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Research Note

Potential groundnut pre-breeding genotypes with resistance to *Aspergillus flavus*

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Abstract
Aflatoxin contaminated groundnut can cause serious health effects to both humans and livestock. Twenty nine pre-breeding genotypes derived from A, B and K genomes of groundnut along with their parents, susceptible, resistant checks, released cutlivars and advanced breeding lines were evaluated under field for various productivity parameters in addition to screening for resistance against aflatoxin in the artificial condition. Among the pre-breeding genotypes only two genotypes (ICGIL 17101 and ICGIL 17124) showed resistance to *A flavus* with colonization severity of 1 and very less incidence percentage (< 7%) and considered as resistant compared to known resistant check, ICG 02207 which had colonization severity of 3 with 95 % incidence. Three pre breeding genotypes, ICGIL 17107, ICGIL 17114, and ICGIL 17128 showed colonization severity of 2 and considered as moderately resistant. The late leaf spot and rust resistant released variety, GPBD 4 showed colonization severity of 3 and classified as susceptible indicating different resistant mechanism operating against different pathogens. The genotype ICGIL 17124 in addition to having resistance to A flavus, also had higher pod yield per plant and hence could be tested widely for its consistent performance before releasing as a variety.

Keywords: Groundnut, afltoxin, pre-breeding, producitivity, resistance

Groundnut is an important oilseed crop mainly cultivated for its edible seeds all over the World. The kernels are rich in protein (20-26 %) and oil content (40 to 49 %) and popularly called as poor man's almond (Jambunathan, 1991) occupying an important position in human diet (Dwivedi *et al.,* 2003). Beside the usage of its seed as boiled, roasted or in raw form, good amount of oil is extracted and used as vegetable oil. The oilcake after oil extraction will be used as concentrates for feeding livestock (Ayele, 2010). One of the problems associated with groundnut seeds is its contamination with aflatoxin due to the infection by *Aspergillus flavus* which can occur at any stage of the cropping period and even after harvest. The consumption of aflatoxin contaminated groundnut can cause serious health hazards to both human and livestock (Kumar *et al.*, 2017; Sarma *et al.*, 2017; Ezekiel *et al.,* 2019). More than five billion people who have been affected by aflatoxin worldwide who have been chronically exposed to higher (> 1000 ppb) amount of toxin (Strosnider *et al.,* 2006). Contamination due to aflatoxin is a food safety concern and also has adverse health and financial implications in groundnut growing areas all over the World. Cooking, drying, sterilization or pasteurization cannot decompose aflatoxin due to its higher decomposition temperature requirement of 237 to 306 °C (Awasthi *et al.,* 2012). The pre harvest aflatoxin contamination can be controlled by using bio control agents, good agronomic practices during the cultivation. But these have certain limitation in terms of proper time of application in addition to increasing cost of cultivation to the farmers. Under such circumstances, most effective way to manage the aflatoxin contamination is through genetic resistance at least for pre-harvest infection during the cropping period. The first and most

important step in breeding for resistance to aflatoxin contamination would be screening available germplasm. Many scientists have worked on screening of diverse groundnut genotypes thereby identifying several promising lines showing resistance or moderate resistance for aflatoxin contamination (Kisyombe *et al*., 1995; Nigam *et al.,* 1991; Anderson *et al*., 1995; Thakur *et al*., 2000; Jiang *et al.,* 2010; Dieme *et al.,* 2018; Yu *et al.,* 2020). But these results could not lead to breeding of resistant cultivars which could be due to their association with one or other undesirable features or having inconsistent expression of stable resistance over location or years. Very fewer efforts were made on screening wild *Arachis* species against aflatoxin contamination. In this regard, present investigation had been carried out to identify resistant sources to aflatoxin contamination in groundnut pre-breeding material derived from wild *Arachis* species vis-à-vis assessing their productivity potential.

The experimental material comprised of pre-breeding genotypes (29), parents (2), checks (2), released varieties (5) and advanced breeding lines (2) and these materials were generated from advanced backcross populations derived from synthetics ISATGR 121250 (*A. Kempffmercadoi* × *A. hoehnei*), ISATGR 278-18 (*A. duranensis* × *A.batizocoi*) and ISATGR 265-5 (*A. duranensis* × *A. ipaensis*) as donors. Diploid wild *Arachis* accessions having A, B, and K genomes were crossed in different combinations, followed by chromosome doubling of the diploid intra and inter-genomic F_1 hybrids using colchicine treatment to generate tetraploid synthetics. Through crossing between these allotetraploid synthetics and popular groundnut cultivars (ICGV 91114 and ICGV 87846), the introgressed lines were developed (Sharma, 2017). Genotypes were evaluated during *kharif,* 2018 at Main Agriculture Research Station, University of Agricultural Sciences, Dharwad (15° 13' N, 75° 07' E, 678 m above MSL, and 800 mm average annual rainfall). Each genotype was sown in a row of two meter length with two replications and spacing of 30 × 10 cm in Randomized Complete Block Design. Normal agronomic practices were followed to raise the crop. All plant protection measures were followed. After harvesting of the crop, the productivity parameters *viz*., number of pods per plant from five random plants, shelling percent, 100 seed weight, pod yield per plant and oil content were recorded. After harvesting of the crop, in each genotype, fifteen well matured seeds with intact seed coat and free from any damage were selected for screening against *Aspergillus* under artificial condition in the laboratory. Seeds were surface sterilized with 1 per cent sodium hypochlorite solution and subsequently washed twice with sterilized distilled water to remove the traces of sodium hypochlorite. Seeds were uniformly wounded by pricking with needle. Spore suspension was prepared. Then wounded seeds were dipped in the spore suspension. Seeds were removed from the suspension and placed in sterilized petri plates and incubated at 28 ^{0}C ±1 ^{0}C in dark for 7 days. Individual seeds were scored for surface colonization by *Aspergillus flavus* and graded by severity rating 1-4 scale (1: < 5 per cent seed surface colonized with scanty mycelia growth and scanty sporulation; 2: 5-25 per cent seed surface colonized with good mycelia growth and scanty sporulation; 3: 26-50 per cent seed surface colonized with good mycelia growth and good sporulation and 4: > 50 per cent seed surface colonized with mycelia growth and heavy sporulation) as given by Thakur *et al.* (2000).

Analysis of variance was carried out using Indostat statistical package. The genotypes were categorized as Resistant (< 5 per cent seed surface colonized with scanty mycelial growth and scanty sporulation), Moderately resistant (5-25 per cent seed surface colonized with good mycelial growth and scanty sporulation), Susceptible (26–50 per cent seed surface colonized with good mycelial growth and good sporulation) and highly susceptible (> 50 per cent seed surface colonized with heavy sporulation).

Mean sum of squares showed highly significant genotypic differences among groundnut pre-breeding genotypes for *A. flavus* severity and incidence besides productivity parameters *viz*., plant height, number of primary branches per plant, number of pods per plant, shelling per cent, hundred seed weight, pod yield per plant and oil content indicating sufficient variability in the pre-breeding material for all these traits (**Table 1**). Wide variation existed for *A. flavus* incidence (3.3-100 %) and *A. flavus* severity (1 - 4) among the pre breeding material (**Table 2**).

Table 1. Mean sum of squares for *A. flavus* severity, incidence and productivity parameters in pre-breeding material of groundnut

Source of variation	Degrees of freedom	*A. flavus* severity	*A. flavus* incidence	Plant height	Number of primary branches per plant	Number of pods per plant	Shelling per cent	Hundred seed weight	Pod yield per plant	Oil content
Replication	1	0.01	0.02	3.61	4.05	8.19	11.92	9.78	18.60	2.54
Genotypes	39	0.08**	0.09**	39.09**	6.00**	16.30**	31.74**	82.62**	71.71**	7.25**
Error	39	0.35	0.41	1.03	1.31	3.53	6.13	2.73	6.24	3.25
Total	79	0.44	0.52	43.73	11.36	28.02	49.79	95.13	97.54	3.69

** Significant at 1 % level

Table 2. Performance of pre-breeding material for resistance to *Aspergillus flavus* and productivity parameters

S. No.	Genotype	Pedigree	*A flavus*		Number of pods per plant	Shelling per cent	Hundred seed weight (g)	Pod yield per plant(g)	Oil content (%)
			Severity	Incidence percentage					
1	ICGIL 17101	ICGV 87846 × ISATGR 265-5	1	3.33	21.1	66.7	32.8	29.6	45.1
2	ICGIL 17124	ICGV 87846 × ISATGR 265-5	1	6.67	20.8	71.4	36.9	36.8	42.9
3	ICGIL 17107	ICGV 87846 × ISATGR 265-5	2	66.67	24.9	65.9	32.8	25.5	42.1
4	ICGIL 17114	ICGV 87846 × ISATGR 265-5	2	73.33	22.3	71.6	50.4	21.9	44.8
5	ICGIL 17128	ICGV 87846 × ISATGR 265-5	2	67.77	21.9	66.8	45.2	35.9	42.6
6	ICGIL 17102	ICGV 87846 × ISATGR 265-5	3	100.00	18.9	71.8	43.7	21.1	49.9
7	ICGIL 17104	ICGV 91114 × ISATGR 121250	3	95.00	16.8	72.7	41.3	25.3	44.1
8	ICGIL 17105	ICGV 87846 × ISATGR 265-5	3	90.00	21.6	70.8	42.1	22.6	44.9
9	ICGIL 17109	ICGV 87846 × ISATGR 265-5	3	95.00	22.3	70.9	41.9	23.1	44.6
10	ICGIL 17111	ICGV 87846 × ISATGR 265-5	3	100.00	18.6	64.4	44.9	35.5	46.1
11	ICGIL 17112	ICGV 91114 × ISATGR 121250	3	100.00	17.4	68.9	39.4	21.8	45.2
12	ICGIL 17113	ICGV 91114 × ISATGR 121250	3	100.00	22.0	65.3	36.9	30.7	44.6
13	ICGIL 17118	ICGV 91114 × ISATGR 121250	3	100.00	21.7	74.7	42.1	30.2	46.2
14	ICGIL 17123	ICGV 87846 × ISATGR 265-5	3	95.00	19.7	75.9	52.0	20.7	43.9
15	ICGIL 17125	ICGV 87846 × ISATGR 265-5	3	95.00	18.2	66.5	43.9	25.8	43.2
16	ICGIL 17126	ICGV 87846 × ISATGR R278-18	3	95.00	18.9	72.7	42.8	32.9	46.2
17	ICGIL 17103	ICGV 87846 × ISATGR R278-18	4	100.00	19.1	65.9	40.9	20.1	44.6
18	ICGIL 17106	ICGV 87846 × ISATGR R278-18	4	95.00	22.9	65.3	52.9	30.1	43.1
19	ICGIL 17108	ICGV 87846 × ISATGR R278-18	4	100.00	14.2	74.1	38.8	20.4	44.3
20	ICGIL 17110	ICGV 87846 × ISATGR R278-18	4	100.00	20.7	67.6	42.6	30.0	44.2
21	ICGIL 17115	ICGV 87846 × ISATGR R278-18	4	95.00	12.5	72.0	38.9	32.4	44.9
22	ICGIL 17116	ICGV 87846 × ISATGR R278-18	4	100.00	24.8	67.8	32.1	25.7	43.5
23	ICGIL 17117	ICGV 87846 × ISATGR R278-18	4	100.00	22.2	66.5	42.9	35.6	44.6
24	ICGIL 17119	ICGV 87846 × ISATGR 265-5	4	100.00	25.6	65.7	31.9	28.4	45.8
25	ICGIL 17120	ICGV 87846 × ISATGR 265-5	4	100.00	26.4	69.9	52.6	28.1	45.8
26	ICGIL 17121	ICGV 87846 × ISATGR 265-5	4	100.00	15.2	68.9	38.8	31.2	44.2
27	ICGIL 17122	ICGV 91114 × ISATGR 121250	4	100.00	25.3	69.2	43.5	33.0	44.6
28	ICGIL 17125	ICGV 91114 × ISATGR 121250	4	95.00	26.5	69.4	53.4	30.5	46.2
29	ICGIL 17129	ICGV 91114 × ISATGR 121250	4	100.00	27.0	72.8	45.7	28.2	44.8
30	ICGV 91114 (P)	ICGV 86055 × ICGV 86533	2	63.33	23.2	66.1	52.7	28.4	44.2
31	ICGV 87846 (P)	CS 9 X ICGS 5	3	100.00	21.4	71.9	42.1	19.8	48.3
32	TMV 2 (SC)	Mass selection from Gudhiatham Bunch	4	100.00	20.9	72.3	43.8	27.3	47.1
33	ICGV 86031 (A)	F 334 A-B-14 × NC Ac 2214	4	100.00	22.1	68.5	31.8	25.7	48.7
34	ICG 2271 (A)	NC Ac 343 ((NC Bunch x PI 121067)	4	100.00	20.3	68.4	34.0	22.8	48.9
35	GPBD 4 (R)	KRG 1 x ICGV 8655 (*A. hypogaea* x *A cardenasii*)	3	95.00	17.8	72.8	40.7	22.9	46.8
36	JL 24 (R)	Selection from EC 94943	3	90.00	22.2	74.7	42.3	27.4	46.6
37	Dh 256 (R)	R2001-2 × GM4-3-12	4	100.00	22.3	74.4	41.8	20.7	45.8
38	Dh 257 (R)	ICGV07211 × ICGV2381	3	90.00	23.1	73.8	41.8	20.6	47.9
39	R 9227 (R)	(ICGS 7 × NC Ac 2214) × ICGV 86031	3	100.00	17.3	65.9	33.1	11.3	45.8
40	ICG 02207 (C)	(F– MIX (X) ICG (FDRS) -20 -1– 45)	3	95.00	17.4	71.8	32.4	12.2	43.8
		Mean	3.30	85.52	20.9	69.8	41.5	26.3	45.3
		CD (5 %)	0.219	1.35	1.5	6.9	2.3	6.6	3.2
		CV (%)	3.42	4.06	4.8	5.1	3.0	14.4	4.1

Note: SC: Susceptible Check; RC: Resistant Check; R: Released variety P: Parent; A: Advanced breeding line

Among the 40 genotypes studied, only two pre-breeding genotypes (ICGIL 17101 and ICGIL 17124) showed colonization severity of 1 with very less incidence percentage (< 7%) and considered as resistant (**Plate 1**) as compared to known resistant check, ICG 02207 which had colonization severity of 3 with 95 % incidence (**Table 2**). Deepa and Kenchanagoudar (2016) reported high level of resistance in ICGV 02207 against *A. flavus* which had shown susceptible reaction in the present study. It indicated that the varied level of seed colonization severity influenced by growing seasons. The genotypes, ICGIL 17101 and ICGIL 17124 have ISATGR 278-18 in its pedigree (**Table 2**) which in turn was derived from 'A' genome (*A. duranensis*) and 'K' genome (*A. batizocoi*) diploid wild species. They were reported as resistant to *Aspergillu flavus* infection (Nigam *et al.,* 1991; Xue *et al*., 2004). Earlier, ICGIL 17101 was also reported as resistant to *Spodoptera litura*, a leaf eating insect in groundnut (Donge and Naidu, 2021) indicating its resistance to multiple pests. Three pre breeding genotypes, ICGIL 17107, ICGIL 17114, and ICGIL 17128 showed colonization severity of 2 and considered as moderately resistant (**Table 3**). The late leaf spot and rust resistant released variety, GPBD 4 showed colonization severity of 3 and classified as susceptible implying that different resistant mechanism could be operating against different pests. Earlier, Ranganathswamy (2014) reported susceptible nature of GPBD 4 and TMV-2 and resistant nature of ICGV-02266 against *A. flavus*. A total of seventeen genotypes each with colonization severity of 3 were categorized as susceptible. A set of another seventeen genotypes showed colonization severity of 4 and categorized as highly susceptible (**Table 3**). Among parents, ICGV 91114 showed colonization severity of 2 with 63.33 per cent incidence and categorized as moderately resistant. ICGV 87846 showed colonization severity of 3 with 100 per cent incidence and categorized as susceptible. Resistant check, ICG 02207 showed colonization severity of 3 with 95 per cent incidence was found to be susceptible. Susceptible checks *viz.,* JL 24 and TMV 2 showed colonization severity of 3 and 4 and categorized as susceptible and highly susceptible, respectively.

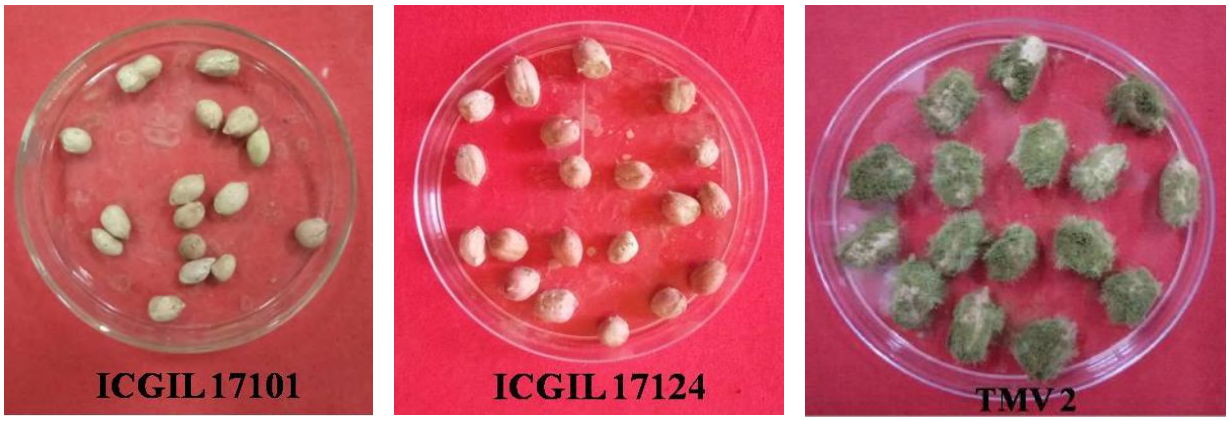

Plate 1. Differential response of pre-breeding groundnut genotypes to *Aspergillus flavus*

Table 3. Grouping of genotypes into resistant, moderately resistant, susceptible and highly susceptible categories based on reaction to *Aspergillus flavus*

Scale	Category & number of genotypes	Genotypes
1	Resistant (2)	ICGIL 17101 and ICGIL 17124
2	Moderately resistant (4)	ICGIL 17107, ICGIL 17114, ICGIL 17128 and ICGV 91114
3	Susceptible (17)	ICGIL 17102, ICGIL 17104, ICGIL 17105, ICGIL 17109, ICGIL 17111, ICGIL 17112, ICGIL 17113, ICGIL 17118, ICGIL 17123, ICGIL 17126, ICGIL 17127, ICGV 87846, GPBD4, JL 24, Dh 257, R 9227 and ICG 02207
4	Highly susceptible (17)	ICGIL 17103, ICGIL 17106, ICGIL 17108, ICGIL 17110, ICGIL 17115, ICGIL 17116, ICGIL 17117, ICGIL 17119, ICGIL 17120, ICGIL 17121, ICGIL 17122, ICGIL 17125, ICGIL 17129, TMV 2, ICGV 86031, ICG2271 and Dh 256

Among the two resistant genotypes identified, ICGIL 17124 was also recorded higher pod yield per plant (36.8 g) with higher shelling per cent (71.4 %) but medium sized pods (36.9 g HSW) and 42.9 % oil content. Among the moderately resistant pre-breeding lines, ICGIL 17128 had higher pod yield per plant (35.9 g) with 42.6 % oil content and 45.2 g of hundred seed weight but lower shelling per cent (66.8 %). Previously reported resistant check, ICGV 02207 had very low pod yield per plant (12.2 g).

The above results suggested that, ICGIL 17124 with resistance to *Aspergillus flavus* both in terms of colonization severity and percentage incidence in addition to having higher pod yield need to be tested over locations and seasons for its resistance and consistent and stable yielding ability besides assessing for the amount of aflatoxin content. The genotype ICGIL 17101 with highest resistance could be employed in aflatoxin resistance breeding program.

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