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IN VITRO VARIETAL SCREENING FOR RESISTANCE TO *ASPERGILLUS FLAVUS* INVASION AND AFLATOXIN PRODUCTION IN GROUNDNUT

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ABSTRACT

Twenty varieties of groundnut were screened for *Aspergillus flavus* invasion and aflatoxin production by dry seed resistance test. The aflatoxin content of infected kernels was measured by indirect competitive procedure. Out of twenty varieties tested, none of the variety was found immune as well as resistant against most virulent isolates of *A. flavus*. TMV-2 showed maximum seed infection (83.33 %) and higher amount of aflatoxin content (7857.42 ppb). Variety TMV-7 showed cent per cent seed germination while the maximum seed mortality was recorded in ICGV-89280 (73.89 %). Minimum seed infection (16.67 %), mortality of germinated seeds (10.74 %) and amount of aflatoxin (955.79 ppb), was recorded in the variety J-11. Out of twenty varieties tested, only variety J-11 was found in low category, where as twelve varieties *viz.,* GG-2, GG-5, GG-7, GG-11, TG-37A, ICGV-89280, ICGV-86564, ICGS-44, ICGS-00440, TAG-24, TMV-7 and MH-1 in moderate and rest of the seven varieties were categorized to have high content of aflatoxin.

***Keywords*:** Aflatoxin, *Aspergillus flavus*, ELISA, Groundnut, Variety

Introduction

Groundnut (*Arachis hypogaea* L) is one of the important oilseed crops of India. One of the serious food quality problems associated with groundnut and its products is the aflatoxin contamination by the fungi, *Aspergillus flavus* not only in the field but also during drying, storage and transit (Mehan, 1989). Aflatoxin causes liver cancer in livestock and human beings. Management of aflatoxin contamination requires both preventive and curative approaches starting from sowing and harvesting to processing and storage. Lack of single effective control measure further enhances the risks of aflatoxin contamination. The use of resistant variety can be the most viable and economical approach to reduce aflatoxin problem (Swindale, 1989). Hence study was undertaken to screen the groundnut variety for resistance to *in vitro* infection by a toxigenic isolate of *A. flavus* (isolate AF-8).

Materials and Methods

Twenty varieties of groundnut listed below were screened for *A. flavus* invasion and aflatoxin production by dry seed resistance test.

Sr.No.	Variety	Sr.No.	Variety
1	GG-2	11	ICGV-89280
2	GG-5	12	ICGV-86564
3	GG-6	13	ICGS-44
4	GG-7	14	ICHG-00440
5	GG-11	15	J-11
6	GG-12	16	JL-24
7	GG-13	17	TAG-24
8	GG-20	18	TMV-7
9	TG-37A	19	MH-1
10	TPG-41	20	TMV-2

Sound, healthy and mature 100 gram kernels of each variety was surface sterilized with 0.1 % aqueous solution of mercuric chloride for 1 minute and was immediately washed thoroughly with sterile distilled water for three times. The seeds of each variety were decanted and were aseptically placed in sterilized Petri plates. The kernels were uniformly inoculated with spore suspension of *A. flavus* (10^6 spores ml^{-1} @ 1ml per 10 g kernels). Ten seeds were placed aseptically in each sterile 15 cm diameter Petri plate with three replication. The seeds were rolled gently around the plates to spread the inoculums evenly over their surface. The spore suspension was prepared from 7 days old culture and spore load counted with haemocytometer. Inoculated seeds were incubated under standard conditions of temperature and humidity. After 10 days of incubation, observations were recorded for per cent seed infection; percent seed germination and percent mortality of germinated seeds. Aflatoxin content was recorded after 30 days of incubation.

Varieties with < 15 per cent seed infection and sparse growth were regarded as resistant, 16-30 per cent seed infection and moderate growth were regarded as moderately resistant, 31-50 per cent seed infection and moderate to dense growth were regarded as susceptible and > 50 per cent seed infection with dense growth were regarded as highly susceptible.

The aflatoxin content of infected kernels was measured by indirect competitive ELISA procedure. The varieties with < 100 ppb were regarded as negligible categories, 100-1000 ppb as low, 1000-5000 ppb as moderate and >5000 ppb as high level categories (Thakur *et al.,* 2000).

Results and Discussion

The use of resistant variety can be the most viable and economical approach to reduce aflatoxin problem. In Gujarat, one of the leading producers of groundnut, very little information is available on the reaction of cultivated varieties to *A. flavus* infection. Hence, this study was undertaken to screen the groundnut varieties of Gujarat along with other popular varieties for resistance to *in vitro* seed infection and aflatoxin content by toxigenic isolate of *A. flavus* (AF-8).

The seed infection, seed germination and mortality of germinated seeds were recorded after eight days of incubation, and aflatoxin content was determined by indirect ELISA procedure after thirty days of incubation.

1. Seed Infection (%)

Among twenty varieties tested, most of varieties having seed infection ranged from 16.67 to 83.33 per cent. The maximum seed infection (83.33 %) was recorded in GG-20 and TMV-2, which were at par with MH-1 (80.00 %). The minimum seed infection (16.67 %) was recorded with J-11 (Table 1). Categorization of different varieties based on seed infection (Table 2) revealed that out of twenty varieties tested, none of the variety was found immune as well as resistant against most virulent isolate of *A. flavus* (AF-8). Four varieties *viz.,* J-11, GG-5, GG-11 and GG-13 were found moderately resistant, whereas five varieties *viz.,* GG-2, GG-12, ICGV-86564, ICGS-44 and ICGS-00440 were found susceptible, and the remaining eleven varieties were highly susceptible against *A. flavus* invasion.

2. Seed Germination (%)

In case of seed germination, cent per cent seed germination was recorded in variety TMV-7, which was at par with varieties GG-13 (96.67 %), GG-6 (93.33 %), J-11 (93.33 %), GG-2 (90.00 %), GG-5 (90.00 %) and ICHG-00440 (90.00 %) (Table 1). The minimum seed germination was recorded in GG-20 (50.00 %) and ICGV-89280 (50.00%), which were at par with GG-7 (53.33 %).

3. Mortality of Germinated Seeds (%)

Per cent mortality of germinated seeds was recorded in the range from 10.74 to 73.89 % (Table 1). The maximum seed mortality was recorded in ICGV-89280 (73.89 %), which was at par with TMV-2 (73.81 %), while the minimum mortality of germinated seeds (10.74 %) was noted in varieties J-11, at par with GG-2 (11.20 %), GG-5 (11.20 %) and GG-11(15.74 %).

4. Aflatoxin Content (ppb)

Aflatoxin content was recorded after thirty days of incubation from the seeds of twenty different varieties and presented in Table 3 ranged from 955.79 ppb to 7857.42 ppb. The variety J-11 produced lowest amount (955.79 ppb) of aflatoxin which was at par with TAG-24 (1052.84 ppb). Higher amount of aflatoxin was recorded in TMV-2 (7857.42 ppb) which was at par with variety GG-20 (7409.85 ppb). Categorization of different varieties based on aflatoxin content (Table 4) revealed that out of twenty varieties tested, only variety J-11 was found in low category, where as twelve varieties *viz.,* GG-2, GG-5, GG-7, GG-11, TG-37A, ICGV-89280, ICGV-86564, ICGS-44, ICGS-00440, TAG-24, TMV-7 and MH-1 in moderate and rest of the seven varieties were categorized to have high content of aflatoxin.

Different groundnut varieties differ in their ability against invasion of *A. flavus.* Nayak *et al.,* (1992) found four different genotypes and the variety J-11 to be resistant against infection and colonization *in vitro.* Waliyar *et al.* (1994) reported three lines (ICGVs 87084, 87094 and 87110) to be resistant against *A. flavus* in India and also abroad. Rao *et al.* (1995) evaluated 472 different germplasm, out of them two lines ICGV 88145 and ICGV 89104 along with J-11 were found resistant against *A. flavus* invasion under field conditions. Upadhyay *et al.* (1997, 2001) have released three lines *viz.,* ICGVs 91278, 91283 and 91284 as improved germplasm. They also found J-11 as resistant and JL-24 as susceptible varieties. Varma *et al.* (2001) evaluated 14 different groundnut cultivars for resistance to *in vitro* seed colonization with toxigenic strains of *A. flavus.* Among them, cultivars JL-24, TAG-24 and TMV-2 were reported as susceptible. Khandar *et al.* (2004) reported 11 genotypes showed very less seed infection, seed colonization and aflatoxin content as compared to GG-20. Babu *et al.* (2004, 2004a) evaluated 15 groundnut cultivars and 30 advanced breeding lines. Among them, they found 5 cultivars had relatively lower seed colonization than TMV-2, whereas 3 lines were superior to J-11. They noted J-11 as resistant and TMV-2 as susceptible variety. Out of 15 large seeded confectioneries grade groundnut genotypes along with resistant (J-11) and susceptible (TMV-2) screened against *A. flavus* seed infection, 4 genotypes *viz.,* TG-19, TG-49, TG-18 and TG-18A showed high level of resistance with low seed colonization.

Different groundnut varieties differ in their ability to produce aflatoxin production. Nagarajan and Bhat (1973) reported higher aflatoxin production in TMV-2 as compared to US-26. Ghewande *et al.* (1989) reported higher aflatoxin in BG-1 followed by JL-24, GG-2, RSB-87, TMV-12, TMV-7, S-230 and KRG-1. Reddy *et al.* (2003) recorded maximum aflatoxin in variety TMV-2 followed by JL-24, GG-2 and TAG-24.

The resistance of groundnut seed to *A. flavus* is associated with certain morphological and biochemical characters. The seeds of damaged pod and that of shell have been considered a barrier to penetration by *A. flavus* (McDonald and Harkness, 1967). Carter (1973) and Mixon and Roger (1975) established protective role of seed testa in case of seed colonization by *A. flavus.* Presence of wax and cuticle layer on testa and phenolic compound in testa have been found as an important character for resistance (Liang *et al.,* 2003). More compact cell structure of testa was one of the important reasons for resistance in genotype PI 337394 and PI 337409 (Dieckert and Dieckert, 1977). Moisture levels in resistant variety (Ketring *et al.,* 1976) and less water soluble amino acid (Amaya *et al.,* 1980) were also the important factor for their resistance. Slower rate of water uptake by resistant varieties was noted by Nayak *et al.* (1992). Thus the present results are in accordance with the reports of above workers.

Table 1: Seed infection (%), seed germination (%) and mortality of germinated seeds (%) of different groundnut varieties after 10 days of incubation with *A. flavus**

Sr. No.	Name of Varieties	Seed infection (%)	Seed germination (%)	Mortality of germinated seeds (%)	Level of resistance
1	GG-2	44.98 (50.00)	74.97 (90.00)	19.53 (11.20)	S
2	GG-5	33.20 (30.00)	74.97 (90.00)	19.53 (11.20)	MR
3	GG-6	46.90 (53.33)	77.68 (93.33)	32.19 (28.52)	HS
4	GG-7	56.97 (70.00)	46.90 (53.33)	25.73 (18.89)	HS
5	GG-11	30.98 (26.67)	66.12 (83.33)	23.17 (15.74)	MR
6	GG-12	44.98 (50.00)	61.20 (76.67)	35.93 (34.52)	S
7	GG-13	32.99 (30.00)	83.82 (96.67)	27.07 (20.74)	MR
8	GG-20	66.12 (83.33)	44.98 (50.00)	44.81 (49.44)	HS
9	TG-37A	56.97 (70.00)	61.20 (76.67)	43.61 (47.62)	HS
10	TPG-41	46.90 (53.33)	56.97 (70.00)	38.34 (38.69)	HS
11	ICGV-89280	54.76 (66.67)	44.98 (50.00)	59.37 (73.89)	HS
12	ICGV-86564	41.14 (43.33)	56.77 (70.00)	28.93 (23.81)	S
13	ICGS-44	44.98 (50.00)	63.90 (80.00)	35.58 (34.19)	S
14	ICHG-00440	39.22 (40.00)	74.97 (90.00)	28.22 (22.41)	S
15	J-11	23.84 (16.67)	77.68 (93.33)	19.12 (10.74)	MR
16	JL-24	52.75 (63.33)	61.20 (76.67)	43.94 (48.21)	HS
17	TAG-24	50.83 (60.00)	68.83 (86.67)	38.26 (38.43)	HS
18	TMV-7	50.75 (60.00)	89.96 (100.00)	44.98 (50.00)	HS
19	MH-1	63.41 (80.00)	54.76 (66.67)	48.23 (55.56)	HS
20	TMV-2	66.12 (83.33)	61.20 (76.67)	59.19 (73.81)	HS
S.Em.±		2.33	4.44	2.78	
C.D. at 5 %		6.67	12.69	7.95	
C.V. %		8.52	11.80	13.47	

*Average of three replication Figures in parenthesis are retransformed value

Level of resistance on the basis of seed infection:

< 15 per cent – Resistant (R), 16-30 per cent - Moderately Resistant (MR),
31-50 per cent – Susceptible (S), > 51 per cent - Highly Susceptible (HS)

Table 2 : Categorization of different groundnut varieties based on seed infection (%)

Sr. No.	Categories	No. of varieties	Per cent varieties	Name of Varieties
1	Resistant < 15 %	0	0	---
2	Moderately Resistant 16-30 %	4	20	GG-5, GG-11, GG-13, J-11
3	Susceptible 31-50 %	5	25	GG-2, GG-12, ICGV-86564, ICGS-44, ICGS-00440
4	Highly Susceptible > 51 %	11	55	GG-6, GG-7, GG-20, TG-37A, TPG-41, ICGV-89280, JL-24, TAG-24, TMV-7, MH-1, TMV-2

Table 3: Aflatoxin content (ppb) in different groundnut varieties after 30 days of incubation with *A. flavus**

Sr. No.	Name of Varieties	Aflatoxin content (ppb)	Level of aflatoxin content
1	GG-2	1384.81	Moderate
2	GG-5	2205.22	Moderate
3	GG-6	6459.89	High
4	GG-7	4152.69	Moderate
5	GG-11	3926.92	Moderate
6	GG-12	5933.77	High
7	GG-13	5453.09	High
8	GG-20	7409.85	High
9	TG-37A	2864.67	Moderate
10	TPG-41	5301.61	High
11	ICGV-89280	3068.16	Moderate
12	ICGV-86564	2738.58	Moderate
13	ICGS-44	1653.84	Moderate
14	ICHG-00440	1474.61	Moderate
15	J-11	955.79	Low
16	JL-24	6959.99	High
17	TAG-24	1052.84	Moderate

18	TMV-7	3187.53	Moderate
19	MH-1	4641.69	Moderate
20	TMV-2	7857.42	High
S.Em.±		81.47	
C.D. at 5 %		232.85	
C.V. %		3.59	

* Average of three replication

Levels of aflatoxin content (ppb):

< 100 – Negligible 100-1000 – Low 1000-5000 – Moderate > 5000 - High

Table 4: Categorization of different groundnut varieties based on aflatoxin content (ppb)

Sr. No.	Categories	No. of varieties	Per cent varieties	Name of Varieties
1	High > 5000 ppb	7	35	GG-6, GG-12, GG-13, GG-20, TPG-41, JL-24, TMV-2
2	Moderate 1000-5000 ppb	12	60	GG-2, GG-5, GG-7, GG-11, TG-37A, ICGV-89280, ICGV-86564, ICGS-44, ICGS-00440, TAG-24, TMV-7, MH-1
3	Low 100-1000 ppb	1	5	J-11
4	Negligible < 100 ppb	0	0	---

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