J. Farm Sci., 34(4): (366-370) 2021

RESEARCH PAPER

Screening of groundnut (*Arachis hypogaea* L.) genotypes for resistance to *in vitro* seed colonization and infection by *Aspergillus flavus*

DEEPA P. SALUNKE AND P. V. KENCHANAGOUDAR

Department of Genetics and Plant Breeding, College of Agriculture, Dharwad
University of Agricultural Sciences, Dharwad - 580 005, Karnataka, India
E-mail: deepa3824@gmail.com

(Received: September, 2021 ; Accepted: November, 2021)

Abstract: Groundnut (*Arachis hypogaea* L.) is one of the most susceptible host crops to *Aspergillus flavus* (Link Ex Fries) invasion and subsequent aflatoxin production before and after harvest. The present experiment comprised 9 local cultivars of Karnataka, 34 germplasm collections and 25 advance breeding lines from ICRISAT along with popular tolerant variety J-11 used as check. The study was conducted to screen for *A. flavus* invasion by following the standard method used at ICRISAT using the aggressive strain *Af* (11-4). Ample amount of variation among cultivars and germplasm lines for seed colonization severity was observed in spore spray as well as in pin prick method of screening. From the present investigation, it is clear that ICGV-02207 and ICGV-02266 exhibited both seed coat and cotyledon resistance towards *A. flavus* infection and less aflatoxin accumulation as compared to susceptible one. These can serve as good source of resistance for future groundnut breeding.

Key words: *Aspergillus flavus,* Groundnut, Screening, Variety

Introduction

One of the serious food quality problems associated with groundnut *(Arachis hypogaea)* and its products is the aflatoxin contamination by *Aspergillus flavus* Link Ex Fries not only in the field but also during drying, storage and transit. This results in significant financial losses to producers due to substantial price reductions received for their crop and also to buyers, as contaminated groundnuts cannot be sold for full value. Aflatoxin causes liver cancer in livestock as well as in human beings (Arapcheska *et al.,* 2015). Aflatoxin contamination has become a serious problem in groundnut since it affects the quality of produce thereby affecting the trade in the international market (Tushar Tanna, 2002).

Attempts to mitigate the problem of aflatoxins using good post-harvest handling practices such as good produce handling and storage has not proved to be effective because aflatoxin contamination can occur in the field as well as in storage and use of fungicides is not cost effective for small and marginal farmers. The practices are further not widely adopted by the small and marginal farmers in the developing countries, which contribute about 60 % of the world groundnut production (Upadhyaya *et al.,* 2002). One of the possible means of reducing aflatoxin contamination of groundnut would be use of cultivars resistant to seed invasion/ colonisation or aflatoxin production by aflatoxin producing fungi (Ozimati *et al*., 2014). Therefore, breeding for resistance to *A. flavus* colonization and infection and/or aflatoxin production will play a very significant role in preventing aflatoxin contamination in groundnut and consequently the associated economic losses and health hazards (Upadhyaya *et al.*, 2002, Ozimati *et al*., 2014).

Breeding for resistant cultivars is only possible when there are available sources of stable high level resistance, reliable assessment methods and an understanding of the inheritance of resistance. An effective screening technique is helpful for identification of sources of resistance that could aid in studying resistance mechanisms. Hence, present study was undertaken to screen 9 local cultivars of Karnataka, 34 germplasm collections and 25 advanced groundnut breeding lines (collected from ICRISAT) for identification of resistance source and less aflatoxin accumulation.

Material and methods

Present study was conducted at the College of Agriculture, Dharwad in summer season. Various methods have been used to screen groundnut genotypes for aflatoxin resistance, but the most prevalent has been evaluation of *in vitro* seed colonization by *A. flavus* (IVSCAF) in the laboratory. Mechanisms of resistance to *Aspergillus* colonization and infection may relate to combinations of physical and chemical characteristics of the seed testa. Mixon and Rogers suggested the use of groundnut cultivars with resistance to seed invasion and colonization by toxigenic *Aspergillus* species as an effective means of preventing aflatoxin contamination and developed a new *in vitro* seed colonization procedure for screening the groundnut genotypes against *A. flavus*.

Inoculation of groundnut seeds

Thirty seeds which are well matured with intact seed coat and free from any damage were selected from each genotype for *in vitro* inoculation in two replications. Seeds were surface sterilized with 1 per cent sodium hypochlorite solution for 1 minute and subsequently washed in two changes of distilled sterilized water to remove any traces of sodium hypochlorite. Half of the seeds (15) were uniformly wounded by pricking with a group of sterile needles (six needles) to facilitate the invasion by fungal spores and hyphae. The remaining fifteen seeds were not wounded and kept as healthy. The wounded and healthy seeds were placed separately in sterilized beakers

and spray inoculated with spore suspension ($1x10^6$ spores/ml) of *AF-(11-4)* using an automizer under strict aseptic conditions. The seeds were placed in sterilized petri dishes in equidistance position and incubated at 28^0 c $\pm 1^0$ C in dark for 7-8 days. Individual seeds were scored for surface colonization by *A. flavus* and graded by following severity rating scale (1-4) given by Thakur *et al*. (2000) and the mean of two replications is expressed as colonisation severity. Mean colonization severity was calculated for each line. Based on the scale / grade (<1 to 4.0) accessions /lines were classified into resistant (grade 0.0-1.0), moderately resistant (grade 1.1-2.0), susceptible (2.1-3.0) and highly susceptible (grade 3.1-4.0). Seeds that showed resistance to seed colonization (seed coat resistance in unwounded seeds) and seed infection (cotyledon resistance in unwounded seeds) could be selected for advancing generation for resistance breeding.

The data/scale obtained from *in vitro* screening studies transformed and subjected to ANOVA for a Completely Randomized Design by using M-STATC programme.

Result and discussion

Screening cultivars of Karnataka

Selected released cultivars of Karnataka collected from MARS, Dharwad, cultivars were grown in *summer* and harvested seeds screened against *A. flavus* by artificial inoculation to identification of resistance source. Mean seed colonisation of the varieties is presented in the table 1. Varieties differed significantly for *A.flavus* seed colonisation.

Among the s*ummer* grown cultivars that were screened for *A. flavus* colonization using spore spray method, variation was observed among cultivars for seed colonization severity with colonization rating of 3.0 to 4.0 in spore spray method. Cultivar JL-24 recorded low seed colonization severity (3.0) and remaining cultivars TAG-24, GPBD-4, TGLPS-3, G-2-52, Dh-86, K-6, TMV-2 which recorded high seed colonization severity (4.0) whereas, popular tolerant check variety J-11 (1.8) recorded moderate degree of resistance reaction. Initially released cultivars of Karnataka recorded high seed colonization severity indicating absence of seed coat resistance to *A. flavus*. All the cultivars along with popular resistant check recorded susceptible and highly susceptible reaction in pin prick method indicating no cotyledon resistance towards *A. flavus* infection.

Table 1. Initial screening of released groundnut varieties by *A. flavus* seed colonization (spore spray method) and seed infection (pin prick method) of *summer* harvested crop

Identity	*A. flavus* colonization severity	
	Spore spray	Pin prick
TAG - 24	4.0	4.0
GPBD-4	4.0	4.0
JL-24	3.0	3.7
TGLPS-3	4.0	4.0
GPBD-5	4.0	4.0
G-2-52	4.0	4.0
Dh-86	4.0	4.0
K-6	4.0	4.0
TMV-2	4.0	4.0
J-11 (Popular tolerant check)	1.8*	3.4
S.Em.±	0.06	0.04
C.D. (0.01)	0.18	0.13
C.D. (0.05)	0.13	0.09
C.V. (%)	1.98	1.08

Crop season -*summer* (January- May)
In vitro screening seed colonization and infection by *A. flavus* (11-4)
* and ** - significance at 0.05 and 0.01 level for probability, respectively.

Screening of groundnut germplasm collections

Germplasm collections collected from ICRISAT and grown during *summer* at MARS, Dharwad were screened for *A. flavus* by artificial inoculation for identification of resistance source (Table 2). Significant variation was observed among accessions with seed colonization rating of 0.8-4.0 in spore spray method. Accessions ICG-2857, ICG-7633 recorded lower seed colonization severity (colonization rating of < 1.00) showing seed coat resistance and ICG-1122, ICG-3336, ICG-14985 recorded lower seed colonization severity with range of 1.0-2.0 seed colonization score showing moderate degree of seed coat resistance reaction and remaining all accessions recorded high seed colonization severity with colonization rating of 2.7-4.0. Whereas, popular tolerant check variety

J-11 (1.6) and resistant check variety ICGV-02266 (0.8) also recorded moderate resistance and resistance reaction, respectively. Remaining all accessions and susceptible check TGLPS-3 recorded no seed coat resistance towards *A. flavus.*

In pin prick method, accessions ICG-1122, ICG-2857 and ICG-7633 recorded low seed colonization rating of 1.0-2.0 indicating moderate degree of seed coat resistance reaction. Remaining germplasm accessions along with popular tolerant check J-11 were recorded seed colonization severity rating of 3.0-4.0 scale indicating susceptible reaction, while resistant check ICGV-02266 recorded resistance reaction with seed colonization rating of 1.0 scale. This indicated absence of cotyledon resistance against *A. flavus* invasion in these susceptible genotypes.

Screening of groundnut advance breeding lines

Advance breeding lines collected from ICRISAT were screened for *A. flavus* by artificial inoculation for identification of resistance source (Table 3).

Significant variation was observed in *A. flavus* seed colonization severity with rating of 0.2 to 4.0 in spore spray method. Among advanced breeding lines, ICGV-02207 and ICGV-02266 recorded very low seed colonization severity (< 1.0) showing seed coat resistance. Another breeding line ICGV-00308 (1.4) and also popular tolerant check variety J-11 (1.9) recorded lower seed colonization severity (1.0-2.0) showing moderate degree of seed coat resistance. Remaining all advance breeding lines and susceptible control TGLPS-3 recorded high seed colonization severity (3.0-4.0), indicating no seed coat resistance towards *A. flavus* in these susceptible lines.

Table 2. Screening of germplasm collections against *A. flavus* seed colonization (spore spray method) and seed infection (pin prick method) of *summer* harvested crop

Identity	*A. flavus* colonization severity	
	Spore spray	Pin prick
ICG -1122	1.4*	2.0*
ICG -1173	4.0	4.0
ICG- 1323	4.0	4.0
ICG- 1326	4.0	4.0
ICG -1859	3.0	4.0
ICG -1994	3.7	4.0
ICG -2857	0.9**	1.5*
ICG- 3263	4.0	4.0
ICG -3267	3.8	4.0
ICG -3336	1.8*	4.0
ICG -3673	4.0	4.0
ICG- 3700	4.0	4.0
ICG -4589	4.0	4.0
ICG -4746	4.0	4.0
ICG -4749	4.0	4.0
ICG -4888	3.8	4.0
ICG- 6025	4.0	4.0
ICG- 6706	4.0	4.0
ICG -7412	2.7	3.0
ICG -7633	0.8**	1.8*
ICG- 8686	3.6	4.0
ICG- 8760	4.0	4.0
ICG -9407	4.0	4.0
ICG- 9610	4.0	4.0
ICG -10020	4.0	4.0
ICG- 10094	4.0	4.0
ICG- 10933	4.0	4.0
ICG- 11088	4.0	4.0
ICG -11426	3.4	4.0
ICG -12625	3.7	4.0
ICG -12672	3.8	4.0
ICG- 13787	4.0	4.0
ICG -14475	3.8	4.0
ICG -14985	2.0*	4.0
TGLPS-3 (Susuceptible check)	4.0	4.0
ICGV-02266 (Resistant check)	0.8**	1.0**
J-11(Popular tolerant check)	1.6*	2.8
S.Em. ±	0.06	0.04
C.D. (0.01)	0.23	0.15
C.D. (0.05)	0.15	0.08
C.V. (%)	4.16	2.61

Collected from ICRISAT, Hyderabad and maintained at MARS, Dharwad

Crop season –*summer* (Jan- April)

Invitro screening seed colonization and infection by *A. flavus* (11-4)

* and ** - significance at 0.05 and 0.01 level for probability, respectively.

In pin prick method, 23 germplasm accessions showed seed colonization severity rating of 4.0 scale and also popular tolerant check variety J-11 recorded with colonization grade 2.8, indicating absence of cotyledon resistance in these susceptible lines. Only two breeding lines ICGV-02207 and ICGV-02266 recorded lower seed colonization severity (<1.0) showing cotyledon resistance towards *A. flavus.*

Harish Babu *et al.* (2005), Kiran Kumar (2005) evaluated genotypes in laboratory against *A. flavus* and found varied level of seed colonization severity in germplasm lines. Ranganath *et al.* (2014) screened genotypes, germplasm collections and advance breeding lines by two methods (spore spray and pin prick). Among all these only ICGV-02266 showed moderate resistant reaction (score 1.0) for both the methods indicating presence of seed coat and cotyledon resistance in this line.

As seed coat is outmost layer of groundnut kernels, presence of seed coat resistance in genotypes due to structure of seed coat (seed coat thickness, palisade like layer, wax and cutin layers) might play role of physical barrier in resistance to *A. flavus* colonization and invasion/infection.

Seed/kernel acts as substance for fungus to produce aflatoxin. Presence of cotyledon resistance in genotypes due

Table 3. Screening of advanced breeding lines against *A. flavus* seed colonization (spore spray method) and seed infection (pin prick method) of *summer* harvested crop

Identity	*A. flavus* colonization severity	
	Spore spray	Pin prick
ICGV- 09040	4.0	4.0
ICGV- 09044	4.0	4.0
ICGV- 09048	4.0	4.0
ICGV -09054	4.0	4.0
ICGV -09072	4.0	4.0
ICGV- 09075	4.0	4.0
ICGV -09078	4.0	4.0
ICGV -09084	4.0	4.0
ICGV- 09085	4.0	4.0
ICGV -09086	4.0	4.0
ICGV- 09087	3.0	4.0
ICGV- 09089	4.0	4.0
ICGV -09091	4.0	4.0
ICGV- 09104	4.0	4.0
ICGV- 09105	4.0	4.0
ICGV- 09111	4.0	4.0
ICGV- 01015	4.0	4.0
ICGV -01105	4.0	4.0
ICGV -02184	4.0	4.0
ICGV- 02207	0.8**	1.0**
ICGV- 06408	4.0	4.0
ICGV -88145	4.0	4.0
ICGV -02266	0.2**	0.8**
ICGV -91114	4.0	4.0
ICGV- 00308	1.4*	4.0
TGLPS-3 (Susceptible check)	4.0	4.0
J-11(Popular tolerant check)	1.9*	2.8
S.Em. ±	0.07	0.03
C.D. (0.01)	0.18	0.08
C.D. (0.05)	0.13	0.04
C.V. (%)	3.36	1.28

Collected from ICRISAT, Hyderabad and maintained at MARS, Dharwad

Crop season - *summer* (Jan- April)

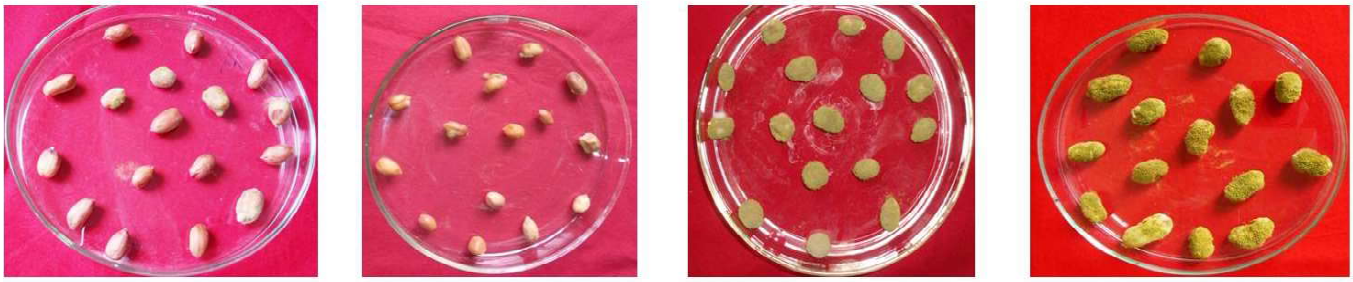
Invitro screening seed colonization and infection by *A.flavus* (11-4)

*and ** - significance at 0.05 and 0.01 level for probability, respectively.

Spore spray method

Resistant parents | Susceptible parents

ICGV -02207 Aflatoxin content : 2.1µg/kg | ICGV -02266 Aflatoxin content : 1.3 µg/kg | GPBD-5 Aflatoxin content : 2908.4µg/kg | TGLPS-3 Aflatoxin content : 3043.9 µg/kg



ICGV -02207 Aflatoxin content : 6.25µg/kg | ICGV -02266 Aflatoxin content: 3.23 µg/kg | GPBD-5 Aflatoxin conten: 2976.15µg/kg | TGLPS-3 Aflatoxin content: 3408.10µg/kg

Plate 1. Resistant and susceptible parents used for breeding programme

to proteins which function to protect against infection of fungi during storage and germination. These antifungal proteins includes enzyme inhibitors, non-specific lipid transfer proteins, ribosome inactivating protein. Their localization and concentration within the kernels may play an important role in preventing fungal invasion. Phytoalexins may be one of the defense mechanism of seeds for *Aspergillus* invasion. Similar study reported by Liang (2002) who observed protein extracted from seeds showed markedly antifungal activity against *A. flavus in vitro* and reported level of resveratrol (phytoalexin) synthesis in resistant and susceptible genotypes. Similar study on seed coat structure reported by Shan *et al*. (2006) and Liang *et al*., (2003) observed significant difference of seed coat structure in resistant and susceptible genotypes.

Conclusion

Breeding resistant cultivars is possible only when there are available sources of stable, high-level of resistance. The objective is to incorporate the resistance to *A. flavus* into the breeding and development of desirable commercial varieties. Among two types of resistance cotyledon resistance was more important than seed coat resistance as seed coat is one of the barrier but cotyledon acts as final substrate for *Aspergillus flavus* to produce aflatoxin in groundnut kernel and this toxin make groundnut kernel unfit for consumption and also barrier in trade market. Hence, cotyledon resistance is more valuable source for aflatoxin resistance. Only two advance breeding lines ICGV- 02207 and ICGV-02266 (plate 1) reported as resistant for both seed coat and cotyledon resistance and none of the local cultivars, accessions showed cotyledon resistant reaction towards *A.flavus*. These lines act as good resistance source for Aflatoxin or *Aspergillus flavus* infection resistance breeding. These genotypes used as resistance source and local cultivar bold seeded GPBD-5, TGLPS-3 used as female parents for our research programme to study the genetics of *A.flavus* colonization and infection and also identification of resistance lines along with good yield traits in advance generation. Breeding *A. flavus* resistant varieties, particularly as part of a groundnut breeding program, appears to be very encouraging. Screening and selecting breeding lines for resistance would insure the development of future peanut/groundnut varieties with high degree of tolerance to invasion by *A. flavus.*

References

Arapcheska M, Jovanovska V, Jankuloski Z, Musliu Z H and Uznov R, 2015, Impact of aflatoxins on animal and human health. *International Journal of Innovative Science, Engineering & Technology*, 2(2): 156-161.

Harish Babu B N, Gowda M V C and Kusuma V P, 2005, Confectionery groundnuts resistant to seed colonization by *Aspergillus flavus*. *International Arachis News Letter,* 25: 10-12.

Kiran Kumar M G, 2005, Variability in *Aspergillus flavus* L. ex Fries infecting ground nut (*Arachis hypogaea* L.). *M. Sc.* (*Agri.) Thesis,* University of Agricultural Sciences, Dharwad, Karnataka, India.

Liang X Q, 2002, Studies on the mechanism and inheritance of resistance to *Aspergillus flavus* invasion and aflatoxin production in peanut (*Archis hypogaea* L.). *Ph.D. Thesis*, University of South China, Guangzhou, China.

Liang X Q, Zhou G Y and Pan R Z, 2003, Study on the relationship of wax and cutin layers in peanut seeds and resistance to invasion and aflatoxin production by *Aspergillus flavus*. *Journal of Tropical and Subtropical Botany*, 11: 11-14.

Ozimati A A, Rubaihayo P R, Gibson P, Edema R, Kayondo I S, Ntare B R, Okello D K, 2014, Inheritance of resistance to kernel infection by *Aspergillus flavus* and aflatoxin accumulation in groundnut. *African Journal of Crop Science,* 2 (1): 051-059.

Ranganthswamy, 2014, Studies on characterization and management of mycotoxin producing organisms in groundnut with special reference to *Aspergillus flavus* Link ex Fries. *Ph.D. Thesis*, University of Agricultural Sciences Dharwad, Karnataka, India.

Shan S, Wang H, Li C, Wan S, Liu H and Jiang G, 2006, Research of seed testa structure and storage material of peanut germplasm with different resistance to *A. flavus Agricultural Sciences China,* 5 (6): 478-482.

Thakur R P, Rao V P, Reddy S V and Ferguson M, 2000, Evaluation of wild *Arachis* germplasm accession for *in vitro* seed colonization and aflatoxin production by *Aspergillus flavus*. *International Arachis News Letter*, 20: 44 - 46.

Tushar Tanna, 2002, Quality considerations for export of oilseeds. In: *Oilseeds and Oils, Research and Development Needs* (Eds. Mangal rai, Harvir Singh D and Hegde D M), Directorate of Oilseed Research, Rajendranagar, Hyderabad, Andhra Pradesh, India, pp. 347-354.

Upadhyaya H D, Nigam S N and Thakur R P, 2002, Genetic enhancement for resistance to aflatoxin contamination in groundnut. *In: Proceedings of the seventh ICRISAT Regional Groundnut Meeting for Western And Central Africa, 6-8 Dec. 2000*, Cotonou, Benin. Patancheru, India, pp. 81-85.