Chapter 5

Inheritance of *Aspergillus flavus* Resistance in Groundnut

5.1 Introduction

The diallel mating design is an important scheme used for crop breeding. Anlysis of diallel experiments is used to estimate heterosis and combining ability to select superior crosses and parents. The type of gene action for a trait in a population can be ascertained by estimation of additive and dominance variance (Burow and Coors, 1994). Griffing (1956) proposed four methods to analyze the combining abilities by using the genetic estimates of the parents and hybrid components of a diallel analysis, represented by general (GCA) and specific combining ability (SCA). Considering the GCA and SCA effects, inferences can be made about additive or non-additive gene effects. The GCA of each parent (*gi*) should be examined when the objective is the development of superior genotypes while the SCA effects (*Sij*) provides information about hybrid performance (Pace *et al.*, 1998). According to Griffing (1956), the SCA of a parent with itself (*Sii*) have great genetic significance and indicates the existence of unidirectional dominance. Negative *Sii* values indicate that deviations are predominantly positive, and vice-versa. The magnitude of *Sii* is indicative of varietal heterosis and their additive values express the mean values of such heterosis.

Therefore, this analysis allows broad inferences on the nature of the gene effect for a characteristic under selection. Breeding programs can take advantage from this information to find the best selection strategy to transfer desirable traits between gene

pools. There are only few published reports on inheritance of resistance to *A. flavus* infection in groundnut, which estimate combining ability. Heritability of resistance to *A. flavus* is very complex with diverse inheritance mechanisms reported, including additive, dominance, modifier genes, epistasis and recessive resistance (Guiyuan and Xuanqiang, 2004; Mongkolsiriwat, 1998; Rao *et al.,* 1989). Nevertheless, the available information on the heritability, dominance, gene interactions and other genetic parameters associated with *A. flavus* resistance in groundnut is far from complete. Therefore, this research was carried out of the following objectives:

- To examine the combining ability of groundnut peg resistance to *A. flavus* infection to understand the type of gene action governing resistance to the fungus infection.
- To identify groundnut lines suitable for use as parents in a disease resistance-breeding program, in crosses between five parental genotypes and hybrid of half-diallel crosses, using peg screening and aniline blue fluorescence and hematoxylin staining (AFHS) methods.

5.2 Materials and Methods

5.2.1 Groundnut germplasms and screening

Five groundnut genotypes, three resistant (J_{11}, ICGX990093 and ICGX990094) and two susceptible (ICGV91066 and KK4) genotypes to *A. flavus* infection were selected from Chapter 4 and hybridized by half-diaelle crosses. The inheritances of resistant to *A. flavus* were evaluated in five parental genotypes and 10 F_1 hybrids using peg screening method and aniline blue fluorescence and hematoxylin staining (AFHS) technique. Five parental genotypes and 10 F_1 hybrids were planted in 5-liter pots soil. A complete randomized design with four replications was used. Each groundnut genotypes was sprayed with aflatoxin producing *A. flavus* conidial suspension (prepared earlier as section 3.2.1.2) at peg stage. Pots of groundnut were kept in growth chamber at 30 °C under 98 ± 2 % relative humidity. The pegs were collected at seven days after inoculation. Each replicated peg was surface sterilized by 3 % sodium hypochlorite for 5 minutes and then soaked three times with sterile deionized water. After that, the sterile pegs were placed on M3S1B medium. A half of peg was also observed with the novel screening technique, aniline blue fluorescence and hematoxylin staining (AFHS) technique as described in Chapter 3.

5.2.2 Statistic analysis

5.2.2.1 Analysis of variance

The randomized block trial were used for analysis of variance for parents and hybrid genotypes following:

DF	SS	MS	VR	EMS
Genotypes				$\sigma_e^2 + b\sigma_g^2$
Replicate				$\sigma_e^2 + a\sigma_r^2$
Error				σ_e^2
Total				

In reference to:

$$\text{SS Genotypes} = \frac{\sum Yi.^2}{b} - \frac{(\sum yij^2}{ab}$$

$$\text{SS Replication} = \frac{\sum Y.j^2}{a} - \frac{(\sum yij^2}{ab}$$

SS Error = SS Total – SS Genotypes – SS Replication

$$\text{SS Total} = \sum\sum yij^2 - \frac{(\sum yij)^2}{ab}$$

Lest significant design (LSD) were used to compare the variance ratio (VR) of genotypes and replication of parents and hybrid genotypes.

5.2.2.2 Combining ability analysis

The mathematical model for the combining ability analysis following Griffing (1956) Method 2 Model II is

$$x_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{b}\sum_k b_k + \frac{1}{b}\sum_k (bv)_{ijk} + \frac{1}{bc}\sum_k \sum_l e_{ijkl}$$

where all effects except μ are considered to be random variables.

μ = mean of population

i, j = 1.....p = number of parents

k = 1.....b = replication or blocks

l = 1.....c = number of individual in each replication

g_i, g_j = effect of GCA (general combining ability) of parents i or j

s_{ij} = effect of SCA (specific combining ability) of the hybrydization between i and j genotypes

bv_{ijk} = interaction between genotypes ij and replication k

b_k = effect of replication k

e_{ijkl} = effect of each observation error

Table 5. 1 Analysis of variance for Method 2 giving expectation of mean square for the assumptions of Model II.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	Expectation of Mean Square
General combining ability	p-1	S_g	M_g	$\sigma^2 + \sigma_s^2 + (p+2)\ \sigma_g^2$
Specific combining ability	$p(p-1)/2$	S_s	M_s	$\sigma^2 + \sigma_s^2$
Error	m	S_e	M_e	σ^2

In accordance with:

$$S_g = \frac{1}{p+2}\left\{\sum_i (X_{i.} + x_{ii})^2 - \frac{4}{p} X_{..}^2 \right.$$

$$S_s = \sum_{i \le j}\sum x_{ij}^2 - \frac{1}{p+2}\sum_i (X_{i.} + x_{ii})^2 + \frac{2}{(p+1)(p+2)} X_{..}^2$$

$$M_e^{'} = \frac{M_e}{b}$$

M_e = mean square of error in analysis of variance

b = replication

The following F ratios are used for testing hypotheses pertaining to the different variance components. To test $\sigma_g^2 = 0$ use

$$F_{[(p-1),p(p-1)/2]} = M_g / M_s$$

and to test $\sigma_s^2 = 0$ use

$$F_{[p(p-1)/2,m]} = M_s / M_e^{'}$$

The variance components may be estimated as followed:

$$\hat{\sigma}_g^2 = \frac{1}{p+2}[M_g - M_s],$$

and

$$\hat{\sigma}_s^2 = M_s - M_e^{'}.$$

The effects of combining ability may be estimated as followed:

$$\hat{\mu} = \frac{2}{p(p+1)} X_{..},$$

$$\hat{g}_i = \frac{1}{p+2}\left[X_{i.} + x_{ii} - \frac{2}{p} X_{..} \right],$$

and

$$\hat{S}_{ij} = x_{ij} - \frac{1}{p+2}\left[X_{i.} + x_{ii} + X_{.j} + x_{jj} \right] + \frac{2}{(p+1)(p+2)} X_{..}.$$

5.3 Results

5.3.1 Analysis of variance

Percent-infected peg and percent-infected peg fluorescence by *A. flavus* of parents and their F1 crosses are given in Table 5.2. Analysis of variance showed highly significant differences for both characters, percent-infected peg and percent infected peg area fluorescence, between parents and their crosses in F1 generations. While, the groundnut genotype J_{11} showed the lowest average for percent-infected peg and percent infected peg area fluorescence being 7.4 and 0.02 % respectively following by ICGX990093 and ICGX990094. Two parents, genotype KK4, of Khon Kaen source, and ICGV91066 had high percent infection as 39.5 and 70.0 % for percent-infected peg and 2.18 and 3.94 % for percent-infected peg area fluorescence.

Among all the crosses for *A. flavus* infection peg, J11 × ICGV91066 showed the highest resistance followed by J11 × ICGX990094 and ICGX990094 × ICGV91066 (Table 5.2), while ICGX990093 × ICGX990094 showed the highest susceptibility followed by ICGX990094 × KK4 (percent infection of 8.4, 21.8, 23.5, 71.8 and 62.4 respectively). In contrast for the percent-infected peg area fluorescence by AFHS method, J11 × ICGV91066 showed high infection of 2.27 % like ICGV91066 parent. The crosses J11 × ICGX990094 showed the highest resistance to *A. flavus* infection, whereas ICGX990094 × KK4 showed the highest susceptibility followed by ICGX990093 × KK4 (percent-infected peg area fluorescence of 0.05, 3.30 and 3.12 respectively).

Table 5.2 Percentage of infected peg of 5 parents and their F1 using peg screening and AFSH method.

Genotypes	Percentage of infection	
	Peg screening method	AFHS method
J11	7.4	0.02
ICGX990093	26.6	0.26
ICGX990094	26.3	0.02
ICGV91066	39.5	2.18
KK4	70.0	3.94
J11 × ICGX990093	31.9	0.45
J11 × ICGX990094	21.8	0.05
J11 × ICGV91066	8.4	2.27
J11 × KK4	42.2	0.48
ICGX990093 × ICGX990094	71.8	0.15
ICGX990093 × ICGV91066	29.0	0.16
ICGX990093 × KK4	46.4	3.12
ICGX990094 × ICGV91066	23.5	0.37
ICGX990094 × KK4	62.4	3.30
ICGV91066 × KK4	43.3	1.72
Mean	34.66	1.23
$LSD_{0.05}$	14.28	1.16
$LSD_{0.01}$	18.96	1.52

5.3.2 Combining ability of *A. flavus* infection pegs by peg screening method and AFHS method.

The diallel analysis of variance based on Griffing's Method 2 (Griffing, 1956), mean square of crosses involving 5 parents and 10 F1 crosses by peg screening method are showed in Table 5.3. The estimates of GCA effects were significant differences ($p < 0.01$) but not for SCA effects of the crosses. The variance components of GCA and SCA on *A. flavus* infection were 148.30 and 24.47 respectively (Table 5.4).

Table 5.3 Analysis of variance of groundnut peg resistance t o *Aspergillus flavus* infection by peg screening method.

	df	SS	MS	F
GCA	4	4359.04	1089.759	21.10**
SCA	10	516.36	51.636	1.90ns
Error	74		27.1643	
CV = 33.87 %				

**, ns: Significant at $p \leq 0.01$; not significant at the $p \leq$ 0:05, by F-test, respectively.

Table 5.4 Diallel crosses: percent-infected peg by *A. flavus* using peg screening method Griffing's+ combining ability, Method II, Random Model.

	Variance*	SE
GCA	148.30	1.762
SCA	24.47	3.597
Error	27.16	5.212

* SCA > GCA means non-additive genetic effect; SCA < GCA means additive genetic effects.
+ Griffing (1956)

For percent-infected peg area fluorescence by AFHS method showed significant differences ($p < 0.01$) in both GCA and SCA effects (Table 5.5) that the variance components were 0.4943 and 0.7566 respectively (Table 5.6).

Table 5. 5 Analysis of variance of groundnut peg resistance t o *A. flavus* infection by AFHS method.

	df	SS	MS	F-value
GCA	4	17.565	4.391	25.1777**
SCA	10	9.307	0.931	5.3383**
Error	107		0.1744	
CV = 60.14 %				

** Significant at $p \leq 0.01$

Table 5.6 Diallel crosses: percent-infected peg area fluorescence by *A. flavus* using AFHS method Griffing's[+] combining ability, Method II, Random Model.

	Variance*	SE
GCA	0.4943	0.141
SCA	0.7566	0.288
Error	0.1744	0.499

* SCA > GCA means non-additive genetic effect; SCA < GCA means additive genetic effects.

[+] Griffing (1956)

The estimated values of GCA effects of each parental population of percent-infected peg and percent- infected peg area fluorescence are presented in Table 5.7. The GCA effects of the parental lines for percent-infected peg ranged from –13.72 (J_{11}) to 20.43 (KK4). Both J_{11} and KK4 were highly significant but the other three genotypes, ICGX990093, ICGX990094 and ICGV91066 were not significant at p = 0.05. However, only KK4 revealed positive GCA effects. For the percent- infected peg area fluorescence for the GCA effects ranged from –0.58 (J_{11}) to 1.30 (KK4). The GCA effects were significant for four of the five parents, three of which were negative. While, only KK4 genotypes were exposed highly significant with the positive GCA effects.

Table 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.

Parents	Percent-infected peg	Percent-infected peg area fluorescence
	GCA	GCA
J11	-13.72**	-0.58*
ICGX990093	-3.27	-0.43*
ICGX990094	-2.27	-0.50*
ICGV91066	-1.17	0.21
KK4	20.43**	1.30**

*, ** Significant at $p \leq 0.05$; significant at $p \leq 0.01$ respectively

The estimates of the GCA and SCA effects of their crosses with respect to the percent-infected pegs area fluorescence by *A. flavus* are presented in Table 5.8. Several of the crosses in the diallel exhibited significant SCA effects for the percent-infected pegs area fluorescence. Among these, three crosses (J_{11} × KK4, ICGX990093 × ICGV91066 and ICGV91066 × KK4) showed significant negative SCA effects. The highest negative SCA effect for percent-infected pegs area fluorescence was observed in the cross, J_{11} × KK4. However, the hybrid J_{11} × ICGV91066 for instance showed the highest positive effects of J_{11} × ICGV91066 SCA. The estimates of the GCA and SCA effects of their crosses with respect to the percent-infected pegs by *A. flavus* are presented in Table 5.9.

Table 5.8 Estimates of general combining ability (GCA), specific combining ability (SCA) of percent-infected pegs area fluorescence by AFHS technique based on half-diallel analysis.

Parents	General combining ability, GCA and Specific combining ability, SCA				
	J_{11}	ICGX990093	ICGX990094	ICGV91066	KK4
J_{11}	-0.58*	0.23	-0.10	**1.41****	**-1.47****
ICGX990093		-0.43*	-0.16	**-0.85***	**1.02****
ICGX990094			-0.50*	-0.57	**1.27****
ICGV91066				0.21	**-1.02****
KK4					1.30**

Diagonals represent GCA effects; figures above diagonals represent SCA effects of the crosses. Correlation coefficient between the SCA effects and the corresponding mean was 0.71. Standard errors: S.E.(g_i) = 0.14; S.E.(g_i- g_j) = 0,22; S.E.(S_{ij}) = o.29; S.E.(S_{ij}- S_{ik}) = 0.55; S.E.(S_{ij}- S_{kl}) = 0.50.

*, ** Significant at $p \leq 0.05$; significant at $p \leq 0.01$ respectively

Table 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.

Parents	General combining ability, GCA and Specific combining ability, SCA				
	J_{11}	ICGX990093	ICGX990094	ICGV91066	KK4
J_{11}	-13.72**	7.09	3.17	**-11.37***	0.84
ICGX990093		-3.27	2.66	-1.23	-5.44
ICGX990094			-2.27	-7.77	**9.60***
ICGV91066				-1.17	6.04
KK4					20.43**

Diagonals represent GCA effects; figures above diagonals represent SCA effects of the crosses. Correlation coefficient between the SCA effects and the corresponding mean was 0.71. Standard errors: S.E.(g_i) = 0.14; S.E.(g_i- g_j) = 0,22; S.E.(S_{ij}) = o.29; S.E.(S_{ij}- S_{ik}) = 0.55; S.E.(S_{ij}- S_{kl}) = 0.50.

*, ** Significant at $p \leq 0.05$; significant at $p \leq 0.01$ respectively

5.4 Discussion

Comparison of percent-infected peg and peg area fluorescence for parents and F1 crosses population indicated that the variation in the infection of *A. flavus* reaction ranged from susceptible to resistant (Table 5.2). The LSD test has also permitted the individuation of those hybrid combinations really superior, at a 0.01 % significance level, compared to the parents for each character analyzed. The parent J_{11} was highest resistant for both percent-infected peg and peg area fluorescence. While, J_{11}can be considered high resistant genotype parent in both peg screening and AFHS methods, ICGX990093 and ICGX990094 had moderate resistant capacity (26.6 and 26.3 % from peg screening method and 0.26 and 0.02 % from

AFHS method respectively). Two parents, KK4 and ICGV91066 had the susceptibility in both screening methods. And J_{11} progeny, the best resistant were observed in the hybrids J11 × ICGV91066 for percent-infected peg and J11 × ICGX990094 for percent-infected peg area fluorescence (8.4 and 0.05% respectively). Hence, genotypes J_{11}, a commercial cultivar in India and is reported to be resistant to *Aspergillus* spp. infection (Kisyombe *et al.*, 1985; Mehan and McDonald, 1980; Mongkolsiriwat, 1998).

The GCA mean squares were significant for both percent-infected peg and percent-infected peg area fluorescence at 0.01 % significance level (Table 5.3 and 5.5). The SCA mean squares were also significant, except for percent-infected peg. As reported by Baker (1978), when the SCA mean squares were not significant, the performance of a single-cross progeny could be adequately predicted on the basis of GCA. Significant of GCA is caused mainly by additive effects, even though non-additive effects may also involved (Baker, 1978). Thus, the percent-infected peg from peg screening method was caused primarily by additive effects. Variance components of GCA and SCA on peg screening method were 148.30 and 24.47 respectively (Table 5.4), confirming that additive genetic effects play a major role in the inheritance of *A. flavus* infection resistance on percent-infected peg. On the contrary, for percent-infected area fluorescence the non-additive gene effects seem more important, in promoting a positive transgressive expression of these parameters in the F_1 hybrid. In addition, the mean squares of GCA were always higher than those of SCA. The ratios of mean squares, 2GCA/(2GCA+SCA), were o.98 for percent-infected peg and 0.90 for percent-infected peg area fluorescence. These ratios were close to 1, confirming the predominance of additive effects over non-

additive effects (Baker, 1978), and giving additional evidence that selection may be effective in the F_2 and later generations for the traits studied in these crosses. Du *et al.* (1999) reported similar finding for genetic analysis of resistance to *Stagonospora nodorum* (Berk) in wheat. It is encouraging that additive effects were prevent for these traits because it would be difficult to exploit the non-additive effects, since hybrids are not used in groundnut. Therefore selection should be delayed to later generation. For this specific characteristic, breeding methods, which explore the additive portion of the genotypical variance, may be utilized for obtaining genetic gains.

The estimates of GCA effects of individual parents are presented in Table 5.7. Negative values indicate a contribution towards resistance while positive values represent that for susceptibility. J_{11} had a negative GCA value both for percent-infected peg and percent-infected peg area fluorescence, showing their capacity to transmit resistance, whereas the GCA effects of KK4 was positive, showing their capacity to transfer susceptibility to *A. flavus* infection groundnut pegs. It was indicated that J_{11} and KK4 were the good combiner for resistance and susceptibility respectively in both peg screening and AFHS methods and ICGX990093 and ICGX990094 were the good combiner only in AFHS screening method. For seed infection by *A. flavus* in groundnut Mongkolsiriwat (1998) also have been reported that J_{11} genotype was the significant negative GCA effects, good combiner for resistance to *A. flavus* infection seed. The GCA value of ICGX990093, ICGX990094 and ICGV91066 were not significant difference in percent seed infection indicated poor combiner for this character resistance.

The SCA effects for the percent infected pegs area fluorescence ranged from -1.47 (J_{11} × KK4) to 1.41 (J_{11} × ICGV91066). This indicates that J_{11} × KK4 had the most resistant combination while J_{11} × ICGV91066 had the most susceptible combination (Table 5.8). Based on these results, J_{11} seem to be good donor for resistance to *A. flavus* infection. Parents with positive GCA effects produced hybrids with negative SCA effects as in case of ICGV91066 × KK4 (-1.02). A complementary gene action might be envisaged to be responsible for such finding (Srivastava *et al.,* 1978). In general, crosses involving high × low general combiners exhibited negative SCA effects, as in ICGV91066 (low) × KK4 (high). Such situation might be due to mutual suppression of heterosis components (Pace *et al.,* 1998). These represent the deviation of a hybrid as compared with the expected performance based on the GCA of the parents. Thus, high SCA values indicate a tendency to influence the hybrid performance besides that expected on the basis of the GCA of the parents. However, also for the performance of the hybrid combinations and estimates of the effects (S_{ij}) of the specific combining ability between the two parents the correlation is very high, confirming the potential of Diallel scheme.