GENETICS OF RESISTANCE TO *ASPERGILLUS FLAVUS* Link ex Fries IN GROUNDNUT *(Arachishypogaea* L.)

BY

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DECLARATION

I hereby declare that this thesis entitled “GENETICS OF RESISTANCE TO *ASPERGILLUS FLAVUS*Link ex Fries IN GROUNDNUT (*Arachishypogaea* L.)” is the result of my own research work. No part of this thesis has previously been submitted for a degree or any other qualification. The information derived from literature has been duly acknowledged in the text and explicit references are given.

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The above declaration is confirmed

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CERTIFICATION

This thesis entitled ‘‘GENETICS OF RESISTANCE TO *ASPERGILLUS FLAVUS* Link ex Fries in groundnut (*Arachishypogaea* L.)by DEBORAH BALA meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is affectionately dedicated to the memories of my late mother, Mrs Alack John Bobai for being the best mother in the whole world.

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ABSTRACT

The mode of inheritance to surface seed colonization by *Aspergillusflavus* Link ex Fries and aflatoxin contamination in groundnut (*Arachishypogaea* L.) was studied from four groundnut varieties. Two varieties were reported to be resistant (J11 and 55-437) and the remaining two susceptible (Samnut23 and Samnut 22). Each resistant variety was crossed to the two susceptible varieties in a bi-parental mating design in the screen house of the Institute for Agricultural Research, Ahmadu Bello University, Samaru Zaria. Six genetic populations (P_1, P_2, F_1, F_2, BCP_1 and BCP_2) were developed which were evaluated for surface seeds colonization by inoculation with conidia of *Aspergillusflavus* in the laboratory. The inoculated seeds were incubated at 28^{o}Cfor 8 days, at the end of which the seeds were rated for surface seeds colonization. Aflatoxins were extracted from the inoculated seeds by column chromatography and analysed for aflatoxin content by thin layer chromatography (TLC). The data collected were subjected to analysis of variance by the general linear model (GLM) of the statistical analysis systems (SAS). The data was also subjected to scaling tests to test the adequacy of the additive –dominance model. The A,B,C scaling tests used to test the adequacy of the additive -dominant model to explain the within population variation were not significant, indicating that the model was adequate. There was significant variation among the parental genotypes for the mean surface seed colonization by *Aspergillusflavus*

and aflatoxin content.The genotypes J11 and 55-437 earlier reported to be resistant to *invitro* seed colonization by *Aspergillusflavus* showed moderate to high surface seed colonization and different levels of aflatoxin contamination. The means of the parents in relation to their progenies revealed preponderance of dominance gene effects.The number of effective factors estimated for surface seed colonization and aflatoxin content ranged from 1 to 2 respectively.Frequency distribution of the F_2 and backcross populations and the number of effective factors segregating for the two parameters studied indicated monogenic inheritance. Heritabilitywas also estimated for surface seed colonization and aflatoxin content.Broad sense heritability estimates ranged from 42.92% to 85.30 % and 56.31 % to 84.46 % for surface seed colonization by *Aspergillusflavus* and aflatoxin content, respectively. Narrow sense heritability also ranged from 9.38 % to 33.91 % and 18.85 % to 54.82 % for the two parameters studied. The moderate to high heritability estimates revealed that the traits are highly heritable and selection can be practiced to improve them.

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ABBREVIATIONS/SYMBOLS

A.B.U	Ahmadu Bello University
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BCP_1	F_1 x Parent 1-Backcross
BCP_2	F_1 x Parent 2-Backcross
CV	Coefficient of Variation
Df	Degrees of freedom
ELISA	Enzyme-Linked Immunosorbent Assay
FAO STAT	Food and Agriculture Organisation Statistics
F_1	First filial generation
F_2	Second filial generation
FSCAF	Field Seeds colonization by *Aspergillusflavus*
GLM	General Linear Model
H^2	Broad sense heritability

…………………………...	
h^2	Narrow sense heritability
……………………………	
HPLC……………………….	High Performance Liquid Chromatography
IAR………………………….	Institute for Agricultural Research
ICRISAT……………………	International Crops Research Institute for the Semi- Arid Tropics
IVSCAF……………………..	*Invitro* Seeds Colonization by *Aspergillusflavus*
PAC…………………………	Pre-harvest Aflatoxin contamination
P_1**…………………………….**	Parent 1
P_2**…………………………….**	Parent 2
$\overline{P}_1, \overline{P}_2, \overline{F}_1, \overline{F}_2, \overline{B}_1 \overline{B}_2$**…….**	Mean values of the measurements in therespective populations
R^2**……………………………**	Coefficient of determination
SAS………………………….	Statistical Analysis System
S. E………………………….	Standard Error
TLC…………………………	Thin Layer Chromatography
UV…………………………...	Ultra violet
V_A, V_B, V_C, V_D…………….	Variances of the A, B,C, D scales
&g/kg………………………..	Microgram per kilogram
µl…………………………….	Micro litre
σ^2 **…………………………...**	Variance

CHAPTER ONE

INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an important annual legume that probably originated in Bolivia, South America. It is now widely grown in many tropical, sub-tropical and temperate countries. It is the sixth most important oilseed in the world, cultivated in over 100 countries on about 23.4 million hectares with a total annual production of 47.77 million tons worldwide (FAO, 2008). Developing countries constitute 97% of the global area and 94% of the global production of groundnut (Ntare *et al*., 2008). The production is concentrated in Asia and Africa (56% and 40% of the global area and 68% and 25% of the global production, respectively).

Groundnut is an important cash crop in several countries. It plays an important role in the livelihoods of poor rural farmers and rural economies of many developing countries (Nigam *et al*., 2009). It generates 60%, 42% and 21% of rural cash earnings among groundnut producers in Senegal, Niger and Nigeria, respectively and accounts for about 70% of rural employment in Senegal (Ndjeunga, *et al.*, 2006). Groundnut is an important source of food worldwide and constitutes an inexpensive source of easily digestible protein (26-28%), high quality edible oil (48-50%), and a rich source of dietary fiber, minerals and vitamins (Ntare *et al.*, 2008).

Groundnuts are frequently infected with fungi that produce mycotoxins during and after harvesting which affect the quality and safety of human food (Martin *et al*., 1999). *Aspergillus*, *Fusarium* and *Penicillin* are the main genera of fungi that produce mycotoxins in food (Pitt, 2000). Groundnuts are prone to infection by two closely related fungal species: *Aspergillus flavus* and *Aspergillus parasiticus*. Both species produce highly toxic mycotoxins known as aflatoxin (Waliyar *et al.,* 2000). However, *Aspergillus flavus* is the most common species found in groundnut and groundnut by-products worldwide. Aflatoxin contamination of groundnut is a serious quality problem in many tropical and sub tropical countries in the world (Narasimhulu, 2007). Field infection by the aflatoxigenic fungus can lead to serious post harvest aflatoxin contamination. The moisture and heat stress during pod development, damage to the pods by insect pests and nematodes and other injury during cultural operations facilitates pre-harvest seed infection (Mehan *et al.,* 1991) while improper harvest and storage practices lead to high levels of post harvest aflatoxin contamination. The infection mainly occurs before the crop is harvested in the semi-arid tropics, particularly under late season drought stress conditions. In wet and humid areas, infection is predominantly post harvest. *Aspergillus flavus* can invade groundnut in the field before harvest, during post harvest drying and curing, and in storage and transportation. According to Klich (2007), *Aspergillus flavus* is an opportunistic pathogen of crops. It is an extremely common soil fungus. In culture, the fungus is characterized by fast growing yellow-green colonies, usually 65-70mm in diameter after 7 days growth in the dark at 25^{o}C on Czapek yeast.

The problem of aflatoxin contamination in peanut was first recognized in 1960 following outbreaks of Turkey –x disease in the United Kingdom (Nigam *et al*., 2009). Aflatoxins are carcinogenic and toxic secondary metabolites. Chemically, there are four major aflatoxins namely: B_1, B_2, G_1 and G_2 with the letters referring to the color of their fluorescence under ultra violet light (Blue or Green) (Klick, 2002). Aflatoxin B_1 is considered to be the most important because it is the most toxic and has been classified by the International Agency for Research on cancer as a probable human carcinogen (Xue 2004). In many developing countries, aflatoxin is a major health risk to humans and animals.

The aflatoxin contamination does not affect crop productivity but it makes the produce unfit for consumption as toxins are injurious to health. The marketability of contaminated produce, particularly in the international trade is diminished to nil due to stringent standards of permissible limits on aflatoxin contamination set by importing countries, (Uphadhyaya *et al.,* 1997). The United States Food and Drug Administration permits maximum aflatoxin levels of 20ppb in peanut products destined for human consumption; the European Union allows 3 ppb of total aflatoxin and 2 ppb on aflatoxin B_1. This has a tremendous impact on the peanut industry (Xue 2004). In the United States alone, the mean economic loss from mycotoxins is estimated to be 932 million United States dollars. Nigeria and most other African countries have lost out in the international market of groundnut due to aflatoxin contamination.

Management of aflatoxin in peanuts is complex. Aflatoxin contamination can be minimized by adopting good cultural, produce handling and storage practices. However, these practices are not widely adopted by the small scale farmers in the developing countries which contribute about 60% to the world groundnut production. One of the possible means of reducing aflatoxin contamination of groundnut is the use of cultivars resistant to seed invasion or aflatoxin production by aflatoxin producing fungi. Therefore, breeding for resistance to *Aspergillus flavus* and/or aflatoxin production will play a very significant role in preventing aflatoxin contamination in groundnut and consequently the associated economic losses and health hazards (Upadhyaya *et al.*, 2002).

Thus, this study was initiated with the following objectives:

1. To determine the mode of inheritance of resistance to surface seed colonization by *Aspergillus flavus* and aflatoxin production.
2. To estimate heritability for resistance to surface seed colonization by *Aspergillus flavus* and aflatoxin production.

The information derived from this study will be useful in planning an effective breeding program in order to incorporate durable resistance to *Aspergillus flavus* and subsequent aflatoxin contamination in groundnut.

CHAPTER TWO

LITERATURE REVIEW

2.1 AFLATOXIN CONTAMINATION

Groundnuts (*Arachis hypogaea L*) are prone to infestation by *Aspergillus flavus*, which produces highly toxic mycotoxins known as aflatoxins (Waliyar *et al.,* 2000). The development and use of resistant varieties is considered the most economic and effective approach of controlling aflatoxin contamination in groundnut. The use of resistant cultivars has an economic impact on more than 200 million dollars annually for US peanut producers. Rao and Tupule (1967) were the first to report varietal differences in resistance to aflatoxin contamination in groundnut. According to Upadhyaya *et al.* (2002), the alleviation of aflatoxin contamination through genetic manipulation has been attempted since mid-1970s. Significant progress has been achieved; however these efforts have not resulted in complete eradication of aflatoxin contamination. Mixon and Rogers (1973) were the first to suggest the use of resistant cultivars to contain the problem of aflatoxin contamination in peanut. They developed a laboratory method to screen peanut genotypes for resistance to seed invasion and colonization by *Aspergillus flavus* and *Aspergillus parasiticus* using rehydrated mature seeds. They reported two Valencia genotypes, PI 337394F and PI 337409 as having high levels of resistance to *in vitro* seed colonization (IVSC) by both pathogens (Nigam *et al.,* 2009).

2.2 TYPES OF RESISTANCE

Resistance to aflatoxin producing fungi may be of three types: resistance to pod infection (pod wall), resistance to seed invasion and colonization (seed coat) and resistance to aflatoxin production (cotyledons) (Upadhyaya *et al.,* 2002). Xue *et al.* (2004) defined four mechanisms of resistance to *Aspergillus flavus* namely: resistance to *in vitro* seed colonization by *Aspergillus flavus* (IVSCAF), field resistance to seed colonization to *Aspergillus flavus* (FSCAF), resistance to pre-harvest aflatoxin contamination (PAC) and resistance to aflatoxin production.

The fungi have to penetrate the pod wall and the seed coat to reach the cotyledon from which they derive their sustenance. Resistance to infection is attributed to pod shell structure, while resistance to seed invasion and colonization is mostly physical and is correlated with thickness, density of palisade layers, absence of fissures and cavities and presence of wax layers (Upadhyaya *et al.,* 2002). According to Nigam *et al.* (2009), the pathogen has to cross two barriers before it can reach the cotyledons to derive its sustenance. The first interaction between the pathogen and the host is at the pod wall which is the physical barrier and resistance is attributed to pod shell structure. The second barrier to the pathogen is the seed coat. This resistance is attributed to thickness and density of palisade layers, presence of wax layers and absence of fissures and cavities (Pettit *et al.,* 1989; Liang *et al.,* 2006). Mechanisms of *in vitro* seed colonization to *Aspergillus flavus* (IVSCAF) resistance is a combination of physical and chemical characteristics of the seed testa.

It was realized that significant invasion of apparently undamaged peanut pods by *Aspergillus flavus* and subsequent aflatoxin contamination can occur in the field before harvest (Davidson *et al.,* 1983; Mehan *et al.,* 1986). The moisture and heat stress during pod development, damage to the pods by insect pests and nematodes, and other injury during cultural operations facilitate pre-harvest seed infection (Mehan *et al.,* 1991). Once infection is established, it cannot be undone through post harvest operations or in storage. Following appropriate drying, curing and storage practices, post harvest aflatoxin contamination can be minimized. However, these practices will fail if the aflatoxin is already present in the seed before drying and the storage. Thus resistance to pre-harvest aflatoxin contamination remains an important issue in breeding for resistance to aflatoxin contamination. If resistance to pre-harvest aflatoxin contamination is found, then much of the problem will be solved because it will also confer resistance to post harvest aflatoxin production.

2.3 SOURCES OF RESISTANCE

Breeding for resistance to *Aspergillus flavus* is predicated on high level resistant sources and reliable assessment methods. In earlier screening of germplasm accessions for reaction to *in vitro* seed colonization at ICRISAT Patancheru, India, resistance of three genotypes PI337394F, PI337409, and UP71513 was confirmed and six new sources of resistance (Ah78223, J11, U4-47-7, Var. 27, Faizpar and Monir 240-30) were identified (Mehan *et al.*, 1989). According to Nigam *et al.* (2009), some accessions such as ICG1326, ICG3336, ICG3700, ICG4749 and ICG7633 showed consistent resistance to seed infection in India and Senegal. Most of these accessions were also screened for resistance to *in vitro* seed colonization. Accessions ICG1326, ICG3263, ICG3700, ICG4749, ICG4888, ICG7633 and ICG9407 also showed resistance to *in vitro* seed colonization. According to Xue (2004), many sources of resistance have been identified by different screening methods. Among the many resistance reported, the resistance in J11, PI337394F, PI337409, UF71513, Ah7223, Faizpur1-5 and Var.27 has been confirmed by testing over locations. All these sources exhibited resistance to *in vitro* seed colonization, field seed colonization and pre-harvest aflatoxin contamination. Drought resistant line 55-437 has also been used in Senegal as a source resistance (Rao *et al.,* 1989).

2.4 RELATIONSHIPS BETWEEN TYPES OF RESISTANCE

There are many conflicting reports on the relationships among different types of resistance. For example the relationships between *in vitro* seed colonization, natural seed infection, aflatoxin production in the field and their contribution in reducing aflatoxin contamination are not understood (Nigam *et al.,* 2009). Majority of the invitro seed colonization resistant lines tested by Mehan *et al* (1987, 1988) did not show resistance to pre-harvest seed infection. *In vitro* seed colonization and seed infection in the field are reported to be two independent genetic events (Utomo *et al.,* 1990; Upadhyaya *et al.,* 2001). Xue *(*2004) in a study to test the interaction between peanut genotypes and strains of *Aspergillus* species reported that strains within species differed significantly from one another with respect to fungal growth and aflatoxin production. A similar report was given by Mehan *et al*. (1982), Wilson and Bell (1984), and Nagarajan and Bhat (1973). Similarly, no correlations were reported between aflatoxin content, colonization of seed or shells and population density of *Aspergillus flavus* in the soil (Will *et al.,* 1994).

Poor correlations were reported between *in vitro* seed colonization to *Aspergillus flavus* (IVSCAF) and field seed colonization by *Aspergillus flavus* (FSCAF) resistance (Kisyombe *et al.,* 1985) or between IVSCAF and pre-harvest Aflatoxin contamination resistance

(Blankenship *et al.*, 1985; Davidson *et al.,* 1983; Anderson *et al.,* 1995). On the other hand, a significant positive correlation was found between IVSCAF resistance and FSCAF resistance (Zambettakis *et al.*, 1981) and between IVSCAF resistance and PAC resistance. Mehan *et al.* (1987) evaluated 11 genotypes (six resistant and five susceptible) to IVSCAF, for field resistance and seed colonization and for aflatoxin contamination. Significant positive correlations were found between IVSCAF resistance and FSCAF resistance and between FSCAF resistance and PAC resistance.

Mehan *et al.* (1989) reported that seven IVSCAF resistant genotypes had significantly greater field resistance to *A. flavus* and lower aflatoxin production but several IVSCAF- resistant breeding lines were highly susceptible in the field, while some IVSCAF- susceptible genotypes U4-7-5 and VRR-245 showed field resistance. They concluded that there was no consistent positive or negative relationship between IVSCAF resistance and FSCAF or PAC resistance. Poor correlation between IVSCAF resistance and post- harvest aflatoxin production was also observed by Mehan and McDonald (1983). It was reported that resistance to IVSCAF, FSCAF and aflatoxin production might be influenced by different genes. Conflicting results could be due to the presence or absence of different genes responsible for different types of resistance or due to differential gene function in different environments. The relationships among various types of resistance need to be better understood to enable breeders assemble the non–allelic genes influencing different resistance mechanisms in a single genotype.

2.5 GENETICS OF RESISTANCE

Very little is known about the inheritance of resistance to pre-harvest infection, *in vitro* seed colonization or aflatoxin production (Nigam *et al.,* 2009). Utomo *et al.* (1990) reported lack of significant relationship among the three resistance mechanisms and concluded that different genes governed them. Upadhyaya *et al.* (2002) also indicated that the three components of resistance are inherited independently. Xue (2004) reported predominantly non-additive genetic variance for aflatoxin production, suggesting that selection for this trait in early generations will be ineffective. The genetics of resistance mechanization has not been clearly established. The allelic relationship among various sources for each resistance trait needs to be elucidated to enable breeders to pyramid the non-allelic genes for each resistance mechanism.

2.6 HERITABILITY

Heritability is the proportion of phenotypic variation in a population that is due to genetic variation between individuals. Phenotypic variation among individuals may be due to genetic and/or environmental factors. Factors including genetics, environment and random chance can all contribute to the variation between individuals in their observable characteristics. Heritability is measured by estimating the relative contributions of genetic and non-genetic differences to the total phenotypic variation in a population. The main purpose of estimating heritability and the genetic parameters that compose heritability is to compare the expected gain from selection based on alternative selection strategies. One can use heritability to predict gain from selection (Holland *et al.,* 2003).

Heritability estimates are useful for comparing the gains from selection under different experimental designs and this information can be used to design optimal breeding strategies (Milligan *et al.,* 1990). Similarly, heritability based on different family structures derived from the same population can be compared to determine which family structure is best for maximizing genetic gain over units of time (Burton and Carver, 1993). Because heritability may vary among population, heritability estimates of different traits can be used to identify indirect selection schemes that may be more effective than direct selection schemes. Heritability could increase if genetic variation increases, causing individuals to show more phenotypic variation. On the other hand, heritability might also increase if the environmental variation decreases, causing individuals to show less phenotypic variation. Heritability increases because genetic factors are contributing more variation, or because non-genetic factors are contributing less variation. Heritability is used to indicate the relative degree to which a character is transmitted from parent to offspring. The magnitude of such estimates also suggests the extent to which improvement is possible (Omoigui, *et al,* 2006). Heritability can be determined with greater accuracy if it is studied with genetic advance. Two specific types of heritability can be estimated, the broad sense and narrow sense heritability. The broad-sense heritability is the ratio of total genetic variance to total phenotypic variance. The narrow-sense heritability is the ratio of additive genetic variance to the total phenotypic variance. Heritability does not indicate the degree to which a trait is genetic; it measures the proportion of the phenotypic variance that is the result of genetic factors.

Heritability of a character is important to the breeder because it provides him an idea of the extent of genetic control for the expression of a particular character. Moreover, heritability serves as a guide to the reliability of phenotypic variability in the selection programme and hence determines its success. According to Upadhyaya *et al.* (2002), there are only few published reports on inheritance of resistance to seed infection, *in vitro* seed colonization and aflatoxin production, which gives estimates of broad sense heritability and combining ability. A few published reports give information on broad sense heritability (low to moderate) and combining ability of resistance sources (Rao *et al.,* 1989; Utomo *et al.,* 1990; Upadhyaya *et al.,* 1997; Xue, 2004). Mixon (1976) estimated broad sense heritability for *in vitro* seed colonization resistance to be 75.5% in the F_2 generation of a cross between resistant genotype PI 337409 and susceptible line PI 331326. Upadhyaya *et al.* (1997) reported heritability estimates of 56-87% for pre-harvest seed infection. Utomo *et al.* (1990) reported broad sense estimates in F_2 derived F_6 population from two crosses between AR4 (Resistant) and NC7 (susceptible) as well as GFA2 (Resistant) and NC7 (susceptible).The heritability estimates from these crosses were 55 and 63% respectively, for seed colonization, 27 and 33% for pre-harvest seed infection, and 23 and 21% for aflatoxin production.

2.7 SCREENING METHODS

Reliable and consistent inoculation techniques are useful in the identification of sources of heritable plant resistance as well as facilitating selection within a population genetically variable for resistance (Innes, 1983). Evaluations of resistance to pre-harvest aflatoxin contamination are performed in the field where care must be taken to avoid damaging the seed and pod. *Aspergillus flavus* is not an aggressive pathogen and its ability to invade undamaged pods and seeds is strongly influenced by environmental conditions such as temperature and moisture during pod maturation and whether the soil contains high populations of toxigenic strains of *Aspergillus*. Thus field screening for pods resistant to invasion and aflatoxin production is difficult due to large variability in *Aspergillus* growth under field conditions, even with intensive management of soil water and inoculum levels (Holbrook *et al.*, 1997).

Artificial inoculation is frequently used when screening germplasm for resistance. The standard method for inoculation with *Aspergillus* has been a spore suspension in water applied at mid bloom. A laboratory inoculation method was developed at ICRISAT to screen peanuts for resistance to aflatoxin production (Mehan and McDonald, 1980). This method has been used in several post harvest screening programs.

Aflatoxin determination in groundnut can be approached in several ways. It is important to have analytical values that represent the total aflatoxin content. The analytical methods for mycotoxins include Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA) (AOAC, 2000).TLC, also known as flat bed chromatography or planar chromatography is one of the most widely used separation techniques in mycotoxins analysis. The aflatoxins are well suited for analysis by TLC since most of the compounds fluorescence strongly under long-wave UV light (Yazdani *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 EXPERIMENTAL SITES

The experiments were conducted at the Institute for Agricultural Research of Ahmadu Bello University, Samaru Zaria and the Federal University of Technology, Minna. The development of populations was carried out in the screen house while the inoculation of seed was done in the Mycology Laboratory of the Institute for Agricultural Research, Samaru Zaria. Zaria is located on latitude 11^{0} $11^{'}$ N and longitude7^{0} $38^{'}$E at altitude 686m above sea level in the Northern Guinea Savannah ecological zone of Nigeria. The study lasted between June 2010 and August, 2011. The extraction of aflatoxin and determination of aflatoxin content was carried out at the Biochemistry Laboratory of the Federal University Technology, Minna.

3. 2 EXPERIMENTAL MATERIALS AND DESCRIPTION

The experimental materials for this study were four groundnut varieties, namely:

55-437 (ICGV 1326), J11, Samnut 22 and Samnut 23. Two of these varieties (J11 and 55-437 were reportedly resistant to *Aspergillus flavus* while the other two (Samnut 22 and Samnut 23) were susceptible. The basic characteristics and distinguishing features of these varieties are given below:

Table1. Description of genetic materials used in this study

Genotype	Description
55-437	It is an early maturing variety with a vegetative cycle of 90 days. It is resistant to drought and seed colonization by *Aspergillus flavus*. The seeds are pink in color with oil content of 49%. It is adapted to the dry zones of West Africa.
J11	It is early maturing with a vegetative cycle of 90-100 days. It is resistant to seed infection and seed colonization by *Aspergillus flavus*. The seed color is tan. The oil content is between 42 and 45%. It is adapted to the dry zones of West Africa
Samnut 23	It is an early maturing variety with vegetative cycle of 90 to100 days. It is susceptible to *Aspergillus flavus.* The seed color is red with oil content of 53%. It is adapted to Sudan and Sahel savannah. Potential pod yield is 2.2 tones/ha. Potential haulm yield is 1.3 tonnes/ha.
Samnut 22	It is medium maturing variety with a vegetative cycle of 110 to 120 days. It is susceptible to *Aspergillus flavus.* The seed color is tan. The oil content is 45%. It is adapted to Sudan and Guinea savannah. Potential pod yield is 2.4 tones /ha while haulm yield is 4.0 tonnes/ha

3.3 POPULATIONS DEVELOPMENT

Crosses were made among the four parents using biparental mating design as described by Comstock and Robinson (1948). The resistant parents (J11 and 55-437) were used as the male parents while the susceptible parents (Samnut 22 and Samnut 23) were used as the female parents. Each resistant parent was crossed to susceptible both parents as shown below:

Susceptible Parents	Resistant Parents	
	J 11	55-437
Samnut 23	Samnut 23 x J 11	Samnut 23 x 55-437
Samnut 22	Samnut 22 x J 11	Samnut 22 x 55-437

The F_1 populations were advanced to F_2 as well as backcrossed to their parents to produce BCP_1 and BCP_2 respectively.

3.4 LABORATORY EVALUATION

The seeds of the parents, F_1, Backcrosses and the F_2 plants were screened in the laboratory for surface seed colonization by *Aspergillus flavus* and aflatoxin production.

3.4.1 Inoculation of Seeds

Thirty matured seeds with intact seed coat were selected from each population (F_1, F_2, BCP_1, BCP_2 and the parents) for inoculation. Seed testas were manually removed to eliminate the potential barrier to *Aspergillus flavus* growth. The experiment was conducted using a Completely Randomized Design (CRD), with three repetitions. Ten (10) seeds were used per each repetition. The seeds were surface sterilized by immersion in sodium hypochlorite solution for three minutes, then rinsed in sterile water. The samples were placed on sterile filter paper moistened with sterile water in Petri dishes. Each sample was inoculated with a suspension containing about $1x10^6$ conidia ml^{-1} of *Aspergillus flavus*. The samples were incubated for (8) eight days at 28ºC.

3.4.2 Scoring for Colonization

After the eight days of incubation, samples were removed from the incubator and rated separately for surface seed colonization by *Aspergillus flavus* and for colonization severity as shown in Table 2 using a scale given by Singh and Oswalt (1992).

Table 2. Scale for surface seed colonization rating.

Scale	Description	Interpretation
1	Sporulating growth on less than 15% of the seeds, with growth and sporulation sparse	Resistant
2	Sporulation on 16-30% of the seeds	Moderately resistant
3	Sporulating growth on 31-50% of seeds with dense sporulation	Susceptible
4	Sporulating growth on over 50% of seeds with dense sporulation	Highly susceptible

3.4.3 Determination of Aflatoxin Content

After the evaluation for colonization, samples were dried for one day at 60ºC and another three (3) days at 50ºC, ground to a friable meal and analyzed for aflatoxin content by Thin Layer Chromatography (TLC) using the procedure of the Association of Official Analytical Chemists (AOAC, 2000).

3.4.4 Extraction of Aflatoxin

Two grams ground sample was weighed and put into 250ml Erlenmeyer flask and 1ml 1M – phosphoric acid and 10ml of methylene chloride were added. The flask was shaken for 30 minutes and the content filtered with 18cm circle filter paper. The filtrate was collected in a flask. A column was set up with glass wool; 30ml methylene chloride (CH_2Cl_2) was poured in column and emptied half way. Anhydrous sodium sulphate (Na_2SO_4) was added, the sides were washed with methylene chloride. Silica gel was then added to the column and 20ml methylene chloride (CH_2Cl_2) added and emptied half way. Three (3) scoops of anhydrous sodium sulphate (Na_2SO_4) was added and drained off to top of column. The filtrate was added, drained off to top of column, 40ml n – Hexane was then added, drained to top of column. 40ml petroleum ether was also added and drained. Petroleum ether: Methanol: Distilled water in the ratio 48: 1.5: 0.5 was added and collected off column in a new beaker. The extract was evaporated to near dryness and put into vials for aflatoxin content determination by Thin Layer Chromatography.

3.4.5 Estimation of Aflatoxin Content by Thin Layer Chromatography

The aflatoxin sample extract was spotted side by side with the aflatoxin standard \`B_1, $B_{2,}$ G_1 and G_2 on pre-coated silica plates (20 x 20cm). 2&l, 5&l, 10&l, 15&l and 20µl of standard containing 2.6mg/kg of aflatoxin $B_{1,}$ 4.2mg/kg $B_{2,}$ 14.4mg/kg G_1 and 2.6mg/kg G_2 and the same quantity (2&l, 5&l, 10&l, 15&l and 20&l) of the sample extract were spotted on the TLC plates and developed for about 45 minutes in a TLC tank at room temperature. The plates were developed in 200mL Methylene chloride: Ethyl acetate: Iso propanol in a ratio 8: 1: 1 as the mobile phase, dried and further developed in 200mL Toluene: Ethyl acetate: Formic acid in a ratio 6: 3: 1. The plates were allowed to dry, and then examined in ultra violet (UV) cabinet, under the UV lamp. Aflatoxin G was not detected as all spots produced blue fluorescence indicating the presence of only aflatoxin B. The intensity of the sample spots was compared with that of the aflatoxin standard. Aflatoxin B concentration was calculated according to the formula of the Association of Official Analytical Chemists (AOAC 2000).

$$\& \text{g/kg} = \frac{S \text{ x } Y \text{ x } V}{X \text{ x } W}$$

Where:

S = Volume, in &l of aflatoxin standard of equivalent intensity to Z &l of sample.

Y = concentration of aflatoxin standard in &g/ml

X = Volume, in &l, of sample extract required, to give fluorescence intensity comparable to that of S &l of the standard.

V = Volume, in &l, of solvent required to dilute final extract.

W = Weight, in g, of original sample contained in final extract.

Aflatoxin data were log-transformed [$Y' = \ln(Y+0.5)$] to stabilize error variance and were subjected to analysis of variance.

3.4.6 Identity Confirmatory Test

Occasionally, fluorescent spots may appear which can be easily mistaken for aflatoxin. Therefore, chemical confirmation of identity of toxin from all positive test samples is essential. In this study, confirmation of the identity of aflatoxins was carried out by spraying the developed and dried plates with diluted sulphuric acid (H_2SO_4) in a ratio 1:3, which changed aflatoxin fluorescence from blue to yellow. Spots which do not turn yellow are positively not aflatoxin.

3.5 DATA COLLECTION

The following observations were recorded:

a. Number of seeds per Petri dish

b. Number of seeds with sporulating growths of *Aspergillus flavus* on their surfaces

c. Percentage of seeds invaded. This was calculated using the formula:

$$\% \text{ of seeds invaded} = \frac{\text{Number of seeds with sporulating growth}}{\text{Number of seeds per Petri dish}} \times 100$$

d. Aflatoxin B_1 content

e. Aflatoxin B_2 content

f. Total Aflatoxin content

3.6 STATISTICAL ANALYSES

3.6.1 Analysis of Variance

Data collected was subjected to Analysis of Variance (ANOVA) to estimate genetic variances using the General Linear Model of SAS (SAS, 2002). The mean values were separated using the Duncan Multiple Range Test.

3.7 GENETIC ANALYSES

3.7.1 Scaling Tests

The data collected were subjected to scaling tests based on the procedure outlined by Singh and Chaudhary (1985). Mather (1949) and Hayman and Mather (1955) proposed four types of scaling tests (A, B, C, and D) to test the adequacy of the additive – dominance model. If the model is adequate the values of A, B and C will each equal to zero within the limits their respective standard errors. These scales have the following effects.

$$A = 2\overline{B_1} - \overline{P_1} - \overline{F_1}$$

$$B = 2\overline{B_2} - \overline{P_2} - \overline{F_1}$$

$$C = 4\overline{F_2} - 2\overline{F_1} - \overline{P_1} - \overline{P_2}$$

$$D = 4\overline{F_3} - 2\overline{F_2} - \overline{P_1} - \overline{P_2}$$

Where:

$\overline{P_1}$ = mean of parent 1

$\overline{P_2}$ = mean of parent 2

$\overline{B_1}$ = mean of backcross to Parent 1

$\overline{B_2}$ = mean of the backcross to Parent 2

$\overline{F_1}$ = mean of the first filial generation

$\overline{F_2}$ = mean of the second filial generation

Their respective variances were computed as follows:

$$VA = 4V(\overline{B_1}) + V(\overline{P_1}) + V(\overline{F_1})$$

$$VB = 4V(\overline{B_2}) + V(\overline{P_2}) + V(\overline{F_1})$$

$$VC = 16V(\overline{F_2}) + 4V(\overline{F_1}) + V(\overline{P_1}) + V(\overline{P_2})$$

$$VD = 16V(\overline{F_3}) + 4V(\overline{F_2}) + V(\overline{P_1}) + V(\overline{P_2})$$

Where:

$VA =$ variance of A

$VB =$ variance of B

$VC =$ variance of C

$VD =$ variance of D

Standard errors (S.Es) were estimated as the square roots of the respective variances:

S.E$_{(A)} = \sqrt{VA}$, S.E.$_{(B)} = \sqrt{VB}$, S.E.$_{(C)} = \sqrt{VC}$, S.E.$_{(D)} = \sqrt{VD}$

The t values were calculated using:

$$t_{(A)} = \frac{A}{S.E.(A)} \qquad t_{(B)} = \frac{B}{S.E(B)} \qquad t_{(C)} = \frac{C}{S.E.(C)} \qquad t_{(D)} = \frac{D}{S.E.(D)}$$

In this study, it was only the A, B, and C scales that were applied because there was no F_3 generation.

3.7.2 Number of Effective Factors

The following formula proposed by Lawrence and Frey (1976) was used to estimate the minimum number of effective factors (N).

$$N = R^2 / 8(\sigma^2_g)$$

Where R is range of F_2 segregates in the cross.

σ^2_g = genetic variance of the F_2.

3.7.3 Heritability

Broad sense and narrow sense heritability estimates were obtained using the variance component methods.

(i) Broad sense heritability

The F_2 variances were used to compute the Broad sense heritability estimates by the method described by Mahmud and Kramer (1951), as given below:

$$H_{(B)} = \frac{\sigma^2 F_2 - \sqrt{\sigma^2 P_1 x \sigma^2 P_2}}{\sigma^2 F_2} x100$$

Where $\sigma^2 F_2 = \sigma^2 G + \sigma^2 E$, $\sigma^2 P = \sigma^2 E$, $\sigma^2 P_1 = \sigma^2 E_1$, $\sigma^2 P_2 = \sigma^2 E_2$

In these relationships,

$H_{(B)}$ = broad sense heritability

$\sigma^2 F_2$ = phenotypic variance of F_2 population of a cross

$\sigma^2 G$ = genotypic variance of F_2 population of a cross

$\sigma^2 E$ = environmental variance of F_2 population of a cross

(ii) Narrow Sense Heritability

Narrow sense heritability estimates were determined using the F_2 and backcross variance method as described by Warner (1952).

$$H_{(N)} = \frac{2\sigma^2 F_2 - (\sigma^2 B_1 + \sigma^2 B_2)}{\sigma^2 F_2}$$

Where $H_{(N)}$= narrow sense heritability

$\sigma^2 F_2 = \frac{1}{2}A + \frac{1}{4} D + E$

$\sigma^2 B_1 + \sigma^2 B_2 = \frac{1}{2}A + \frac{1}{2}D + E$

$2\sigma^2 F_2 = \sigma^2 B_1 - \sigma^2 B_2 = A + \frac{1}{2}D + 2E - (\frac{1}{2}A + \frac{1}{2}D + 2E) = \frac{1}{2}A$

A = Additive variance

D = Dominance variance

E = Environmental variance

B_1 = Backcross to parent 1(P_1)

B_2 = Backcross to parent 2 (P_2)

F_2 = Second filial generation

CHAPTER FOUR

RESULTS

4.1 MEAN PERFORMANCE FOR POPULATIONS

The mean scores for surface seed colonization by *Aspergillus flavus* for all the populations evaluated and the values for their aflatoxin contents are presented in Tables 3 and 4 respectively. All the genotypes supported extensive fungal growth. Means for surface seed colonization ranged from 30% to 90% for all crosses. The parental genotypes varied in the levels of surface seed colonization by *Aspergillus flavus.* Although all the parents showed colonization in the susceptible range, J11 was comparatively less susceptible having the lowest mean surface seed colonization (43%). The highest mean surface seed colonization was recorded for Samnut 22 (90%).The means of both parents were significantly different from the F_1s for all crosses except in the cross between Samnut 23 and 55-437 where the means of the F_1 and the better parent were not different. The means of the F_1s for all the crosses were lower than at least one of the parents, indicating presence of dominance. The mid-parent values were higher than their corresponding F_1 means for most of the crosses which indicates the presence of dominance. In all crosses, the F_1 progenies had means that were within the range of the parents. Also in two out of the four crosses, (Samnut 23 x J11 and Samnut 23 x 55-437), the means of the F_1 progenies were lower than their corresponding F_2 mean values. Significant difference was observed between the mean of F_2 progeny and the parents in the cross Samnut 22 x 55-437. The F_2 exhibited a mean seed colonization that was considerably lower than both parents, indicating resistance despite the high levels of seed colonization exhibited by both parents. The mid-parental means for most of the crosses were outside the range of the F_2 means. The mean values for the backcrosses for most of the crosses fell between the values of the F_1 progenies and their recurrent parents.

The aflatoxin content of the various generations is presented in Table 4. Aflatoxin was produced in all genotypes but differences between them were significant. Significant differences were observed among the parental genotypes, with the means of J11 for aflatoxin B_1, B_2 and the total aflatoxin content being considerably low. The lowest total aflatoxin content was recorded for the BCP_2 progeny in the cross between Samnut 23 and J11. In all the crosses, the F_1 mean values for total aflatoxin content were lower than the means of the lower parents, showing partial dominance. Also, the means of the F_1s in all crosses were lower than the means of the mid-parents, indicating dominance. In most of the crosses, the means of the F_2 progenies were higher than the means of their corresponding F_1 progenies. The backcross populations had means that fell within the range of their respective recurrent parents and the means of their F_1 progenies.

4.2 MEAN SQUARES, COEFFICIENT OF VARIATION AND COEFFICIENT OF DETERMINATION FROM ANALYSIS OF VARIANCE

The mean squares, coefficient of variation (CV) and coefficient of determination (R^2) for surface seed colonization and aflatoxin content for all the populations are presented in Table 5. The mean square values were highly significant (p= 0.01) for most of the crosses. The mean squares for aflatoxin B_1 were highly significant (p=0.01) for all the crosses except in the cross between Samnut 23 and 55-437 which had a non-significant mean squares. Aflatoxin B_2 on the other hand had highly significant (p=0.01) mean squares in two out of the four crosses, significant (p=0.05) in one of the crosses and a non significant mean square in the remaining cross. The mean squares for total aflatoxin content were highly significant (p=0.01) for three out of the four crosses and significant (p=0.05) for the remaining cross. For the surface seed colonization, the mean squares were highly significant (p=0.01) in the cross Samnut 22 x J11, significant (p=0.05) in the cross Samnut 22 x 55-437 and non-significant in the other two crosses. There was significant variation among all the genotypes within each population.

The coefficient of variation (CV) for surface seed colonization was considerably low and ranged from 12.99% to 21.508% for all the crosses. For aflatoxin B_1, the coefficient of variation ranged from 5.198 to 8.477, while that for aflatoxin B_2 ranged from 4.555 to 19.222. The coefficient of variation for total aflatoxin content was also low and ranged from 2.53% to 4.40%. The lowest coefficient of variation estimate was recorded for total aflatoxin content in the cross Samnut 22 x 55-437, while the highest estimate (21.508%) was recorded for surface seed colonization in the cross between Samnut 22 x 55-437. In this study, the coefficient of determination (R^2) was considerably high for most of the crosses and ranged from 0.174 to 0.978. The highest estimate was recorded for total aflatoxin content (TAC) in the cross between Samnut 22 and 55-437 while the lowest was recorded for aflatoxin B_2 in the cross between Samnut 22 and J11.

Table 3. Means for surface seed colonization by *Aspergillus flavus* for crosses of groundnut,2011

	Crosses			
Population	Samnut 23 x J11	Samnut 22 x J11	Samnut 23 x 55-437	Samnut 22 x 55-437
P_1	67^a	90^a	67^b	90^a
P_2	43^b	43^c	80^a	80^a
F_1	53^{ab}	63^b	67^b	67^b
F_2	63^a	43^c	70^b	30^c
BCP_1	76^a	70^{ab}	70^b	73^{ab}
BCP_2	70^a	63^b	80^a	53^{bc}
Mid parent	55^{ab}	66.5^{ab}	73.5^{ab}	85^a

Means within the columns followed by same letter(s) are not significantly different from each other by DMRT

Table 4. Means for Aflatoxin content for groundnut crosses, 2011

Population	Crosses											
	Aflatoxin Content (µg/kg)											
	Samnut 23 x J11						Samnut 23 x 55-437					
	B_1	ln $B_1+0.5$	B_2	ln $B_2+0.5$	B_1+B_2	ln B_1+B_2	B_1	ln $B_1+0.5$	B_2	ln $B_2+0.5$	B_1+B_2	ln B_1+B_2
P_1	469	6.61^{a}	68	4.72^{bc}	538	6.76^{a}	469	6.60^{b}	68	4.72^{d}	538	6.76^{b}
P_2	71	5.15^{c}	44	4.67^{bc}	115	5.63^{b}	964	7.37^{a}	327	6.12^{a}	1291	7.53^{a}
F_1	91	4.96^{c}	65	4.49^{cd}	156	5.52^{bc}	664	6.64^{b}	144	5.43^{bc}	809	7.18^{ab}
F_2	224	5.90^{b}	112	5.21^{a}	336	6.31^{a}	974	7.38^{a}	252	5.32^{c}	1226	7.44^{a}
BCP_1	260	6.04^{ab}	89	4.96^{ab}	349	6.34^{a}	818	7.20^{ab}	148	5.47^{bc}	966	7.36_{a}
BCP_2	55	4.46^{c}	45	4.27^{d}	100	5.10^{c}	937	7.18^{ab}	215	5.87^{ab}	1152	7.55^{a}
Mid parent	270	5.88^{b}	56	4.69^{bc}	327	6.20^{a}	717	6.99^{ab}	198	5.42^{bc}	915	7.15^{ab}

Means within columns followed by same letter(s) are not significantly different from each other by DMRT

Table 4 Continued

Population	Crosses											
	Aflatoxin Content (µg/kg)											
	Samnut 22 x J11						Samnut 22 x 55-437					
	B$_1$	ln B$_1$+0.5	B$_2$	ln B$_2$+0.5	B$_1$+B$_2$	ln B$_1$+B$_2$	B$_1$	ln B$_1$+0.5	B$_2$	ln B$_2$+0.5	B$_1$+B$_2$	ln B$_1$+B$_2$
P$_1$	1444	7.75^{a}	214	5.50^{ab}	1659	7.89^{a}	1444	7.75^{a}	214	5.81^{a}	1659	7.89^{a}
P$_2$	71	4.15^{d}	44	4.67^{c}	115	5.63^{c}	964	7.37^{a}	327	6.12^{a}	1291	7.56^{a}
F$_1$	702	6.87^{b}	94	5.04^{bc}	796	7.16^{b}	861	5.67^{b}	223	5.90^{a}	1084	7.32^{a}
F$_2$	954	7.36^{ab}	456	5.58^{ab}	1410	7.75^{a}	78	4.75^{b}	33	5.62^{a}	111	5.13^{b}
BCP$_1$	992	7.39^{ab}	242	5.97^{a}	1234	7.62^{a}	1010	7.42^{a}	250	6.00^{a}	1259	7.64^{a}
BCP$_2$	154	5.49^{c}	89	4.82^{bc}	243	5.80^{c}	956	7.36^{a}	171	5.46	1128	7.59^{a}
Mid parent	758	5.95^{bc}	129	5.09^{bc}	887	6.76^{b}	1204	7.56^{a}	271	5.97^{a}	1475	7.73^{a}

Means within columns followed by same letter(s) are not significantly different from each other by DMRT.

Table 5. Mean squares, CV and R^2 from analysis of variance for surface seed colonization by *Aspergillus flavus* and Aflatoxin content

Crosses	Source of variance	Df	Mean squares			
			B_1	B_2	TAC	% SSC
Samnut 23 x J 11	Genotype	3	1.374**	0.277**	0.828**	344.728
	Error	6	0.122	0.046	0.046	130.556
	C V (%)		6.311	4.555	4.085	17.986
	R^2		0.897	0.823	0.916	0.673
Samnut 23 x 55-437	Genotype	3	0.285	0.535**	0.232*	111.905
	Error	6	0.135	0.065	0.061	86.667
	C V (%)		5.198	4.629	3.391	12.989
	R^2		0.597	0.853	0.726	0.475
Samnut 22 x 55-437	Genotype	3	3.434**	0.508*	1.913**	892.647*
	Error	6	0.162	0.146	0.032	197.222
	C V (%)		6.069	7.205	2.533	21.508
	R^2		0.943	0.730	0.978	0.779
Samnut 22 x J 11	Genotype	3	3.410**	0.376	2.263**	919.841**
	Error	6	0.324	1.247	0..99	178.889
	C V (%)		8.477	19.222	4.397	20.231
	R^2		0.880	0.174	0.941	0.783

*Significant at 5%, **Significant at 1% probability level

B_1 = Aflatoxin B_1

B_2 = Aflatoxin B_2

TAC = Total Aflatoxin Content
% SSC = Percentage Surface Seed Colonization
CV (%) = Coefficient of variation
R^2 = Coefficient of determination

4.3 SCALING TESTS

Scaling test estimates and standard errors for surface seed colonization and aflatoxin content are presented in Table 6. The results of the various scaling tests for all the crosses for both surface seed colonization by *Aspergillus flavus* and aflatoxin content showed that the additive–dominance genetic model was adequate to explain the within population variation. The A and B scaling tests which estimate the variation between the means of the backcrosses, the F_1s and the parental generations were not significant for the parameters studied for all the crosses. Similarly, the C scaling test, which tests the relation of the F_2 means to the F_1 and the parental generation means was not significant in three out of the four crosses for the two parameters examined. These confirm the adequacy of the additive – dominance model to explain the variations.

4.4 MODE OF INHERITANCE

The frequency distribution of surface seed colonization by *Aspergillus flavus* for the parents, F_1, F_2 and backcross populations are presented in figures 1, 2, 3 and 4. In all the crosses, the parents did not represent phenotypic extremes for resistance and susceptibility to seed colonization by *Aspergillus flavus,* as the mean surface seed colonization values were 67% for Samnut 23, 43% for J11, 90% for Samnut 22 and 80% for 55-437. The observed frequency distributions of the parents for all crosses overlapped, revealing that none of the parents was absolutely resistant or susceptible. The F_1 distribution curves for all the crosses skewed towards either of the parents showing dominance. In three out of the four crosses, (Samnut 23 xJ11, Samnut 23 x 55-437 and

Samnut 22 x 55-437) the F_1 skewed toward the better parents suggesting partial dominance of these parents. The distribution of the F_2 was bimodal in all the crosses, which suggests major gene inheritance. The frequency distributions for the backcrosses in all crosses skewed towards their recurrent parents, indicating the presence of dominance gene effects.

The frequency distributions of aflatoxin content for the parents, F_1, F_2, and backcross populations are presented in figures 5 - 8. The frequency distribution for all the parents overlapped in all the crosses. The F_1 distributions for all the crosses were skewed towards their parents. In three out of the four crosses (Samnut 22 x 55-437, Samnut 23 x 55-437 and Samnut 23 x J11) the F_1s skewed towards the better parents, suggesting partial dominance of the better parents. Bimodal F_2 frequency distributions expected of major gene inheritance were observed in all the crosses. The backcrosses were also skewed towards their recurrent parents in all the crosses,

Table 6. Estimates of scaling tests and associated standard errors (S.E. ±) for surface seed colonization and aflatoxin content

	Colonization			Aflatoxin content		
Crosses	A-Scale	B-Scale	C-Scale	A-Scale	B-Scale	C-Scale
Samnut23 x J11	2.2306 ±0.897	2.3394 ±1.367	2.0788 ±1.732	2.2084 ±0.838	2.3312 ±1.329	2.3309 ±1.867
Samnut 22 x J 11	-1.6782 ±0.775	2.2361 ±0.894	0.2773 ± 1.082	0.9977 ±0.221	1.4291 ±1.869	2.3309 ±0.897
Samnut 23 x 55-437	2.3540 ±1.195	-0.5035 ±1.390	-0.0559 ±1.789	-0.7881 ±0.241	-2.1783 ±0.987	-0.1131 ±1.414
Samnut 22 x 55-437	-2.1310 ±0.5162	-2.2450 ±0.9354	-1.9720 ±0.8306	2.0369 ±0.6235	-1.4295 ±1.3571	2.3153 ±0.6856

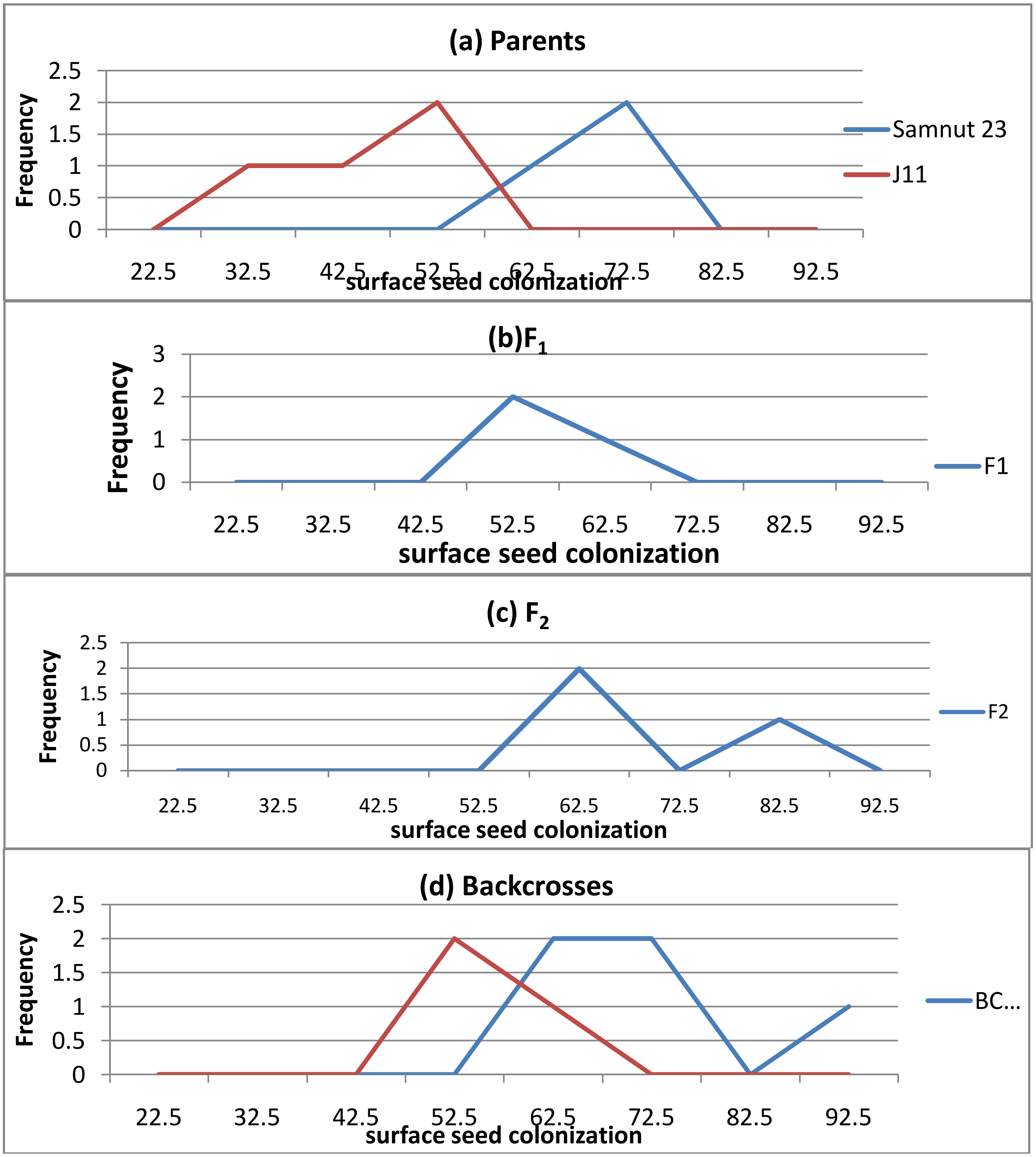

FIG. 1: Frequency distribution of surface seed colonization by *Aspergillus flavus* for (a) parents, (b) F_1, (c) F_2 and (d) backcross populations for the cross Samnut 23 x J 11

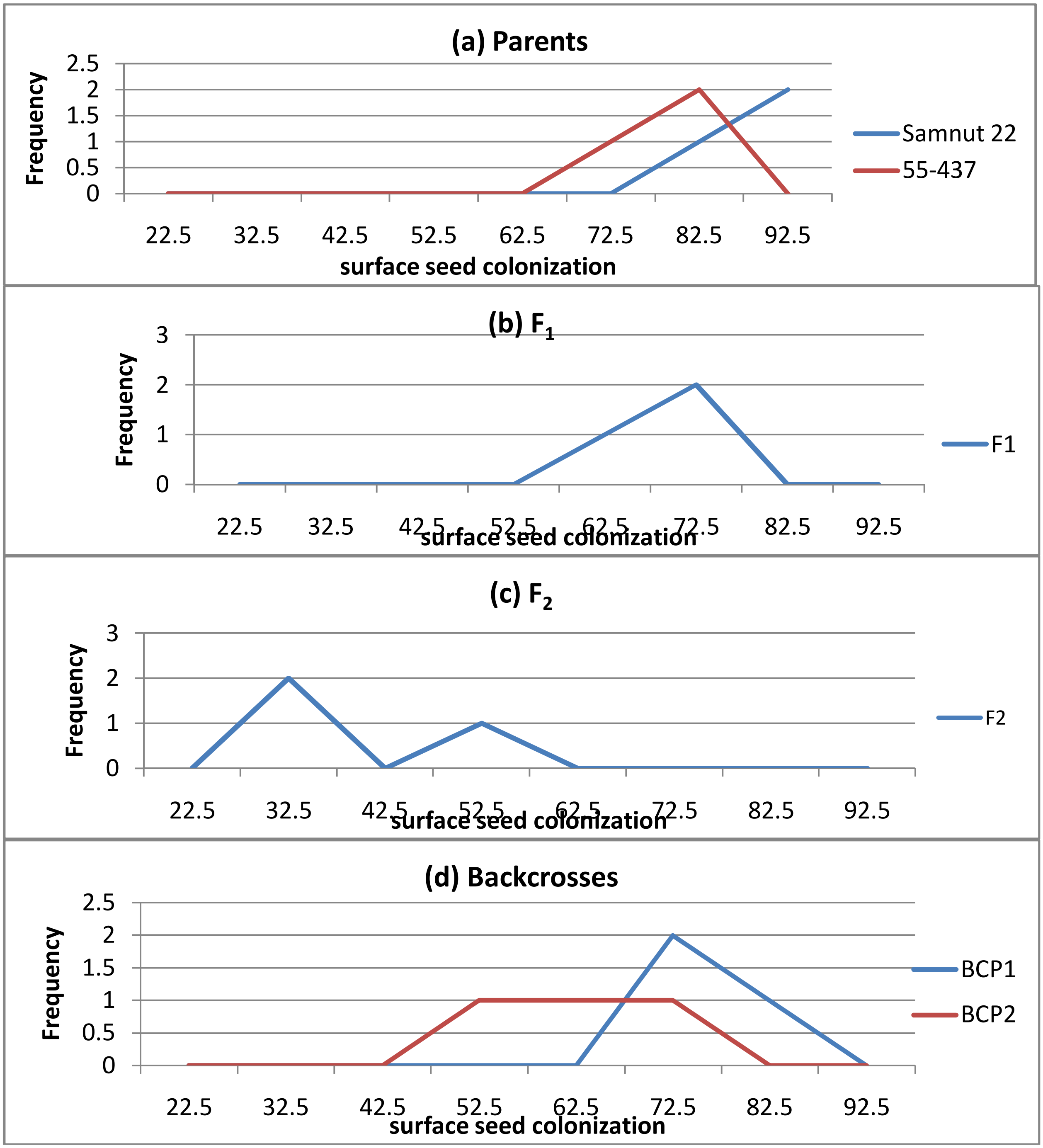

FIG. 2: Frequency of distribution of surface seed colonization by *Aspergillus flavus* for (a) parents, (b) F_1, (c), F_2 and (d) backcross populations for the cross Samnut 22 x 55-437

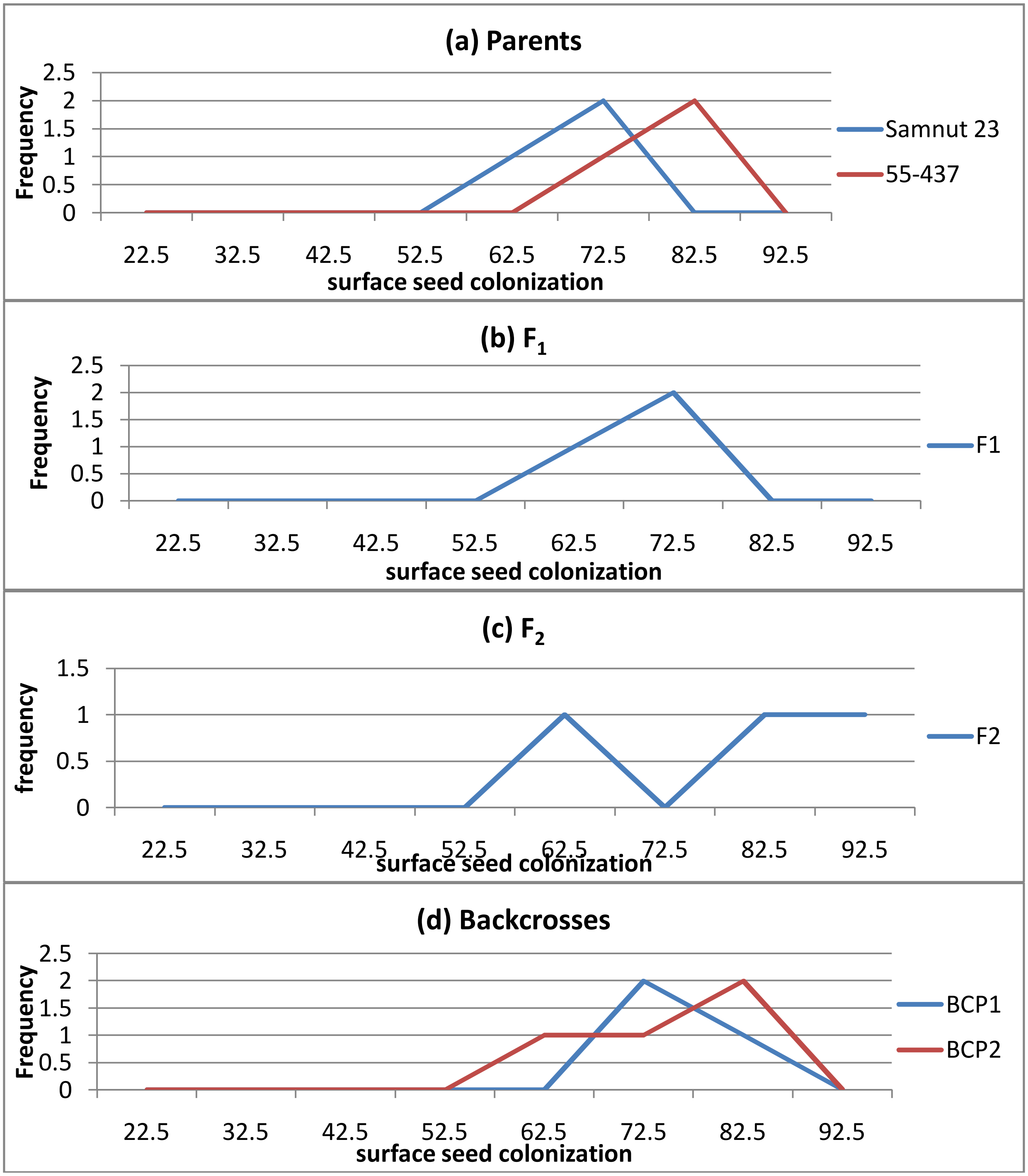

FIG. 3: Frequency of distribution of surface seed colonization by *Aspergillus flavus* for (a) parents, (b) F_1, (c) F_2 and (d) backcross, populations for the cross Samnut 23 x 55-437.

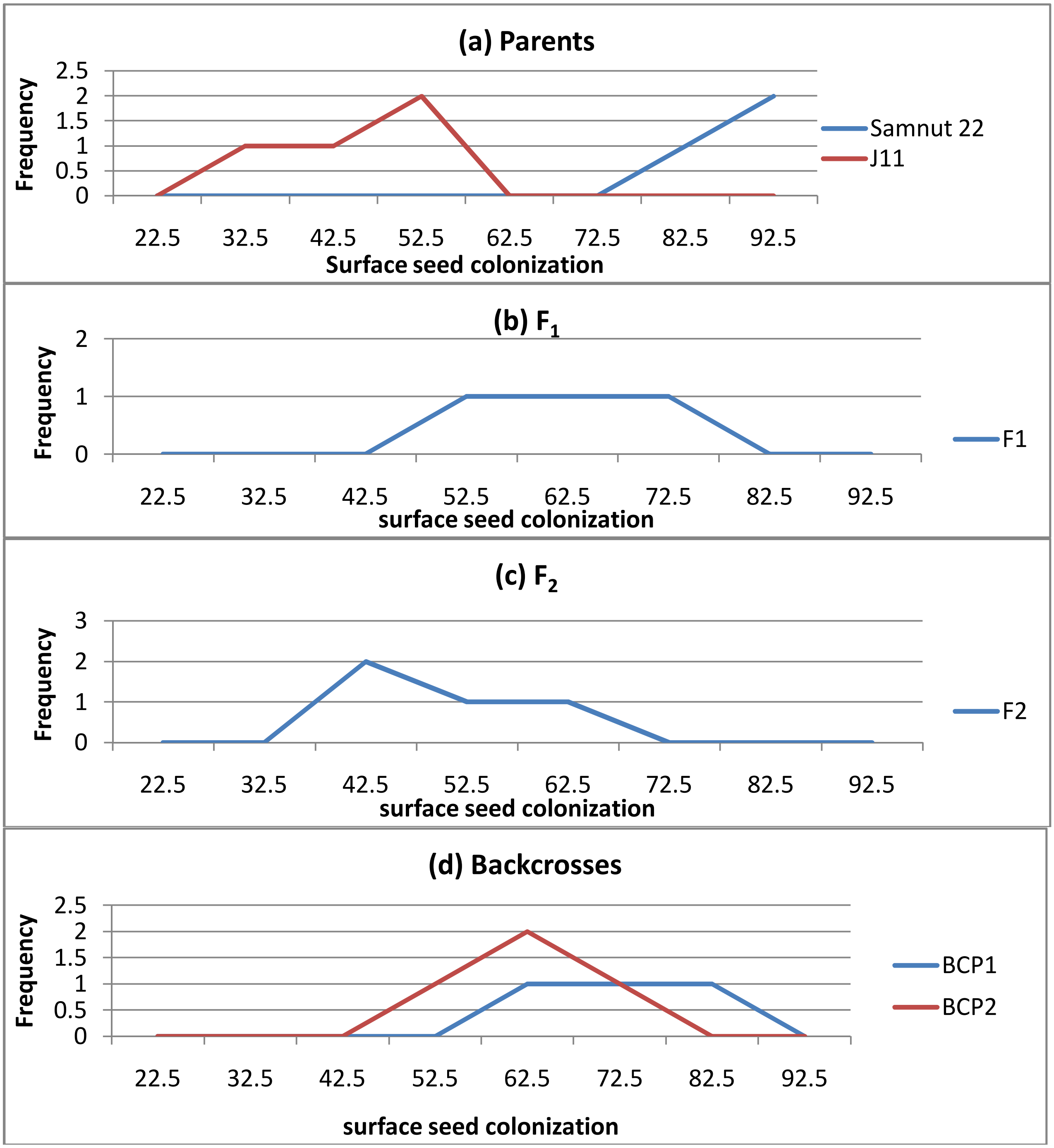

Figure 4: Frequency of distribution of surface seed colonization by *Aspergillus flavus* for (a) parents, (b) F_1, (c) F_2 and (d) backcross populations, for the cross Samnut 22 x J11.

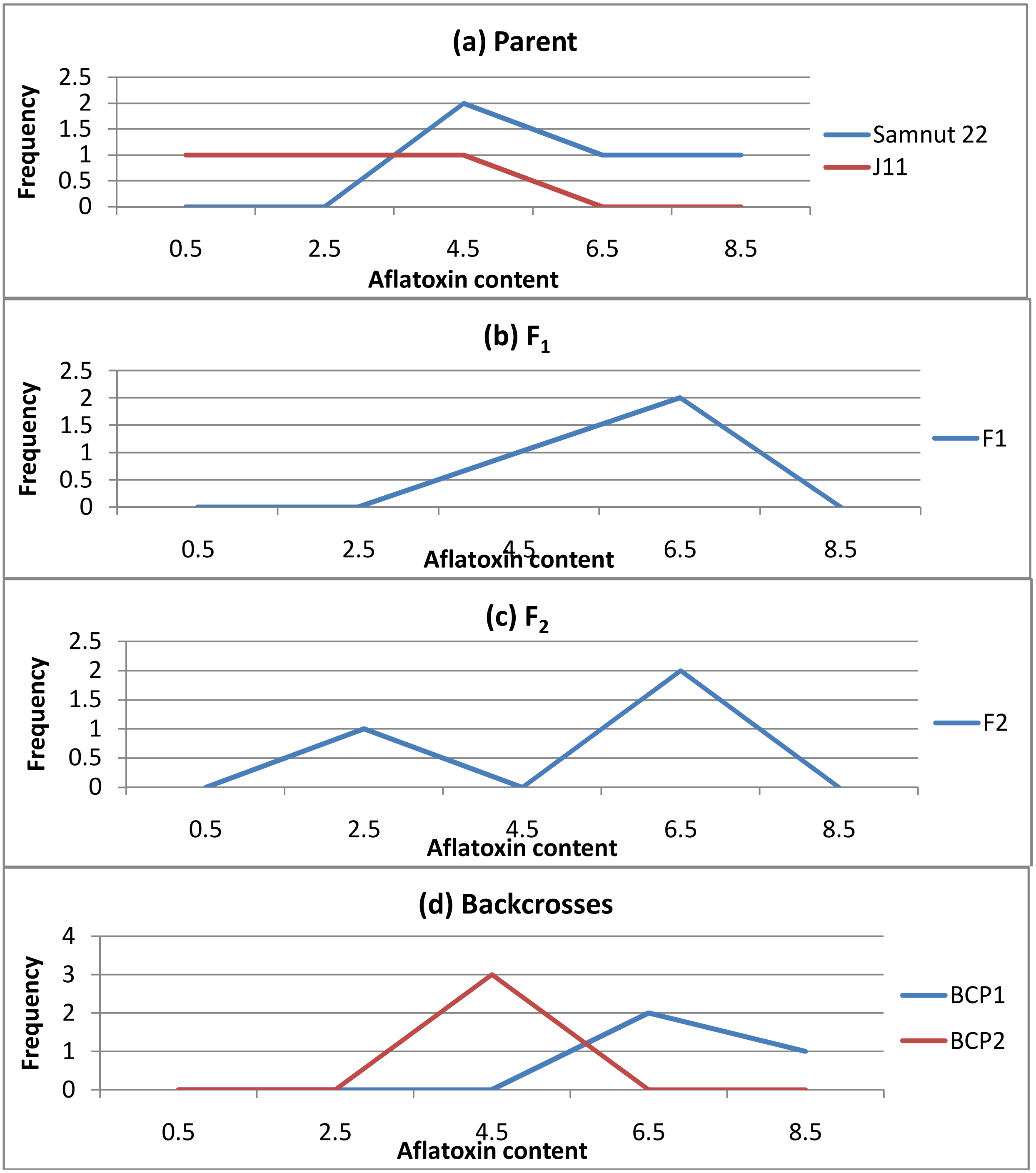

Figure 5: Frequency distribution of aflatoxin content for (a) parents, (b) F_1, (c) F_2 and (d) backcross populations for the cross Samnut 22 x J11

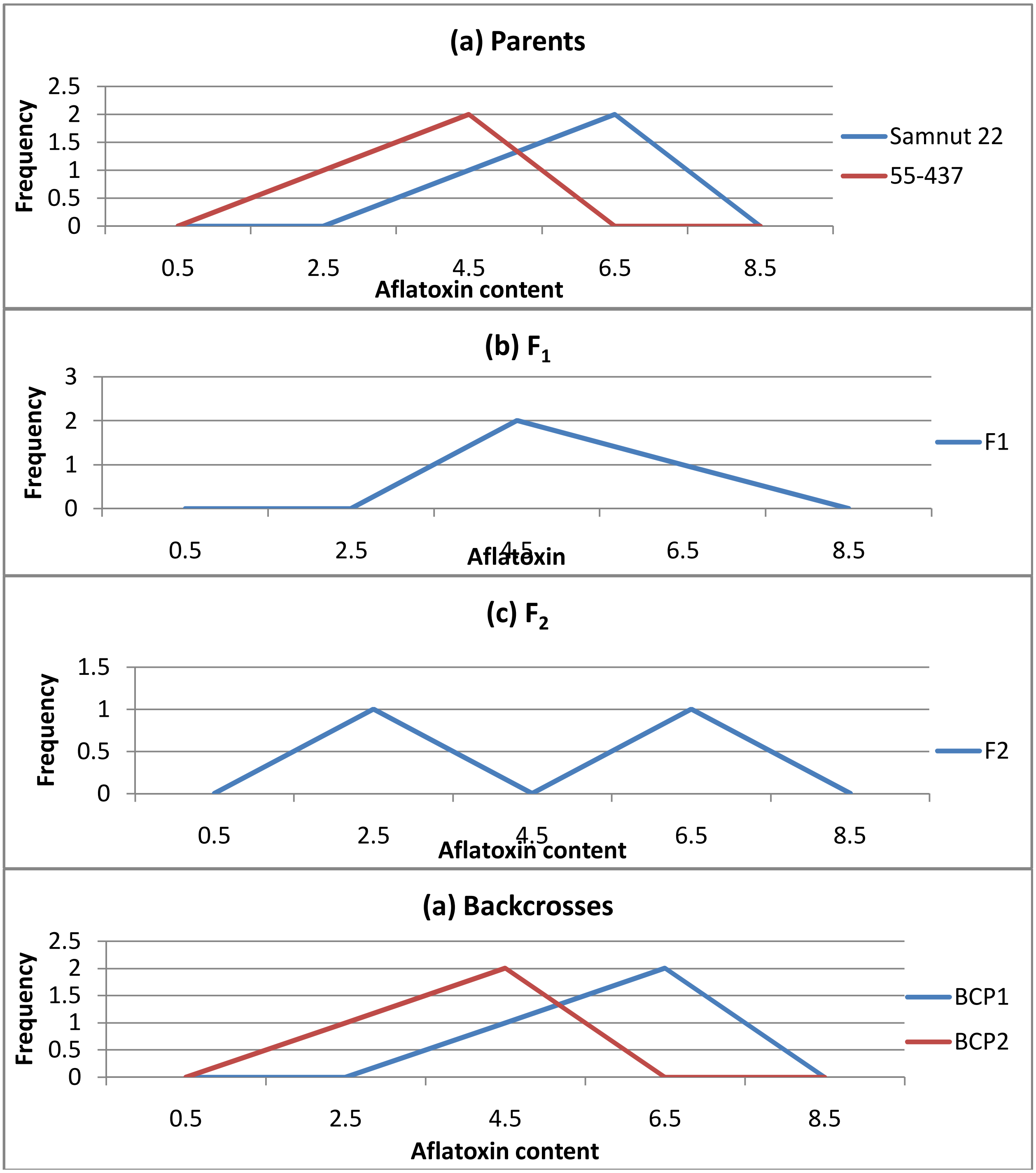

Figure 6: Frequency distribution of aflatoxin content for (a) parents, (b) $F_{1,}$ (c) F_2 and (d) backcross populations for the cross Samnut 22 x 55-437.

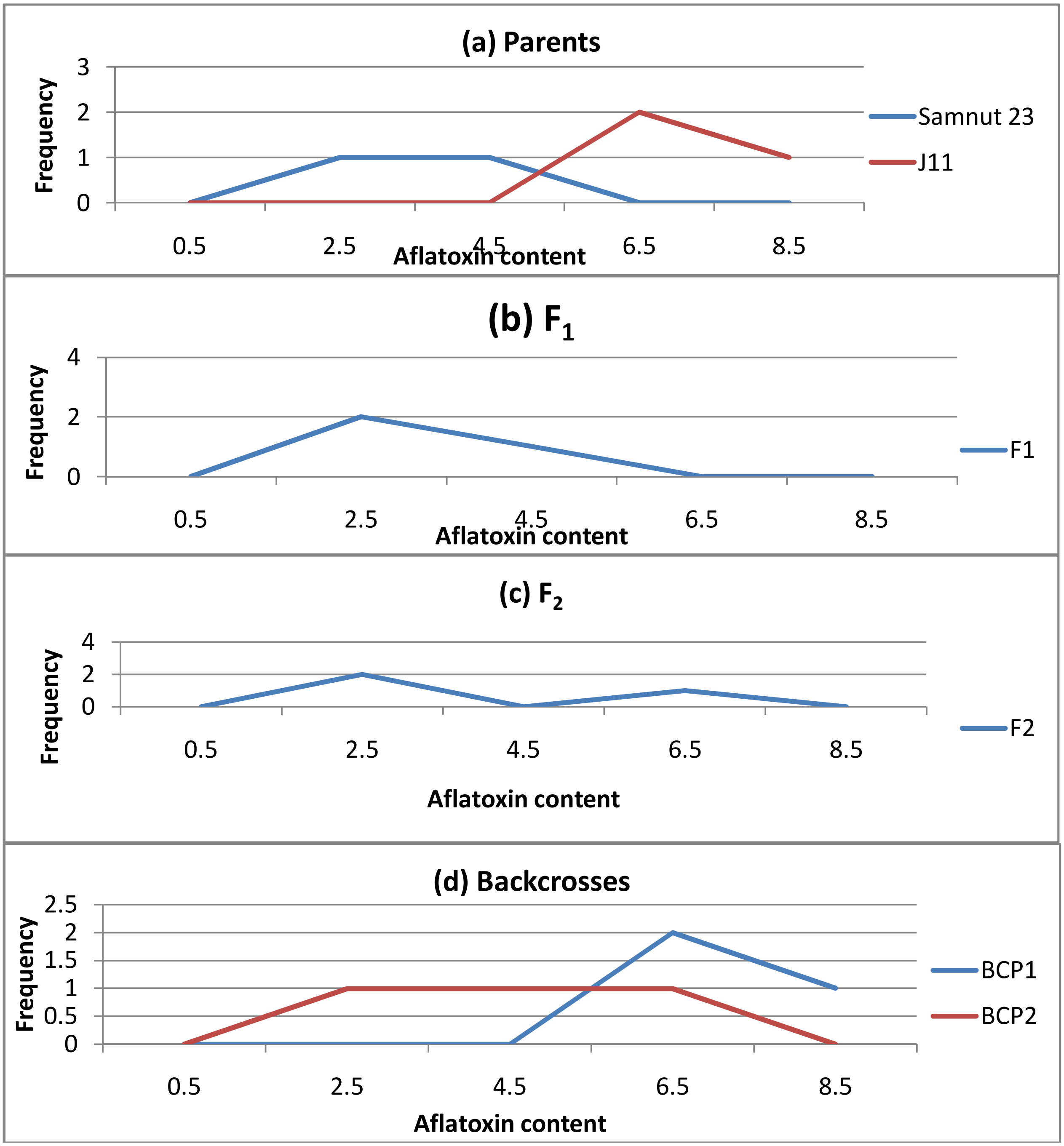

Figure 7: Frequency distribution of aflatoxin content for (a) parents, (b) F_1, (c) F_2 and (d) backcross populations, for the cross Samnut 23 x J 11.

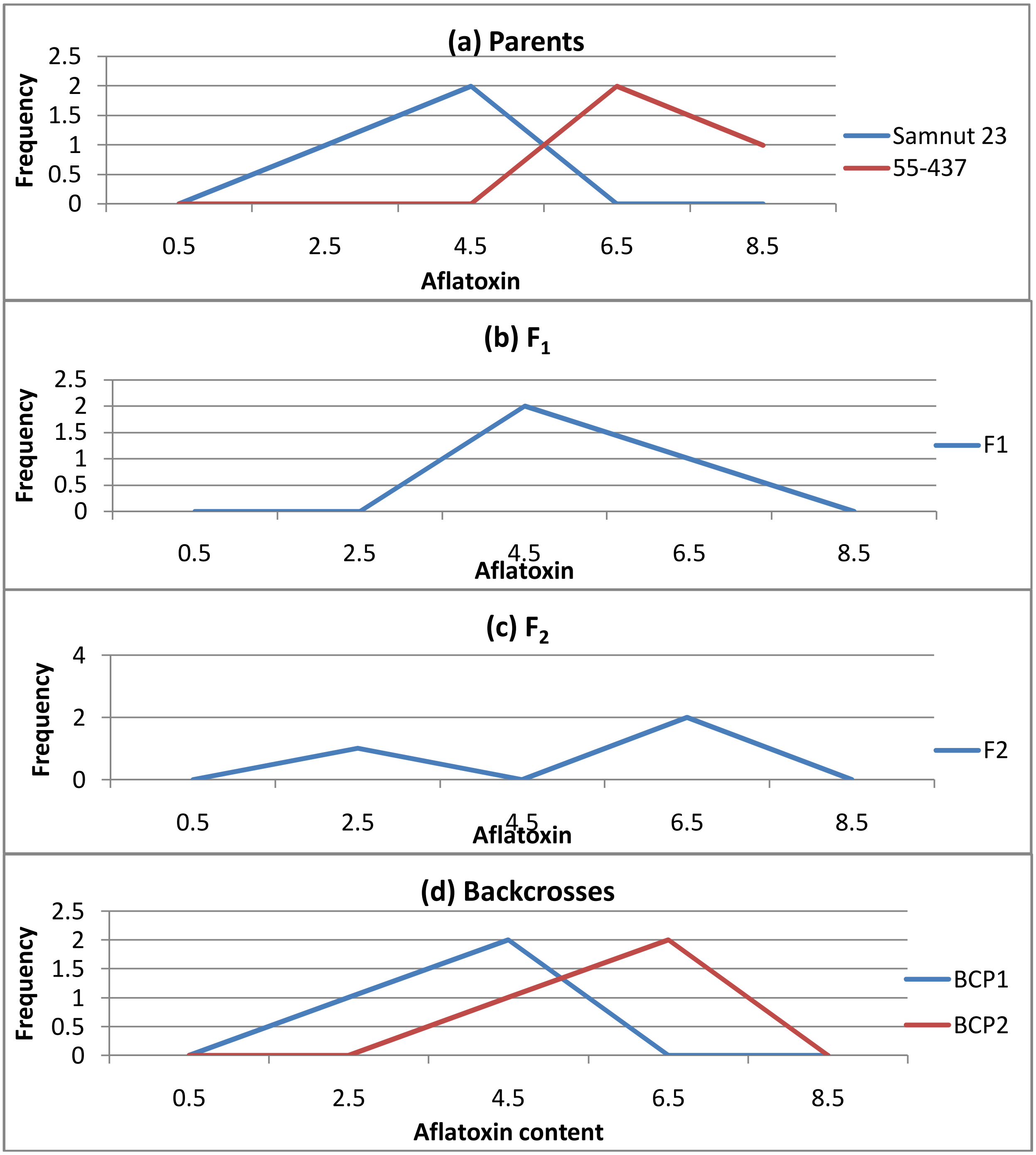

Figure 8: Frequency distribution of aflatoxin content for (a) parents, (b) F_1, (c) F_2 and (d) backcross populations for the cross Samnut 23 x 55-437.

4.5 NUMBER OF EFFECTIVE FACTORS

The estimates of the number of effective factors for surface seed colonization by *Aspergillus flavus* and aflatoxin content are presented in Table 7. The minimum number of effective factors segregating in the F_2 generations of all the crosses for surface seed colonization and total aflatoxin content ranged from 1 to 2. The estimates for surface seed colonization was 1, obtained for all the crosses. The highest estimate for aflatoxin content was 2 and was obtained in the crosses between Samnut 23 x J11 and Samnut 23 x 55-437.

4.6 HERITABILITY

Heritability estimates for all the crosses for surface seed colonization and aflatoxin content are presented in Table 8. In this study, all the traits showed high estimates of broad sense heritability indicating that environmental effects may not reduce the inheritance of these traits. The broad sense heritability ranged from 42.92% to 85.30% and 56.31% to 84.46% for surface seed colonization and aflatoxin content, respectively. The narrow sense heritability ranged from 9.38% to 33.91% and 18.85% to 54.82% for surface seed colonization and aflatoxin content respectively. Surface seed colonization by *Aspergillus flavus* exhibited the highest estimates of broad sense heritability considering the four crosses with the highest value (85.30%) obtained in the cross Samnut 23 x 55-437 and aflatoxin content exhibited the highest narrow sense heritability estimate (54.82%) in the cross Samnut 23 x J11.

Table 7. Number of effective factors

Crosses	Colonization	Total Aflatoxin

Samnut 23XJ11	1	2
Samnut 23X55-437	1	1
Samnut 22XJ11	1	2
Samnut 22X55-437	1	1

Table 8. Heritability estimates for surface seed colonization and Aflatoxin content

	Colonization		Aflatoxin Content	
	H^2 (%)	h^2 (%)	H^2 (%)	h^2 (%)

Crosses				
Samnut 23 X J11	52.25	9.38	84.46	54.82
Samnut 23 X 55-437	85.30	12.02	80.17	52.05
Samnut 22 X 55-437	57.08	24.03	80.63	18.85
Samnut 22 X J11	42.92	33.91	56.31	29.00

H^2 (%) = Broad sense heritability. h^2 (%) = Narrow sense heritability

CHAPTER FIVE

DISCUSSION

Aflatoxin contamination produced by aflatoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* is a very serious food quality problem associated with groundnut and its products. It has great impact on the groundnut industry. Breeding for resistant cultivars is

the most effective and reliable means of aflatoxin control and it is only possible when there are available sources of stable high level resistance, reliable assessment methods, and an understanding of the inheritance of the traits. Many sources of resistance have been identified by different screening methods (Xue, 2004). Among
these are J11 and 55-437 which were used as the resistant parents for this study.

In this study there was significant variation among the parental genotypes for the mean surface seed colonization by *Aspergillus flavus* with the intensity of colonization ranging from 43% to 90%. The same trend was observed in the progenies which also demonstrated varying degrees of mean seed colonization for all crosses (Table 4). The genotypes J11 and 55-437 earlier reported to be resistant to *in vitro* seed colonization by *Aspergillus flavus* showed moderate to high surface seed colonization (43% - 80%) respectively, almost at par with the susceptible varieties. This corroborated the findings of Narasimhulu (2007). Xue (2004) reported that no peanut germplasm has been found to have complete resistance to aflatoxin contamination. Similar result was obtained by Kiran et al. (1988).The conflicting results could be due to the presence or absence of different genes responsible for different types of resistance or due to differential gene function in different environments (Xue *et al.,* 2004). Another reason for the fluctuation in the resistance of J11 and 55-437 may be due to the strains of the *Aspergillus flavus* used for inoculation. Not all strains of *Aspergillus* are able to produce mycotoxins; therefore, there is a need for screening for their toxin production abilities (Younis and Kamal, 2003). There were significant differences in the means of the progenies for most of the crosses and between the means of the progenies and their respective parents. Considering the means of the parents in relation to their F_1s, it is revealed that dominance is important in the inheritance of resistance to surface seed colonization by *Aspergillus flavus.* The F_1s means were lower than that of either a single parent or both parents in all the crosses. Similarly, the means of the F_1s were lower than the means of the mid-parents in all the crosses, thus confirming the preponderance of dominance of the lower scoring parent.

Similarly, there was significant variation for aflatoxin concentration. A similar report was given by Xue *et al.* (2003). The variation in the mean aflatoxin content among the parental genotypes and between the parents and their progenies was highly significant. The mean performance of the F_1 progenies in relation to the means of their parents and mid-parents for the crosses Samnut 23 x J11 and Samnut 22 x 55-437 also revealed the preponderance of dominance in the inheritance of this trait.

The reaction of the parental genotypes and the F_1s to seeds colonization and aflatoxin production and the segregation pattern of the F_2 and the backcross progenies in relation to resistance were used to determine the mode of inheritance of resistance in each cross.

The F_1 progenies were skewed in almost all the crosses for the two parameters towards either of the parents thus confirming the presence of dominance. This suggestion was strengthened by the segregation of backcross generations in almost all the crosses evaluated. The bimodal distribution of the F_2 population in all the crosses for surface seed colonization by *Aspergillus flavus* and aflatoxin content suggests major gene inheritance for these traits.

The estimates of the mean squares, coefficient of variation and coefficient of determination revealed that variability exists among the populations for most of the crosses. The significant mean squares in all for total aflatoxin and in two out the four crosses (Samnut 22 x 55-437 and Samnut 22 x J11) for surface seed colonization are indications of the presence of considerable amount of genetic variability in the genotypes studied. The low coefficient of variation indicated precision in the conduct of the experiment and suggests that indirect selection can be used to improve the characters studied. Coefficient of determination is the ratio of the explained variation to the total variation. It gives the proportion of the variance of one variable that is predictable from the other variable. The coefficient of determination is such that $0 \leq r^2 \leq 1$, and denotes the strength of the linear association between the variables. The considerably high coefficient of determination showed that the variability in the populations for all crosses was adequately explained. The results of the ABC scaling tests confirmed the adequacy of the additive–dominance model to explain the variations in the populations.

In this study, the estimates obtained for the minimum number of effective factors controlling the inheritance of traits studied for most of the crosses were one and did not exceed a value of two. The minimum values for surface seed colonization was one in all crosses while a value of one was obtained in two out of the four crosses (Samnut 23 x 55-437 and Samnut 22 x 55-437) and two in the remaining two crosses (Samnut 23 x J11 and Samnut 22 x J11) for aflatoxin content. These estimates suggest that a few major genes control the resistance to surface seed colonization and aflatoxin content. This corroborated the findings of Mixon

(1986), who revealed that the resistance to seed infection by *Aspergillus flavus* is controlled by a pair of major genes.

Estimate of heritability are very important to the plant breeder as it determines progress in selection. It's most important value lies in its predictive role in expressing the reliability of the phenotypic value as a measure of the breeding value (Nuhu, 2006). In this study, two types of heritability estimates were reported. The results indicated that there were differences in the heritability obtained among crosses for the traits studied. Based on the findings, it can be concluded that selection will be effective in the test materials used in this study as revealed by the significant substantial variations among the genotypes for all the characters observed. The estimates of heritability (Broad and Narrow sense) for surface seed colonization and aflatoxin production ranged from low to high. Dabholkar (1992) classified heritability estimates as low (5 to 10%), medium (10 to 30%) and high (> 30%). High heritability estimates indicate that the genotypic variances (σ^2g) were higher than the phenotypic variances (σ^2 ph). It can also be interpreted that their genetic variation is large due to major genes controlling the characters which can easily be inherited, and that selection would be effective in the desired direction for each of the traits in the population (Aba, 1998). This suggestion was further strengthened by the low estimates of narrow sense heritability which signifies the predominance of non-additive gene influence. The variation shown in the heritability estimates may also be due to the fact that only one environment was used thus the genotype x environment estimates was not obtained. This had been reported to cause bias in the estimate of heritability (Obilana and Fakorede, 1981). The high heritability values indicated that the traits are highly heritable and selection can be done to improve the characters. This finding corroborated the report of Upadhyaya *et al.* (1997). High heritability is useful to plant breeders for making effective selection on a phenotypic basis (Arunkumar *et al.*, 2003).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY AND CONCLUSIONS

Groundnuts *Arachis hypogaea* L are prone to infestation by two closely related fungal species, *Aspergillus flavus* and *Aspergillus parasiticus.* Both produce a highly toxic group of mycotoxins known as aflatoxins (Waliyar *et al.*, 2000). Aflatoxins are hazardous to human and animals health and cause economic losses. The use of resistant cultivars is very paramount. Breeding for resistance is one of the best means of reducing economic losses and health risks due to aflatoxin contamination.

The means of surface seed colonization by *Aspergillus flavus* and the estimates of aflatoxin content were the criteria used. The analysis of variance (ANOVA) for surface seed colonization by *Aspergillus flavus* and aflatoxin content of all populations was carried out using the General Linear Model (GLM) of the statistical analysis system (SAS) procedure. The ANOVA revealed significant variation among the genotypes used. The two parental genotypes previously reported to be resistant to seed colonization and aflatoxin production supported varying levels of seed colonization with low levels of aflatoxin contamination. There were significant differences among the means of the parents, the progenies and between the parents and their progenies for both traits.

The frequency distribution of the progenies in relation to their parents revealed the presence of dominant gene effects. The F_2 distribution of all the crosses observed were bimodal, indicating major gene inheritance of resistance to surface seed colonization by *Aspergillus* *flavus* and aflatoxin production. The non significance of the ABC scales revealed the adequacy of the additive-dominance model. The minimum number of effective factors responsible for resistance to surface seeds colonization and aflatoxin production ranged from one to two, revealing that the resistance is controlled by a few major genes. The estimates of both broad sense and narrow sense heritability ranged from low to high. The high genetic variation and heritability obtained in the present study suggests that substantial genetic variability is available to ensure good progress from selection.

6.2 RECOMMENDATIONS

This study revealed that the genotypes used had no complete resistance to surface seed Colonization by *Aspergillus flavus* and aflatoxin production. Although researchers have not located germplasm lines which show complete resistance to the fungi, it is expected that the level of resistance can be improved further by pyramiding resistance genes from different and diverse sources. Based on the findings in this study, I wish the following recommendations are made:

1. Re- validation of the parental genotypes (J11, 55-437, Samnut 23 and Samnut 22) should be carried out to ascertain their levels of resistance or susceptibility to surface seed colonization by *Aspergillus flavus* and aflatoxin production.
2. Pyramiding of resistance genes from different sources should be done in order to combine the different types of resistance into one genetic background.
3. Efficient and reliable screening techniques should be readily available for proper verification of resistance in genotypes.

4. Genotypes reported to be resistant to seed colonization by *Aspergillus flavus* and aflatoxin production should be extensively screened across diverse growing environments to identify stable sources of resistance.
5. The use of biotechnology to understand the mechanisms governing the resistance pathways should be harnessed and the information should be used in breeding programs for the control of *Aspergillus flavus* in groundnut and its products.

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