EVALUATION OF RESISTENCE TO *Aspergillus flavus* Link ex Fries IN GROUNDNUT

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1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an annual legume grown primarily for high quality edible oil (36 – 54% on dry matter basis) and easily digestible protein (12 – 36%) in its seeds. It is cultivated worldwide in tropical, sub-tropical and warm temperature areas located between 40^0N to 40^0S with a world production of 35.9 million tonnes at an area of 25.2 million ha. In India, it is spread over an area of 6.6 million ha with production of 5.9 million tonnes (FAO, 2006).

Groundnut is believed to have originated in the Bolivian region of South America where the greatest diversity is found (Krapovickas, 1969; Gregory and Gregory, 1976). The distinguishing morphological features of this genus are aerial flowers that give rise to subterranean fruits. There are about 70 species, most of them diploid (2n = 2× = 20). Two are allotetraploid (2n = 4× = 40) with two genomes, A and B. Only *A. hypogaea* (2n = 40) has been domesticated and grown extensively for seeds and oil (Stalker, 1992). The gene pool of cultivated groundnut is divided into two subspecies *fastigiata* and *hypogaea*. The subspecies *fastigiata* is sub-divided into four botanical varieties, *fastigiata*, *peruviana*, *aequatoriana* and *vulgaris* whereas, subspecies *hypogaea* includes varieties *hypogaea* and *hirsuta* (Krapovickas and Gregory, 1994).

One of the serious food quality problems associated with groundnut and its products is the aflatoxins contamination produced by *Aspergillus flavus* Link ex Fries and *Aspergillus parasiticus* spear, which are proved to be potent carcinogens to animals and humans leading to liver cancer. Of the four types of aflatoxins (B1, B2, G1 and G2), aflatoxin B1 is the most toxic and regarded as the most potent naturally occurring carcinogen by IARC (International Agency for Research on Cancer). Other harmful effects posed by aflatoxins are teratogenicity (deformation of developing fetus) (Dipaolu *et al.*, 1967), reduction in RBC, WBC and hemoglobin content in blood (Panda *et al.*, 1975), delayed blood clotting (Clark *et al.*, 1986) and suppression of immune system in case of chronic poisoning. Aflatoxins reach human beings through food chain as they were detected in milk, egg and meat when the animals were fed on contaminated diet.

Aflatoxin contamination had a tremendous impact on the peanut industry. Because of human health concerns many countries, have set maximum levels of aflatoxin allowed in food and feed (Stoloff *et al.*, 1991). FAO and drug administration permits maximum total aflatoxin levels of 20 ppb in the peanut products destined for human consumption; the European Union allows 3 ppb of total aflatoxins and 2 ppb on aflatoxin B1. The Ministry of Commerce, Government of India vide public notice No. 68 (8) (RE)/1997-2002 dt. 03/02/1999 announced that exports to European Union (EU) will be allowed subject to compulsory registration of contracts with Agriculture and Processed Food Products Export Development Authority (APEDA) along with a controlled aflatoxin level certificate given by agencies/laboratories nominated by APEDA and this certificate will be a compulsory of export documents (Tushar Tanna, 2002).

Aflatoxin contamination in food and livestock feed is particularly severe in developing countries of Africa, Southeast Asia, where food safety and security systems are not well developed to protect poorer community and livestock against unsafe food products. It is increasingly an important issue in India because of its greater role as a food crop rather than an oil crop (Vasanthi and Bhat, 1998 and Varma and Agarwal, 2000) and wide range of environmental conditions in sub-tropical regions often favour the *A. flavus* infection both in field as well as in storage. Resource poor conditions of the farmers in our country to exploit recommended management practices further enhance the chances of aflatoxin contamination.

Management of aflatoxin contamination requires both preventive and curative approaches starting from sowing and harvesting to processing and storage. Hence, resistant cultivars should be an effective and low-cost part of an integrated aflatoxin management program and it is the most viable and economical solution to aflatoxin problem.

Groundnut is prone to several biotic and abiotic stresses. Among several biotic stress, diseases like Late Leaf Spot (LLS) caused by *Phaeoisariopsis personata* (Berk and Curt) or *Cercosporidium personatum* and rust caused by *Puccinia arachidis* (Speg.) are the major constraints for groundnut cultivation. These are the most destructive diseases occurring commonly throughout the world wherever groundnut is grown and cause substantial yield losses (Subrahmanyam *et al.*, 1984).

Foliar disease management by using fungicides has been reported but fungicides are not eco-friendly and also pose many health problems in addition to their adverse effect on quality. Breeding for resistance to LLS and rust is a cost effective and viable option.

In India, only limited efforts have been made to identify resistant genotypes because one of the major problems with screening is lack of reliable method. The yield potential under the disease pressure has been rarely investigated. It is evident that diseases have significant effect on the yield. Hence, the simultaneous evaluation of the genotypes for the disease as well as yield potential would be more useful.

Identification of the resistant genotypes needs careful, repeated and through screening under ideal epiphytotic conditions, which is time consuming and laborious. Molecular tools such as DNA markers are increasingly becoming important as effective tool in crop breeding programs. Molecular markers associated with diseases would hasten the process of identification of resistant genotypes. But, their application in groundnut enhancement is lagging behind because of limited knowledge of genome.

Hence, a systemic attempt was made to identify genotypes with resistance to *A. flavus* seed colonization, LLS and rust in order to utilize such genotypes effectively in further breeding program.

Keeping this in view, the present study was formulated with the following objectives.

1. To evaluate the groundnut lines for resistance to *Aspergillus flavus*, late leaf spot, rust and yield traits

2. To assess segregating material for resistance to *A. flavus* and other traits

2. REVIEW OF LITERATURE

Groundnut is affected by several production constraints. Since the crop is mostly grown under rainfed and low input condition, it is essential that new groundnut varieties carry multiple resistance to different constraints operating in a region in the appropriate maturity backgrounds. Since groundnut is also used as food, it is essential that quality traits receive adequate attention in genetic enhancement.

2.1 Germplasm collection and utilization

Collection and assembly of groundnut genetic resources has been extensive. Expeditions to various groundnut-growing regions of the world and to the centers of diversity in South American have collected thousands of accessions. Over 14,310 accessions are maintained at the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) (Upadhyaya *et al*., 2001a), and over 9,027 accessions of *A. hypogaea* and 684 accessions of *Arachis* species in the United States Department of Agriculture (USDA) germplasm collection (Holbrook, 2001).

Germplasm collections have been screened for many different purposes. The ICRISAT collection has been examined for resistance to major biotic and abiotic stresses. More than 9,000 entries have been evaluated for resistance to groundnut rust (*Puccinia arachidis* Speg.) and late leaf spot (*Phaeoisariopsis personata* (Berk and Curt) V. Arx.) (Moss *et al*., 1989; Singh *et al*., 1992).

A significant proportion of the U.S. germplasm collection has also been evaluated for several economically important groundnut diseases and high levels of resistance have been identified, such as resistance to *C. personatum* (Anderson *et al*., 1993), *Sclerotinia* blight (*Sclerotinia minor* Jagger) (Smith *et al*., 1991), and root-knot nematode (*Meloidogyne* spp.) (Holbrook *et al*., 2000a). The development of a groundnut core collection has further improved the efficiency of germplasm evaluation (Holbrook, 2001).

In addition to *A. hypogaea*, the wild *Arachis* species have been evaluated for quality parameters and disease and insect resistance (Subrahmanyam *et al*., 1985; Wynne *et al.,* 1991; Lynch, 1990). Resistance sources found in *Arachis* species are summarized by Stalker (1992).

Despite the considerable genetic resources of groundnut, utilization of available genetic variability is still limited. Breeders prefer to use sources of resistance, even those with less potent resistance, that conform to market and industry standards instead of new sources with poor agronomic characteristics.

Breeding objectives are different from region to region and time to time. The primary objectives of groundnut breeding are to develop cultivars with high yield potential, resistance or tolerance to environmental stress, resistance to diseases and insects, and adaptation to specific environments and production systems. Breeding resistant cultivars is one of the best means of reducing crop yield losses from leaf spots, but resistance levels of cultivars are not sufficient to eliminate the use of foliar fungicides. However, progress in breeding for resistance to the soil borne fungi has been difficult and slow.

2.2 Aflatoxin

Aflatoxin contamination of groundnut is a serious quality problem in many tropical and sub-tropical countries in the world. India is no exception to this because groundnut is cultivated in varied environmental conditions in different states.

2.2.1 Historical background

In the 1960, more than 100,000 young turkeys on poultry farms in England died in the course of a few months from an apparently new disease that was termed “Turkey × disease”.

Histopathological examination of birds revealed the degeneration of liver cells and proliferation of bile duct epithelium.

Subsequent surveys demonstrated that the toxic manifestations were caused by ingestion of certain mold contaminated seeds containing imported Brazilian groundnut meal. Allcroft *et al.* (1961) isolated the toxic principle produced by molds growing on the groundnut meal. Sargeant *et al.* (1961) for the first time isolated the toxin producing fungus, *Aspergillus flavus* and gave the name 'aflatoxin' to the toxin principle in view of its origin.

2.2.2 Economic importance of aflatoxins

A variety of contaminants are found naturally occurring in foods. Of these, mycotoxins are the major contaminants and 25 per cent of foods are contaminated with mycotoxins. Among them aflatoxins are the major mycotoxins produced by toxigenic strains of *A. flavus* and *A. parasiticus* in the suitable environment.

In August 1981, the Ministry of Agriculture in the United Kingdom banned the feeding of groundnut products to diary cows because of the possible hazards of aflatoxins to the health of milk drinkers (Swindale, 1989).

Aflatoxins are highly carcinogenic immunosuppressive, highly toxic and fatal to humans and animals particularly affecting liver and digestive track. Katiyar *et al.* (2000) reported the risk of aflatoxin with hepatitis B. Infection to human and livestock populations in India. It is currently known that there are synergistic effects between aflatoxin and hepatitis B virus infection causing liver cancer.

During 1983-1993 in India, 4818 samples of cereals, oilseed cakes, compound feeds and other ingredients showed high amounts of aflatoxin in groundnut cake, deoiled groundnut cake, maize and mixed feeds (Dhavan and Choudary, 1995). Surveys conducted in different parts of India (Ghewande *et al.*, 1989, Sahay and Rajan, 1990, Kolhe *et al.*, 1994 and Verma *et al.*, 1997) have revealed that groundnuts and groundnut products were high-risk commodities for aflatoxin contamination. Levels of aflatoxin contamination varied from 0.8 to 2200 mg per kg in groundnut kernel, traces to 200 mg per kg in edible flour, 786 mg per kg in unrefined oil, 27 to 1122 mg per kg in cake. In survey, 18 per cent groundnut based snack products carried aflatoxin B1 beyond permissible limit of 30 ppb (Rati and Santha, 1994) and 21 per cent of groundnut samples (Bhat *et al.*, 1996). A recent survey by Vijay Krishna Kumar *et al.* (2001) in Tumkur district of Karnataka revealed natural seed infection of groundnut by *A. flavus* was in the range of 10 to 22 per cent and aflatoxin content in commercial market samples were in the range of 3 to 18 µg per kg. Regulation set by European Union (EU) for maximum permissible limits of aflatoxins in foodstuffs is presented in Table 1.

In Japan, aflatoxin B1 was detected in the exports from 20 of 31 countries, five lots of large type raw shelled and 269 lots of small type raw shelled groundnuts were rejected as having above the regulation level (10 ppb) of aflatoxin B1 (Itoh *et al.*, 2001).

2.2.3 Genetic resistance to *A. flavus* invasion and aflatoxin production

Besides adopting certain cultural, harvest, and storage practices, resistant varieties should be an effective and low-cost part of an integrated aflatoxin management program. Alleviation of aflatoxin contamination through genetic manipulation has been attempted in several groundnut producing countries since the late 1960s. Breeding resistant cultivars is possible only when there are available sources of stable, high-level of resistance. It is very important that screening methods provide reliable information on the responses of various genotypes. Many efforts have been made to better understand the interactions between plant and pathogen, and four mechanisms of resistance to *Aspergillus* spp. have been defined, including resistance to *in vitro* seed colonization by *A. flavus* (IVSCAF), resistance to field seed colonization by *A. flavus* (FSCAF), resistance to preharvest aflatoxin contamination (PAC), and resistance to aflatoxin production.

2.2.4 Screening studies

Mechanisms of resistance to *Aspergillus* colonization and infection may relate to combinations of physical and chemical characteristics of the testa. Mixon and Rogers (1973) first suggested that use of groundnut cultivars with resistance to seed invasion and colonization by toxigenic *Aspergillus* species would be an effective means of preventing aflatoxin contamination and developed a new *in vitro* seed colonization procedure for screening the groundnut genotypes against *A. flavus*. Their results indicated that Valencia type genotypes *viz.,* PI 337394F and PI 337409 were resistant to two toxin-producing strains of the fungus.

Bartz *et al.* (1978) performed an *in vitro* experiment of seed colonization of 15 cured and hand shelled groundnut genotypes and showed that Florunner was the most tolerant cultivar and Tifspan was the most susceptible to seed colonization by *A. flavus* even though tolerance to *A. flavus* seems to be a varietal characteristic, it is too variable to be used readily in groundnut breeding program. Some of the lines that were tolerant in one assay were susceptible in another. Florunner was the most tolerant cultivar but highly variable. Since, the percentage seed colonized was in the range of 22 to 39 per cent across the assays.

Priyadarshini and Tulpule (1978) studied the reaction of different varieties of maize and groundnut and stated that there was no direct correlation between fungal growth and aflatoxin production, suggesting that the genotypes produced different amount of aflatoxin per unit growth of the fungus.

The value of a resistance source depends upon the level and stability of its resistance. Resistance to pod infection was highly variable and of a low level. Similarly, IVSCAF-resistance is not absolute because even the best sources showed up to 15 per cent seed colonization (Upadhyaya *et al*., 1997a).

Davidson *et al.* (1983) reported that levels of infection and aflatoxin contamination were related primarily to environmental conditions especially to drought stress during pod maturation.

Blankenship *et al.* (1985) evaluated groundnut genotypes to *A. flavus* infection under laboratory conditions and found that all were resistant. However, these genotypes when evaluated under field condition by imposing the drought conditions were found to be susceptible.

Kisyombe *et al.* (1985) reported that genotypes J-11 and Lamoang were characterized as resistant to *A. parasiticus* under both dry and moist field conditions. They also evaluated 34 genotypes for dry seed resistance in laboratory and found that there was no correlation between genotype for resistance to dry seed infection and resistance under field conditions.

Mehan *et al.* (1986) evaluated several groundnut genotypes in four rainy seasons (1979-82) and found that IVSCAF – resistant genotypes had significantly lower seed infection with *A. flavus* than the IVSCAF-susceptible genotypes at both normal harvest (at optimum maturity) and late harvest (10 days after maturity). Aflatoxin contamination increased with increase in maturity of pods indicating the importance of lifting the groundnut crop at optimum maturity.

Kiran Kalia *et al.* (1988) evaluated 53 groundnut cultivars and found that high yielding lines were susceptible to invasion by *A. flavus* and aflatoxin contamination. These results also indicated that line Oh 53-1 showed highest resistance with low yield potential and J-11 showed resistance to aflatoxin production and moderately susceptible reaction to *A. flavus* invasion.

Mehan *et al.* (1988) evaluated 11 groundnut genotypes and showed that six were resistant and five susceptible. They were evaluated under field conditions in seven environments in South India. Five of the IVSCAF resistant genotypes had significantly greater

Table 1: Maximum permissible limits of aflatoxins in food stuffs and animal feeds

Sl. No.	Products	Aflatoxins : Maximum admissible level (µg/kg)	
1.	Human consumption: Commission regulation (EC) No. 1525/98 dt. 16/07/1998	B1	B1+B2+G1+G2
	✓ Peanuts intended for direct human consumption or as ingredient in food stuffs	2	4
	✓ Peanuts to be subjected to sorting or other physical treatment before human consumption or use as an ingredient in food stuffs	8	15
2.	Animal and bird feed council directive: 1999/29/EC dt. 22/04/1999		
	✓ Peanuts intended for direct usage as nutrient dietary for animal and bird (maximum content in µg/kg related to a feeding stuff with a moisture content of 12%)	20	
	✓ Peanuts to be subjected to further processing before animal and bird consumption or use as an ingredient in feeding stuff (maximum content in µg/kg related to a feed material with a moisture content of 12%)	200	

resistance to infection of seed by *A. flavus* and had lower aflatoxin contamination than the susceptible genotypes.

Groundnut genotypes are more susceptible to *A. flavus* and *A. parasiticus* colonization under both long and short drought stress condition compared to non-stress conditions as reported by Azaizeh *et al.* (1989).

More than 80 per cent of the tested groundnut cultivars were susceptible to seed colonization *in vitro* by *A. flavus* at a concentration of 6.3×10^3 spores per ml. Only five cultivars were resistant with 17.5 to 27 per cent infection. Li (1989) reported that cultivars with crinkled and split shells were 100 per cent infected.

Cultivars such as RS 1, J 11, Chitra, GG 11 and koyana can be adopted in areas where aflatoxin contamination is a serious problem (Desai *et al.*, 1991 and Ghewande *et al.*, 1993).

Desai *et al.* (1991) tested 39 different groundnut varieties and breeding lines to *A. flavus* infection and found that tested groundnuts were significantly differed in infection and aflatoxin production, infection and seed colonization were strongly correlated and no correlation was found between infection and aflatoxin content.

Using seed inoculation method, Nayak *et al.* (1992) screened 46 genotypes *in vitro* for resistance to *A. flavus* and reported considerable variation in resistance reaction of genotypes to this disease.

Cole *et al.* (1993) found that enhanced resistance of groundnut genotypes was partially associated with improved drought tolerance as measured by the ability to maintain high kernel moisture under extended drought conditions.

Anderson *et al.* (1995) evaluated 12 potentially resistant genotypes for pre-harvest aflatoxin contamination and found that none of the genotypes were more resistant to pre-harvest aflatoxin contamination than the genotype Florunner.

Rao *et al.* (1995) assessed the Spanish groundnut germplasms ICGV-88145 and ICGV-89104 for seed colonization by *A. flavus* under artificial inoculation conditions and it averaged 22.2 and 24.0 per cent compared with 15.6 per cent in the best resistant control.

Anderson *et al.* (1996) developed an effective procedure i.e., *in vitro* seed colonization for screening the individual plants for resistance to invasion by *A. flavus*.

Nahdi (1996) screened four groundnut genotypes TMV-2, NCAC-17090, Robust 33-1 and EC 76446 in two seasons by creating early and midseason drought and found increased infection of seeds by *A. flavus* and aflatoxin contamination was found only in second season.

Groundnut genotype resistance to late leaf spot and white mold can not be used as an indirect selection tool for resistant to aflatoxin contamination (Holbrook *et al.* 1997).

Holbrook *et al.* (2000) evaluated 20 genotypes of groundnut having drought tolerance and susceptibility. These results indicated that susceptible genotypes had greater pre-harvest aflatoxin contamination and drought tolerant genotypes had less pre-harvest aflatoxin contamination.

Thakur *et al.* (2000) evaluated 35 wild *Arachis* germplasm belonging to 24 species in six sections for *in vitro* seed colonization with a highly aggressive and toxigenic strain of *A. flavus* (isolate Af11-4) and for aflatoxin production. Large variation existed for seed colonization severity (1.0 – 4.0) and aflatoxin production.

Nageshwara Rao *et al.* (2001) have suggested that management of drought by either escape tolerance or avoidance mechanisms may therefore have a significant impact on a genotype ability to reduce aflatoxin contamination.

Upadhyaya *et al.* (2001b) studied seed colonization by *Aspergillus flavus* under artificial inoculation conditions in the laboratory and found average 18.4, 15.4 and 16.7 per cent colonization in ICGV-91278, ICGV-91283 and ICGV-91284, respectively compared with 13.6 per cent in J 11 and 46.6 per cent in JL-24.

Varma *et al.* (2001) reported that most of the cultivated varieties of Karnataka exhibited colonization comparable to the most susceptible variety, TMV-2. Three genotypes S-206, KRG-1 and GPBD-4 recorded relatively low levels of colonization indicating their tolerance to *A. flavus* (isolate UASD-1).

Gowda *et al.* (2002) reported that mutant 28-2 was early maturing resistance to late leaf spot on a 1-9 disease rating scale scored 5 for late leaf spot and it had a colonization severity of 3.0 for *Aspergillus flavus* compared to 3.7 for JL-24 on a 1 to 4 scale. Mutant 28-2 also gave mean pod yield ranging from 1.54 to 3.86 t per ha with yield increases of 9.6 to 31.9 per cent over the control JL-24.

Liao Boshou *et al.* (2003) studied thirty groundnut cultivars with different levels of bacterial wilt resistance to *Aspergillus flavus* and aflatoxin production and found that the cultivar Niaohongmao recorded *A. flavus* resistance Similar to that of J-11, the Taishan Zhenzhu and 93-76 recorded the lowest aflatoxin content.

Mohan *et al.* (2003) screened 13 confectionary groundnut genotypes against *A. flavus* seed colonization. None of the genotypes of cultivated groundnut showed stable resistance to *A. flavus* although there was certain degree of resistance to seed colonization in the genotypes studied.

Harish Babu *et al.* (2004) reported that ICGV 86155, ICGV 86699 and ICGV 96266 were significantly more resistant to *A. flavus* compared to resistance check J11.

Rahmianna *et al.* (2004) reported the evaluation of fourteen groundnut genotypes for drought tolerance and aflatoxin contamination. Three genotypes ICGV 86590, ICGV 93280 and ICGV 95322 had pod yields of more than 2.5 t per ha and low aflatoxin contamination was observed inspite of seed infection by *Aspergillus flavus*, which ranged between 3.3 and 14.7 per cent.

Wang Sheng Yu *et al.* (2004) observed four groundnut lines that showed resistance to aflatoxin contamination *in vitro* for pre-harvest contamination by aflatoxins under end of season drought stress and the results confirmed that aflatoxin resistance *in vitro* could reduce pre-harvest contamination of seeds by aflatoxins.

Chen-Shuang Long *et al.* (2005) evaluated five new groundnut cultivars for their resistance to *A. flavus* in a field experiment. All the cultivars were resistant to the pathogen and recorded high yield during regional trial.

Harish Babu *et al.* (2005) reported that Trombay groundnut genotypes TG-19, TG-49, TG-18A and TG-18 showed high level of resistance with very low seed colonization by *A. flavus* compared to resistant check J 11.

Yugandhar (2005) reported that ICG-6027, ICG-13787, ICG-8760, ICG-14985 and GPBD-6 has resistant to *A. flavus* infection in *in vitro* conditions.

2.2.5 Genetics of resistance and breeding

Breeding for resistance to *Aspergillus* is predicted on the existence of high-level resistance sources, reliable assessment methods and an understanding of the inheritance of the traits. Many sources of resistance have been identified by different screening methods (Table 2). Of the many resistance sources reported, J 11, PI 337394F, PI 337409, UF 71513, Ah 7223, Faizpur 1-5 and Var. 27 have been confirmed by testing over locations (Rao *et al.,* 1989). All these sources exhibited resistance to IVSCAF and FSCAF. Two genotypes U 4-7-5 and VRR 245 were reportedly resistant to aflatoxin production and J 11, PI 337394F, PI 337409, UF 71513-1, Faizpur 1-5 and U 4-7-5 have been used in breeding programs to develop cultivars with desirable resistance to *A. flavus*. Lines with resistance to IVSCAF and

Table 2: Sources of resistance to *Aspergillus flavus* or *A. parasitius* (based on Isleib *et al*, 1994)

Source of resistance	PI number	Type of resistance	Country where used	References
1-4		IVSCAF	India	Ghewande *et al.* (1989)
1-7		IVSCAF	India	Ghewande *et al.* (1989)
55-457	360862	FSCAF	Senegal	Waliyar and Bockelee-Morvan (1989)
	363058			Zambettakis *et al.* (1981)
	407492	IVSCAF	Senegal	Zambettakis *et al.* (1981)
73-30		FSCAF	Senegal	Waliyar and Bockelee-Morvan (1989)
				Zambettakis *et al.* (1981)
		IVSCAF	Senegal	Zambettakis *et al.* (1981)
73-33		FSCAF	Senegal	Waliyar and Bockelee-Morvan (1989)
				Zambettakis *et al.* (1981)
		IVSCAF	Senegal	Zambettakis *et al.* (1981)
A. cardenasii		AP	India	Ghewande *et al.* (1989)
		IVSCAF	India	Ghewande *et al.* (1989)
A. duranensis		AP	India	Ghewande *et al.* (1989)
		IVSCAF	India	Ghewande *et al.* (1989)
A purilla	497572	IVSCAF and AP	India	Thakur *et al.* (2000)
A chiquilana	476004	IVSCAF and AP	India	Thakur *et al.* (2000)
A. triseminata	338449	IVSCAF and AP	India	Thakur *et al.* (2000)
A. triseminata	ICG 14875	IVSCAF and AP	India	Thakur *et al.* (2000)
Acc 63		IVSCAF	Philippines	Pua and Medalla (1986)
Ah 6487	590327	IVSCAF	China	Tsai and Yeh (1985)
Ah 7223	590343	FSCAF	India	Mehan *et al.* (1986, 1987))
		IVSCAF	India	Mehan and McDonald (1980)
				Ghewande *et al.* (1989)
AR-1	565480	IVSCAF	USA	Mixon (1983b)
AR-2	565481	IVSCAF	USA	Mixon (1983b)
AR-4	565483	IVSCAF	USA	Mixon (1983b)
Basse	229553/298858	IVSCAF	China	Tsai and Yeh (1985)
C 116®	590295	IVSCAF	China	Tsai and Yeh (1985)
C 184	590299	IVSCAF	China	Tsai and Yeh (1985)
Celebes		IVSCAF	Philippines	Pua and Medalla (1986)
CES 48-30		IVSCAF	Philippines	Pua and Medalla (1986)
CGC 7		IVSCAF	India	Ghewande *et al.* (1989)
CGC 2		IVSCAF	India	Ghewande *et al.* (1989)
Darou IV		Pod infection	Senegal	Zambettakis *et al.* (1981)
F-7		IVSCAF	China	Tsai and Yeh (1985)
Faizpur	590321	IVSCAF	India	Mehan and McDonald (1980) *Contd……*
GE 652		IVSCAF	China	Tasi and Yeh (1985)
GFA-1	565478	IVSCAF	USA	Mixon (1983a)
GFA-2	565479	IVSCAF	USA	Mixon (1983a)
J 11		FSCAF	India	Mehan *et al.* (1986, 1987)
		IVSCAF	India	Mehan and McDonald (1980)

(Contd)

				Ghewande *et al.* (1989)
			USA	Kisyombe *et al.* (1985)
M 395	590300	IVSCAF	China	Tsai and Yeh (1985)
Muna B	590284	IVSCAF	China	Tsai and Yeh (1985)
Monu 240-30	590325	IVSCAF	India	Mehan and McDonald (1980)
NC 449		IVSCAF	China	Tsai and Yeh (1985)
NC 482		IVSCAF	China	Tsai and Yeh (1985)
PI 196621		IVSCAF	China	Tsai and Yeh (1985)
PI 196626		IVSCAF	China	Tsai and Yeh (1985)
PI 337394 F		FSCAF	India	Mehan *et al.* (1986, 1987)
			Senegal	Walvar and Bockelee-Morvan (1989)
				Zambettakis *et al.* (1981)
		IVSCAF	India	Mehan and McDonald (1980)
			Senegal	Zambettakis *et al.* (1981)
			USA	Mixon and Rogers (1973, 1975)
Rosado	337409	FSCAF	Senegal	Zambettakis *et al.* (1981)
		IVSCAF	India	Mehan and McDonald (1980)
			Senegal	Zambettakis *et al.* (1981))
			USA	Kisyombe *et al.* 1985
				Mixon and Rogers, 1973, 1975
			Senegal	Waliyar and Bockelee-Morvan, 1989
RMP 12	443080	IVSCAF	China	Tsai and Yeh, 1985
Roxo (Sal)		IVSCAF	China	Tsai and Yeh, 1985
S 230		IVSCAF	India	Ghewande *et al.*, 1989
Shalanth		Pod infection	Senegal	Zambettakis *et al.* (1981)
SO 218		IVSCAF	China	Tsai and Yeh, 1985
SO 424		IVSCAF	China	Tsai and Yeh, 1985
U4477	362144	FSCAF	India	Mehan *et al.*, 1986b, 1987
		IVSCAF	India	Mehan and McDonald, 1980
U4-7-5		AP	India	Mehan *et al.*, 1986a

(Contd)

U4-7-3	590353			
U4-7-25	590331			
U4-47-2	590332			
UF-71513	590374	FSCAF	India	Mehan *et al.* (1986, 1987)
		IVSCAF	India	Mehan and McDonald (1980)
UPL Ph4		IVSCAF	Philippines	Pua and Medalla (1986)
Var 27		IVSCAF	India	Mehan and McDonald (1980)
VRR 245		AP	India	Mehan *et al.* (1986a)
ICGV 88145		FSCAF	India	Rao *et al.* (1995)
ICGV 89104		FSCAF	India	Rao *et al.* (1995)
ICGV 91278		FSCAF	India	Upadhyaya *et al.* (2001b)
ICGV 91283		FSCAF	India	Upadhyaya *et al.* (2001b)
ICGV 91284		FSCAF	India	Upadhyaya *et al.* (2001b)

IVSCAF = *In vitro* seed colonization by *A. flavus*
FSCAF = Field seed colonization by *A. flavus*
AP = Aflatoxin production

FSCAF [GFA-1, GFA-2, AR-1, -2, -3, and -4 (Mixon, 1983a, 1983b, 1986)], or resistance to FSCAF (ICGV 88145, ICGV 89104, ICGV 91278, ICGV 91283, and ICGV 91284) have been bred (Rao *et al*., 1995; Upadhyaya *et al*., 2001b). AR-1, -2, -3, and -4 have been used as resistance sources in Thailand, and drought-resistant line 55-437 has been used in Senegal (Rao *et al*., 1989). U4-7-5 is the only genotype supporting low levels of aflatoxin that has been used in a breeding program.

ICGV 89104 was selected from a cross between U4-7-5 and J 11 and was reported to be resistant to seed infection in field, and seed colonization in artificial inoculation conditions. Its natural aflatoxin contamination was zero, compared with 2.1 µg kg-1 in J 11 (Rao *et al*., 1995).

ICGV 91283 was selected from a cross between U4-7-5 and JL 24. JL 24 is susceptible to seed infection and seed colonization by *A. flavus*. ICGV 91283 was reported resistant to seed infection in field and seed colonization after artificial inoculation. Aflatoxin content in this line was not reported (Upadhyaya *et al*., 2001b). The results suggested that a low aflatoxin production trait could be transferred to other lines. Inheritance of resistance has not been studied extensively. Mixon (1976) estimated the broad sense heritability for IVSCAF resistance to be 75.5% in the F2 generation of a cross between resistant genotype PI 337409 and susceptible line PI 331326.

Upadhyaya *et al.* (1997a) reported heritability estimates of 56 to 87% for preharvest seed infection. Utomo *et al.* (1990) reported broad-sense heritability estimates in F_2-derived F_6 populations from two crosses, AR-4 / NC 7 and GFA-2 / NC 7. AR-4 and GFA-2 are IVSCAF-resistant genotypes, and NC 7 is a susceptible cultivar. The heritability estimates from those two crosses were 55 and 63%, respectively, for seed colonization, 27 and 33% for preharvest seed infection, and 23 and 21% for aflatoxin production. There were no significant correlations among types of resistance, and it was concluded that different genes controlled them.

2.3 Correlation studies

Mishra and Yadav (1992) reported that pod yield showed positive association with pod yield per plant indicating that pod yield per plant could be used as criteria for selecting high yielding genotypes. Similar association with yield per plant was reported by Bhagat *et al.* (1993).

Vasanthi *et al*. (1998) reported that pod weight per plant showed significant positive correlation with sound matured kernel percentage and shelling percentage showed significant positive association with sound matured kernel, late leaf spot and rust severities. Also reported high heritability and genetic advance for 100-kernel weight, late leaf spot and rust.

Lakshmidevamma *et al*. (2004) carried out correlation and path coefficient analysis for pod yield and oil yield with some of their component characters in 81 genotypes of groundnut pod yield possessed significant positive association with kernel yield and test weight at both genotypic and phenotypic levels.

2.4 Late leaf spot

The foliar diseases *viz.,* late leaf spot and rust are commonly present wherever the groundnut is cultivated. Each disease alone is capable of causing substantial yield loss but when they occur together losses are further increased up to 70 per cent in India (Subrahmanyam *et al*., 1980). These diseases also have an adverse influence on the recovery of pods, quality of seeds and haulms.

Rust is one of the most destructive fungal diseases in almost all groundnut-growing areas of the world. It is caused by *Puccinia arachidis* Speg. and first noted by Spegazzini (1884). The disease occurs in most of the groundnut growing Indian states and more intensively in South Indian states as conditions favours the development and spread of the disease (Subrahmanyam and McDonald, 1982). Pod loss caused by rust reach 50-80 per cent in epidemic year (Sandhikar *et al*., 1989).

The *Cercospora* or tikka leaf spots (Early and late leaf spots) are the most important foliar fungal diseases of groundnut. Late leaf spots distributed throughout the world and more predominant compared to early leaf spot because of its fast spreading nature. It is caused by *Cercospora personatum* (Berk and Curt) and was described in USA in 1875. The perfect stage *Mycosphaeselles berkeleyii* was described by Jenkins (1938). But, recently, it was renamed as *Phaeoisariopsis personata* (Berk and Curt) V. Arx.

Reys and Romasata (1940) reported that leaf spot lesions are not only confined to the leaf lamina, but may occur on petioles, stems and pegs leading to direct deterioration of the developing pods. Leaf spot causes damage by causing lesion formation, reduction in photosynthetic area by way of defoliation (Boote *et al*., 1980) and premature leaflet abscission. Generally, 10 to 15 per cent yield losses were reported due to late leaf spot (McDonald *et al*., 1985) worldwide and reduced seed yield could be due to reduction in dry weight, chlorophyll, protein and sugar (Ghosh and Biswas, 1995).

Sources of resistance to both early and late leaf spot have been identified in *Arachis hypogaea* (Chiteka *et al.* 1988; Anderson *et al.* 1993). Very high level of resistance to late leaf spot has been found in wild species of groundnut (Stalker and Simpson 1995) and used to develop breeding lines with resistance (Gorbert *et al.* 1982; Melouk *et al.* 1984; Wells *et al.* 1994; Xue and Holbrook 1998). Programmes are going on to introgress this resistance into *A. hypogaea* (Stalker and Beute 1993).

Dinakaran *et al.* (1992) conducted separate field evaluations of disease damage caused by late leaf spot and rust with 28 groundnut genotypes and the standard cultivars JL-24 and TMV-2. Their study revealed that PI-215696, NCAC-927, EC76446 (292), PI350680 and PI259747 were resistant to both diseases.

Gopal *et al.* (1993) screened 56 groundnut genotypes for late leaf spot and rust diseases during the rainy season (1990). The results showed that 2 Spanish, 3 Virginia bunch and 5 Virginia runners were resistant to both diseases.

Gopal *et al.* (1994) evaluated six genotypes and four susceptible controls Girnar 1, JL-24, TMV-2 and KRG-1 for late leaf spot and rust resistance. R 8972 was the most resistant to LLS and rust with scores of 3.0 and 2.5, respectively.

Aquino *et al.* (1995) found that latent period and maximum percentage of lesion that sporulated were the components of resistance most highly correlated with late leaf spot diseases development. They suggested that using either of these two components to evaluate breeding populations might facilitate more rapid selection of lines with improved levels of rate reducing resistance.

Vasanthi *et al.* (1998) reported that a significant and positive association of leaf spot and rust severity with shelling percentage. By studying the eleven elite lines developed at ICRISAT and three varieties for late leaf spot and rust, which showed high heritabilities of 96.55 and 93.28 per cent, respectively.

Until the release of ‘Southern Runner’ in 1984, no commercial cultivar was available with meaningful resistance to late leaf spot (Gorbet *et al.* 1999). Holbrook and Isleib (2001) observed that screening of 13000 accessions at IAC led to the identification of 69 genotypes resistance to LLS (Mehan *et al.* 1996). Forty-nine of these resistance sources were land races from Peru. Out of 69 LLS resistant genotypes only 19 were being used in breeding programme. Only one of them (ICG 4747) had resulted in release of resistant cultivars as ICG (FDRS) 4 and ICGV 86590 from IAC and Girnar 1 from Indian national programme. Some resistant accessions from Peru are ICGS-10920, ICGS-11182, ICGS-12720.

Gowda *et al.* (2002) developed GPBD-4 an early maturing resistant variety of late leaf spot and rust from the cross KRG-1 × ICGV-86855.

Reddy *et al.* (2004) studied molecular diversity among genotypes for resistance to late leaf spot and rust in groundnut. The susceptible lines clustered distinctly away from the resistant group and GPBD-4 occupied a distinct cluster.

Mondal *et al.* (2005) studied nineteen groundnut genotypes with varying resistance to late leaf spot and rust VG-9514, TFDRG-5, GPBD-4, DTG-27, DTG-57, DTG-58, DTG-60, TDG-56 and Mutant 28-2 resistant to both diseases.

2.5 Molecular diversity

Molecular markers allow selection for the traits to be done on the basis of a simple laboratory tests on a small amount of plant tissue, rather than direct measurement of the character itself.

Markers based on DNA sequence variations are increasingly being utilized in crops for construction of genetic maps and marker-assisted selection. Application of molecular markers in plant breeding has established the need for information on varieties in DNA sequence even in those crops where little genetic and cytogenetic information is available; DNA markers provide a reliable means of estimating the genetic relationship between genotype compared to morphological markers (Gepts, 1993).But, their application in groundnut enhancement is lagging behind because of limited knowledge of genome.

Hopkins *et al.* (1999) with an objective of identifying simple sequence repeats (SSR) markers in cultivated groundnut and to test these markers for their ability to discriminate among genotypes and he developed six fluorescent SSR (simple sequence repeat) primer pairs, which discriminated 19 accessions into 17 primer genotypes. Subsequently more number of SSR primers were developed and polymorphism among cultivated groundnut genotypes was reported (He *et al.*, 2003; Ferguson *et al.*, 2004 and Moretzsohn *et al.*, 2004).

Previous studies have shown little or no DNA polymorphism in cultivated groundnut (*Arachis hypogaea*). Subrahmanyam *et al.* (2000) selected 70 genotypes exhibiting variation for several morphological, physiological and other characters and studied polymorphism using random amplified polymorphic DNA (RAPD) assay with only seven out of 48 oligonucleotide primers were polymorphic. Out of total 408 bands, 27 (6.6%) bands were polymorphic.

Dwivedi *et al.* (2001) selected 26 accession and 8 primers for random amplified polymorphic DNA assay to determine genetic diversity. The genetic similarity (S_{ij}) ranged from 59.0 to 98.8 per cent with an average of 86.2 per cent. Both multidimensional scaling and

unweighted pair group method with arithmetic averages (UPGMA) dendrogram revealed the existence of five distinct clusters. Some accessions with diverse DNA profile (ICG 1448, 7101, 1471, 99106 and 99014) were identified for mapping and genetic enhancement in groundnut.

Raina *et al.* (2001) used 71 random and 29 SSR primers to assess genetic variation and inter relationships among subspecies and botanical varieties of cultivated groundnut. They reported that 42.7 and 54.4 per cent polymorphism from RAPD and SSR primers, respectively. Also the dendrogram based on RAPD, ISSR and RAPD + ISSR data precisely organized the five botanical varieties of two subspecies into five clusters and established phylogenetic relationships among cultivated groundnut and *Arachis* wild species.

Sixteen accessories possessing varying levels of resistance to early leaf spot could show polymorphism ranging from 11.7 to 55 per cent using RAPD assay, with average of 27.4 per cent per primer (Dwivedi and Gurtu, 2002).

Krishna *et al.* (2004) evaluated 48 cultivated Valencia groundnut genotypes using 18 fluorescent-labeled SSR (f-SSR) primers. This study showed considerable genetic variation among selected genotypes. Bhagwat *et al.* (1997) studied difference in banding pattern between Spanish improved and its X-ray induced mutants using 12 random primers. The large selected mutant and dwarf mutant showed greater variation with Spanish improved. He and Prakash (1997) reported the presence of DNA polymorphism in cultivated groundnut using the amplified fragment length polymorphism (AFLP) technique.

Lei-Yong *et al.* (2005) identified that two AFLP markers are closely linked to the gene conferring resistance to *A. flavus* were identified. Tests to verify the reliability of the markers using 20 groundnut genotypes showed a high correlation between the molecular markers and the resistant of the crop to *A. flavus*.

3. MATERIALS AND METHODS

The information regarding the materials and methodology used and statistical procedures followed in each experiment are described in this chapter.

Experiments conducted

3.1 EXPERIMENT No. I: Evaluation of selected groundnut genotypes for resistance to *Aspergillus* seed colonization, yield traits and diversity

Materials used: Eighteen selected genotypes (Table 3a) comprising highly resistant, moderately resistant and susceptible cultivars identified by *in vitro* screening were evaluated in field for yield characters. In rainy season, the experimental material was sown on 26th June 2006, in a Randomized Complete Block Design (RCBD) with two replications. Each genotype was grown in three rows of 2.25m length with spacing of 30 cm between rows and 10 cm between plants respectively. The crop was harvested on 16th October 2006. The experiment was repeated during 2007 post rainy season. The material was sown on 17th January and harvested on 18th May.

3.1.1 Observations recorded

1. Pod yield
2. Pod yield per plant
3. Kernel yield per plant
4. Test weight
5. Shelling per cent
6. Sound mature kernels
7. Disease resistance (late leaf spot and rust)
8. *Aspergillus* score
9. Genetic diversity

3.2 EXPERIMENT No. II: Assessing the segregating material for resistance to *Aspergillus* seed colonization and other traits.

Materials used: The experimental materials for present study comprised of segregating populations in the F_3 (selfing series) of TG 19 x GPBD 4, TG 49 x GPBD 4 and their reciprocal crosses. In rainy season, each segregating line sown on 29th June 2006 in a randomized design with two replications each line was grown in one-meter length with a spacing of 30 $\times$ 10 cm. The crop was harvested on 10th October 2006. The parents used in the generation of experimental materials for the present investigation have been given in Table 3b.

3.2.1 Observations recorded

1. Pod yield per plant
2. Kernel yield per plant

Table 3a: Pedigree of selected genotypes

Sl. No.	Genotypes	Botanical group	Pedigree/origin	Remarks
1	ICG 14985	Spanish bunch	Unknown	Germplasm
2	ICG 8760	Virginia runner	Sudan	Germplasm
3	ICG 13787	Virginia bunch	Niger	Germplasm
4	ICG 6027	Valencia	Sudan	Germplasm
5	ICGV 86155	Spanish bunch	ICGS 30 × (TMV10 × Chico F6)	Germplasm
6	ICGV 86699	Virginia bunch	(*Arachid batizocoi* × *A. duranensis*) × *A. hypogaea* (NC2 × CS29)	Germplasm
7	GPBD 5	Spanish bunch	TG 49 × D 39d, 10-1	Breeding lines
8	GPBD 6	Valencia	Mutant × NcAc 343, 1-35	Breeding lines
9	TG 19	Virginia bunch	TG 17 × TG1	Breeding lines
10	TG 49	Spanish bunch	TG 28A × TG 26	Breeding lines
11	TG 41	Spanish bunch	TG 28 × TG 22	Cultivar
12	TGLPS 3	Valencia	TAG 24 × TG 19	Cultivar
13	M 28-2	Valencia	EMS treated mutant derived from VL-1 (2002)	Cultivar
14	GPBD 4	Spanish bunch	Cross between KRG-1 and ICGV 86855	Cultivar
15	TAG 24	Spanish bunch	TGS 2 × TGE 1	Cultivar
16	JL 24	Spanish bunch	Selection from EC 94943 (1878)	Cultivar
17	TMV 2#	Spanish bunch	Mass selection from Gudhiathan bunch (1940)	Cultivar
18	J 11##	Spanish bunch	Ah 4218 × Ah 4354	Cultivar

- Susceptible check ## - Resistant check

Table 3b: Salient features of the parents

Genotype	Botanical type	Duration (days)	Salient features
TG 49	Spanish bunch	110	✓ Early maturing ✓ Large seeded ✓ Resistant to *Aspergillus* seed colonization ✓ Susceptible to late leaf spot and Rust
TG 19	Virginia bunch	120	✓ Large seeded ✓ Resistant to *Aspergillus* seed colonization ✓ Susceptible to late leaf spot and Rust
GPBD 4	Spanish bunch	100 - 105	✓ High yielder, early maturing ✓ Resistant to late leaf spot and rust

3. Test weight
4. Shelling per cent
5. *Aspergillus* score
6. Disease resistance (late leaf spot and rust)

3.3 Experimental site

The research work was conducted at Botanical garden of University of Agricultural Sciences, Dharwad for two seasons, rainy and post rainy. This location is situated in the Northern Transitional Tract of Karnataka at $15^0$13', at latitude of $75^0$07' E longitudes at an altitude of 678m above sea level.

3.4 Cultivation practices

The seedbed was prepared to the fine tilth before taking up the sowing. The recommended package of practices for cultivation of groundnut crop was adopted.

3.5 Environmental seasons

The monthly meteorological data obtained from meteorological department of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, with regard to temperature, relative humidity, rainfall and number of rainy days during the course of investigation is presented in (Appendix I). Disease incidence (Late leaf spot and Rust) was scored during rainy season as well as in post rainy season.

3.6 Description of the observation

3.6.1 Pod yield (PYH)

Well-dried and cleaned pods from each plot were weighed. The pod weight (g) per plot was expressed in kg ha^{-1}.

3.6.2 Pod yield per plant

Pod yield per plant was calculated by dividing total pod yield per plot by number of plants in the plot expressed as g / plant.

$$\text{Pod yield per plant (g)} = \frac{\text{Pod yield per plot (g)}}{\text{Number of plants per plot}}$$

3.6.3 Kernel yield per plant

Kernel yield per plant was calculated by dividing total kernel yield per plot by number of plants in the plot expressed as g / plant.

$$\text{Kernel yield per plant (g)} = \frac{\text{Kernel yield per plot (g)}}{\text{Number of plants per plot}}$$

3.6.4 Test weight

The well-dried and cleaned pods from each genotype were shelled and 100 kernels at random were counted and weight was recorded in grams.

3.6.5 Shelling per cent

Hundred grams of pods were taken and shelled. The weight of kernels gave shelling percent.

3.6.6 Sound mature kernels

After shelling the known quantity of pods, the kernels obtained were sorted out into well-matured and shriveled kernels and the number of good kernels was counted and expressed as percent of total number of kernels to obtain SMK.

$$\text{SMK (\%)} = \frac{\text{Number of well-matured kernels}}{\text{Total number of kernels}} \times 100$$

3.7 Disease resistance

3.7.1 Rust and Late leaf spot

The modified 9- point scale for rust (Table 4a and Fig. 1) and late leaf spot (Table 4b and Fig. 2) diseases as given by Subbarao *et al.* (1990) was used for screening genotypes. The visual scores (1-9) and extent of leaf area destroyed (1-100%) are linearly related. The field disease scores are mainly based on the extent of leaf area damaged. For late leaf spot, the extent of defoliation is also incorporated into the scale. Genotypes were scored 100 days after sowing.

3.7.2 *Aspergillus seed colonization*

3.7.2.1 Biological material of *Aspergillus flavus:*

Pure culture of *Aspergillus flavus* strain *Af* 11-4 was used in the study. This strain was procured from ICRISAT (International Crop Research Institute for semi Arid Tropics), Patancheru, Hyderabad, and Andhra Pradesh, India and is considered to be highly aggressive and toxigenic strain (Thakur *et al*., 2000).

3.7.3 *In vitro* seed colonization

3.7.3.1 Chemicals and Glass wares:

Mercuric chloride - 0.1 % (w/v) aqueous solution was used for surface sterilization of seeds.

Ethyl alcohol - 70% (v/V) was used for surface sterilization of inoculation chamber and Inoculation loop.

Tween - 80 - One or two drops of this is added to the inoculum (spore suspension) for uniform distribution of spores in the suspension.

Petridishes - Borosil Petridishes (9 cm diameter) were used to place the seed for incubation after inoculation with spore suspension. Semi-rigid plastic boxes were used to place Petridishes for incubation in an incubator.

Automizer - 50 ml capacity used for spray inoculation of seeds with spore Suspension.

3.7.4 Maintenance of the fungus culture

The pure culture of the fungus, *Aspergillus flavus* (*Af* 11-4) was sub cultured on the potato Dextrose Agar (PDA) slants and allowed to grow at 25 ± 1^0 C temperature. The culture so obtained was stored in refrigerator at 4^0 C for further use. The sub culturing was done regularly at one-month interval. Standard procedure was followed for preparation of PDA.

Table 4a. Modified 9-point scale used for field screening groundnut genotypes for resistance to rust disease

Diseas e score	Description	Disease severity (%) ψ
1.	No disease	0
2	Pustules sparsely distributed, largely on lower leaves	1-5
3	Many pustules on lower leaves, necrosis evident, very few pustules on middle leaves	6-10
4	Number of pustules on lower and middle leaves, severe necrosis on lower leaves	11-20
5	Severe necrosis of lower and middle leaves, pustules may be present on top leaves but less severe	21-30
6	Extensive damage to lower leaves, middle leaves, necrotic with dense distribution of pustules, pustules on top leaves	31-40
7	Severe damage of lower and middle leaves, pustules densely distributed on top leaves	41-60
8	100 per cent damage to lower and middle leaves, pustules on top leaves, which are severely	61-80
9.	Almost all leaves withered, bare stems seen	81-100

ψ Percentage leaf area damaged by rust

Table 4b: Modified 9-point scale used for screening groundnut genotypes for resistance to late leaf spot disease

Diseas e score	Description	Disease severity (%) ψ
1.	No disease	0
2	Lesions present largely on lower leaves; no defoliation	1-5
3	Lesions present largely on lower leaves, very few on middle leaves; defoliation of some leaf lets evident on lower leaves	6-10
4	Lesions present on lower and middle leaves but severe on lower leaves; defoliation of some leaf lets evident on lower leaves	11-20
5	Lesions present on lower and middle leaves, over 50 % of defoliation of lower leaves	21-30
6	Severe; eosins on lower and middle leaves; lesions present but less severe on top leaves; extensive defoliation of lower leaves; some defoliation on middle leaves	31-40
7	Lesions on all leaves but less severe on top leaves; defoliation of all lower and middle leaves	41-60
8	Defoliation of all lower and middle leaves; severe lesions on top leaves evident.	61-80
9	Almost all leaves defoliated, leaving bare stem; some leaflets my remain, but show severe leaf spot	81-100

ψ Percentage leaf area damaged by late leaf spot (Subbarao *et al* 1990)

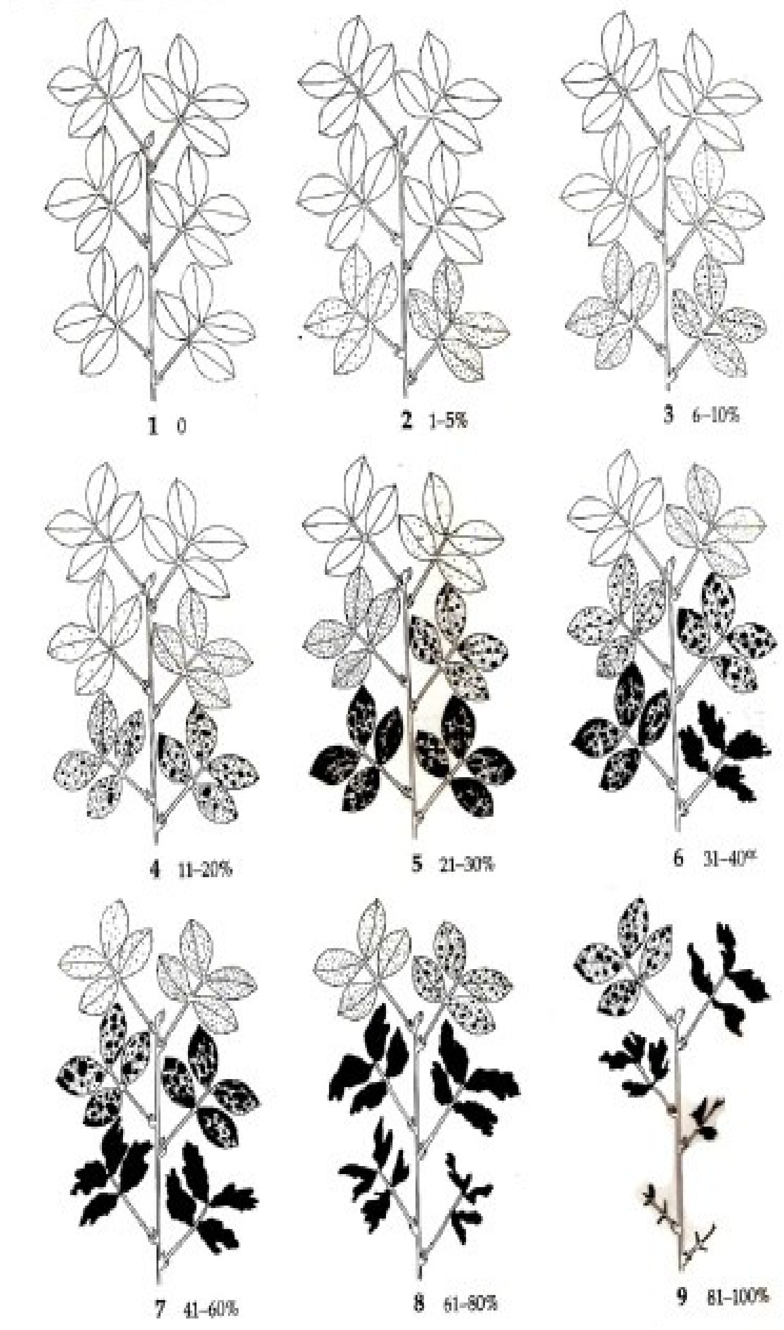


Fig 1: The modified 9-point scale for field evaluation of rust of groundnut

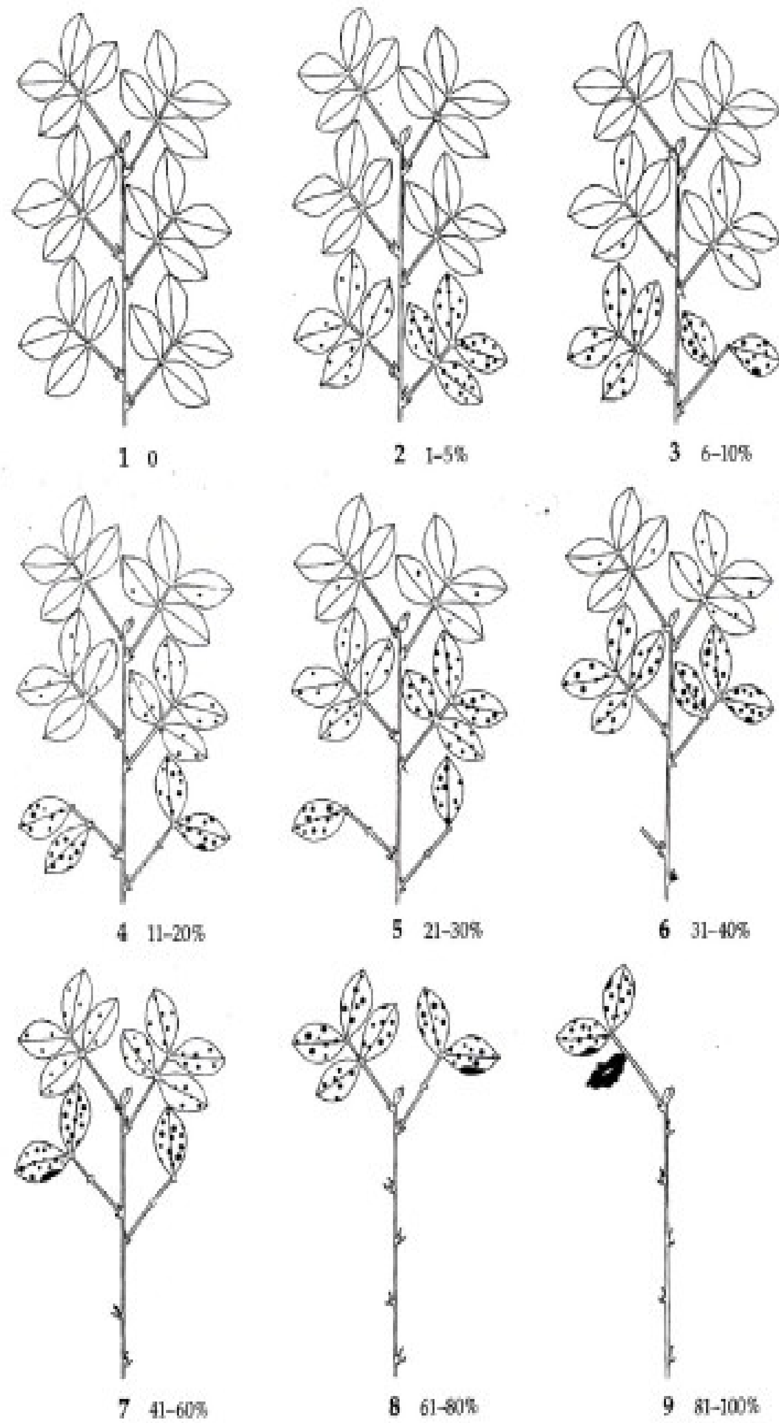


Fig2 The modified 9-point scale for field evaluation of late leaf spot of groundnut

3.7.5 Preparation of conidial suspension (inoculum)

Aspergillus flavus was grown on PDA slants. The culture was incubated at room temperature for 7 days from the time of inoculation to ensure maximum sporulation. The test tubes having thick/heavy sporulation were irrigated with 5 ml of distilled sterile water and the conidia were harvested into suspension by gentle brushing using an inoculation loop without disturbing the agar. This suspension was added to a test tube containing 5 ml of distilled sterile water to make up 10 ml of spore suspension. To this, about one to two drops of Tween-80 were added for uniform dispersal of conidia in the suspension. Serial dilution and pour plate method was used for enumeration of spores or conidia per ml of spore suspension. Accordingly the original spore suspension was diluted using distilled sterile water to get final concentration of 1×10^6 spore/ml and was used for inoculation of groundnut seeds.

3.7.6 Inoculation of groundnut seeds

Twenty seeds (weighing 4-10 g depending on the seed size) which are well matured with intact seed coat and free from any damage were selected from each genotype for *in vitro* inoculation by *A. flavus*. Seeds were surface sterilized with 0.1% (w/v) aqueous solution of mercuric chloride for 2 minutes and subsequently washed in two changes of distilled sterilized water to remove any traces of mercuric chloride.

Each seed was uniformly wounded by pricking with a sterile needle to facilitate the invasion by *A. flavus* spores. Seeds were placed in a sterilized petridish (9 cm diameter) and spray inoculated with *A flavus* spore suspension (1×10^6 spores/ml) using an automizer under strict aseptic conditions. The petridishes were shaken vigorously to roll the seeds allowing uniform distribution of inoculum on the seeds. The experiment was conducted in two replication with 10 seeds per replication.

3.7.7 Incubation

The petridishes were placed at high humidity (>95% RH) in semi-rigid plastic boxes lined with cotton wool and blotting paper with closely fitting lids and incubated at 25 ± 1^0 C in dark for 7-10 days.

3.7.8 Scoring for colonization severity

Individual seeds were scored for surface colonization by *A. flavus* and for colonization severity following rating scale given by Thakur *et al.*, (2000).

Scale	Description
1	< 5 per cent seed surface colonized with scanty mycelial growth and scanty sporulation.
2	5-25 per cent seed surface colonized with good mycelial growth and scanty sporulation.
3	26 – 50 per cent seed surface colonized with good mycelial growth and good sporulation
4	> 50 per cent seed surface colonized with heavy sporulation.

3.8 Genetic Diversity

3.8.1 DNA isolation

DNA was extracted based on previously reported cetyltrimethylammonium bromide (CTAB) method (Saghai-Maroof *et al.*, 1984) with some modifications. Leaves were ground to fine powder in the presence of liquid nitrogen and transferred to sterile tube containing 9 ml of preheated (65^0C) 2x CTAB extraction buffer (1 M Tris-Hcl buffer pH 8, 4 M NaCl, 500 mM ethylene di-aminetetraacetic acid (EDTA) pH 8 and 1 per cent b-mercaptoethanol, 200 mg polyvinylpyrolidone) 10 per g of leaf tissue was added and mixed gently. The contents were incubated for 90 minutes at 65^0C in a water bath with occasional shaking during incubation. The tubes were kept for 10 minutes to allow them to return to room temperature. An equal quantity of (9 ml) of chloroform and amyl alcohol solution, prepared in a ratio of 24:1 was added to the tubes and they were centrifuged at 8000 rpm at 4^0C for 20 min. The aqueous phase was transferred to clean tube and the chloroform and amyl alcohol solution step was repeated. Nucleic acids were precipitated by adding 0.6 ml chilled isopropanol to the aqueous phase and incubating at -20^0C for 20 min. The DNA was hooked out and transferred to new sterile tube containing 2 ml of $T_{10}E_1$ buffer (50 ml $T_{10}E_1$ + 1 ml of Rnase 10 mg/ml) and left over night at room temperature. Phenol, chloroform, and amyl alcohol prepared in the ratio of 25:24:1 was added to the tube and mixed gently and tube was centrifuged at 8000 rpm at 10^0C. The clear phase was once again cleaned using another phenol, chloroform amylalcohol solution, then washed and spun at 2^0C. The aqueous phase was transferred to new tube and DNA was precipitated using 2-4 ml of pure chilled ethanol. Tubes were kept at -20^0C for 10 min. The DNA was precipitated by centrifuge for 5 min. The DNA pellet was further washed with 70 per cent alcohol for 1 min. The tubes were allowed to drain and dried at room temperature for 2 to 3 hr, then resuspended in 200 to 500 microlitre of $T_{10}E_1$ buffer. The quantity and concentration of DNA was assessed by spectrophotometer and also by gel electrophoresis using 0.8 percent agarose with known concentration of uncut lambda DNA.

3.9 Polymerase chain reaction

3.9.1 Requirements for polymerase chain reaction

1 Random primers: Commercial kits of random decamer DNA primers were obtained from operon Technologies Inc. Almedas, USA. A total of 20 random primers were used for the assay. The sequence details of the primers are presented in the Table 5.

2. dNTPs: The four dNTPs *viz* dATP, dCTP, dGTP and dTTP were obtained from M/s Bangalore Genei Pvt. Ltd., Bangalore.

3 *Taq* DNA polymerase: Taq DNA polymerase and 10x Taq assay buffer were obtained M/s Bangalore Genei Pvt. Ltd., Bangalore.

3.9.2 Preparation of master mix for PCR

Master mix was prepared by mixing different components in the proportion as shown in the Table 6, and master mix was distributed to each tube (19 microlitre /tube) and 1 microlitre of template DNA from respective genotypes was added making the final volume 20 microlitre.

3.9.3 The thermo profile for PCR

The thermo profile for PCR reaction was set as shown in Table 7. After completion of the PCR, the products were stored at 4^0 until the gel- electrophoresis was done.

3.9.4 Agarose gel electrophoresis

The PCR product was mixed with 2 microlitre of loading dye (Bromphenol blue) and was loaded in 1.4 per cent agarose gel of 1x TAE buffer containing Ethidium bromide (5

Table 5: List of primers used for RAPD analysis

Sl. No.	Primers	Sequence (5’-3’)
1	OPK-09	CCCTACCGAC
2	OPK 14	CCCGCTACAC
3	OPA 19	CAAACGTCGG
4	OPC 15	GACGGATCAG
5	OPC 09	CTCACCGTCC
6	OPC 13	AAGCCTCGTC
7	OPB 11	GTAGACCCGT
8	OPF 09	CCAAGCTTCC
9	OPJ 06	TCGTTCCGCA
10	OPV 16	GGGCCAATGT
11	OPA 15	TTCCGAACCC
12	OPA 20	GTTGCGATCC
13	OPF 07	CCGATATCCC
14	OPA 12	TCGGCGATAG
15	OPJ 17	ACGCCAGTTC
16	OPC 03	GGGGGTCTTT
17	OPV 15	AGTCGCCCTT
18	OPC 06	GAACGGACTC
19	OPF 10	GCAAGCTTGG
20	OPA 17	GACCGCTTGT

Table 6: Components of master mix

Components	Quantity (microlitre/tube)
10x Assay buffer	2
dNTPs (10mM)	1
Primer (5 pM)	2
Taq DNA Polymerase	0.33
Nanopure water	13.67
DNA template	1
Total	20.0

Table 7: Thermo profile for PCR

Sl. No.	Step	Temperature (^{0}C)	Duration (min)	No. of cycles
1	Denaturation	94	5	1
2	Denaturation Annealing Primer extension	94 36 72	2 1 2	38
3	Final extension	72	10	1
4	Dump	4		

µl/100 ml). Gel was run at 50 volts. The gel was photographed by using documentation system (Uvitech, Cambridge, England).

3.9.5 Scoring the amplified fragments

The amplified fragments were scored as ‘1’ for presence and ‘0’ for the absence of a band generating the 0 and 1 matrix.

3.10 Statistical analysis

3.10.1 Analysis of variance (ANOVA)

The data on different characters were subjected to analysis of variance for Randomized Complete Block Design (RCBD) as detailed by Panse and Sukhatme (1967).

$$\text{Coefficient of variation (CV \%)} = \times 100 \; \frac{\sqrt{\text{EMSS}}}{\bar{X}}$$

Critical difference (CD) = $S.E_d$ x t at 0.05 for (r-1) (g-1) d f

3.10.2 Phenotypic and genotypic variances

These were calculated according to the formula given by Lush (1940) and Choudhary and Prasad (1968).

Genotypic variance $\sigma^2 g$ = (GMSS-EMSS)/r

Phenotypic variance $\sigma 2p$ = $\sigma 2g + \sigma^2 e$

Error variance $\sigma^2 e$ = EMSS

3.10.3 Coefficient of variance

The components of variance *viz.*, phenotypic and genotypic variance were used for the estimation of phenotypic and genotypic co-efficient of variation as per the methods suggested by Singh and Choudhary (1979).

$$\text{Phenotypic coefficient of variation (PCV \%)} = \times 100 \; \frac{\sqrt{\sigma^2 p}}{\bar{x}}$$

$$\text{Genotypic coefficient of variation, (GCV \%)} = \times 100 \; \frac{\sqrt{\sigma^2 g}}{\bar{X}}$$

Where,

$\sigma^2 p$ = Phenotypic variance

$\sigma^2 g$ = Genotypic variance

X = Mean of the character

The GCV and PCV values were classified as described by Sivasubramanian and Menon (1973).

GCV and PCV values	Classification
0 – 10	Low
10 – 20	Medium
20 and above	High

3.10.4 Heritability

Heritability in broad sense (H) was computed as a ratio of genotypic variance to the total variance (Hanson *et al.*, 1956)

$$H = \frac{\sigma^2_g}{\sigma^2_p} \times 100$$

Where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

Heritability estimates were classified into low, moderate and high by following Hanson *et al.* (1956).

Heritability	Classification
0 – 30%	Low
30 – 60%	Medium
60% and above	High

3.10.5 Genetic advance

Genetic advance was estimated by using the formula given by Johnson *et al.* (1955).

$$GA = h^2 \times k \times \sigma_p$$

Where,

h^2 = Heritability estimate

k = Selection differential which is equal to 2.06 at 5% intensity of selection (Lush, 1940)

σ = Phenotypic standard deviation

3.10.6 Genetic advance percentage over mean

$$GAM = \frac{GA}{\overline{X}} \times 100$$

Where,

GA = Genetic advance

$\overline{X}$ = General mean of the character

Classification of GAM is as follows (Johnson *et al.*, 1955)

GAM	Classification
0 – 10	Low
10 – 20	Medium
20 and above	High

3.11 Correlation Analysis

Genotypic and Phenotypic correlation was computed (Singh and Chaudury, 1979) to determine the degree of association among the characters by using the formula.

3.11.1 Genotypic correlation

$$r_{xy}(g) = \frac{Cov_{xy}(g)}{\sqrt{V_x(g) \times V_y(g)}}$$

3.11.2 Phenotypic correlation

$$r_{xy}(p) = \frac{Cov_{xy}(p)}{\sqrt{V_x(p) \times V_y(p)}}$$

Where,

$Cov_{xy}(g)$ = Genotypic covariance between characters x and y

$Cov_{xy}(p)$ = Phenotypic covariance between characters x and y

$Cov_{xy}(e)$ = Error covariance between characters x and y

$V_x(p)$ = Phenotypic variance of character x

$V_y(p)$ = Phenotypic variance of character y

$V_x(g)$ = Genotypic variance of character x

$V_y(g)$ = Genotypic variance of character y

r_{xy} (p) = Phenotypic correlation coefficient between characters x and y

r_{xy} (g) = Genotypic correlation coefficient between characters x and y

3.11.3 Duncan's Multiple Range Test (DMRT)

The means for different traits in experiment I were subjected to Duncan's Multiple Range Test (DMRT) using MSTAT C Software to know the significant differences among the selected genotypes.

3.11.4 Polymorphic information content (PIC)

The polymorphic information content (PIC) (Bolstein *et al*., 1980) of the primer was estimated by,

$$PIC = 2 \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} P_i P_j (1 - P_i P_j)$$

Where,

P_i = Frequency of ith allele

K = Number of alleles

3.11.5 Dice similarity coefficient

Pair wise genetic similarity (Sij) between genotypes was estimated using Dice similarity coefficient (Okuno *et al*., 1998). Clustering was done using symmetric matrix of similarity coefficient. A dendrogram was constructed based on Sij values using clustering technique of unweighted pair group arithmetic mean (UPGMA) using SHAN module of NTSYSpc version 2.0 (Rohlf, 1998).

3.11.6 Superior segregates (%)

In all segregating generations separately the number of plants that exceeded the performance of the better parents were noted as superior segregates for that particular trait and expressed as per cent.

4. EXPERIMENTAL RESULTS

The results obtained from Experiment 1 and Experiment 2 are presented under different headings *viz.,* i) analysis of variance, ii) components of variation, iii) association among traits and iv) mean performance of the genotypes.

4.1 Analysis of variance (ANOVA)

Analysis of variance for individual seasons as well as pooled data over seasons is presented in Table (8a, 8b and 8c). Genotypes exhibited significant variation for all the traits during rainy season as well as post- rainy season. Pooled analysis revealed a highly significant variation due to seasons, genotypes and genotype x season interaction for all the characters (except shelling percentage due to seasons).

4.2 Components of variance

The nature and magnitude of the variation was assessed by phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H %) and genetic advance over mean (GAM) for each season as well as pooled data (Table 9a, b and c).

In rainy season, high GAM (29.97 – 45.99) was observed for pod yield(kg ha^{-1}), test weight (g), pod yield per plant(g) and kernel yield per plant(g) coupled with high heritability (55.90 – 89.80), PCV (20.08 – 26.02) and moderate to high GCV (19.03 – 23.91), whereas shelling percentage and sound mature kernel showed low GAM (7.08 – 10.50) due to low magnitude of variation GCV (4.57 and 6.96) and PCV (6.08 and 9.51) respectively with moderate heritability (56.50 and 53.60) in yield traits.

All diseases showed high GAM (49.46 – 121.19) due to high magnitude of variation PCV (26.66 – 59.74), GCV (25.31 – 59.27) coupled with high heritability (90.20 – 98.40).

In post-rainy season, all yield traits including shelling percentage exhibited high GAM (20.32 – 47.85 coupled with high heritability (49.45 – 91.00) and moderate to high magnitude of variation PCV (11.21 – 29.19) and GCV (10.52 – 26.03) except sound mature kernel which exhibited low GAM (7.38) due to low magnitude of variation GCV (4.60) and PCV (5.91) with high heritability (60.50).

In post-rainy season also, all diseases showed high GAM (63.70 – 118.96) due to high variation GCV (31.96 – 57.97) and PCV (33.02 – 58.23) coupled with high heritability (93.70 – 99.10).

When pooled over a season, among yield traits, only test weight and pod yield per hectare had high GAM (35.50 and 20.84) coupled with high to moderate heritability (87.80 and 43.20) and moderate magnitude of variation GCV (18.40 and 15.4), PCV (19.63 and 23.44), respectively whereas all other yield traits showed low GAM (0.00 – 7.15) due to low magnitude of variation GCV (0.04 – 6.91) and PCV (3.95 – 13.78) coupled with low heritability (0.00 – 25.20).

Diseases had high GAM (48.50 – 120.00) coupled with high heritability (89.90 – 98.80), PCV (26.18 – 59.07) and GCV (24.82 – 58.71).

4.3 Genotypic performance

The results pertaining performance of genotypes for yield traits and diseases during two seasons and average performance over seasons is presented in Table 10 and 11.

4.3.1 Pod yield (kg ha^{-1})

The mean pod yield was high in post- rainy season (2560 kg ha^{-1}) compared to rainy season (2279 kg ha^{-1}). However, it ranged from 1732 (ICG 6027) to 4018 kg ha^{-1} (GPBD 5)

Table 8: ANOVA of selected genotypes for different traits

a) Rainy season

Source	D.F.	Pod yield (kg/ha)	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	Sound mature kernel (%)	*A. flavus* score (1-4 scale)	Late leaf spot	Rust
Genotype	17.00	548611.76**	13.82**	7.19**	190.50**	27.60**	97.29**	3.06**	9.17**	4.42**
Error	17.00	155166.12	0.77	0.49	10.20	7.68	29.43	0.02	0.15	0.23

b) Post-rainy season

Source	D.F.	Pod yield (kg/ha)	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	Sound mature kernel (%)	*A. flavus* score (1-4 scale)	Late leaf spot	Rust
Genotype	17.00	597209.41**	17.73**	14.38**	342.34**	109.92**	46.14**	3.01**	7.18**	7.67**
Error	17.00	202191.06	2.95	1.64	16.19	7.04	11.34	0.01	0.23	0.25

c) Pooled

Source	D.F.	Pod yield (kg/ha)	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	Sound mature kernel (%)	*A. flavus* score (1-4 scale)	Late leaf spot	Rust
Season (S)	1.00	922176.00**	149.41**	75.07**	678.70**	13.47	880.25**	0.02**	82.35**	1.68*
Genotype (G)	17.00	844400.94**	16.26**	11.51**	456.21**	68.65**	52.06**	6.06**	14.02**	10.30*
G x S	17.00	301415.53**	15.29**	10.07**	76.62**	68.88**	91.38**	0.01**	2.32**	1.80*
Error	34.00	178682.35	1.86	1.07	13.20	7.36	20.38	0.02	0.19	0.24

*, ** : Significant at 5% and 1% level of probability, respectively

Table 9: Components of variation for different traits in selected genotypes

a) Rainy season

Characters	MEAN	MIN	MAX	GCV	PCV	H (%)	GA M
Pod yield (kg/ha)	2279.41	1206.00	3432.00	19.46	26.02	55.90	29.97
Pod yield / plant (g)	11.08	7.95	17.08	23.06	24.39	89.40	44.96
Kernel yield / plant (g)	7.65	5.35	12.09	23.91	25.61	87.10	45.99
Test weight (g)	49.88	35.38	66.99	19.03	20.08	89.80	37.17
Shelling percentage	69.06	63.65	74.74	4.57	6.08	56.50	7.08
Sound mature kernel (%)	83.66	73.40	96.83	6.96	9.51	53.60	10.50
A. flavus score (1-4 scale)	2.08	1.00	4.00	59.27	59.74	98.40	121.19
Late leaf spot	6.14	2.00	9.00	34.59	35.15	96.90	70.21
Rust	5.72	2.00	7.00	25.31	26.66	90.20	49.46

b) Post-rainy season

Characters	MEAN	MIN	MAX	GCV	PCV	H (%)	GA M
Pod yield (kg/ha)	2505.75	1732.00	4018.00	17.74	25.23	49.40	25.68
Pod yield / plant (g)	13.96	8.50	20.65	19.48	23.04	71.50	33.96
Kernel yield / plant (g)	9.70	3.58	15.53	26.03	29.19	79.50	47.85
Test weight (g)	56.02	35.94	81.85	22.79	23.90	91.00	44.78
Shelling percentage	68.20	42.46	74.91	10.52	11.21	88.00	20.32
Sound mature kernel (%)	90.65	77.97	96.79	4.60	5.91	60.50	7.38
A. flavus score (1-4 scale)	2.11	1.00	4.00	57.97	58.23	99.10	118.96
Late leaf spot	4.00	2.00	7.00	46.60	48.11	93.80	93.00
Rust	6.03	2.00	8.00	31.96	33.02	93.70	63.70

c) Pooled

Characters	MEAN	MIN	MAX	GCV	PCV	H (%)	GA M
Pod yield (kg/ha)	2392.58	1626.00	3254.00	15.40	23.44	43.20	20.84
Pod yield / plant (g)	12.52	9.40	17.00	3.94	11.58	11.60	2.80
Kernel yield / plant (g)	8.68	5.50	12.39	6.91	13.78	25.20	7.15
Test weight (g)	52.95	35.66	74.42	18.40	19.63	87.80	35.50
Shelling percentage (%)	68.63	57.24	74.68	0.05	3.95	0.00	0.00
Sound mature kernel percentage (%)	87.15	80.00	93.31	0.04	5.18	0.00	0.00
A. flavus score (1-4 scale)	2.10	1.00	4.00	58.71	59.07	98.80	120.00
Late leaf spot	5.07	2.00	8.00	33.75	34.81	94.00	67.46
Rust	5.88	2.00	7.50	24.82	26.18	89.90	48.51

and from 1206 (ICG 13787) to 3432 kg ha^{-1} (GPBD 4) in post-rainy season and rainy season, respectively. Average yield over seasons ranged from 1626 (ICG 8760) to 3254 kg ha^{-1} (GPBD 5) Table (10).

During rainy season, GPBD 4 gave highest pod yield (3432 Kg ha^{-1}) followed by M 28-2 (2680 kg ha^{-1}) and JL 24 (2654 kg ha^{-1}). Whereas during post-rainy season, GPBD 5 gave highest pod yield (4018 kg ha^{-1}) followed by GPBD 6 (3294 kg ha^{-1})and GPBD 4 (2888 kg ha^{-1}). But, over seasons GPBD 5 recorded highest pod yield (3254 kg ha^{-1}) followed by GPBD 4 (3160 kg ha^{-1}) and GPBD 6 (2894 kg ha^{-1}).

All genotypes performed better in post- rainy season except TMV 2 and TGLPS 3, which did not show any seasonal variation.

4.3.2 Pod yield per plant (g)

As an indicator of yield potential, pod yield per plant was more in post-rainy (13.96 g) compared to rainy season (11.08 g). In rainy season, it ranged from 7.95 g (TG 19) to 17.08 g (GPBD 5). GPBD 4 was found on par with GPBD 5 which was followed by GPBD 6 (14.93 g). In post-rainy season, average pod yield per plant was 13.96 g. However pod yield per plant ranged from 8.5g (ICG 13787) to 20.65 g (TAG 24). TAG 24 recorded highest (20.65 g) pod yield per plant followed by ICGV 86155 (17.61 g), GPBD 5 (16.91 g), JL 24 (15.74 g), ICG 14985 (15.65 g) and GPBD 6 (15.56 g) and ICGV 86155 was found on par with TAG 24. When pooled over seasons, the average pod yield per plant was 12.52 g. The range was from 9.4 g (ICG 8760) to 17.0 g (GPBD 5), GPBD 6 was on par with GPBD 5. This was followed by GPBD 4 (14.77 g) (Table 10).

A seasonal difference among the varieties was found except for GPBD 5. But TAG 24, ICGV 86155 performed better in summer than in rainy season.

4.3.3 Kernel yield

The mean kernel yield was more in post-rainy (9.70 g) compared to rainy season (7.65 g). In rainy season, it ranged from 5.35 g (TG 19) to 12.09 (GPBD 5). GPBD 4 was found on par with GPBD 5 which was followed by GPBD 6 (9.88 g). In post-rainy, mean kernel yield per plant was 9.7 g. but, kernel yield ranged from 5.38 g (ICG 8760) to 15.53 g (TAG-24). GPBD 5, J-11, ICGV 86155 and JL-24 followed TAG-24 and were found on par with each other. When pooled over seasons, the mean kernel yield per plant was 8.68 g. The range was from 5.50 g (ICG 8760) to 12.39 g (GPBD-5). GPBD-5 was followed by GPBD 4 (10.87 g) and TAG-24 (10.59 g).

4.3.4 Test weight

The mean test weight was more in post-rainy season (56.02 g) than in rainy (49.88 g). Test weight ranged from 35.38 g (J 11) to 66.99 g (TG-19) in rainy season. Three genotypes, ICG-8760, GPBD-6 and ICG-6082 were found on par with TG-19.

In post-rainy season, the mean test weight of varieties was 56.02 g however, the test weight ranged from 35.94 g (J 11) to 81.85 g (TG-19). GPBD-6 was on par with TG-19 which was followed by TG-41, GPBD-5, ICG-6027, TGLPS-3 and TG-49 which were on par with each other.

When pooled over seasons, test weight ranged from 35.66 g (J 11) to 74.42 g (TG-19). GPBD-6 was on par with TG-19 which was followed by ICG-6027, ICG-8760, TG-41, GPBD-5 and TG-49 which were on par with each other.

Seasonal differences were observed among the genotypes for test weight in most of the cases except in case of ICG-14985, J 11, TMV-2, ICG-6027 and GPBD-4 whose performance was almost same in both the seasons. Reduction in test weight was more conspicuous in TG-41, GPBD-6, TGLPS-3 and TG-19.

4.3.5 Shelling percentage

Shelling percentage was found high in rainy season (69.06%) compared to post-rainy season (68.2%). In rainy season, it ranged from 63.65 (ICGV 86155) to 74.74 per cent (J 11). TMV-2, GPBD-4 and TAG-24 were found on par with J 11. In post-rainy season, shelling percentage ranged from 42.46 (ICG 8760) to 74.91 per cent (GPBD-5). J 11, GPBD-4, TAG-24 and TG-41 were observed on par with GPBD-5. When pooled over seasons, shelling percentage ranged from 57.24 (ICG 8760) to 74.68 per cent (J 11). GPBD-4 was found on par with J 11, which was followed by TAG-24 and GPBD-5, and on par with each other.

Seasonal differences were observed among the varieties, but performance of J 11, GPBD-4, TAG-24 and GPBD-5 remained the same in both the seasons. In rainy season, TMV-2, ICG 8760, ICG 14985 were on par with J 11, but in post-rainy, the performance of ICG 8760 was incomparable to J 11. Whereas, TG-41 performed better in post-rainy than in rainy season.

4.3.6 Sound mature kernel (%)

The mean sound mature kernel was more in post-rainy (90.65) compared to rainy season (83.66). In rainy season, it ranged from 73.40 (ICGV 86155) to 96.83 (ICG-14985). ICG-14985 recorded highest sound mature kernel followed by ICG-6027 (94.38), ICG-8760 (92.78) and TAG-24 (90.85), which were on par with each other. In post-rainy season the mean ranged from 77.97 (ICG-8760) to 96.79 (TG-49). TG-49 recorded highest sound mature kernel followed by TAG-24 (96.28), GPBD-6 (94.85), M 28-2 (94.79), GPBD-4 (94.51) and JL-24 (93.86) were on par with each other.

When pooled over seasons, the mean sound mature kernel was 87.15. The range was from 80.0 (ICGV-86155) to 93.31 (TAG-24). ICG-14985 (92.06), GPBD-6 (91.78) and M 28-2 (90.26) were found on par with TAG-24, which recorded highest sound mature kernel.

4.3.7 Reaction to diseases

4.3.7.1 *Aspergillus* seed colonization

Performance of selected genotypes for *A. flavus* seed colonization severity ranged from 1 (TG 19) to 4 (TMV-2) with mean performance of 2.10 is presented in Table 11.

The genotype, TG-19, TG-49 and ICG-8760 recorded lowest seed colonization severity (1.0) on 1 to 4 scale followed by ICG-14985 (1.08), ICG-6027 (1.10), ICG-13787 (1.11) and ICGV-86699 (1.20) which were on par with each other and with TG-19, whereas GPBD-6 (1.35), TG-41 (1.38) and M28-2 (1.48) little higher seed colonization than ICGV-86699. TGLPS-3 (2.03), ICGV-86155 (2.10) and GPBD-5 (2.25) recorded moderate seed colonization. On the contrary, varieties TAG-24 (4.00), GPBD-4 (4.00), J 11 (3.95) and JL-24 (3.70) in descending order showed higher seed colonization and were on par with susceptible check TMV-2 (4.00)

4.3.7.2 Rust

Rust was observed high in post-rainy season (6.03) when compared to rainy season (5.72). In rainy season, it ranged from 2 (ICGV-86699) to 7 (TGCPS-3). While the genotypes GPBD-4, ICG-13787, ICG-8760 and ICG-14985 followed ICGV-86699 for resistance and were on par with each other and all other genotypes were susceptible and on par with TGCPS-3.

In post-rainy season, the rust ranged from 2 (ICGV-86699) to 8 (TGLPS-3). Whereas the genotypes GPBD-4 (2.51), ICG-13787 (4), ICG-8760 (4.5), 6027 (4.5) followed ICGV-86699 and GPBD-4 on par with ICGV-86699 for the resistance.

When pooled over seasons, rust ranged from 2 (ICGV-86699) to 7.5 (TGLPS-3). GPBD-4 (3), ICG13787 (4), ICG-8760 (4.25) followed the ICGV-86699 for resistance. While

Table 10: Mean performance of selected genotypes for productive parameters

Sl. No	Genotype	Pod yield (kg/ha)			Pod yield per plant (g)			Kernel yield per plant (g)		
		Rainy	Post-rainy	Pooled	Rainy	Post-rainy	Pooled	Rainy	Post-rainy	Pooled
1	ICG 14985	1711^{c-f}	1930^{de}	1821^{fg}	10.54^{eg}	15.65^{bd}	13.09^{bf}	7.53^{c-f}	11.70^{d-c}	9.30^{d-d}
2	ICG 8760	1428^{ef}	1824^{e}	1626^{c}	10.31^{f-g}	15.65^{bd}	9.40^{i}	7.24^{c-f}	3.58^{c}	5.50^{c}
3	ICG 13787	1206^{f}	2054^{c-e}	1630^{g}	10.51^{c-g}	8.50^{g}	10.80^{g-i}	6.90^{d-g}	6.66^{ff}	6.78^{fg}
4	ICG 6027	2192^{b-e}	1732^{e}	1962^{e-g}	11.91^{df}	11.10^{e-g}	10.53^{g}	8.31^{cd}	5.88^{fg}	7.09^{f-g}
5	ICGV 86155	2559^{a-d}	2606^{d-c}	2582^{b-d}	9.77^{f-i}	17.61^{ab}	13.69^{b-f}	6.22^{fg}	11.35^{bc}	8.79^{cd}
6	ICGV 86699	2301^{d-c}	2450^{d-c}	2376^{c-f}	13.53^{cd}	14.00^{d-c}	13.86^{d-d}	8.77^{bc}	9.81^{b-d}	9.29^{b-d}
7	GPBD 5	2490^{d-d}	4018^{a}	3254^{a}	17.08^{a}	16.91^{a-c}	17.00^{a}	12.09^{a}	12.68^{b}	12.39^{a}
8	GPBD 6	2494^{b-d}	3294^{ab}	2894^{a-c}	14.93^{dc}	15.56^{d-d}	15.24^{ad}	9.88^{b}	10.88^{b-d}	10.38^{bc}
9	TG 19	2172^{b-e}	2499^{b-e}	2336^{c-f}	7.95^{fj}	14.88^{be}	10.98^{f-1}	5.35^{c}	9.96^{b-d}	7.65^{b-f}
10	TG 49	2590^{a-d}	2775^{b-d}	2683^{a-d}	10.17^{e-h}	12.88^{c-f}	11.52^{e-i}	6.86^{d-g}	9.20^{c-f}	8.03^{d-f}
11	TG 41	2413^{b-d}	2223^{c-e}	2318^{c-f}	8.65^{g-j}	11.16^{c-g}	9.91^{hi}	5.61^{g}	7.90d-^{f}	6.76^{fg}
12	TGLPS 3	2632^{ac}	2642^{b-e}	2637^{b-d}	10.07^{e-h}	9.16^{f-g}	11.20^{f-i}	6.47^{e-g}	8.74^{cf}	7.60^{d-f}
13	M 28-2	2680^{ab}	2368^{b-e}	2524^{c-e}	11.10^{ef}	14.24^{b-e}	12.67^{c-c}	7.74^{c-f}	9.76^{b-d}	8.75^{c-e}
14	GPBD 4	3432^{a}	2888^{bc}	3160^{ab}	15.50^{ab}	14.03^{b-e}	14.77^{bc}	11.39^{a}	10.35^{b-d}	10.87^{b}
15	TAG 24	1649^{ab}	2798^{b-d}	2224^{d-g}	9.69^{j}	20.65^{a}	14.17^{bc}	5.64^{c}	15.53^{a}	10.59^{b}
16	JL 24	2654^{ac}	2512^{b-e}	2583^{b-d}	10.54^{e-c}	15.74^{b-d}	13.14^{b-f}	7.35^{c-f}	11.22^{b-d}	9.28^{b-d}
17	TMV 2#	2241^{b-e}	2249^{c-e}	2245^{d-f}	10.91^{ef}	12.66^{d-f}	11.79^{d-h}	8.11^{ce}	8.44^{cf}	8.28^{df}
18	J 11##	2185^{b-e}	2240^{c-e}	2213^{d-g}	8.22^{hj}	12.33^{d-c}	11.55^{e}	6.14^{fg}	11.51^{bc}	8.83^{cd}
	GM	2279	2560	2393	11.08	13.96	12.52	7.65	9.70	8.68
	CV	17	18	18	7.90	12.30	10.90	9.20	13.20	11.90
	CD 5%	831	949	631	1.90	3.60	2.00	1.50	2.70	1.50

- Susceptible check ## - Resistant check

Values followed by same letter do not differ at 5% level of probability

Contd……

Sl. No	Genotype	Test weight (g)			Shelling percentage			Sound mature kernel (%)		
		Rainy	Post-rainy	Pooled	Rainy	Post-rainy	Pooled	Rainy	Post-rainy	Pooled
1	ICG 14985	52.93^{de}	53.18^{e-h}	53.06^{de}	71.43^{ad}	69.64^{ad}	70.54^{ad}	96.83^{a}	87.29^{cd}	92.06^{ab}
2	ICG 8760	64.26^{ad}	54.27^{c-g}	59.27^{dc}	72.01^{a-c}	42.46^{e}	57.24^{c}	92.78^{a-c}	77.97^{c}	85.38^{d-c}
3	ICG 13787	57.57^{b-d}	45.62^{gi}	51.59^{de}	65.60^{c-f}	60.06^{e}	62.83^{f}	87.35^{ae}	88.14^{ad}	87.74^{ad}
4	ICG 6027	60.82^{a-c}	63.23^{b-d}	62.02^{b}	69.79^{a-f}	64.42^{de}	67.10^{de}	94.38^{ab}	84.59^{df}	89.48^{a-c}
5	ICGV 86155	44.70^{fh}	53.87^{d-g}	48.97^{ef}	63.65^{f}	67.25^{b-d}	65.45^{ef}	73.40^{f}	86.61^{cd}	80.00^{c}
6	ICGV 86699	41.53^{g-i}	55.35^{c-f}	48.44^{ef}	64.74^{a-f}	68.53^{a-d}	66.64^{d-f}	74.79^{ef}	91.74^{a-d}	83.26^{c-e}
7	GPBD 5	54.14^{c-e}	63.73^{bc}	58.93^{bc}	70.78^{a-e}	74.91^{a}	72.85^{a-c}	84.13^{a-f}	92.93^{a-c}	88.53^{a-d}
8	GPBD 6	62.50^{ab}	81.57^{a}	72.04^{a}	66.11^{c-e}	69.82^{a-d}	67.96^{df}	88.71^{a-d}	94.85^{a-c}	91.78^{ab}
9	TG 19	66.99^{a}	81.85^{a}	74.42^{a}	67.11^{b-f}	70.83^{a-c}	68.97^{c-e}	81.31^{bf}	88.54^{bd}	84.93^{be}
10	TG 49	51.79^{de}	61.72^{b-e}	56.75^{b-d}	67.54^{b-f}	70.95^{a-c}	69.25^{b-e}	82.06^{b-f}	96.79^{a}	89.42^{a-c}
11	TG 41	49.33^{ef}	68.71^{b}	59.02^{bc}	64.83^{df}	71.35^{ac}	68.09^{de}	78.57^{d-f}	92.93^{a-c}	85.41^{b-e}
12	TGLPS 3	47.96^{e-c}	62.97^{b-d}	55.47^{cd}	64.19^{ef}	74.86^{a-c}	67.53^{de}	73.96^{f}	88.77^{a-d}	81.37^{de}
13	M 28-2	49.56^{ef}	53.69^{d-h}	51.62^{df}	69.53^{a-f}	68.43^{a-d}	68.98^{c-e}	85.74^{a-f}	94.79^{a-c}	90.26^{a-c}
14	GPBD 4	38.93^{hi}	41.35^{h}	40.41^{hi}	73.52^{ab}	73.71^{ab}	73.62^{ab}	77.77^{d-f}	94.51^{a-c}	86.14^{a-e}
15	TAG 24	43.70^{hi}	47.90^{hi}	45.80^{fg}	73.32^{ab}	7325^{ab}	73.29^{a-c}	90.35^{a-d}	96.28^{ab}	93.31^{a}
16	JL 24	39.42^{hi}	44.37^{h-j}	41.90^{gh}	69.74^{a-f}	70.67^{a-d}	70.20^{b-d}	79.96^{c-f}	93.86^{a-c}	86.91^{ae}
17	TMV 2#	37.02^{hi}	39.10^{h}	38.06^{hi}	74.49^{a}	65.76^{ce}	70.12^{bd}	82. 46^{b-f}	88.21^{b-d}	85.34^{b-f}
18	J 11##	35.38^{i}	35.94^{j}	35.66^{i}	74.74^{a}	67.25^{b-d}	74.68^{a}	81.29^{d-f}	93.59^{a-c}	87.44^{a-c}
	GM	49.88	56.02	52.95	69.06	68.20	68.63	83.65	90.65	87.15
	CV	6.40	7.20	6.90	4.00	3.90	4.00	6.5	3.7	5.2
	CD 5%	6.70	8.50	5.40	5.80	5.60	4.00	11.4	7.1	6.7

- Susceptible check ## - Resistant check

Values followed by same letter do not differ at 5% level of probability

Table 11: Mean performance of selected genotypes for disease reaction

Sl. No	Genotype	IVSCAF			Rust			Late leaf spot		
		Rainy	Post-rainy	Pooled	Rainy	Post-rainy	Pooled	Rainy	Post-rainy	Pooled
1	ICG 14985	1.08^{gh}	1.09^{ef}	1.08^{g}	4.50^{b}	5.50^{c-f}	5.00^{f}	7.50^{d}	2.00^{c}	4.75^{f}
2	ICG 8760	1.00^{h}	1.00^{f}	1.00^{g}	4.00^{b}	4.50^{ef}	4.25^{c}	$600.^{c}$	3.50^{cd}	4.75^{f}
3	ICG 13787	1.10^{gh}	1.12^{ff}	1.11^{g}	4.00^{b}	4.00^{f}	4.00^{g}	6.00^{c}	2.50^{df}	4.25^{fg}
4	ICG 6027	1.85^{d}	1.10^{ef}	1.10^{g}	6.50^{a}	4.50^{ef}	5.50^{ef}	2.00^{f}	2.00^{e}	2.00^{k}
5	ICGV 86155	2.10^{c}	2.10^{df}	2.10^{cd}	7.00^{a}	7.50^{ab}	7.25^{ab}	5.00^{d}	4.50^{c}	4.75^{f}
6	ICGV 86699	1.20^{b}	1.20^{de}	1.20^{fg}	2.00^{c}	2.00^{g}	2.00^{i}	3.50^{e}	2.00^{e}	2.75^{j}
7	GPBD 5	2.25^{b}	2.25^{b}	2.25^{c}	7.00^{a}	5.50^{c-e}	6.25^{de}	5.00^{d}	2.50^{de}	3.75^{gh}
8	GPBD 6	1.35^{cf}	1.35^{c-c}	1.35^{cf}	7.00^{a}	5.00^{d-f}	6.00^{de}	3.50^{e}	2.00^{e}	2.75^{j}
9	TG 19	1.20^{df}	1.00^{f}	1.00^{c}	7.00^{a}	7.50^{ab}	7.25^{ab}	7.50^{b}	7.00^{a}	7.25^{bc}
10	TG 49	1.00^{h}	1.00^{a}	1.00^{g}	6.00^{a}	8.00^{a}	7.00^{a-c}	8.00^{d}	6.50^{a}	7.25^{de}
11	TG 41	1.30^{f-g}	1.45^{cd}	1.38^{ef}	6.00^{a}	8.00^{a}	7.00^{a-c}	8.00^{b}	7.00^{a}	7.50^{ab}
12	TGLPS 3	4.00	2.20^{b}	2.03^{d}	7.00^{a}	8.00^{a}	7.50^{a}	8.00^{b}	5.50^{b}	6.75^{e}
13	M 28-2	1.45^{f}	1.50^{c}	1.45^{f}	6.50^{a}	7.50^{ab}	7.00^{a-c}	3.50^{e}	3.50^{cd}	3.50^{hi}
14	GPBD 4	4.00^{a}	4.00^{a}	4.00^{a}	3.50^{b}	2.50^{c}	3.00^{h}	4.00^{e}	2.00^{e}	3.00^{ij}
15	TAG 24	4.00^{a}	4.00^{a}	4.00^{a}	6.00^{a}	8.00^{a}	7.00^{ac}	9.00^{a}	7.00^{a}	8.00^{a}
16	JL 24	3.65^{b}	$3 .75^{a}$	3.70^{b}	6.00^{a}	8.00^{a}	7.00^{a-c}	8.00^{b}	4.00^{c}	6.00^{e}
17	TMV 2#	4.00^{a}	4.00^{a}	4.00^{a}	6.00^{a}	6.50^{bc}	6.25^{c-e}	8.00^{b}	4.50^{c}	6.25^{de}
18	J 11##	1.00^{h}	3.90^{a}	3.95^{a}	7.00^{a}	6.00^{cd}	6.50^{b-d}	8.00^{d}	4.00^{c}	6.00^{c}
	GM	2.08	2.11	2.10	5.72	6.03	5.88	6.40	4.00	5.07
	CV	7.50	5.50	6.50	8.40	8.30	8.30	6.20	12.00	8.50
	CD 5%	0.30	0.20	0.20	1.00	1.10	0.70	0.80	1.00	0.60

- Susceptible check ## - Resistant check IVSCAF – *In vitro* seed colonization by *A. flavus*

Values followed by same letter do not differ at 5% level of probability

ICGV-86155 was on par with TGLPS-3 for susceptibility followed by TG-49 (7), TAG-24 (7), JL-24 (7) and M28-2 (4).

Seasonal differences were observed among the genotypes for rust except for ICGV-86699 (2) and ICG-13787 (4), but GPBD-4 was superior than ICG-13787 for resistance. Likewise TGLPS-3, TG-19 and ICGV-86155 was almost same in both seasons for susceptibility.

4.3.7.3 Late leaf spot

Scoring for disease incidence (late leaf spot) was done in two seasons. Incidence of late leaf spot was more in rainy season (6.4) compared to post-rainy season (4.0). In rainy season, it ranged from 2 (ICG-6027) to 9 (TAG-24). ICGV-86699, GPBD-4, M28-2 and GPBD-4 followed ICG-6027 for resistance and were found on par with each other. ICG-13787 and ICG-8760 shown moderate resistance and remaining lines were susceptible to LSS and on par with each other following the TAG-24 (9).

In the post-rainy season, it ranged from 2 (ICG-6027) to 7 (TAG-24). The genotypes ICGV-86699, GPBD-5, GPBD-6, GPBD-4 and ICG-14985 were on par with ICG-6027 for resistance and TG-41, TG-19 and TG-49 were on par with TAG-24 for susceptibility.

When pooled over seasons, disease incidence ranged from 2 (ICG-6027) to 8 (TAG-24). Three genotypes, TG-41 (7.5), TG-19 (7.25) and TG-49 (7.25) followed TAG 24 and TG-41 was on par with TAG-24 for susceptibility.

There were seasonal differences among the genotypes for resistance to disease except ICG-6027 and M 28-2. ICGV-86699, GPBD-6 and GPBD-4 have shown seasonal variation but were significantly superior to all other genotypes for resistance.

4.3.8 Character associations

Correlation coefficients measure the mutual relationship between various characters, which help in devising efficient strategies for indirect selection using component characters and simultaneous selection of multiple traits. It also reveals favorable associations, which could be exploited, and unfavorable associations that should be modified employing appropriate breeding strategies.

4.3.9 Associations with pod yield

The association of all the traits with the most important productivity trait *viz.,* pod yield per plant was examined. Kernel yield and pod yield showed strong and positive association in both the seasons (Table 12). The association was positive with shelling percentage (0.67), sound mature kernel (0.65) and *Aspergillus* seed colonization (0.51) in post-rainy season, but not in rainy season. Test weight had negative correlation in post-rainy and positive in rainy season, but no significant association between them. LLS showed desirable negative association (-0.69) in rainy season.

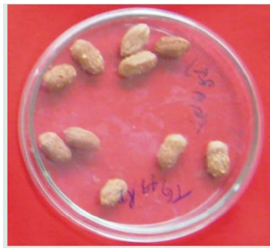
4.3.10 Association with test weight

The association of all the characters with test weight was assessed using phenotypic and genotypic correlations. None of the productive traits *viz.,* pod yield per plant, kernel yield per plant and shelling percentage showed any significant association. But, pod yield per hectare showed negative association (-0.51) in rainy season and sound mature kernel had strong positive association in rainy season (0.66). Test weight showed strong desirable negative association with *Aspergillus* seed colonization in both the seasons (Table 13).

4.3.10 Association with disease

Among diseases, significant variation among genotypes was observed for *Aspergillus* seed colonization and late leaf spot, hence its association with other traits was examined (Table 14). The *Aspergillus* seed colonization was positively associated with shelling

Plate 1 : Reaction to late leaf spot and rust in selected groundnut genotypes



TG 49

TG 19

ICG 8760

ICG 14985

TMV 2

J 11

Plate2: selected genotypes resistant o in vitro seed colonization by Aspergillus flavus

percentage (0.77) in rainy season and kernel yield (0.55) in post-rainy. LLS exhibited desirable negative association with kernel yield (-0.63) in rainy season and positive with rust (0.81) in post-rainy season.

4.4 Molecular diversity

Eighteen varieties were subjected to RAPD assay using 20 primers OPK-09, OPK-14, OPA-19, OPC-15, OPC-09, OPC-13, OPB-11, OPF-09, OPJ-06, OPV-16, OPA-15, OPA-26, OPF-07, OPA-12, OPJ-17, OPC-03, OPV-15, OPC-06, OPF-10 and OPA-17.

Twenty primers generated a total of 150 amplified fragments (Table 15), out of which 60 (3.00) showed polymorphic bands. 1 to 3 major fragments were amplified along with bands of less intensity. An example of RAPD variation is shown in Plate 3. The polymorphism percentage for primers ranged from zero (OPA-19) to 100 per cent (OPA-12) with an overall average of 42.32 per cent. Number of amplified fragments ranged from 3 to 10 in a given primer. On an average 7.5 bands per primer were amplified and 3.00 bands per primer were polymorphic. Primer OPA-17, OPA-19 and OPJ-06 showed monomorphic bands in all varieties. Primer OPK-14, OPJ-17, OPA-12, OPF-09 and OPF-10 showed polymorphism. The band profile obtained from 20 primers is summarized in Table 15. PIC (polymorphic information content) values were calculated to identify most polymorphic primer. PIC values ranged from 0 (OPA-19) to 0.2 (OPF-10) with mean PIC of 0.08 per primer. OPF-10 had high PIC values followed by OPA-12 (0.19) and OPF-09 (0.18).

Based on the Dice Coefficients (Okuno et al., 1998), the mean similarity indices for 18 varieties ranged from 0.86 to 0.99 (mean 0.93) indicating that accessions had 93 per cent of their RAPD fragments in common (Table 16). The genotypes ICG 13787 and GPBD 4 were most diverse in comparison with other genotypes. The dendrogram revealed three distinct clusters at S_{ij} of 0.94. All cultivars were clustered together, distinct from germplasm lines except for ICG 14985, which clustered in cultivars. GPBD 4 is distinct among the cultivars whereas resistant lines for *Aspergillus* seed colonization are distributed through out the dendrogram (Fig: 3)

4.5 Segregating population

4.5.1 Analysis of variance (ANOVA)

The analysis of variance revealed significant differences for pod yield per plant, kernel yield per plant, test weight, shelling percentage, *Aspergillus* seed colonization score, rust and LLS score in both the F_3 populations *viz.,* TG 49 × GPBD 4 (Table 17a) and TG 19 × GPBD 4 (Table 17b).

4.5.2 Components of variance

The estimates of range, mean, genotypic and phenotypic coefficients of variability of F_3 populations indicated substantial range of variation, as presented in Table 18a & b.

In TG 49 × GPBD 4 population, the mean for pod yield per plant, kernel yield per plant, test weight and shelling percentage were significantly more compared to TG 19 × GPBD 4. The mean of *Aspergillus* seed colonization score and rust score were comparable and on par in both the populations. Whereas, mean for LLS is better in TG 19 cross. The range for all the parameters was wider and beyond the parents in both the populations.

4.5.3 Variability in segregating population

In both the populations GAM estimates were high (35.26 - 88.78) for all the characters coupled with high heritability (63.10 – 97.80), PCV (23.40 – 55.02) and GCV (20.01 – 47.31) except for shelling percentage. Shelling percentage exhibited low to moderate

Table 12: Genotypic and phenotypic correlation coefficient of pod yield per plant with other characters

Character	Rainy		Post-rainy	
	Genotypic correlation	**Phenotypic correlation**	**Genotypic correlation**	**Phenotypic correlation**
Pod yield/ha (kg)	0.51*	0.37	0.66**	0.54*
Kernel yield/plant (g)	0.98**	0.97**	0.97**	0.97**
Test weight (g)	0.04	0.07	-0.18	-0.04
Shelling percentage (%)	0.02	0.04	0.67**	0.59**
Sound mature kernel (%)	0.04	-0.04	0.65**	0.54
A. flavus score (1-4 scale)	-0.01	-0.03	0.51*	0.42
Late leaf spot	-0.69**	-0.65**	0.14	0.13
Rust	-0.23	-0.23	0.28	0.27

*, ** : Significant at 5% and 1% level of probability, respectively

Table 13: Genotypic and phenotypic correlation coefficient of test weight with other characters

Character	Rainy		Post-rainy	
	Genotypic correlation	**Phenotypic correlation**	**Genotypic correlation**	**Phenotypic correlation**
Pod yield/ha (kg)	-0.51*	-0.32	0.28	0.29
Pod yield/plant (g)	0.04	0.07	-0.18	-0.04
Kernel yield/plant (g)	-0.04	0.02	-0.15	-0.05
Shelling percentage (%)	-0.42	-0.23	0.06	0.05
Sound mature kernel (%)	0.66**	0.44	-0.05	-0.01
A. flavus score (1-4 scale)	-0.80**	-0.76**	-0.68**	-0.65**
Late leaf spot	-0.29	-0.25	0.20	0.20
Rust	0.14	0.10	0.17	0.16

*, ** : Significant at 5% and 1% level of probability, respectively

Table 14: Genotypic and phenotypic correlation coefficient of diseases with other characters

	Character	Rainy		Post-rainy	
		Genotypic correlation	**Phenotypic correlation**	**Genotypic correlation**	**Phenotypic correlation**
A. flavus score (1-4 scale)	Pod yield (kg/ha)	0.35	0.25	0.29	0.19
A. flavus score (1-4 scale)	Kernel yield / plant (g)	0.13	0.10	0.55*	0.48*
A. flavus score (1-4 scale)	Shelling percentage	0.77**	0.56*	0.41	0.39
A. flavus score (1-4 scale)	Sound mature kernel (%)	-0.29	-0.21	0.45	0.33
Late leaf spot	Pod yield (kg/ha)	-0.33	-0.20	0.00	0.01
Late leaf spot	Kernel yield / plant (g)	-0.63**	-0.59*	0.18	0.16
Late leaf spot	Shelling percentage	0.21	0.16	0.22	0.17
Late leaf spot	Sound mature kernel (%)	-0.16	-0.07	0.21	0.18
Late leaf spot	*A. flavus* score (1-4 scale)	0.33	0.33	0.12	0.10
Rust	Pod yield (kg/ha)	0.24	0.19	0.08	0.07
Rust	Kernel yield / plant (g)	-0.23	-0.23	0.29	0.28
Rust	Shelling percentage	-0.03	-0.04	0.30	0.26
Rust	Sound mature kernel (%)	-0.06	-0.04	0.27	0.20
Rust	*A. flavus* score (1-4 scale)	0.12	0.11	0.14	0.13
Rust	Late leaf spot	0.21	0.20	0.81**	0.78**

*, **: Significant at 5% and 1% level of probability, respectively

Table15: Analysis of RAPD banding pattern in varieties

	No of amplified bands	No of Polymorphic bands	Percent polymorphism	PIC Values
OPA12	5	5	100.00	0.19
OPA15	3	2	66.66	0.10
OPA17	7	0	0.00	0.00
OPA19	10	0	0.00	0.00
OPA20	6	3	50.00	0.07
OPB11	7	4	57.14	0.13
OPC03	8	5	62.50	0.11
OPC06	6	2	33.33	0.04
OPC09	10	4	40.00	0.12
OPC13	8	5	62.50	0.15
OPC15	9	2	22.22	0.07
OPF07	7	3	42.85	0.05
OPF09	8	7	87.50	0.17
OPF10	8	7	87.50	0.20
OPJ06	7	0	0.00	0.00
OPJ17	7	1	14.28	0.03
OPK09	8	4	50.00	0.12
OPk14	8	1	12.50	0.05
OPV15	10	2	20.00	0.04
OPV16	8	3	37.50	0.06
Total	150	60	--	--
Average	7.5	3.0	42.32	0.0840

Table 16: Similarity matrix of selected genotypes based on RAPD banding pattern

	TG 49	ICG 14985	TMV 2	TAG 24	GPBD 4	JL 24	GPBD 6	GPBD 5	M 28-2	ICG 6027	ICG 8760	TGLPS 3	TG 19	ICGV 86699	J 11	TG 41	ICGV 86155	ICG 13787
TG 49	1.000																	
ICG 14985	0.983	1.000																
TMV 2	0.972	0.969	1.000															
TAG 24	0.962	0.966	0.954	1.000														
GPBD 4	0.936	0.940	0.935	0.946	1.000													
JL 24	0.951	0.955	0.957	0.947	0.934	1.000												
GPBD 6	0.969	0.973	0.969	0.965	0.953	0.961	1.000											
GPBD 5	0.986	0.983	0.966	0.976	0.950	0.951	0.983	1.000										
M 28-2	0.983	0.986	0.969	0.972	0.947	0.962	0.979	0.990	1.000									
ICG 6027	0.928	0.925	0.912	0.916	0.902	0.926	0.938	0.935	0.932	1.000								
ICG 8760	0.936	0.940	0.935	0.953	0.933	0.949	0.946	0.943	0.947	0.895	1.000							
TGLPS 3	0.980	0.983	0.972	0.969	0.957	0.965	0.990	0.986	0.990	0.935	0.957	1.000						
TG 19	0.986	0.983	0.972	0.969	0.943	0.951	0.976	0.986	0.990	0.928	0.950	0.986	1.000					
ICGV 86699	0.983	0.980	0.962	0.965	0.940	0.947	0.972	0.990	0.986	0.924	0.940	0.983	0.983	1.000				
J 11	0.983	0.986	0.976	0.966	0.954	0.969	0.986	0.983	0.986	0.932	0.954	**0.997**	0.983	0.980	1.000			
TG 41	0.986	0.990	0.973	0.976	0.951	0.958	0.983	0.993	**0.997**	0.936	0.951	0.993	0.993	0.990	0.990	1.000		
ICGV 86155	0.944	0.947	0.943	0.939	0.919	0.920	0.961	0.958	0.954	0.918	0.904	0.951	0.951	0.954	0.947	0.958	1.000	
ICG 13787	0.900	0.889	0.875	0.894	**0.864**	0.889	0.902	0.907	0.896	0.949	0.879	0.900	0.892	0.896	0.896	0.900	0.888	1.000

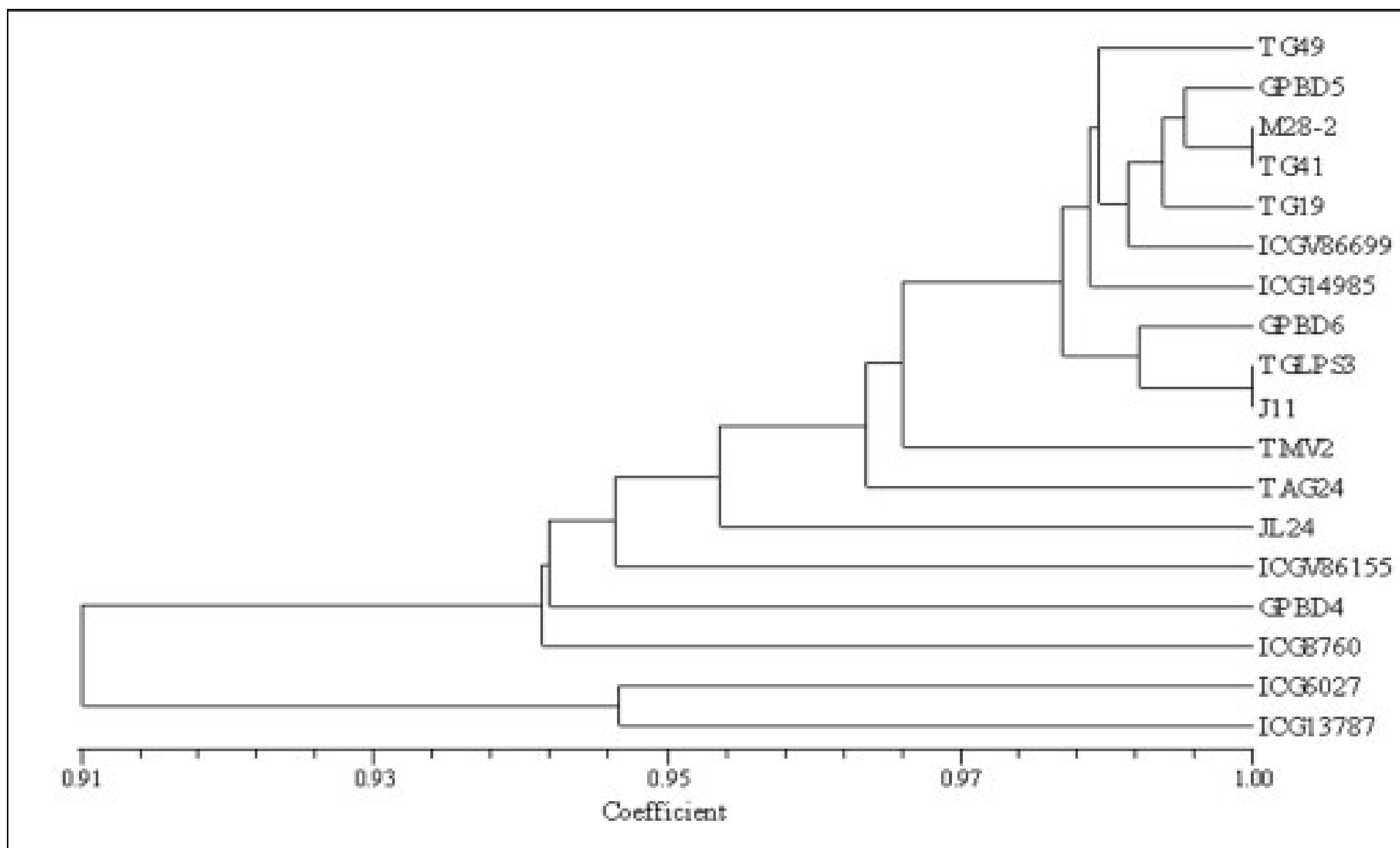
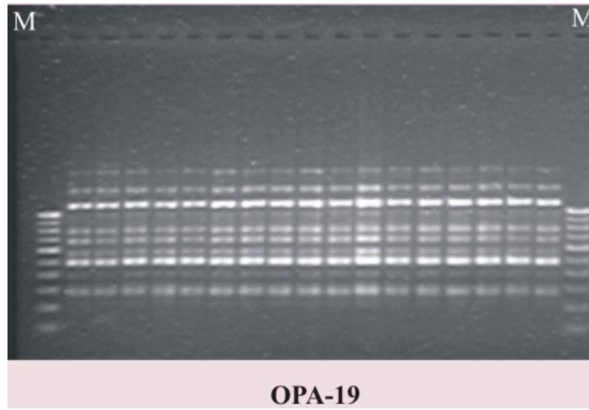
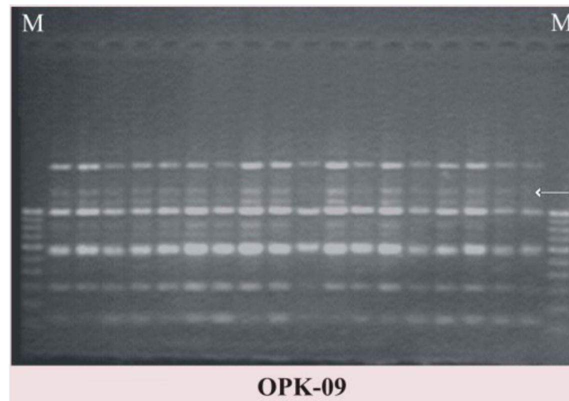
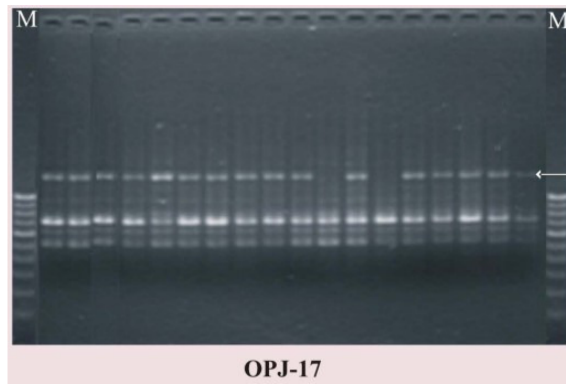


Fig.3: Dendrogram depiciting the genetic diversity of selected genotype







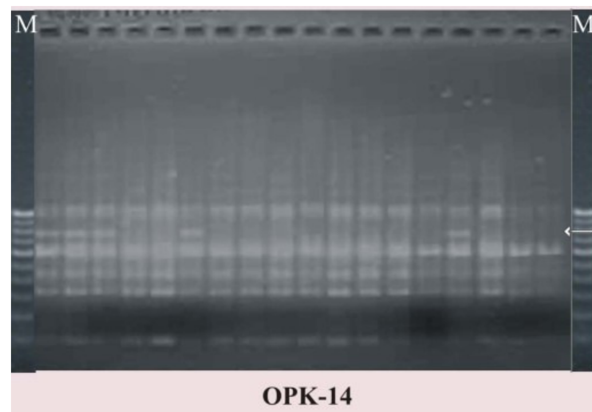


Plate3: RAPD profile in selected groundnut genotypes

Table 17: ANOVA for different traits in parents and F_3 populations

a) TG 49 × GPBD 4

Source	D.F.	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
Genotype	161.00	215.81**	112.67**	294.70**	97.68**	2.00**	3.12**	5.61**
Error	161.00	44.88	25.13	14.04	51.60	0.02	0.29	0.98

b) TG 19 × GPBD 4

Source	D.F.	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
Genotype	55.00	120.96**	68.29**	285.71**	139.91**	2.33**	2.58**	4.73**
Error	55.00	27.38	14.37	20.30	63.54	0.03	0.30	0.73

*, ** indicate the significance of 5% and 1% level of probability, respectively

GAM (8.00 – 11.89) coupled with moderate heritability (30.90 – 37.50), due to moderate PCV (12.59 – 15.37) and low GCV (6.99 – 9.42).

4.5.4 Superior Segregants (%)

The frequency of superior segregants across two populations were estimated over better parent and it revealed that the cross, TG 49 × GPBD 4 has responded very well for yield traits such as pod yield (65.62%), kernel yield (62.5%) and test weight (32.5%) compared with TG 19 × GPBD 4 population.

4.5.5 Diseases

The segregants scoring low grade than the resistant parent was considered as superior, to workout the percentage superiority. It was observed that the frequency of superior segregants was more in cross TG 19 × GPBD 4 for *Aspergillus* seed colonization (12.96%) and rust (18.51%) whereas TG 49 × GPBD 4 was better for LLS resistance (6.25%).

4.6 Association studies

Genotypic and phenotypic correlations were estimated at 5 and 1 per cent level of significance (Table 19a and 19b), which revealed a strong positive association of pod yield per plant with kernel yield and shelling percentage. Kernel yield per plant also exhibited positive association with test weight and shelling percentage in both the populations.

In TG 49 × GPBD 4, test weight showed positive association with pod yield per plant. Whereas in TG 19 × GPBD 4 its association was with shelling percentage.

Among the diseases *Aspergillus* seed colonization had a desirable significant negative association with test weight in TG 49 × GPBD 4 cross and with shelling percentage in TG 19 × GPBD 4 cross. Whereas, late leaf spot showed negative association with *Aspergillus* seed colonization (TG 49 × GPBD 4) and rust (TG 19 × GPBD 4).

4.7 Breeding potential of crosses for resistance and yield/seed size at F_3 generation

The segregating populations were raised during rainy season, to assess the resistant lines for three diseases (late leaf spot, rust and *Aspergillus* seed colonization) and yield characters.

A range of variation existed among two populations for diseases and yield parameters. TG-19 involving population was superior for *Aspergillus* seed colonization and rust resistance. The population involving TG-49 was better for the late leaf spot resistance and it contributed resistant lines for all three diseases. But, TG-19 cross had no resistant line for LLS (Table 20a). About two (1%) lines exhibited *Aspergillus* and rust resistance, rust and LLS and one (1%) line exhibited *Aspergillus* and LLS. When individual disease resistance combined with pod yield, the frequency was 3 to 9 per cent, which reduced drastically ranging from 0.5 to 1 per cent when combination of stresses with pod yield and none of the line exhibited combining resistance to all three diseases with higher yield.

The two populations were assessed for the lines possessing large seed types combined with resistant to three diseases and better yield. Higher proportion (>10%) of lines exhibited large seed size coupled with higher yield and individual disease resistance. Only one line observed for each combinations *i.e.*, *Aspergillus* and rust resistance, late leaf spot and rust resistance with higher yield and large seeded type. (Table 20b).

Table 18: Components of variation for different traits in F_3 population

a) TG 49 × GPBD 4

Characters	GPBD 4	TG 49	MEAN	MIN	MAX	GCV	PCV	HER	GA M	Superior segregants (%)
Pod yield / plant (g)	21.00	10.17	25.54	7.02	63.51	36.19	44.69	65.60	60.37	65.62
Kernel yield / plant (g)	15.14	6.86	17.65	4.29	45.38	37.49	47.03	63.50	61.54	62.50
Test weight (g)	38.93	51.79	47.86	20.68	82.44	24.75	25.96	90.90	48.62	32.50
Shelling percentage	72.06	67.53	68.63	41.50	86.94	6.99	12.59	30.90	8.00	22.50
A. flavus score (1-4 scale)	4.00	1.00	2.63	1.00	4.00	37.77	38.20	97.80	77.11	6.87
Rust	1.00	4.40	2.51	1.00	7.50	47.31	52.02	82.70	88.78	10.00
Late leaf spot	2.80	8.50	6.24	2.00	9.00	24.40	29.10	70.30	42.15	6.25

b) TG 19 × GPBD 4

Characters	GPBD 4	TG 19	MEAN	MIN	MAX	GCV	PCV	HER	GA M	Superior segregants (%)
Pod yield / plant (g)	21.00	8.94	23.04	8.59	43.92	29.69	37.38	63.10	48.57	57.40
Kernel yield / plant (g)	15.14	5.87	15.24	6.05	30.19	34.07	42.19	65.20	56.70	44.44
Test weight (g)	38.93	66.99	44.78	26.65	76.73	25.73	27.63	86.70	49.36	3.70
Shelling percentage	72.06	65.62	65.63	44.16	82.86	9.42	15.37	37.50	11.89	24.07
A. flavus score (1-4 scale)	4.00	1.00	2.67	1.00	4.00	40.17	40.75	97.20	81.74	12.96
Rust	1.00	2.00	2.29	1.00	5.50	46.67	52.52	79.00	85.31	18.50
Late leaf spot	2.80	7.00	7.06	4.00	9.00	20.01	23.40	73.10	35.26	0.00

Table 19: Genotypic and phenotypic correlation for different traits in segregating population

a) TG 49 × GPBD 4

Characters	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
Pod yield / plant (g)	1.00	0.97**	0.28**	0.12	-0.05	-0.09	-0.09
Kernel yield / plant (g)	0.99**	1.00	0.33**	0.33**	-0.04	-0.09	-0.11
Test weight (g)	0.31**	0.37**	1.00	0.27**	-0.28**	-0.14	-0.02
Shelling percentage	0.23**	0.34**	0.45	1.00	0.04	-0.04	-0.02
A. flavus score (1-4 scale)	-0.05	-0.04	-0.30**	0.04	1.00	-0.03	-0.12
Rust	-0.10	-0.10	-0.15*	-0.01	-0.03	1.00	-0.06
Late leaf spot	-0.12	-0.13	-0.01	0.01	-0.15*	-0.05	1.00

b) TG 19 × GPBD 4

Characters	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
Pod yield / plant (g)	1.00	0.94**	0.22	0.13	-0.08	-0.11	-0.08
Kernel yield / plant (g)	0.98**	1.00	0.26*	0.44**	-0.14	-0.14	-0.10
Test weight (g)	0.24	0.27*	1.00	0.21	-0.17	0.09	0.00
Shelling percentage	0.30*	0.48**	0.26*	1.00	-0.14	-0.13	-0.09
A. flavus score (1-4 scale)	-0.07	-0.15	-0.17	-0.28*	1.00	0.09	-0.03
Rust	-0.15	-0.16	0.14	-0.07	0.08	1.00	-0.20
Late leaf spot	-0.03	-0.08	-0.05	-0.13	0.00	-0.27*	1.00

Below diagonal - Genotypic correlation, Above diagonal – Phenotypic correlation

*, ** indicate the significance of 5% and 1% level of probability, respectively

Table 20a: Frequency of superior lines in F_3 generation for productivity and stress

Crosses	**PY**	**HMS**	**AF**	**RUST**	**LLS**	**AF + PY**	**RUST + PY**	**LLS + PY**	**AF + RUST**	**RUST + LLS**	**AF + LLS**	**AF + RUST + PY**	**RUST + LLS + PY**	**AF + LLS + PY**	**AF + RUST + LLS**
GPBD 4 × TG 19 (54)	31 (57)	4 (7)	7 (13)	10 (19)	0 (0)	5 (9)	8 (15)	0 (0)	2 (4)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
GPBD 4 × TG 49 (160)	105 (66)	25 (16)	11 (7)	16 (10)	10 (6)	9 (6)	12 (8)	7 (4)	0 (0)	2 (1)	1 (1)	0 (0)	2 (1)	1 (1)	0 (0)
TOTAL (214)	136 (64)	29 (14)	18 (8)	26 (12)	10 (5)	14 (7)	20 (9)	7 (3)	2 (1)	2 (1)	1 (1)	2 (1)	2 (1)	1 (1)	0 (0)

Table 20b: Frequency of superior lines in F_3 generation for seed size vis-à-vis pod yield and resistance to stress

Crosses	**HMS**	**Number of superior lines (seed size) combining resistance / high yield**													
		PY	**AF**	**RUST**	**LLS**	**AF + PY**	**RUST + PY**	**LLS + PY**	**AF + RUST**	**RUST + LLS**	**AF + LLS**	**AF + RUST + PY**	**RUST + LLS + PY**	**AF + LLS + PY**	**AF + RUST + LLS**
GPBD 4 × TG 19	4.00	3 (75)	2 (50)	1 (25)	0 (0)	2 (50)	1 (25)	0 (0)	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
GPBD 4 × TG 49	25.00	21 (84)	2 (8)	3 (12)	3 (12)	2 (8)	3 (12)	3 (12)	0 (0)	1 (4)	0 (0)	0 (0)	1 (4)	0 (0)	0 (0)
TOTAL	29.00	24 (83)	4 (14)	4 (14)	3 (10)	4 (14)	4 (14)	3 (10)	1 (3)	1 (3)	0 (0)	1 (3)	1 (3)	0 (0)	0 (0)

PY – Pod yield/plant HSM – 100-seed mass AF – *Aspergillus* seed colonization LLS – Late leaf spot
Figures in parentheses indicate the number of lines evaluated in each cross/percentage of total lines evaluated in each cross

Some of the superior segregants in F_3 generation selected based on seed size and *Aspergillus* seed colonization with other traits have been listed in Table 21 & 22. All the selected segregants were superior to their parents for important component characters.

Table 21: Desirable segregants from F_3 populations of TG 49 × GPRD 4 and TG 19 × GPBD 4

Genotype	Test weight (g)	Pod yield / plant (g)	Kernel yield / plant (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
						Score (1-9 scale)	
1-9	68.96	32.10	24.19	75.22	2.95	2.00	3.00
1-13	71.43	19.40	16.37	84.49	1.05	2.00	8.00
1-16	68.18	41.25	32.19	77.70	3.00	3.00	2.50
1-17	66.39	19.02	15.23	80.29	2.30	2.50	6.00
1-26	74.50	16.62	12.07	72.62	2.60	2.00	4.00
2-1	65.58	40.02	28.13	70.31	4.00	5.00	6.50
2-3	64.66	22.44	14.40	65.33	1.00	4.50	7.50
2-5	68.60	22.22	15.75	70.93	1.00	1.50	6.00
2-19	66.00	31.59	21.00	66.44	2.45	2.00	8.00
2-24	67.62	22.87	16.40	71.71	3.60	5.00	7.00
2-26	65.44	34.36	24.29	69.07	2.50	2.50	4.00
2-27	68.08	30.72	20.42	66.43	2.65	2.00	4.50
3-19	60.05	25.22	17.81	70.63	1.15	2.00	8.00
3-2	76.15	27.92	18.26	64.94	4.00	1.00	4.00
3-26	76.15	22.26	15.99	71.84	1.15	2.00	7.00
4-5	78.46	42.49	32.10	75.44	1.35	1.00	5.00
4-10	82.44	34.61	23.14	67.22	1.05	2.00	6.00
4-16	66.44	34.74	27.19	78.46	2.90	2.50	5.50
5-3	61.15	33.54	22.39	67.05	1.05	3.00	5.50
5-11	66.25	21.76	16.47	74.50	2.00	2.50	8.00
5-12	61.78	15.18	12.54	82.32	2.00	2.00	8.00
5-16	68.18	25.14	17.25	69.64	2.10	1.00	3.00
6-14	61.24	25.23	17.11	67.51	2.85	2.00	7.50
6-41	76.73	20.08	13.00	64.76	4.00	4.50	9.00
6-43	65.18	26.00	17.26	66.45	1.00	1.00	6.50
6-44	73.98	36.14	27.06	74.85	1.00	1.50	8.00
8-6	69.35	31.38	26.35	83.49	2.30	3.00	4.00
8-8	60.11	22.88	17.09	74.27	2.70	3.50	8.00
8-15	61.01	37.55	25.51	65.27	1.90	2.00	5.50
GPBD 4	38.93	21.00	15.14	72.06	4.00	1.00	2.80
TG 19	66.99	8.94	5.87	65.62	1.00	2.00	7.00
TG 49	51.79	10.17	6.86	67.53	1.00	4.40	8.50
CD1	**13.23**	**9.90**	**7.40**	**14.19**	**0.30**	**1.07**	**1.95**
CD2	**10.49**	**7.60**	**9.03**	**15.94**	**0.36**	**1.10**	**1.72**

Table 22: *Aspergillus* seed colonization resistant lines in segregating population

Genotype	*A. flavus* score (1-4 scale)	Pod yield / plant (g)	Kernel yield / plant (g)	Shelling percentage	Test weight (g)	Rust	Late leaf spot
						Score (1-9 scale)	
1-6	1.00	12.28	5.58	46.86	26.73	5.00	3.00
2-3	1.00	22.44	14.40	65.33	64.66	4.50	7.50
2-5	1.00	22.22	15.75	70.93	68.60	1.50	6.00
2-13	1.00	35.23	23.88	67.93	51.00	2.00	7.50
2-33	1.00	13.17	9.12	78.44	37.99	2.00	6.00
3-9	1.00	41.81	28.49	68.43	42.03	2.00	7.00
3-15	1.00	34.27	22.81	65.56	53.74	4.00	7.50
4-7	1.00	36.60	22.36	61.21	57.85	2.00	8.50
4-24	1.00	43.03	32.12	74.94	56.54	3.50	8.00
4-25	1.00	24.90	17.27	69.27	45.44	2.00	5.00
5-5	1.00	44.03	29.76	68.51	49.69	2.50	8.00
6-18	1.00	30.83	21.49	44.17	69.47	1.50	7.00
6-29	1.00	20.06	15.01	40.26	74.90	1.00	5.50
6-32	1.00	22.97	14.38	40.01	61.18	4.50	4.00
6-43	1.00	26.00	17.26	65.18	66.45	1.00	6.50
6-44	1.00	36.14	27.06	73.98	74.85	1.50	8.00
6-50	1.00	22.14	16.35	45.88	74.20	5.00	7.00
6-52	1.00	18.40	13.40	47.85	72.83	2.00	5.50
TG 19	1.00	8.94	5.87	66.99	65.62	2.00	7.00
TG 49	1.00	10.17	6.86	67.53	51.79	4.40	8.50
GPBD 4	4.00	21.00	15.14	72.06	38.93	1.00	2.80
CD1	**13.23**	**9.90**	**7.40**	**14.19**	**0.30**	**1.07**	**1.95**
CD2	**10.49**	**7.60**	**9.03**	**15.94**	**0.36**	**1.10**	**1.72**

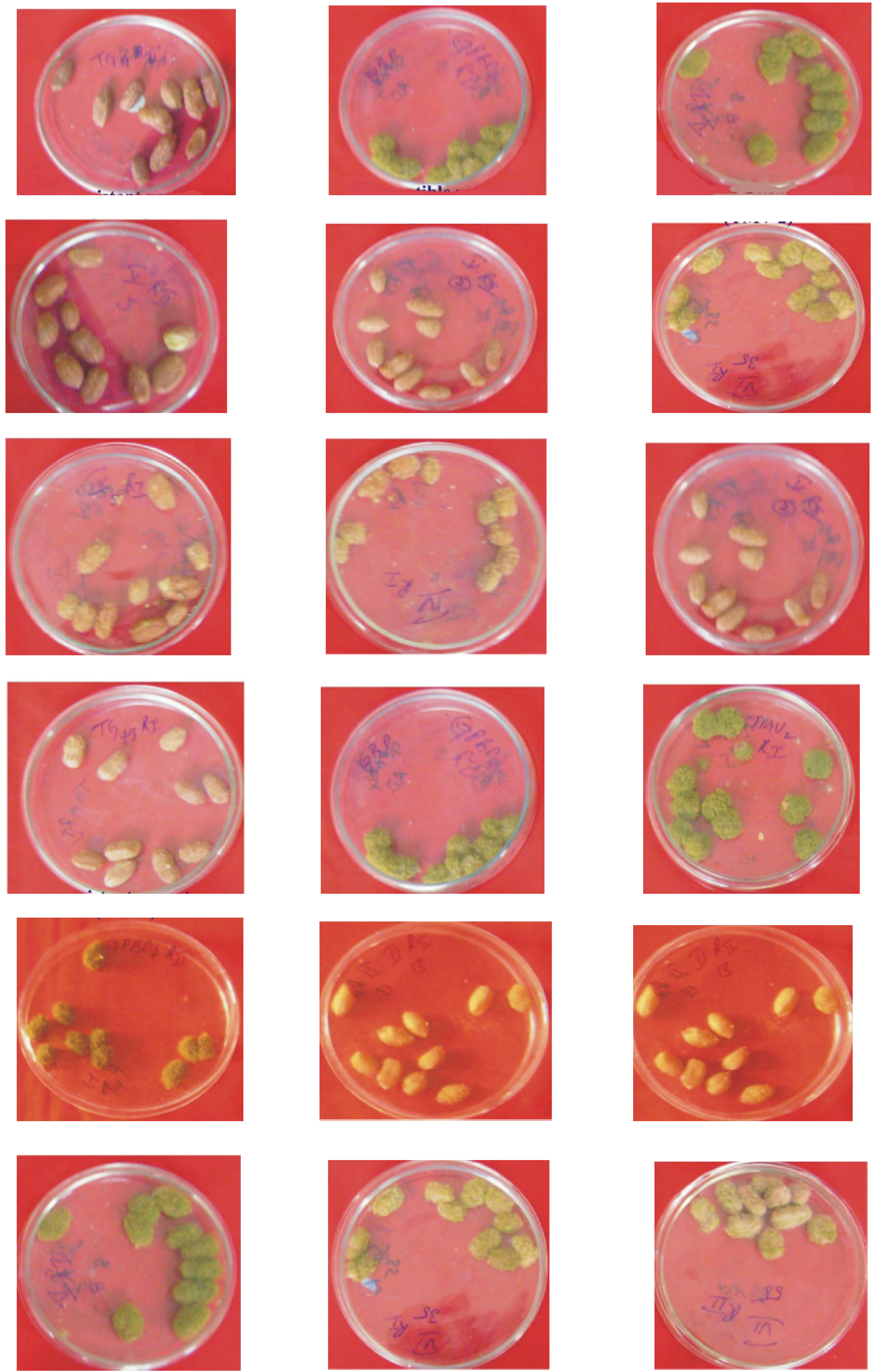


Plate4: Accessions resistant to in vitro seed colonization by Aspergillus flavus in segregating populations

5. DISCUSSION

One of the most profound multifaceted challenges being faced by human kind is not only the need to ensure adequate quantity but also to ensure high quality food, which was rightly summed up by the German proverb "*A man is what he eats*".

Aflatoxin contamination seriously affects the edible quality of groundnut and its products rendering them unfit for human and livestock consumption. Elimination of aflatoxin from the human food chain is a goal of many countries. Several recommendations have been made on cultural practices, curing and drying procedures and storage conditions to minimize seed invasion by *A. flavus*. However, these remain mostly unadopted in many countries where groundnut cultivation is also subject to vagaries of climate. One of the possible reasons of reducing the risk of aflatoxin contamination is the use of cultivars resistant to seed invasion by aflatoxin producing fungi or aflatoxin production (Swindale, 1989). These cultivars will be of great value to the farmers as there is no cost input and can play a significant role in preventing economic losses and health hazards associated with aflatoxin. Late leaf spot (LLS) and rust two economically important foliar fungal diseases normally occur together and can cause yield losses up to 70 per cent (Subrahmanyam *et al*., 1980). Although, these diseases can be controlled very effectively by certain fungicides (Smith and Littrell, 1980), these are costly and are not readily available to small farmers, who generally lack the resources and technical expertise to effectively use chemical control methods. Breeding for resistance is therefore one of the best means of reducing disease related yield losses. This indicates the need for varieties with multiple disease resistance and high yield potential to solve the problems of important diseases of groundnut.

A total of 18 selected genotypes were screened under *in vitro* condition to know their reaction to artificial seed inoculation by *A. flavus.* In the past, Mixon and Rogers (1973) first suggested that use of peanut cultivars with resistance to seed invasion and colonization by toxigenic *Aspergillus* species would be an effective means of preventing aflatoxin contamination. A significant positive correlation between *in vitro* resistance and field resistance was observed (Mixon, 1986; Zambettakis *et al*., 1981, Mehan *et al*., 1987, Wang Sheng Yu *et al*., 2004). Besides ease in screening large germplasm and the cost effectiveness make the *in vitro* seed colonization as the most commonly used method (Anderson *et al*., 1996; Dan *et al*., 1997, Upadhyaya *et al*., 2001b, Varma, 2001, Thakur *et al*., 2000 and Harish Babu *et al*., 2005).

The present study clearly demonstrated genotypic differences in the level of seed colonization by *A. flavus*. The variation observed was highly heritable as indicated by high genetic advance due to high phenotypic and genotypic coefficient of variation (Upadhyaya *et al*., 1997a). This clearly indicated a greater scope for selection of resistant genotypes. TG 19 (1), TG 49 (1), ICG 8760 (1), ICG 14985 (1.08), ICG 6027 (1.10), ICG 13787 (1.11) and ICGV 86699 (1.20) showed high level of resistance to *in vitro* seed colonization by *A. flavus*, which is in accordance with Harish Babu *et al.* (2005) and Yugandhar *et al.* (2005) followed by GPBD 6 (1.35), TG 41 (1.38) and M 28-2 (1.48). The genotypes TGLPS 3 (2.03), ICGV 86155 (2.10) and GPBD-5 (2.25) showed moderate level of resistance to *in vitro* seed colonization by *A. flavus*.

Some of the genotypes, TG 41, M 28-2, TG 49, TGLPS 3 exhibited low level of infection to *Aspergillus* than reported by Harish Babu *et al.* (2005); which may be due to more incubation period in that study. Whereas in the present study observations were recorded whenever the susceptible check, TMV 2 exhibited a severity scale of 4 for seed colonization, which may give precise scoring results. The cultivar J 11 was susceptible (Thakur *et al*., 2000) which is in contrast with Harish Babu *et al*., (2005) report. The varying response of some genotypes to *in vitro* seed colonization by *A. flavus* in different screening experiments gives an indication that IVSCAF resistance (seed coat resistance) is highly influenced by the experimental (environment) conditions prevailing in the screening assays. Influence of environment (incubation temperature, kernel moisture content, concentration of inoculums, damage to seed coat) on resistance was reported by Mixon (1976). However, low error co-efficient of variation (<7%) observed in the course of present screening experiments indicate

the reliability of the methods used and *in vitro* conditions maintained for screening and the stability of the resistant sources identified.

5.1 Performance of genotypes for productivity and other diseases

The selected genotypes were subjected to field evaluation to know their response to other foliar fungal diseases (LLS and Rust) and productivity parameters.

The genotypes studied differed significantly for most of the productivity parameters (pod yield, kernel yield per plant, shelling percentage, test weight and sound mature kernel) and reaction to diseases (LLS and Rust). This indicated usefulness of these materials and their appropriateness in the study. Over seasons, significant seasonal variations and genotype × season interaction was observed for majority of the traits revealing the role of environment and genotype × environment interactions. Wynne and Isleib (1978) reported extensive prevalence of G × E interactions for productivity traits while, Knauft and Gorbert (1993), Wynne and Coffelt (1983) and Wynne and Isleib (1978) for physical traits.

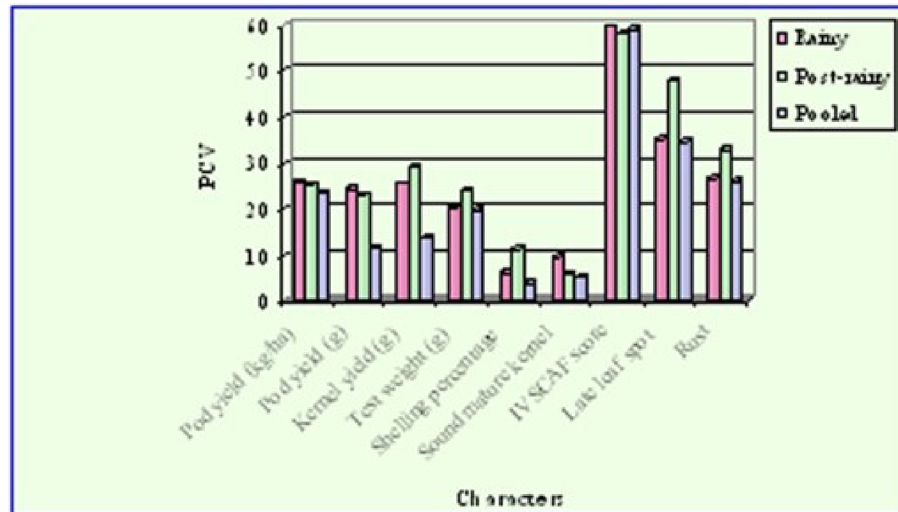
In general, variance estimates were relatively high in individual seasons as compared to pooled data indicating the role of G × E interactions. Among productivity parameters, pod yield and test weight over seasons had high GAM due to high variation coupled with high heritability. A similar trend was evident for LLS and Rust and it is in accordance with Vasanthi *et al.* (1998) who observed high heritability for LLS (96.55) and rust (93.28). This entails better scope for selection of genotypes with high plant yield and test weight along with disease resistance. Whereas, shelling percentage and sound mature kernel exhibited zero genetic advance coupled with zero heritability and low PCV (Fig. 4) indicating the predominance of unpredictable components in the expression of these two traits.

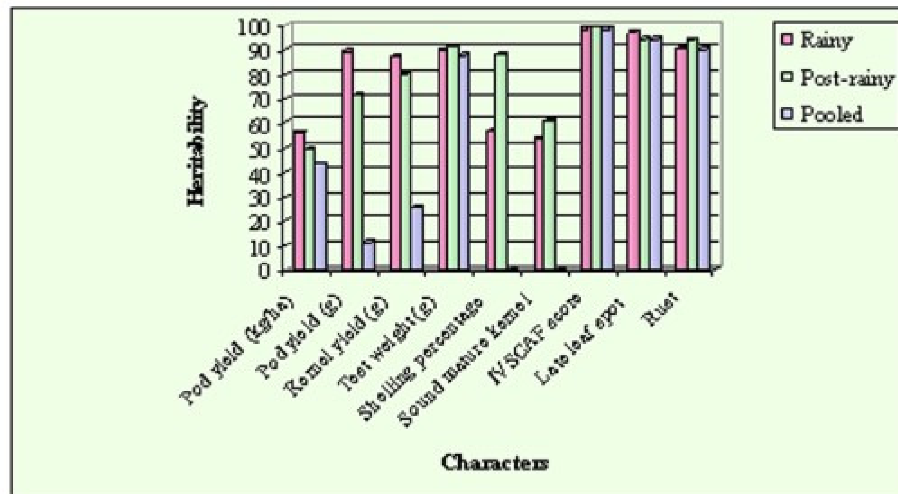
The genotypes with high level of resistance to IVSCAF *viz.,* TG 19, ICG 8760, TG 49, ICG 14985, ICG 6027, ICG 13787 and ICGV 86699 showed low yield potential but larger seed size. Other large seeded genotypes *viz.,* GPBD 6, TG 41, M 28-2 and TG 49 were distinct in having high yield, higher test weight with high levels of resistance to *in vitro* seed colonization by *A. flavus*.

The genotypes, TGLPS 3, ICGV 86155 and GPBD 5 had moderate level of resistance with high yield and test weight. Whereas, the high yielders *viz.,* GPBD 4, TAG 24, TMV 2 and JL 24 were susceptible to seed colonization by *A. flavus* and have high shelling percentage and sound mature kernel. The genotypes *viz.,* TG 19, GPBD 6, TG 49, TG 41 and M 28-2 exhibited high-test weight and high yield along with resistance to *in vitro* seed colonization by *A. flavus*, can be profitably exploited for confectionary purpose in view of increasing demand for confectionary items in the international market.

In both the seasons, pod yield per plant showed positive association with pod yield (kg ha^{-1}), kernel yield per plant indicating that pod yield per plant could be used as a preliminary criteria for selecting high yielding genotypes (Mishra and Yadav 1992 and Bhagat *et al.,* 1993). Shelling percentage and sound mature kernel also showed positive association with pod yield in post rainy season. It is in accordance with the findings of Vasanthi *et al.*, (1998). Lakshmidevamma *et al*., (2004) reported negative association of test weight with pod yield in rainy season revealing that the genotypes with large seeds have yielded less in rainy season. Poor performance in rainy season may be due to lack of resistance to diseases. Test weight showed significant positive association with sound mature kernel, which reveals that larger seed types have high proportion of sound mature kernels.

Late leaf spot is the most widely prevalent disease during rainy season and causes significant loss in yield of groundnut. Late leaf spot had negative association with pod yield per plant and kernel yield per plant in rainy season indicating that the resistance will naturally enhance these traits. Late leaf spot also showed positive association with rust indicate that some of the genotypes have resistance to both the diseases. *Aspergillus* seed colonization exhibited significant desirable negative association with test weight revealing that the resistant genotypes in general had higher seed size.





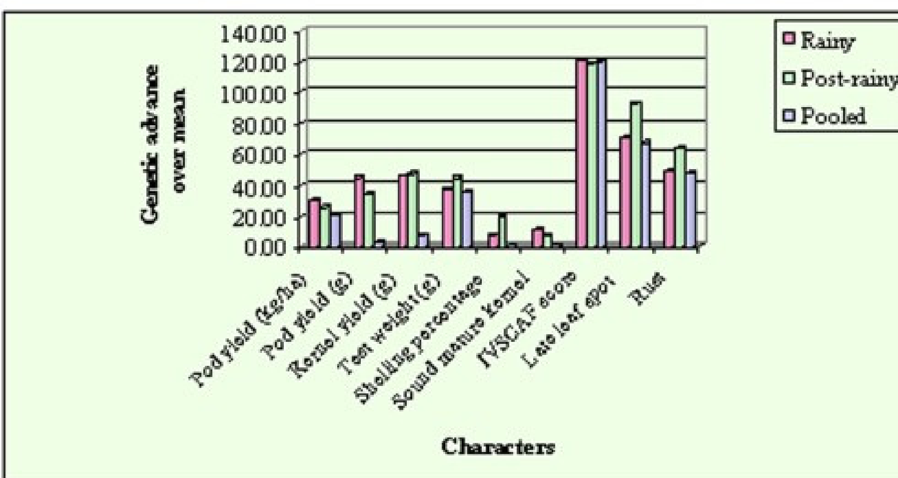


Fig 4: PCV heritability and genetic advance for different traits in selected genotypes

5.2 Genotypes potential for multiple traits

Generally, a genotype is resistant to either one or two diseases. Nevertheless, in nature we do find occasionally, some genotypes resistant/tolerant to many diseases i.e., multiple disease resistant genotypes which are of great significance in integrated disease management systems.

A genotype of great potential in this respect is an interspecific derivative ICGV 86699, which registered as resistance to late leaf spot, rust, bud necrosis, iron chlorosis and *Sclerotium* (Reddy *et al.*, 1996; Naidu, 2002). It showed low seed colonization by *A. flavus* in the present study thus confirming the observation made by Harish Babu *et al.*, (2005).

ICG 8760 (a Virginia runner from Zambia) and ICG 13787 (Virginia bunch from Nigeria) having high level of *in vitro* seed colonization by *A. flavus* resistance also showed resistance to LLS and Rust.

The IVSCAF resistant cultivar, M 28-2 (Gowda, *et al.*, 2002), the accession ICG 6027 (Valencia from Sudan) and ICG 14985 (Spanish bunch of unknown origin) also showed high sound mature kernel, shelling percentage and resistance to LLS but were susceptible to rust.

GPBD 6 having resistance to *in vitro* seed colonization by *A. flavus* and LLS also showed moderate resistance to rust coupled with high test weight, sound mature kernel and pod yield.

ICGV 86155 registered as a Spanish bunch dormant line with high yielding potential (Upadhyaya *et al.*, 1997b) also possessed moderate resistance to late leaf spot and *Aspergillus* colonization, but it was susceptible to rust.

5.3 Molecular diversity

DNA based markers are increasingly recognized as useful tools for assessing genetic diversity among the genotypes because they are not influenced by the environment (Lee, 1995), but their application in groundnut is lagging behind because of limited knowledge of the genome. Extensive variation for morphological and physiological characteristics exists in both wild and cultivated groundnut (Halward *et al.*, 1993), but low DNA polymorphism has been observed in cultivated species (Kochert *et al.*, 1991 and Halward *et al.*, 1993). But, recently, using RAPDs, SSRs and AFLPs techniques significant polymorphism has been reported in groundnut (Dwivedi and Gurtu, 2002; Hopkins *et al.*, 1999 and He *et al.*, 1997).

The RAPD assay was chosen in the present study since the procedure involved was simple, does not require southern blot hybridization as in case of RFLP. Molecular diversity analysis by RAPD assay was carried on selected genotypes with 20 oligonucleotide primers.

A total of 150 bands were amplified across genotypes revealing an average of 7.5 bands per primer with 42.32 per cent polymorphism. In spite of using primers which were polymorphic (Subramanian *et al.*, 2000; Dwivedi *et al.*, 2001; Dwivedi *et al.*, 2002; Pattanashetti, 2005 and Ajay, 2006), only moderate level of polymorphism was observed, which is in accordance with Dwivedi *et al.* (2001), who had observed polymorphism ranging from 8.73 to 33.08 per cent among eight primers in 26 accessions including interspecific derivatives, land races and released cultivars.

The Dice similarity coefficient was generated from pooled data of all primers. The genetic similarity of selected genotype ranged from 0.86 to 0.99 indicating very less diversity. ICG 13787 and GPBD 4 exhibited highest genetic diversity among the genotypes.

Among the different primers, polymorphism ranged from zero to 100 per cent. The primer OPA-12 showed 100 per cent polymorphism followed by OPF-09, OPF-10 and OPA-15. The primer OPF-10 showed highest PIC (polymorphism information content), which is an index of diversity (0.20). Similarly Yugandhar (2005) also reported more polymorphism while evaluating these primers on a germplasm set. In the dendrogram, 18 selected genotypes were divided into three distinct clusters. TG 49 and ICG 13787 were found to span the

extremes of the entire dendrogram with the remaining types distributed between them. All germplasm except ICG 14985 (Spanish bunch) clustered distinctly away from cultivars and all the cultivars clustered together indicating narrow genetic diversity among the cultivars and germplasm as source for wider genetic diversity.

5.4 Suggested crosses

ICG 13787 (*Virginia* bunch) is a bold seeded type with resistance to *Aspergillus*, late leaf spot and rust. It is also superior in oil quality with low saturated fatty acid, high oleic acid and high O/L ratio (Ajay, 2006). It is more diverse (Fig.3) with the popular varieties, GPBD 4 and TMV 2 and hence, it could be hybridized with them.

ICG 8760 (Virginia bunch) is a bold seeded type, resistant to *Aspergillus*, late leaf spot and rust. It has low oil content, low in saturated fatty acid, high oleic acid and high O/L ratio (Ajay, 2006) and it is more diverse (Fig.3) with GPBD 4, GPBD 5, TMV 2 and M 28-2 and hence it could be crossed with these varieties.

ICGV 86699 (Virgnia bunch) is resistant to *Aspergillus*, late leaf spot and rust. It is reported to be resistant to bud necrosis, iron chlorosis and *Sclerotium* (Reddy *et al.*, 1996 and Naidu, 2002). Since ICGV 86699 is diverse (Fig.3) with ICG 6027 and GPBD 4, it can be crossed with either of these genotypes.

In future breeding programme, these crosses can be exploited to develop multiple disease resistant varieties with good confectionary attributes and high yielding potential.

5.5 Segregating populations

The two crosses (TG 49 × GPBD 4 and TG 19 × GPBD 4) were made with the objective to get multiple disease resistant lines coupled with high yield and large seeds using parents, which are resistant to foliar diseases with high yielding capacity (GPBD 4) and resistant to *Aspergillus* seed colonization with large seed type (TG 19 and TG 49).

Segregating F_3 populations were evaluated during rainy season (2006) for disease resistance and productive traits. Analysis of variation for different lines revealed significant variation for all the traits indicating usefulness and appropriateness of the material for the study. In both the populations, all the yield traits except shelling percentage and reaction to diseases had high GAM (>35%) due to high variation (>23%) coupled with high heritability (>63%) indicating scope for selection. Heritability was high in both the population for *Aspergillus* seed colonization, it is in accordance with report from USA where, they observed high estimate (75.5%) of broad sense heritability for seed colonization in a cross involving PI 337409 (resistant) and PI 331326 (susceptible) (Mixon., 1976); 63 per cent in a cross between GFA 2 (resistant) and NC 7 (susceptible) and 55 per cent in the cross involving AR 4 (resistant) and NC 7 (susceptible) (Utomo *et al.*, 1990). In India, the heritability was 60 per cent in a cross involving J 11 (resistant) and OG 43-4-1 (susceptible) and 59 per cent in a cross between two resistant parents, J 11 and Ah 7223 (Upadhyaya *et al.*, 1997a).

In both the populations pod yield per plant revealed a strong positive association with kernel yield and shelling percentage; kernel yield per plant also exhibited positive association with test weight and shelling percentage. All these indicate there is possibility of simultaneous improvement in different productivity traits.

In TG 49 × GPBD 4 test weight exhibited positive association with pod yield indicating lines with large seed size are also high yielders. Where as in TG 19 × GPBD 4 test weight showed positive association with shelling percentage revealing scope for combining these two traits which were antagonistic in the parents.

The diseases had shown desirable negative association with yield traits especially *Aspergillus* seed colonization had significant desirable negative association with test weight (TG 49 × GPBD 4) and with shelling percentage (TG 19 × GPBD 4) indicating that the resistant genotypes had higher seed size and high kernel out put.

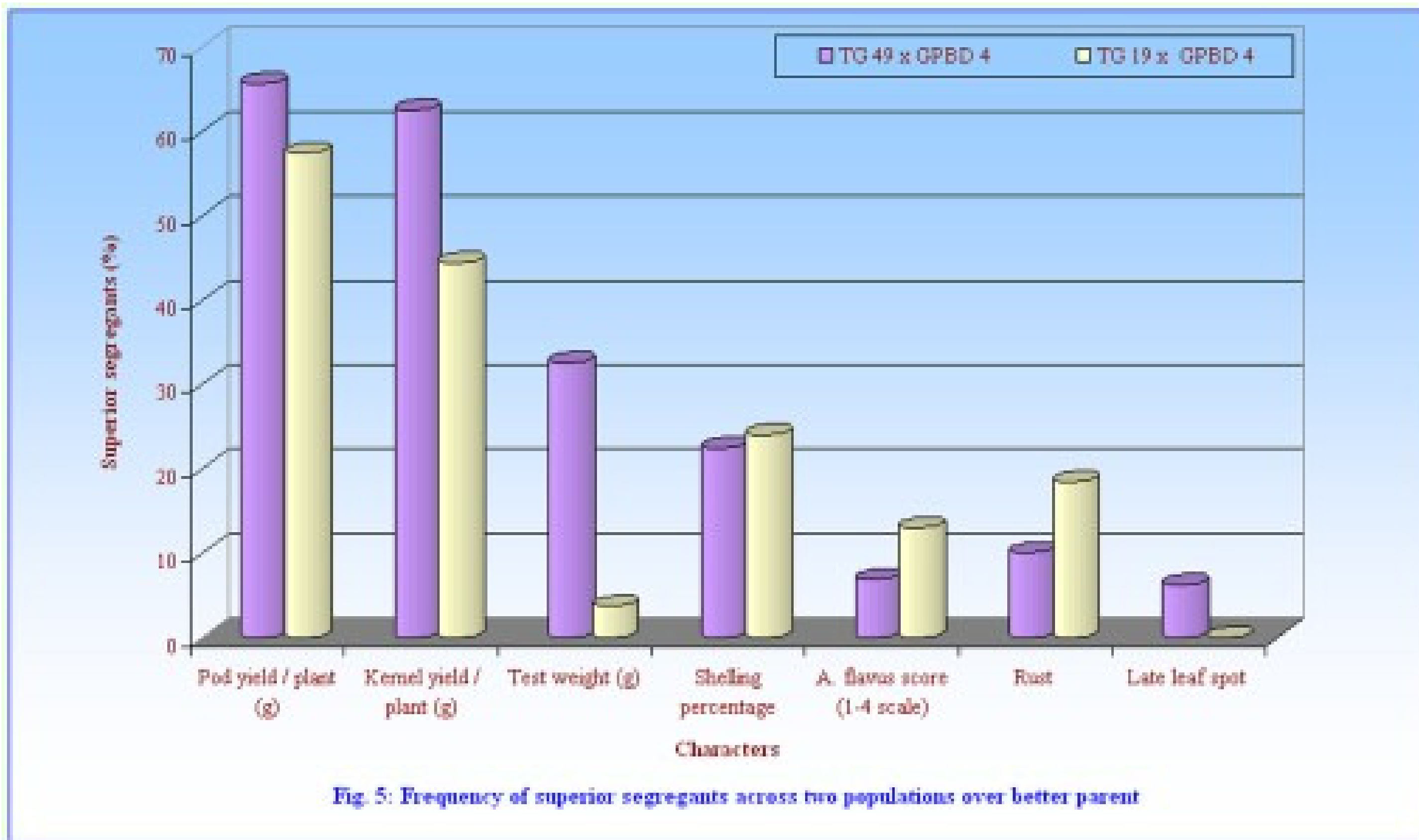


Fig5: Frequency of superior segregants across two population over better percent

LLS showed negative association with *Aspergillus* seed colonization in TG 49 × GPBD 4 and with rust in TG 19 × GPBD 4 indicating a need for breaking these undesirable associations.

5.6 Superior segregants

The resistant germplasm lines belonging to Virginia or Valencia land races, suffer from poor agronomic features. Early maturing Spanish bunch cultivars are popular because of ease in cultivation and harvesting but are susceptible to most of the biotic and abiotic stresses. Same trend was observed in the present study *i.e.,* the frequency of superior segregants (%) across two populations were estimated over better parent, which reveals that the cross involving spanish bunch parents (TG 49 × GPBD 4) responded very well for yield traits and the cross involving a Virginia parent (TG 19 × GPBD 4) was better for disease resistance (Fig: 5).

5.7 Pattern of distribution for diseases in F_3 populations

Distribution for *Aspergillus* seed colonization, LLS and rust were studied in F_3 populations. The distribution pattern for *Aspergillus* revealed that extreme categories are high in population otherwise, it is a normal distribution indicating inheritance may be quantitative nature. Whereas, for rust almost normal distribution was observed indicating quantitative nature of inheritance. It is in accordance with Zheng (1987), Liao Boshou *et al.* (1988) and Subramanyam *et al.* (1993). But, distribution for late leaf spot was more towards susceptible parents TG 19 and TG 49 revealing the contribution of negative alleles from the resistant parent GPBD 4 (Fig. 6). Inheritance of LLS resistance is controlled by multiple recessive genes (Nevill, 1982; Motagi *et al.* 2000).

5.8 Breeding potential of crosses

Range of variation for disease resistance (*Aspergillus* seed colonization, late leaf spot and rust) revealed better scope for selection among crosses at F_3 generation. Frequency of superior lines existed for stress resistance combining higher yield. But, the frequency of superior segregants for various stresses were distributed among crosses. For example cross involving TG 19 × GPBD 4 was better for *Aspergillus* seed colonization and rust resistance, while cross involving TG 49 × GPBD 4 was better for late leaf spot resistance. This suggests the need for intermating among selected lines to combine multiple disease resistance and high yield.

The most important component of edible groundnut export consists of the bold seeded, hand picked and selected (HPS) types, which have great demand all over the world and fetch higher prices in domestic and international markets. In India, groundnut trade is restricted to hand picked selected kernels in the absence of genotypes with large kernels and good quality (Chandrashekhar, 1991 and Dwivedi and Nigaim, 1995). Existing large seeded genotypes lack disease resistance and demand is for aflatoxin and pesticide residue free foods, thereby emphasizing incorporation of multiple disease resistance. The crosses involving TG 49 and TG 19 were superior for seed size as both the genotypes had higher seed size. Hence, the crosses were analyzed for lines with higher hundred seed mass combining disease resistance. Only one line with resistance to *Aspergillus* seed colonization and rust and another line with resistance to late leaf spot and rust having large seed size coupled with high yield were observed. None of the lines combined resistance to *Aspergillus* seed colonization, late leaf spot and rust (Table 20b). This suggests the need for large population size to get better segregants (Sarala and Gowda, 1998) or through inter mating among the selected lines. Selected lines could be evaluated in large scale trials for their suitability in commercial cultivation and future breeding programmes to combine multiple stress resistance.

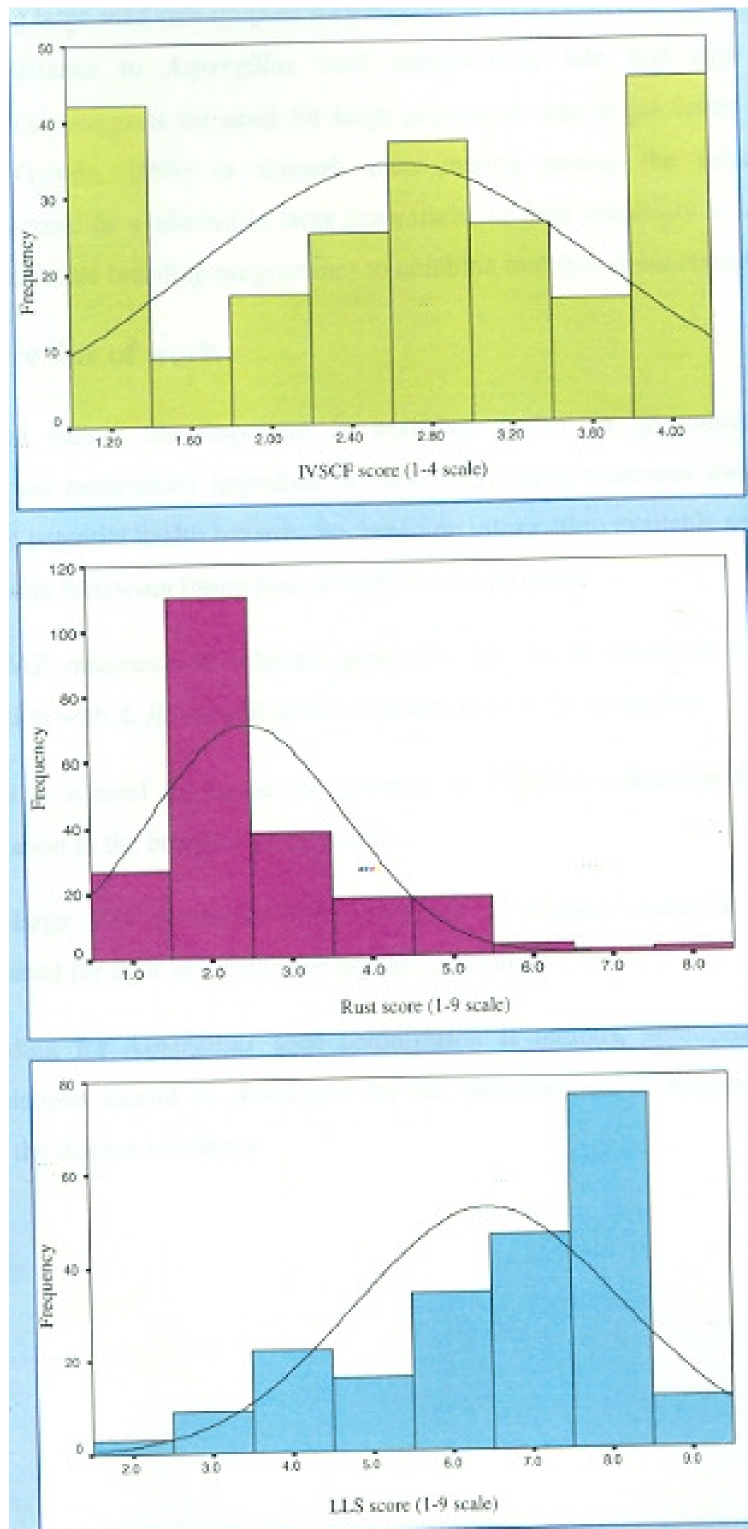


Fig 6 : Pattern of distribution for diseases in segregating population

5.9 Future line of work

Several factors arin ensuring quality in groundnut products. These issues are particularly important in view of growing consumer awareness and concern about potential health hazards. So, based on information available and results of the present study following future line of work can be proposed.

1. IVSCAF resistance in selected genotypes has to be tested by natural field infection with *A. flavus* and aflatoxin levels have to be quantified.

2. There is a need to define the genetics of IVSCAF resistance for effective utilization in the breeding programmes.

3. The large seed genotypes with resistance to diseases could be extensively evaluated for their suitability for organic cultivation.

4. Breeding for *Aspergillus* seed colonization is tedious, appropriate mapping populations should be developed for the identification of markers associated with the disease resistance.

6. SUMMARY AND CONCLUSIONS

Groundnut is one of the most important oilseed crops of India. Nowadays with the availability of cheaper oil from non-conventional sources, groundnut oil has lost its pre-eminent position. Edible groundnuts have become major attention in the international groundnut trade. But, it should meet very stringent standards regarding aflatoxin contamination and pesticide residues. Aflatoxin contamination of groundnut is a widespread serious problem in most groundnut producing countries where, the crop is grown under rainfed conditions. The aflatoxin contamination does not affect crop productivity, but it makes the produce unfit for consumption and it has resulted in loss of export earnings for many groundnut producing countries.

Developing resistant cultivars is one of the effective and low-cost components of an integrated aflatoxin management program. Hence, an effort was made to identify resistance to *in vitro* seed colonization by a toxigenic isolate (*Af* 11-4) of *Aspergillus flavus* in groundnut with good agronomic characters and resistance to other foliar diseases. The results of the investigation are summarized as follows.

Eighteen selected genotypes were screened under *in vitro* condition for reaction to *Aspergillus* seed colonization and also subjected to field evaluation to know their response to other foliar fungal diseases (LLS and Rust) and productive traits. Analysis of variance indicated significant influence of genotype, season and genotype × seasonal interaction. Over seasons, yield traits *viz*., test weight and pod yield (kg/ha) had shown high genetic advance indicating better scope for selection for these traits. Whereas, shelling percentage and sound mature kernel exhibited higher role of unpredictable components in the expression of these two traits.

All the three diseases *viz*., *Aspergillus* seed colonization, late leaf spot and rust showed high genetic advance coupled with high level heritability over seasons, indicating better scope for selection.

The present study revealed high level of resistance to *Aspergillus* seed colonization in TG 19, TG 49, ICG 8760, ICG 14985, ICG 6027, ICG 13787 and ICG 86699. All resistant lines possessed higher seed size but suffered from low yield. The high yielders exhibited low to moderate level of resistance to *Aspergillus* seed colonization. The accessions *viz*., ICGV 86699, ICG 8760 and ICG 13787 showed moderate to high-level resistance to all the three diseases.

Molecular diversity through RAPD analysis was assessed on 18 selected genotypes for better exploitation of genetic resources by identifying diverse genotypes. Out of 20 primers only OPF 10 (0.20) showed high polymorphism. The average polymorphism per primer was 42.32 per cent, and maximum genetic diversity was observed between a germplasm accession ICG 13787 and a cultivar GPBD 4.

Based on complementary traits and molecular diversity, hybridization between ICG 13787 × GPBD 4/TMV 2, ICG 8760 × GPBD 4/GPBD 5/TMV 2 /M 28-2 and ICGV 86699 × ICG 6027/GPBD 4 can be suggested to develop varieties specifically for multiple disease resistance with high yielding potential.

In the two segregating populations (TG 19 × GPBD 4 and low in TG 49 × GPBD 4), the analysis of variance indicated significant variation for all the yield traits and diseases. In both the populations, except shelling percentage, all other yield traits and diseases showed high genetic advance revealing better scope for selection. Whereas, the shelling percentage showed moderate genetic advance in TG 19 × GPBD 4 and low in TG 49 × GPBD 4 population. The three diseases showed a desirable negative association with all yield characters, especially test weight showed a desirable significant negative association with *Aspergillus* seed colonization indicating that the resistant segregants were high yielding and bold seeded types.

Most of the superior segregants for *Aspergillus* seed colonization and rust were observed in the cross involving *Virginia* parent (TG 19). Whereas, the cross involving a Spanish bunch parent(TG 49) was superior for late leaf spot and yield traits.

The two crosses were also assessed for bold seeded types and it revealed that TG 49 cross was better than the TG 19 cross. Though, there were many lines combining higher pod yield and/or seed size with resistance to single disease, none of the lines showed multiple disease resistance. This suggests the need for intermating among selected lines to combine multiple disease resistance and higher yield potential.

- The present study revealed TG 19, TG 49, ICG 8760, ICG 14985, ICG 6027, ICG 13787 and ICG 86699 had high level of resistance to *Aspergillus* seed colonization. Among them ICGV 86699, ICG 8760 and ICG 13787 showed moderate to high level resistance to all the three diseases.

- Based on complementary traits and molecular diversity, hybridization between ICG 13787 × GPBD 4/TMV 2, ICG 8760 × GPBD 4/GPBD 5/TMV 2 /M 28-2 and ICGV 86699 × ICG 6027/GPBD 4 can be suggested to develop varieties specifically for multiple disease resistance with high yielding potential.

- Superior segregants over better parent for *Aspergillus* seed colonization and rust were observed in high percentage in the cross involving Virginia parent (TG 19). Whereas, the cross involving a Spanish bunch parents (TG 49 and GPBD 4) was superior for late leaf spot and yield traits.

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APPENDIX

Appendix I: Monthly Meteorological data for the experimental year (2006-07) of Main Agricultural Research Station, University of Agricultural Sciences, Dharwad

Months	Rainfall	No. of rainy days	Temperature (^0C)		Relative humidity (%)
			Mean max.	Mean min.	
June 2006	212.4	14	29.5	20.6	78
July	176.1	18	26.6	20.4	87
August	115.2	17	26.3	19.6	85
September	91.4	10	29.2	19.2	77
October	38.6	3	30.0	19.1	67
November	55.4	3	29.2	18.1	70
December	-	-	29.1	12.8	61
January 2007	-	-	30.4	14.0	52
February	-	-	31.9	15.7	62
March	12.8	-	35.3	19.7	45
April	1.5	-	37.1	20.3	49
May	166.8	4	35.1	20.9	61
Total	**870.2**	**69**	**-**	**-**	**-**

Appendix II: Performance of lines of TG 49 × GPBD 4 cross for productivity and stress parameters in rainy season

Genotype	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
TG 49	10.17	6.86	51.79	67.53	1.00	4.40	8.50
GPBD 4	21.00	15.14	38.93	72.06	4.00	1.00	2.80
1-1	19.86	17.27	41.57	86.94	4.00	1.50	3.00
1-2	29.24	21.25	42.81	72.51	1.25	2.00	8.00
1-3	17.01	9.18	41.53	53.98	1.25	2.50	8.00
1-4	23.78	19.26	41.76	81.20	2.40	2.00	6.00
1-5	32.02	21.80	48.55	68.43	3.00	1.00	3.00
1-6	12.28	5.58	26.73	46.86	1.00	5.00	3.00
1-7	25.92	15.73	51.57	60.24	2.75	2.50	7.50
1-8	25.11	19.33	55.76	77.36	2.60	3.50	7.00
1-9	32.10	24.19	68.96	75.22	2.95	2.00	3.00
1-10	31.37	23.04	38.56	73.42	3.00	4.50	4.50
1-11	17.92	13.44	52.77	74.78	3.60	2.00	7.50
1-12	21.05	15.73	39.17	74.01	2.80	2.00	7.00
1-13	19.40	16.37	71.43	84.49	1.05	2.00	8.00
1-14	40.70	26.74	49.57	65.33	3.10	2.00	7.00
1-15	14.00	10.06	40.15	71.77	4.00	3.00	8.50
1-16	41.25	32.19	68.18	77.70	3.00	3.00	2.50
1-17	19.02	15.23	66.39	80.29	2.30	2.50	6.00
1-18	30.72	22.19	52.55	72.33	3.25	1.50	7.50
1-19	7.39	4.29	46.94	57.67	3.55	1.00	5.00
1-20	47.39	31.53	38.62	67.10	2.45	2.00	6.50
1-21	11.32	4.87	47.83	41.50	2.90	2.50	6.50
1-22	41.06	28.78	41.29	71.84	4.00	1.50	3.50
1-24	21.86	14.89	36.04	67.13	2.00	1.50	7.00
1-25	23.31	13.65	48.36	57.86	1.90	1.00	4.00
1-26	16.62	12.07	74.50	72.62	2.60	2.00	4.00
1-28	23.72	17.62	57.60	74.05	4.00	1.00	7.00
1-29	27.06	18.98	47.47	70.14	1.05	4.50	7.50
1-30	25.15	16.00	56.82	64.41	3.95	2.50	5.00
2-1	40.02	28.13	65.58	70.31	4.00	5.00	6.50
2-2	28.63	19.32	42.15	66.76	4.00	5.00	5.50
2-3	22.44	14.40	64.66	65.33	1.00	4.50	7.50
2-4	11.12	7.76	31.35	69.35	3.95	5.00	4.00
2-5	22.22	15.75	68.60	70.93	1.00	1.50	6.00
2-6	16.33	12.08	48.07	72.58	1.05	1.50	6.50
2-7	18.40	11.47	30.59	61.54	2.55	6.00	6.00
2-8	29.17	17.40	33.72	59.51	2.75	5.00	7.00
2-9	14.56	7.63	25.51	52.18	2.95	2.50	6.50

Contd…..

12-0	12.26	8.91	33.19	72.95	2.75	2.50	8.00
2-11	20.81	13.02	39.82	62.71	2.60	3.00	7.50
2-13	35.23	23.88	51.00	67.93	1.00	2.00	7.50
2-14	18.19	11.98	37.19	65.81	4.00	2.00	7.00
2-15	23.49	16.43	33.85	69.86	1.40	2.50	6.00
2-16	23.96	12.73	22.38	55.63	2.85	4.00	6.50
2-17	35.18	23.49	55.74	66.78	1.45	4.00	5.00
2-18	23.34	17.94	53.18	76.98	4.00	2.50	6.00
2-19	31.59	21.00	66.00	66.44	2.45	2.00	8.00
2-20	25.96	18.04	39.25	69.49	2.85	4.00	6.00
2-21	29.78	22.81	58.65	76.61	2.75	2.50	7.50
2-22	20.83	14.32	45.60	68.54	1.60	2.00	8.00
2-23	17.30	12.01	51.40	69.30	2.50	3.00	7.00
2-24	22.87	16.40	67.62	71.71	3.60	5.00	7.00
2-25	20.61	13.69	55.24	67.09	2.50	2.00	7.00
2-26	34.36	24.29	65.44	69.07	2.50	2.50	4.00
2-27	30.72	20.42	68.08	66.43	2.65	2.00	4.50
2-28	8.10	6.33	38.43	78.25	2.75	2.00	7.00
2-29	26.94	18.62	56.33	69.42	2.65	3.50	7.00
2-30	10.21	9.26	32.64	86.85	3.00	2.00	7.00
2-31	11.43	5.79	38.60	51.42	1.40	2.00	4.00
2-32	13.82	9.35	46.38	66.53	4.00	2.00	6.00
2-33	13.17	9.12	37.99	78.44	1.00	2.00	6.00
2-34	27.18	18.55	39.76	68.01	1.05	2.00	8.00
2-35	20.91	14.37	37.20	67.02	3.00	2.00	5.50
2-36	9.41	6.35	30.22	67.54	2.95	3.00	7.00
2-37	16.65	9.17	32.29	55.20	2.65	2.00	8.00
2-38	13.73	9.16	45.95	66.79	1.15	2.00	7.00
3-1	34.15	24.28	58.71	71.10	1.10	1.00	8.00
3-2	27.92	18.26	76.15	64.94	4.00	1.00	4.00
3-3	35.08	23.19	42.32	66.34	3.80	3.50	3.50
3-4	28.37	17.61	51.17	62.70	3.20	2.00	7.00
3-5	18.08	12.37	36.31	68.59	3.90	2.00	8.00
3-6	17.30	11.73	41.35	67.70	2.85	2.50	4.00
3-7	24.55	14.12	53.63	58.04	3.00	1.50	8.00
3-8	38.17	26.98	34.76	70.61	4.00	2.00	3.50
3-9	41.81	28.49	42.03	68.43	1.00	2.00	7.00
3-10	38.98	24.32	48.25	63.34	4.00	2.00	8.00
3-35	28.82	18.86	48.33	65.56	4.00	2.00	7.00
3-34	43.11	28.37	33.28	65.90	4.00	2.00	7.00
3-33	27.17	18.24	40.76	67.24	3.70	2.00	7.50
3-32	20.08	13.48	39.52	67.10	2.40	2.00	9.00
3-31	13.06	6.39	28.08	48.94	4.00	2.00	7.00
3-30	28.08	18.09	35.51	64.45	4.00	2.00	3.00
3-29	28.58	19.89	58.88	69.60	3.65	2.00	7.50

Contd…..

3-11	22.95	15.63	49.60	68.09	3.55	2.00	6.50
3-12	24.11	16.76	34.71	69.11	3.95	2.00	7.00
3-13	26.71	17.09	47.32	63.97	2.25	2.00	7.50
3-14	7.35	4.62	34.18	63.78	3.45	2.00	7.50
3-15	34.27	22.81	53.74	65.56	1.00	4.00	7.50
3-16	32.86	20.65	47.21	63.76	2.50	2.00	4.50
3-17	9.09	4.55	20.68	50.26	2.75	2.50	5.50
3-18	16.68	11.03	46.67	64.31	1.30	2.00	5.50
3-19	25.22	17.81	60.05	70.63	1.15	2.00	8.00
3-20	19.64	13.85	58.92	70.51	2.45	2.00	7.50
3-21	11.44	7.24	41.40	63.26	2.45	1.00	6.00
3-22	39.47	28.66	46.20	72.57	3.65	2.00	6.00
3-23	9.55	6.03	39.80	61.71	2.95	2.00	8.00
3-24	31.34	20.15	53.97	64.80	1.15	2.00	7.50
3-25	21.51	15.14	42.76	70.41	3.95	2.00	7.00
3-26	22.26	15.99	76.15	71.84	1.15	2.00	7.00
3-27	25.91	18.67	41.79	71.94	3.90	2.00	5.50
3-28	19.81	13.43	55.97	67.75	2.80	2.00	7.50
4-1	37.70	27.30	31.70	71.73	4.00	3.50	5.50
4-3	31.12	21.60	53.78	69.47	2.15	2.00	8.00
4-4	35.67	25.87	51.17	72.53	2.30	2.00	7.00
4-5	42.49	32.10	78.46	75.44	1.35	1.00	5.00
4-6	34.28	22.93	43.28	65.74	1.90	1.00	7.50
4-7	36.60	22.36	57.85	61.21	1.00	2.00	8.50
4-8	35.54	23.23	48.55	64.48	2.75	2.00	7.50
4-9	31.44	22.79	47.84	72.49	2.50	2.00	4.00
4-10	34.61	23.14	82.44	67.22	1.05	2.00	6.00
4-11	12.70	9.49	49.62	73.45	1.90	1.00	7.00
4-12	25.31	18.23	49.69	72.77	2.65	1.00	8.00
4-13	25.36	18.22	45.28	71.82	2.90	2.00	4.00
4-14	16.40	11.12	43.04	67.43	1.30	4.00	5.00
4-15	41.19	29.39	48.11	71.68	2.60	1.00	6.00
4-16	34.74	27.19	66.44	78.46	2.90	2.50	5.50
4-17	38.93	26.99	55.97	69.35	3.40	1.50	6.00
4-18	50.39	34.29	49.88	68.16	1.90	2.00	5.50
4-19	28.83	20.05	46.87	70.12	3.15	4.00	8.00
4-20	27.40	19.17	48.97	70.64	1.40	4.50	2.00
4-21	19.80	13.15	43.28	66.56	1.15	4.50	5.00
4-22	22.78	13.30	47.92	58.32	3.00	1.50	6.00
4-23	10.83	6.62	29.20	61.53	4.00	1.50	4.00
4-24	43.03	32.12	56.54	74.94	1.00	3.50	8.00
4-25	24.90	17.27	45.44	69.27	1.00	2.00	5.00
4-27	25.14	17.03	56.04	66.96	2.75	4.50	4.00
4-28	31.42	21.92	42.51	69.69	2.60	2.50	8.00
5-1	13.69	9.75	25.89	71.31	3.95	2.00	8.00

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5-2	21.71	16.33	58.36	75.69	2.00	2.00	8.00
5-3	33.54	22.39	61.15	67.05	1.05	3.00	5.50
5-4	24.00	17.47	44.98	72.89	3.00	2.00	8.00
5-5	44.03	29.76	49.69	68.51	1.00	2.50	8.00
5-6	63.51	45.38	46.54	71.48	3.00	1.00	8.00
5-7	30.43	22.17	46.85	72.69	3.90	3.00	6.00
5-9	41.44	25.19	43.67	61.26	1.20	2.00	2.00
5-11	21.76	16.47	66.25	74.50	2.00	2.50	8.00
5-12	15.18	12.54	61.78	82.32	2.00	2.00	8.00
5-13	28.26	20.28	55.56	71.41	3.00	2.00	8.00
5-14	23.20	18.36	30.76	78.48	3.00	1.00	8.00
5-15	19.05	11.75	56.94	61.71	2.45	1.00	5.00
5-16	25.14	17.25	68.18	69.64	2.10	1.00	3.00
5-17	35.71	27.26	54.12	76.35	2.40	2.50	4.00
8-1	22.76	15.52	34.82	68.29	4.00	2.00	7.50
8-2	41.06	24.48	29.34	59.62	3.40	2.00	8.00
8-4	8.70	5.75	37.62	65.58	2.25	2.00	8.00
8-5	31.50	24.89	55.39	78.78	3.25	2.00	7.00
8-6	31.38	26.35	69.35	83.49	2.30	3.00	4.00
8-7	28.56	18.60	49.00	64.37	2.50	2.00	7.00
8-8	22.88	17.09	60.11	74.27	2.70	3.50	8.00
8-9	16.26	12.40	46.30	73.59	1.25	3.00	4.00
8-10	13.50	9.76	25.48	71.93	4.00	7.50	6.00
8-11	14.14	9.40	44.83	66.19	2.35	7.50	7.00
8-12	7.02	5.07	28.16	72.22	3.75	4.50	3.00
8-13	32.08	23.22	53.33	72.40	2.05	6.50	7.00
8-15	37.55	25.51	61.01	65.27	1.90	2.00	5.50
8-16	17.09	12.60	47.96	73.63	4.00	2.00	5.00
8-17	13.44	9.72	47.31	72.26	4.00	4.50	7.50
8-18	38.72	28.02	46.98	70.83	2.70	4.00	4.00
8-19	13.25	9.16	47.15	69.28	3.50	2.00	4.50
8-20	29.73	21.64	44.22	72.83	2.10	5.50	7.00
8-21	56.16	41.58	40.57	73.99	3.90	2.50	2.00
GM	25.51	17.63	47.86	68.64	2.63	2.51	6.24
CV	26.20	28.44	7.83	10.47	5.69	21.62	15.85
CD	13.23	9.90	7.40	14.19	0.30	1.07	1.95

Appendix III: Performance of lines of TG 19 × GPBD 4 cross for productivity and stress parameters in rainy season

Genotype	Pod yield / plant (g)	Kernel yield / plant (g)	Test weight (g)	Shelling percentage	*A. flavus* score (1-4 scale)	Rust	Late leaf spot
TG 19	8.94	5.87	66.99	65.62	1.00	2.00	7.00
GPBD 4	21.00	15.14	38.93	72.06	4.00	1.00	3.00
6-1	19.26	10.69	43.22	55.94	4.00	2.00	8.00
6-5	8.59	6.43	32.34	74.18	3.00	1.50	8.00
6-7	23.99	13.75	29.35	57.25	3.00	2.00	7.00
6-9	26.42	15.80	46.35	58.95	3.60	2.00	8.00
6-10	20.24	15.51	57.78	76.41	2.10	4.00	4.50
6-12	19.81	11.45	37.64	57.94	4.00	1.00	5.50
6-13	23.93	14.74	38.58	62.02	4.00	3.00	8.00
6-14	25.23	17.11	61.24	67.51	2.85	2.00	7.50
6-15	19.33	12.10	44.07	61.48	4.00	4.00	7.50
6-16	29.52	18.47	56.41	63.16	3.00	2.00	8.00
6-17	21.51	15.70	58.93	73.13	2.00	2.00	9.00
6-18	30.83	21.49	44.17	69.47	1.00	1.50	7.00
6-20	23.26	17.93	32.65	77.13	2.40	1.00	7.00
6-21	22.84	16.58	44.96	72.55	1.65	2.00	8.00
6-22	14.93	9.70	40.90	65.63	1.50	2.00	8.50
6-23	20.11	13.84	53.17	68.79	3.45	1.50	7.00
6-25	31.77	22.90	50.04	71.17	3.10	5.50	4.50
6-26	18.99	14.77	41.10	78.12	4.00	2.50	7.00
6-27	17.12	8.66	52.35	53.69	2.85	3.50	8.00
6-28	38.68	30.19	43.44	78.15	1.95	1.00	8.00
6-29	20.06	15.01	40.26	74.90	1.00	1.00	5.50
6-30	43.92	29.44	55.63	67.18	4.00	1.00	6.00
6-32	22.97	14.38	40.01	61.18	1.00	4.50	4.00
6-33	21.53	16.56	28.84	78.71	1.45	1.00	8.00
6-34	17.19	7.56	31.38	44.16	2.25	2.50	8.00
6-35	32.07	21.83	46.08	68.66	3.00	2.00	8.00
6-36	13.97	6.64	35.39	47.36	2.80	2.00	8.00
6-37	31.44	19.19	57.14	61.05	1.15	2.00	8.00
6-39	22.52	14.27	32.78	63.13	3.00	3.00	8.00
6-40	28.41	19.90	30.88	69.58	4.00	2.50	7.00
6-41	20.08	13.00	76.73	64.76	4.00	4.50	9.00
6-42	38.00	25.06	41.22	67.22	3.35	1.00	8.00
6-43	26.00	17.26	65.18	66.45	1.00	1.00	6.50
6-44	36.14	27.06	73.98	74.85	1.00	1.50	8.00
6-45	9.81	7.90	42.72	82.86	3.40	2.50	8.00
6-46	19.07	10.44	46.68	49.71	2.25	1.50	6.00

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6-47	23.38	12.27	43.21	52.43	2.90	5.00	4.50
6-48	10.40	6.05	36.67	58.31	3.75	3.00	4.00
6-49	20.50	11.56	57.80	56.80	4.00	2.00	8.00
6-50	22.14	16.35	45.88	74.20	1.00	5.00	7.00
6-51	23.99	17.03	47.39	70.83	4.00	4.00	4.00
6-52	18.40	13.40	47.85	72.83	1.00	2.00	5.50
6-53	37.78	25.26	50.42	66.31	2.45	1.00	4.00
6-54	16.03	11.32	58.72	71.60	2.90	2.50	5.50
6-55	24.08	16.98	51.60	70.57	2.10	2.00	9.00
6-56	25.88	17.78	54.88	69.45	3.00	2.00	8.50
6-57	21.26	13.32	27.62	62.36	4.00	1.00	8.00
6-58	10.48	6.40	26.65	60.07	1.30	2.00	8.00
6-59	18.56	10.43	28.46	56.08	4.00	2.00	7.50
6-60	29.54	18.76	42.65	62.58	1.15	2.00	8.00
6-61	15.27	8.55	27.35	56.71	3.00	2.00	9.00
6-62	20.85	12.45	34.67	59.76	2.90	2.00	9.00
6-63	36.90	24.85	37.67	67.88	1.60	3.00	6.00
6-64	25.35	16.32	28.42	64.22	3.20	3.00	8.00
GM	22.92	15.09	44.78	65.26	2.67	2.29	7.06
CV	22.84	25.13	10.06	12.19	6.81	24.09	12.14
CD	10.49	7.60	9.03	15.94	0.36	1.10	1.72

EVALUATION OF RESISTANCE TO *Aspergillus flavus* Link ex. Fries IN GROUNDNUT

R. NARASIMHULU 2007 Dr. P. V. KENCHANAGOUDAR

CHAIRMAN

ABSTRACT

In recent times, edible groundnuts have attracted major attention in the international market. Aflatoxin contamination and pesticide residues are the major obstacles in its export. Development of cultivars with resistance to multiple diseases viz., *Aspergillus*, late leaf spot and rust is a major objective in groundnut breeding.

Evaluation of 18 groundnut genotypes over two seasons revealed significant variation among the genotypes for all the traits. Diseases resistance, test weight and pod yield exhibited high genetic advance due to moderate to high magnitude of variation and heritability indicating scope for selection *Aspergillus* seed colonization had desirable negative association with test weight indicating more resistance in the bold seeded types. The genotypes TG 19, TG 49, ICG 8760, ICG 14985, ICG 6027, ICG 13787 and ICGV 86699 had high level of resistance to *Aspergillus* seed colonization. Among them ICGV 86699, ICG 8760 and ICG 13787 showed moderate to high level of resistance to all the three diseases. Popular cultivars TMV 2, JL 24 and TAG 24 were susceptible to all the three diseases. Molecular diversity analysis with 20 RAPD primers revealed more diversity in germplasm than varieties and ICG 13787(germplasm) and GPBD 4(cultivar) as the most divergent genotypes.

The two segregating populations (GPBD 4 x TG 19 and GPBD 4 x TG 49) exhibited significant variation for all the yield traits and diseases. Components of variance revealed scope for selection for all the traits except shelling percentage. GPBD 4 x TG 19 was superior for resistance to *Aspergillus* and rust while GPBD 4 x TG 49 was superior for late leaf spot and yield traits. There were many lines combining high yield and/or seed size with resistance to single disease. None of the lines showed multiple disease resistance indicating a need for large segregating population and selective intermating.