.1.3 Post-harvest determination 57

3.2.1.4 Preharvest determination 58

3.2.2 Peg screening method 60

3.2.2.1 Optimum time for surface sterile 60

3.2.2.2 Appropriate time after inoculation for assessing resistant genotypes by peg screening method 61

3.2.2.3 Appropriate time after inoculation for assessing resistant genotypes by AFHS technique 62

3.2.3 Seed screening method 63

3.2.3.1 Optimum time for surface sterile 63

3.2.3.2 Appropriate time after inoculation for assessing resistant genotypes by seed screening method. 63

3.3 Results **65**

3.3.1 Aniline blue fluorescence and hematoxylin staining (AFHS) method 65

3.3.2 Peg screening method 69

3.3.2.1 Optimum time for surface sterile 69

3.3.2.2 Appropriate time after inoculation for assessing resistant genotypes by peg screening method 70

3.3.2.3 Appropriate time after inoculation for assessing by AFHS technique 72

3.3.3 Seed screening method 75

3.3.3.1 Optimum time for surface sterile 75

3.3.3.2 Appropriate time after inoculation for assessing resistant genotypes by seed screening method 76

3.4 Discussion **79**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Chapter 4 Genotypic variation for resistance to *Aspergillus flavus* of groundnut germplasms **83**

4.1 Introduction **83**

4.2 Materials and Methods **85**

4.2.1 Source of groundnut genotypes 85

4.2.2 Pre-harvest determination: Peg screening method 85

4.2.3 Post-harvest determination: Seed screening method 88

4.2.4 Minerals analysis in groundnut peg and seedcoat 88

4.2.5 Tannins analysis in groundnut peg and seedcoat 90

4.3 Results **91**

4.3.1 Pre-harvest determination 91

4.3.2 Post-harvest determination 101

4.4 Discussion **109**

Chapter 5 Inheritance of *Aspergillus flavus* Resistance In Groundnut **112**

5.1 Introduction **112**

5.2 Materials and Methods **114**

5.2.1 Groundnut germplasms and screening 114

5.2.2 Statistic analysis 115

5.2.2.1 Analysis of variance 115

5.2.2.2 Combining ability analysis 116

5.3 Results **118**

5.3.1 Analysis of variance 118

5.3.2 Combining ability of *Aspergillus flavus* infection pegs by peg screening method and AFHS method 121

5.4 Discussion **125**

Chapter 6 General Discussion **129**

Screening methods **129**

Variation of resistance to *Aspergillus flavus* **131**

Inheritance of resistance to *Aspergillus flavus* **135**

Reference **139**

Curriculum Vitae **156**

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

List of Tables

Table	Description of Table	Page
2.1	Oral acute toxicity LD_{50} value of aflatoxin B_1 for anumber of animal species	19
2.2	Interspecies differences in liver cancer induction by aflatoxin B_1 ingestion (Wogan, 2000)	19
2.3	A selection of maximum tolerated levels of aflatoxin B_1 in food (Moss, 2002)	20
3.1	Dehydrate and transfer to paraffin using TBA as the intermediate solvent, 1 night / step.	58
3.2	Percentage of *Aspergillus flavus* fluorescence in seed coat tissues after staining with aniline blue fluorescence and hematoxylin and separate the fluorescent position by Quacos program	67
3.3	The percentage *A. flavus* contamination after varies time for surface sterile pegs by 3 % sodium hypochlorite in J_{11} and Tainan9 groundnut genotypes	70
3.4	Percentage of contaminated peg after vary the incubation times (days) from 0 to 15 days of six groundnut genotypes	72
3.5	Percentage of infected peg area fluorescence after vary the incubation times (days) of six groundnut genotypes	74
3.6	Percentage of contaminated seed after surface sterilized by 3 % sodium hypochlorite of J11 and Tainan9 groundnut genotypes.	76
3.7	Percentage of contaminated seed after vary the incubation times (days) of 11 groundnut genotypes	78
4.1	Groundnut genotypes reaction to colonization by *Aspergillus flavus* and obtainable source	86
4.2	Percentages and Resistant Rate of groundnut pegs contaminated by *Aspergillus flavus* under uninoculation and inoculation conditions	92
4.3	Percentage of pegs infection and area fluorescence of peg tissue of 43 groundnut genotypes	94
4.4	Concentration of calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), ion (Fe), zinc (Zn) and total tannins content in groundnut peg	97

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Table	Description of Table	Page
4.5	Percentages and Resistant Rate of groundnut seed contaminated by *Aspergillus flavus* under uninoculation and inoculation conditions	103
4.6	Percent infected seed, calcium, copper, magnesium and total tannins content in seedcoat of groundnut genotypes	106
5.1	Analysis of variance for Method 2 giving expectation of mean square for the assumptions of Model II	116
5.2	Percentage of infected peg of 5 parents and their F1 using peg screening and AFSH method	120
5.3	Analysis of variance of groundnut peg resistance t o *Aspergillus flavus* infection by peg screening method	121
5.4	Diallel crosses: percent-infected peg by *A. flavus* using peg screening method Griffing's^{+} combining ability, Method II, Random Model	121
5.5	Analysis of variance of groundnut peg resistance t o *A. flavus* infection by AFHS method	122
5.6	Diallel crosses: percent-infected peg area fluorescence by *A. flavus* using AFHS method Griffing's^{+} combining ability, Method II, Random Model	122
5.7	Estimates of general combining ability (GCA) of percent-infected pegs by peg screening method and percent-infected pegs area fluorescence by AFHS method	123
5.8	Estimates of general combining ability (GCA), specific combining ability (SCA) and percent-infected pegs area fluorescence by AFHS method based on diallel analysis	124
5.9	Estimates of general combining ability (GCA), specific combining ability (SCA) of percent-infected pegs by peg screening method based on half-diallel analysis	125
6.1	Correlation coefficient of percent infection with calcium and tannins in seedcoat and peg of groundnut	132

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

List of Illustrations

Figure	Description of Figure	Page
2.1	Groundnut center of origin (solid line), area of intensive cultivation (dotted line) and areas of maximum cultivation (shaded) (Weiss, 2000)	6
2.2	Groundnut harvest area, yield and production trend in the world since 1961 to 2001 (FAO, 2003)	7
2.3	World area of groundnut production in year 2003 (FAO, 2003)	7
2.4	World production of groundnut in year 2003(FAO, 2003)	8
2.5	Groundnut plant	9
2.6	Compound leaves (A), flower (B), and pegs structure(C), pods (D) and seed color (E) of groundnut	10
2.7	Colonies of *Aspergillus flavus* on M3S1B selective medium	14
2.8	Conidiophores (A), conidial head (B) and conidia (C) of *Aspergillus flavus*	14
2.9	The aflatoxins biosynthesis pathway (Bennett *et al.*, 1994)	17
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)	22
2.11	Vertical sections through leaf tissue to show the arrangement of cell and intercellular spaces (Isaac, 1992)	24
2.12	Distribution and relative concentration of each cell-wall component in the mature cell wall (Huang, 2001)	26
2.13	Chemical structures of some phenolic compounds that are induced in plants as a response to fungal attraction	32
2.14	Signal molecules implicated in the induction of plant defense, systemin, salicylic acid, acetylsalicylic acid and dichloroisonicotinic (DCINA)	33

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Figure	Description of Figure	Page
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)	35
2.16	Quadratic check of gene combinations and the resulting different interaction types in gene-for-gene interaction (Keller *et al.*, 2000)	41
2.17	Molecular model of the gene-for-gene interaction (Keller *et al.*, 2000).	43
2.18	Vertical (A) and horizontal (B) resistance	44
3.1	Long-section by hand of groundnut embryos after flowering 3 days, A; under normal light and B; under UV light microscope	54
3.2	Cross (A.) and long (B.) section by rotary microtrome and stained with hematoxylin of groundnut embryos under normal light microscope	54
3.3	Long-section of groundnut flower after stained with aniline blue (A.) and safanin O (B.) and groundnut embryos after stained with aniline blue (C.) and Hematoxylin (D.) under UV light microscope	55
3.4	The 10 days *Aspergillus flavus* growth on M3S1B selective medium	56
3.5	Infected seedcoat of groundnut by *Aspergillus flavus* were cut	57
3.6	Inoculated pegs were incubated in growth chamber at 30 °C and 100 % relative humidity	59
3.7	Inoculated pegs on M3S1B selective medium under normal light (A) and UV light (B)	61
3.8	Inoculated seeds on M3S1B selective medium under normal light (A) and UV light (B)	64
3.9	The hyphae (H), conidia (C) and conidiophores (CP) of *Aspergillus flavus* under normal light (A) and UV light (B) microscope	65

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Figure	Description of Figure	Page
3.10	Infected seed coats after staining with aniline blue fluorescence and hematoxylin of J_{11} (A) and Tainan9 (B) under normal light and under UV light of J_{11} (C) and Tainan9 (D), outer epidermis (OE), inner epidermis (IE), *Aspergillus flavus* fluorescence (AF) and dark plant position (DP)	66
3.11	Cross section of groundnut pegs and stained with aniline blue fluorescence and hematoxylin under UV light microscope, *Aspergillus flavus* infected positions: AI, vascular bundles: VB and embryo: EM	68
3.12	Percent infection by *Aspergillus flavus* of J_{11} and Tainan9 groundnut genotypes under inoculation and uninoculation condition	69
3.13	The effects of incubation time after inoculation to percent-infected pegs by *Aspergillus flavus* in six groundnut genotypes	71
3.14	The effects of incubation time after inoculation to percent-infected peg area fluorescence by *Aspergillus flavus* in six groundnut genotypes	73
3.15	Percentage of groundnut seed contaminated by *Aspergillus flavus* after varies the time for surface sterile by 3 % sodium hypochlorite under uninoculation and inoculation treatments	75
3.16	The effects of incubation time after inoculation to percent-infected seeds by *Aspergillus flavus* in eleven groundnut genotypes	77
4.1	Inoculated seeds on M3S1B selective medium under normal light (A) and UV light (B)	89
4.2	Percentages of groundnut peg contaminated by *Aspergillus flavus* under uninoculation and inoculation conditions	91
4.3	The correlation of percent peg infection with calcium (Ca) concentration in groundnut aerial pegs and soil pegs	100

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Figure	Description of Figure	Page
4.4	Percentages of groundnut seed contaminated by *Aspergillus flavus* under uninoculation and inoculation conditions	102
4.5	The correlation of calcium (Ca) and total tannins (Tannins) concentration of groundnut seed coat with percent seed infection	108
6.1	Correlation of percent seed infection with calcium and tannins in seedcoat and peg of groundnut	132

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Abbreviations

AA	Atomic absorption
AFHS	Aniline blue fluorescence and hematoxylin staining
Ca	Calcium
CT	Condensed tannins
Cu	Copper
Fe	Iron
GCA	General combining ability
GFP	Green fluorescence protein
GUS	β-glucuronidase
HS	Highly susceptible
Mg	Magnesium
Mn	Manganese
MR	Moderate resistance
R	Resistance
S	Susceptible
SCA	Specific combining ability
UV	Ultraviolet
Zn	Zinc

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved