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SEED COAT RESISTANCE OF GROUDNUT TO *Aspergillus flavus* AND THEIR STABILITY PERFORMANCE IN THE FIELD

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ABSTRACT

One of the weaknesses of the groundnut is the easiness to be infected by fungi, especially *Aspergillus flavus* that produces aflatoxin. Seed from the field experiments for all step of selection (F5 until to multilocation yield test) after processed then stored for 3 and 6 months, further tested their respons to *A. flavus* using a standard method. Examined for seed coat resistance to colonization of *A. flavus* were carried out in laboratory ILETRI (Indonesian of Legume and Tuber Crops Research Institute) since years of 2002 to 2006. Stability performance of resistance to *A. flavus* was analyzed with regression technique. Performance of resistance to *A. flavus* of selected lines tested were not consistant among 16 of testing envoronments. Among genotypes were also sigficantly different response to *A. flavus* invasion from location to location, indicated that those performance of some lines were not stable, except line of MHS/91278-99-C-180-5. The highest pod yield was occupied by line of J/91283-99-C-90-8 and stable, however it's resistance to *A. flavus* did not stable. The resistance of J/91283-99-C-90-8 to *A. flavus* antil to three months after after seed strored similar with variety of J-11.

Keywords: groundnut, *Aspergillus flavus,* aflatoxin

INTRODUCTION

Groundnut as healthy food, in the reality have weakness so that its benefit become less optimal otherwise handled well. One of the weakness of groundnut is easy to infected by toxigenic fungi, especially *Aspergillus flavus* who produce aflatoxin. Aflatoxin was a carcinogenic substances in foods and in animal feeds. Groundnut meal contaminated with aflatoxin could developed liver cancer (Swindle, 1994).

Studies revelead that the test groundnut crop was infected by aflatoxin producing fungi while it still in the field. Thus the source of aflatoxin contamination may be in the field. Mixon and Rogers (1975) first suggested that use of groundnut varieties resistant to seed invasion and colonization by *A. flavus* could be an affective means of preventing aflatoxin contamination. The existence of seed coat resistance was a logical assumption, considering that seeds with damage testa more easily and rapidly invaded by fungus than that were seeds with intact testa, and colored testa conferred greater resistance to invasion by *A. flavus* than white or variegated testa (Carter 1973 *in* Mehan, 1994). The mean seed colonization levels in the resistant genotype by *A. flavus* tested overall several years ranged from 8-13% (Mixon 1986 *in* Mehan 1994). Sanders *et al.* (1993) reported that high *A. flavus* invasion percentages may be found without the presence of aflatoxin, suggesting that invasion and subsequent growth and aflatoxin contamination in groundnut may be separate process or at least regulated in different ways. Furher, invasion and aflatoxin contamination groundnut grown under drought conditions usually occur first and to a greater degree in small, immature pods. At least two kinds on resistance have been discovered in groundnut, ie. resistance to *A. flavus* invasion and resistance to aflatoxin production even though invasion occur (Rao *et al.,* 1994). Cotty (1988 *in* Daren, 1992), and Daren (1992) sugesting used TF (Tube Fluorescence) methods for large-scale screening of groundnut genotypes for resistance to aflatoxin production due to a simple, rapid and inexpensive. Waliyar

e*t al.* (1994) found the positive correlation between resistance in field and resistance in laboratory inoculation test and suggested that either methods could be used in evaluating groundnut cultivars or breeding lines for seed coat resistance to *A. flavus* infection by genotypes 15% or fewer seeds colonization were regarded as resistant.

This paper describes some studied of evaluating groundnut lines of F5-F8 derivated from population of crossed between seed coat resistance parents to *A. flavus* wich were introduced from ICRISAT in 1998 *(*J 11, ICGV 91278, ICGV 91279, ICGV 91283, ICGV 91284 and ICGV 91315) with Mahesa and Jerapah varieties, and their performance their stabilitilty performance in the field of yied and seed coat resistace to. *A. flavus*.

Groundnut varieties of Mahesa and Jerapah were commercial variety of excelent agronomic characters, moderately tolerant to leaf diseases and tolerant to drought stressed at reproductive phase, respectively.

MATERIALS AND METHODS

Line selection for seed coat resistance to colonization by *A. flavus* in laboratory come from groundnut population of F5 derivated from crossing combination of two commercial parents (Jerapah and Mahesa) varieties with five genotypes as donors for seed-coat resistance (J11, ICGV 91278, ICGV 91279, ICGV 91283, ICGV 91284 and ICGV 91315) in 1999. Variety of J 11 was used extensively as gene donors for seed-coat resistance, and their stability of their resistance to seed colonization by *A. flavus*. J11 and other genotypes were introduced from ICRISAT in 1988. Hybridization and lines development were done in Malang, Jambegede and Pasuruan from years of 2002-2003. Selected lines were tested of their performance for yield and seed coat resistance to *A. flavus* at various sites (East Java: Pasuruan, Lamongan, Lumajang, Blitar; Central Java: Wonogiri, Blora, Tayu, Pati; Yogyakarta: Sleman, Bantul, and Lampung: Punggur, Central Lampung) from years of 2004 to 2006 (Table 7).

Culture practice in the field for various generation or researches were : optimal of soil tillage, groundnut seed grown at plant spacing 40 cm x 10 cm, 1 seed/hole, basal fertilization with 50 kg of Urea, 100 kg of Super phosphate, and 100 kg of KCl per hectare, respectively; two times weeding at 14 dan 21 days after sowing (DAS), leaf diseases controll at 7, 9 weeks after sowing (WAS), and harvesting at optimum maturity (95-105) DAS.

After groundnut harvested for all of the breeding steps then pods were handpicked in the field and as soon as possible naturally dried by the sun. After drying, the dried pods were stored in well-ventilated room for 3-6 months with protection from insects. From the stored, intact seeds of each lines 25 seeds samples were taken, placed in clean beaker and covered with a 0.5% aqueous solution of sodium hypochlorite. Seed were soaked for 3 minutes, the exess solution drained of, and seeds rinsed in two changes of sterile distilled water. The water was then drained off and the seeds hydrated to about 20% moisture containt by soaking them for 10-15 minutes in sterile distilled water. Seeds placed in a sterile in semi rigid plastick boxes then inoculated with 1 mL of spore suspension of the toxigenic *A. flavus* isolate of Pasuruan (approximately 4 x 10^6 conidia m $L^{-1)}$. The spore suspension was prepared from 8-10 days-old culture. Plastick boxes were then placed in a AC room with temperature at 25^o C. After 8 days, the seeds were visually examed for invasion and colonization by *A. flavus by* recording. All the activities were carried out in the laboratory of ILETRI (Indonesian of legume and Tuber crops Research Institute) since years of 2002 to 2006.

The percentage of seeds invaded and colonized as shown by the presence of sporulating surface growth were calculated using method as suggested by Mehan (1989 in Mehan, 1994) seed sporulating (0%,) half seed sporulated (50%), seed fully sporulated (100%).

Stability performance of seed coat resistance using the regression appoarch of Eberhart and Russell (1966). The regression coefficient (b) for resistance plotted against the mean percentage seed colonization. In other hand the regression coefficient (b) for yield were plotted against the environmetal index. Performance of colonization of *A. flavus, or pod yield were* considered stable when the coefficient of regression and deviation from regression were equal than unity and zero, respectively.

RESULTS AND DISCUSSION

Lines Selection to *A. flavus* Resistance in the Laborarory

Line selection of groundnut for seed coat resistance was started at F5 generations, there were selected a number of families, namely 88 families of eight crosses combination (Table 1).

Value of skewness were positive for all of cross combinations, its mean that population had a right skewed distribution, showing mode<median<mean for seed colonization of *A. flavus*. So, mostly line were susceptible (Wannacott and Wannacott, 1972). 0f these selected families produced 140 lines and tested for their yield in preliminary yield test. There were selected 80 lines which have seed coat resistace to *A. flavus* and selected for advanced yield test. Total of 17 lines were selected for main diseases and excelent agronomic-characters and yield using variety Jerapah as the check.

Groundnut genotypes tend to loss their resistance to *A. flavus* after six months storaged included J11, exception for lines of MHS/91283-99-C-168-16, ICGV 91278, ICGV 9315 and Jerapah. The promissing line of J/91283-99-C-90-8 showed resistance to *A. flavus* until three months storaged, as well as the control variety of J11 (Table 2). These line were tested for their yield stability and resistance to *A. flavus* across environments (Table 3).

Table 1. *A. flavus* seed colonization (%) of F5 families. Laroratory of ILETRI (Indonesian of legume and Tuber crops Research Institute) Malang, 2002

No	Combination of crossing	No. of families tested	Seed colonization of *A. flavus* (%)		Skew.	No.of selected families	Reaction			
			Range	Mean			R	MR	S	HS
1	Jerapah/J-11	42	0-24.0	3.5	2.4**	13	3	9	0	1
2	Jerapah/ICGV 91279	31	2-31.3	9.8	1.3**	5	0	5	0	0
3	Jerapah/ICGV 91279	13	0-31.3	9.0	1.2	5	3	2	0	0
4	Mahesa/ICGV 91315	59	0-24.9	9.6	0.6	10	2	5	3	0
5	Mahesa/J-11	38	0-22.6	3.5	2.7**	18	4	8	5	1
6	Mahesa/ICGV 91279	53	0-25.0	4.8	2.2**	10	3	6	0	1
7	Mahesa/ICGV 91283	35	0-7.0	1.0	2.5**	8	6	2	0	0
8	J/ICGV 91283	33	0-11,0	4.0	0.3	19	8	7	3	1
	Total	160				88	29	44	11	4
	Controls[a)]									
	Jerapah			2.2						
	Mahesa			5.6						
	J 11[b)]			1.3						
	ICGV 91279			8.3						
	ICGV 91315			1.2						

Remarks = a) Check of commercial varieties ; b) Resistant to *A. flavus* (control variety) R = Resistant; MT= Moderately reisistant S = susceptible HS = Highly susceptible; * significant at P 0,05; ** highly significant at P 0.01.

Table 2. Colonization of *A. flavus* of some lines of groundnut selected from F5–F7 followed with pod yield at various pod storage*)

Lines	Colonization of *A. flavus* (%)				Yield (t/ha) dry pod		
	Pasuruan DS 2002 F5	Pasuruan WS 02/03 F6	J.Gede DS 2003 F7	Pasuruan DS 2003 F7	Pasuruan	J. Gede	Average
MHS/91315-99-C-140-1	3.3	9.1	4.75	62.3	1.60	1.80	1.70
J/91278-99-C-120-4	0.0	2.8	5.25	23.3	1.90	2.80	2.35
J/91283-99-C-90-8	0.6	-	0.75	59.3	1.40	1.60	1.50
MHS/91278-99-C-152-8	1.0	7.8	0.60	21.7	1.70	1.90	1.80
MHS/91283-99-C-164-12	0.0	18.1	8.75	71.3	1.60	2.10	1.85
MHS/91283-99-C-168-16	0.0	16.3	11.75	**7.00**	1.90	1.70	1.80
MHS/91278-99-C-174-6	0.0	3.4	8,75	11.7	1.70	1.80	1.75
MHS/91278-99-C-174-7	0.0	6.9	7.0l	64.0	1.80	2.60	2.20
MHS/91315-99-C-127-8	11.3	32.2	5.00	56.0	1.30	1.80	1.55
MHS/91315-99-C-131-8	0.0	0.0	1.50	50.7	1.50	1.70	1.60
MHS/91278-99-C-173-6	0.0	21.3	4.25	50.0	1.90	1.70	1.80
Jerapah	1.3	33.1	10.75	6.70	2.10	2.70	2.40
J-11	1.3	10.0	2.00	33.7	0,60	2.50	1.95
ICGV 91279	8.3	17.8	6.75	22.3	1,90	1.50	1.70
ICGV 91278	-	35.3	2.00	16.7	1,50	2.00	1.75
Mahesa	5.6	27.2	5.75	72.7	2.20	2.20	2.20
ICGV 91315	1.2	1.9	2.75	10.0	1.50	1.30	1.40
Average	3.6	21.5	4.5	30.2			
Time stored (month)	3	3	3	6			

Remarks= *) WS = weet season; DS = dry season

Stability Performance of Colonozation to *A. flavus* in the Field

Total of 20 genotypes od groundnut were tested at 16 locations for their stability performance to *A. flavus* and yield in the field. The combined variance analyses of seed colonization of *A*. *flavus* showed that among lines were different significantly, and Vx E (linear) interaction was also significant indicated that among lines have different coefficient of regression. Deviation from regression mainly were significant, except for J/911227-99-C-120-4 and MHS/91278-99-C-180-5 (Table 4). Lines of MHS/91278-99-C-180-5, MHS/91278-99-C-180-13, J/91283-99-C-196-1**,** J/91283-99-C-200-8, J/91283-99-C-90-8, J/91283-99-C-87-5, ICGV 91315 and Jerapah have coefficient of regression (bi) were similar with unity, but not stable because deviation from regression were different significant than zero, in exception of MHS/91278-99-C-180-5 was stable (Table 4 and 5). The regression coefficient for seed colonization of it's variety on the colonization index which measure the response of this variety to varying environments). The seed colonization index which defined as the deviation on the deviation of the mean seed colonization of all the varieties at a given location/environment from the overall mean of colonization).

The seed colonization for J11 tend to higher in location that favorable for *A. flavus* due to drought stressed and lodging at harvesting time (Table 7). Deviation from regression of lines MHS/91278-99-C-180-5 and J/911227-99-C-120-4 were negative it's mean very small, but MHS/91278-99-C-180-5 smaler than that of J/911227-99-C-120-4. so that coefficient of regression of MHS/91278-99-C-180-5 bigger and similar with unity than that of J/911227-99-C-120-4. According to Subandi (1992), deviation from regression was mostly important among parameters of stability.

Table 3. Selected lines of F8 who resistant to rust, leaf spot, wild diseases and *A. flavus* followed by pod yield. Pasuruan (Psrn) and Jambegede (Jbgd), growing season of 2003-2004*)

No	Galur	LS 75 das	Rust 75 das	% of wilt	Yield Psrn (t/ha)	Yield Jbgd (t/ha)	Mean of pod yield (t/ha)	*A. flavus (%)*
1	MHS/91315-99-C-140-1 s	6.5	5.5	14.0	1.60	1.80	1.70	16.00
2	J/91278-99-C-120-4 s	6.0	4.5	17.8	1.90	2.80	2.35	7.00
3	MHS/91278-99-C-174-6 s	6.0	4.5	28.9	1.70	1.80	1.75	0.80
4	MHS/91278-99-C-180-5 s	5.5	5.0	16.6	1.50	2.10	1.80	0.75
5	MHS/91278-99-C-180-13 s	5.5	5.0	40.6	1.60	2.10	1.85	2.10
6	J/91283-99-C-192-17 s	6.0	5.0	18.1	1.80	2.70	2.25	3.65
7	J/91283-99-C-194-10 s	6.0	4.0	20.7	2.00	2.30	2.15	2.25
8	J/91283-99-C-195-2 s	6.0	5.0	13.7	2.00	1.60	1.80	2.90
9	J/91283-99-C-196-1 s	6.0	5.5	17.5	2.10	2.50	2.30	4.75
10	J/91283-99-C-196-7 s	6.0	5.0	13.8	2.40	1.70	2.05	1.85
11	J/91283-99-C-197-13 s	6.0	4.0	13.5	1.90	1.50	1.70	4.05
13	MHS/91278-99-C-180-6 s	6.0	5.0	18.5	2.00	2.00	2.00	4.70
14	J/91283-99-C-195-5 ss	6.0	5.5	11.6	1.70	2.30	2.00	0.55
15	J/91283-99-C-200-8 ss	6.0	5.0	21.0	2.10	2.20	2.15	4.00
16	J/91283-99-C-90-8 s	6.0	5.0	13.3	1.90	2.40	2.15	1.35
17	J/91283-99-C-87-5 s	6.0	5.0	12.4	2.60	2.20	2.40	3.95
18	Jerapah	6.5	4.5	16.2	2.10	2.70	2.40	3.10
19	J-11	6.0	6.0	51.6	1.40	2.50	1.95	0.60
20	ICGV 91279	6.0	5.5	16.0	1.90	1.50	1.70	1.00
21	ICGV 91278	6.0	5.5	43.0	1.50	2.00	1.75	3.35
22	Mahesa	6.0	4.5	18.7	2.20	2.20	2.20	6.35
23	ICGV 91315	6.0	6.0	91.2	1.50	1.30	1.40	7.95

Remarks = *) Jbgd= Jambegede; Psrn = Pasuruan s = selected; LS = leaf spot; das = days after sowing

The regression coefficient for pod yield plotted against environmental index (the deviation of the mean of all the varieties at a given location/environment from the overall mean) indicated that some breeding lines were also responsive to the environment (Table 6). Genotypes used as parents in this breeding were reported as seed coat resistance. The genotype, mainly J-11 have been used extensively as gene donors for seed-coat resistance (Rao *et. al*., 1994). Breeding lines were tested in multilocational trials to evaluate the stability of their resistance. Identification of stable resistance because past finding have indicated that environment factors could influence seed coat resistance (Mehan *et al.,* 1983 *in* Mehan, 1994, Rao *et al.,* 1994). Several factors such as low testa permeability, increased surface wax accumulation, uniform wax coating, thin testa with compact and tight cell structure, campact palosase like layer, small hilum, presense of tannins and inhibitory compound, and differences in amino acid composition have been reported to contribute towards *A. flavus* resistance. No effort have been made to breed these traits, because information on the contribution of these mechanisms to resistance traits is not fully available and may be highly influenced by environment variations (Pettit *et. al.,*1994; Rao *et al.,* 1994).

Table 4. Analisis of varianceof stability for seed colonization by *A. flavus*

Source of Variation	Degree of Freedom	Sum Square	Mean Square	F test
Total	319	66295.64		
Varietas	19	5557.70	292.51	3.19**
E + (VxE)	300	60737.94	202.45	2.21**
E liniar	16	31912.53	1994.53	
V x E liniar	19	4060,67	213,72	2.33**
Pooled deviation	280	25596,30	91,42	
MHS/91315-99-C-140-1	14	1082.76	77.34	3.91**
J/911227-99-C-120-4	14	221.05	15.79	0.79
MHS/91278-99-C-174-6	14	1531.12	109.37	5.52**
ICGV 91227	14	883.29	63.09	3.20**
MHS/91278-99-C-180-5	14	271.84	19.42	0.98
MHS/91278-99-C-180-13	14	1312.69	93.76	4.74**
J/ ICGV 9128399-C-192-17	14	1070.22	76.44	3.86**
J/91283-99-C-194-10	14	3086.56	220.47	11.14**
J/91283-99-C-195-2	14	2141.31	152.95	7.73**
J/91283-99-C-196-1	14	1954.27	139.59	7,05**
J/91283-99-C-196-7	14	425.41	30.39	1.53*
J/91283-99-C-197-13	14	1181.94	84.42	4.26**
MHS/91278-99-C-180-6	14	986.19	70.44	3.56**
J/91283-99-C-195-5	14	1640.96	117.21	5.92**
J/91283-99-C-200-8	14	1539.41	109.96	5.55**
J/91283-99-C-90-8	14	1857.68	132.69	6.78**
J/91283-99-C-87-5	14	1184.20	84.59	4.27**
J-11	14	739.19	52.80	2.66**
ICGV 91315	14	1551.03	110.79	5,60**
Jerapah	14	935.19	66.80	3.37**
Pooled error	304	39.55	19.78	

The importance of the stability of seed coat resistance has been stressed by many previous workers. Rao *et. al.* (1994) reported that the regression coefficient (bi) for resistance plotted against the mean pecentage seed colonization indicated that the selected resistant breeding lines were stable as the resistant source lines and had similar levels of seed colonization to the resistance source lines. Drought stress was one the environment factor who affect significantly on seed colonization by *A. flavus.* The association of high aflatoxin contamination and drought stress was reported by previous researchers.

Extensive studies have been conducted to define the environmental condition associated with pre harvest and aflatoxin contamination on groundnut. Cole *et al.* (1994), indicated that *A.flavus* **i**nvasion and aflatoxin production were separate event and suggested that some inherent mechanism prevent aflatoxin formation broke down under stress in response to increase growth of the fungus after invasion. It is possible that such a resistance mechanism operates, in fact, at the level of fungus invasion and those indirectly regulates aflatoxin production. That along fact that an increase in the percent of kernels colonized under stress condition. Less stress that optimum soil temperatures coupled with water stress for longer period of time (>50 days) at the end of the growing season may results in *A.flavus* invasion and aflatoxin production in pre harvest groundnuts (Sanders *et al.*,1993). *A.flavus* invasion and aflatoxin production in preharvest groundnuts, determined by duration of end-of season water stess and

when mean soil temperatures were in the optimum range (28-30.5^{o} C) (Sanders e*t al.,* 1993). Wilson and Stansell (1983), reported that in 2 of 4 years found significantly more aflatoxin in groundnuts when stress was imposed at least 40 days immediately preceeding harvest. There were relationship between water stresses and soil temperatures to *A.flavus* invasion and aflatoxin production.(Sanders *et. al.,* 1993).

Water stress during the last 40 to 75 days of the season contributed to aflatoxin contamination of sound mature kernels three of the four years on one and on both cultivars. Because of years to years variation, drought stress alone does not consistently effect field aflatoxin contamination. In some years other environmental factors must have interacted with drought stress to promote on inhibite preharvest aflatoxin contamination. In all treatments where irrigation was applied during the last 40 days of the season, no significant aflatoxin contamination was detected in any cultivar any years of the test (Wilson and Stansell, 1983). Pettit e*t al.* (1989 *in* Pettit *et al*., 1994) demonstrated that groundnuts grown under dry land conditions, where drought stress occurred, contained more aflatoxin before digging that groundnuts under irrigation.

Table 5. Stability analyses for seed colonization of *A.flavus*

No.	Genotype	bi	S^2di
1	MHS/91315-99-C-140-1	1.33**	57.56473**
2	J/911227-99-C-120-4	0.59**	-3.98601
3	MHS/91278-99-C-174-6	0.98	89.59084**
4	ICGV 91227	1.32**	43.31699**
5	**MHS/91278-99-C-180-5**	**0.83**	**-0.3579**
6	MHS/91278-99-C-180-13	0.96	73.9886**
7	J/ ICGV 9128399-C-192-17	0.64**	56.66912**
8	J/91283-99-C-194-10	1.52**	200.6937**
9	J/91283-99-C-195-2	1.35*	133.1758**
10	J/91283-99-C-196-1	1.26	119.8155**
11	J/91283-99-C-196-7	0.75**	10.61115**
12	J/91283-99-C-197-13	0.35**	64.64951**
13	MHS/91278-99-C-180-6	1.52**	50.66692**
14	J/91283-99-C-195-5	0.32**	97.43624**
15	J/91283-99-C-200-8	0.91	90.18275**
16	**J/91283-99-C-90-8**	**0.97**	112.9167**
17	J/91283-99-C-87-5	1.12	64.81061**
18	**J-11**	**1.40****	33.02437**
19	**ICGV 91315**	**1.05**	91.01259**
20	**Jerapah**	**0.89**	47.02453**

Remarks= **) Signicant than unity and zero, respectivelly for bi and $S^2di = (ó^2 - b_l \sum Y_{ij} l_j) S^2di$ (negative, meaning very small)

Tabel 6. Stability parameters of pod yield of groundnut at 16 locations in years of 2004-2006*)

Genotypes	Stability parameters			
	Yi (t/ha)	R	bi	S^2_{di}
1. MHS/91315-99-C-140-1	1.17 ± 0.04	0.85	1.11 ± 0.12*	0.019
2. J/911227-99-C-120-4	1.19 ± 0.05	0.75	0.99 ± 0.14	0.036
3. MHS/91278-99-C-174-6	1.21 ± 0.07	0.72	1.04 ± 0.17	0.054*
4. ICGV 91227	1.22 ± 0.02	0.88	1.00 ± 0.09s	-0.072
5. MHS/91278-99-C-180-5	1.18 ± 0.06	0.78	1.10 ± 0.15	-0.003
6. MHS/91278-99-C-180-13	1.23 ± 0.01	0.90	1.01 ± 0.08	-0.079
7. J/91283-99-C-192-17	1.17 ± 0.02	0.91	1.00 ± 0.10	-0.069
8. J/91283-99-C-194-10	0.18 ± 0.03	0.88	1.02± 0.11	-0.062
9. J/91283-99-C-195-2	1.17 ± 0.02	0.85	1.06 ± 0.10	0.007
10. J/91283-99-C-196-1	1.10 ± 0.02	0.88	0.79 ± 0.09*	0.0005
11. J/91283-99-C-196-7	1.21 ± 0.01	0.84	0.97 ± 0.07s	-0.005
12. J/91283-99-C-197-13	1.15 ± 0.06	0.91	0.88 ± 0.16	0.048*
13. MHS/91278-99-C-180-6	1.22 ± 0.03	0.67	0.89 ± 0.11	0.011
14. J/91283-99-C-195-5	1.08 ± 0.03	0.82	0.99 ± 0.10s	0.006
15. J/91283-99-C-200-8	1.18± 0.03	0.86	0.98± 0.08s	-0.002
16. J/91283-99-C-90-8	1.35 ± 0.03	0.82	1.09 ± 0.13	0.019
17. J/91283-99-C-87-5	1.34 ± 0.02	0.86	1.04 ± 0.14	0.027
18. J-11	1.03 ± 0.05	0.90	1.12 ± 0.10*	0.007
19. CGV 91315	1.04 ± 0.04	0.82	0.85± 0.13*	0.028
20. Jerapah	1.31 ± 0.03	0.89	0.96 ± 0.12	0.017
Overall Mean	1.18	0.80		

Remarks= *) bi different from unity : S^2_{di} = different from 0

Table 7. Description of location for yield and tolrence performance to *A. flavus* testing of groundnut in 2004-2006

No	Locations	Elevation	Soil type/ climate	Growing season	Problems
1	Pasuruan, East Java	LLE	Alfisol/D3	WS 04	Weed
2	Wonogiri, Central Java	LLE	Entisol/D3	WS 04	Flooded at harvesting time
3	Lamongan, East Java	LLE	Alfisol/D3	DS, 04	Drought and leaf diseases
4	Blora, Central Java	LLE	Alfisol/D3	WS, 04/05	Flooded at harvesting time
5	Tuban, East Java	LLE	Alfisol/D3	DS, 04	Nutrients disorder
6	Lumajang, East Java	LLE	Alfisol/D3	WS' 04	Viruse
7	Tayu, Pati, Jateng	LLE	Alfisol/D3	WS'04	Flooded at harvesting time, wilt disease
8	Blitar, East Java	LLE	Alfisol/D2	EDS ,05	Drought and rust disease
9	Nglegok, Blitar, East Java	LLE	Entisol/D2	DS, 06	Leaf diseases, drought
11	Tuban, East Java	LLE	Alfisol/D3	LDS, 05	Dought at reproductive stages
12	Lamongan, East Java	LLE	Alfisol/D3	LDS, 05	Dought at reproductive stages
13	Sleman, Yogyakarta	LLE	Alfisol/C3	EDS, 05	Weed and rust
14	Bantul, Yogyakarta	LLE	Alfisol/C3	LDS, 05	Flooded at harvesting time
15	Punggur, Central Lampung	LLE	Entisol/B1	LDS, 05	Acid soil, rust and drought
16	Pasuruan, East Java	LLE	Alfisol/D3	DS, 06	Weed, drought, rust

Remarks = LLE = low elevation, EDS = early dry season; LDS = late dry season

Sanders *et al*. (1993) reported that high *A.flavus* invasion percentage may be not without the presence of aflatoxin, suggesting that invasion and aflatoxin contamination may be separate processes or at least regulated in different ways. In fact, invasion of *A. flavus* and aflatoxin contamination occured when groundnut grown under drought conditions. Mainly accured on first and to a greater degree in small immature pods (Sanders *et al.,* 1993). Dorner e*t al.* (1989) demonstrated an association between timing of in vitro loss of the capacity of seed to produce phytoalexins was a compound responsible for fungal resistance mechanisme in storaged groundnuts. Seed water activity appeared to be the most important factor controlling the seed capacity of seed to produce phytoalexins.

Moisture contents of seed within a maturity seed from drought stress conditions were not uniform and some seed within a maturity seed possilibly become contaminated before other. Moisture and temperature thus appeared to serve as the mechanism causing moisture loss from seed associated with pre-harvest aflatoxin contaminations (Dorner *et al.* (1989). Hill *et al.* (1983) reported that aflatoxin contamination in damage category groundnuts from irrigated plot when adjacent plot were in drought stress condition and high insect infestation.

The positive correlation between resistance to natural infection in the field and resistance in laboratory inoculation test indicates that either method could be used in evaluating groundnut cultivars and breeding lines resistance to infection by *A.flavus* (Waliyar *et al.,* 1994**;** Zambettakis *et al.,* 1981). No correlation between aflatoxin containt, colonization of seed or shells and population densities of *A. flavus* in soil (Will *et al.,* 1994). The nature and degree of invasion of *A. flavus* were dependent on the soil environment during growth and development of the groundnut pod, and *A. flavus* invade groundnut pod and produce afatoxin before plant harvested, during post harvest handling, drying ang storage. Pre harvest aflatoxin contamination has been associated with severe late season drought stress and with insect damage (Cole *et al.,* 1994).

Screening for *A. flavus* seed infection for drought resistance was particularly useful as drought stress strongly influences seed infection by *A. flavus.* Resistance to *A. flavus* seed infection may used as an index of possible resistance to aflatoxin contamination, but not all strain have a similar aflatoxin-producing ability (Mehan, 1994). No correlation between fungal growth with aflatoxin production. Genotypes resistant to seed colonization by aflatoxigenic fungi were good substrates for aflatoxin production. J11 was resistant to seed colonization support high level of aflatoxin B1 production, on the contrary VRR 245 is susceptible to seed colonies support only low level of aflatoxin B1 production. The percentage of seeds with colonies of *A. flavus* observed of their surfaces, indicating that the shell acts as a barrier to fungal infection of seed. Internal infections of seed with *A. flavus* may be present without visible external growth of fungus. Genotypes with 15% or fewer seeds colonized were regarder as resistant Resistance to *A. flavus* infection was also important in order to maintain seed quality as the fungus also causes seed rots and aflaroot seedling disease. Cultivars with resistance to *A. flavus* invasion are also likely have resistance to seed invasion by other soilborne pathogens that reduce quality and cause seed and seedling disease (Mehan, 1994). Aflatoxin originates mainly from the soil and not from the air via floral invasion (Cole *et al.,* 1994).

CONCLUSIONS AND SUGGESTION

Lines tested were sigficantly different response to *A. flavus* invasion from location to location, and one line could be identified was stable, namely MHS/91278-99-C-180-5. Yield of MHS/91278-99-C-180-5 as hight as overall mean, but lower than control variety of Jerapah. The highest was accupied by J/91283-99-C-90-8 and stable, but the performances of colonization to *A. flavus* across environments did not stable. The seed coat resistance to *A. flavus* of J/91283-99-C-90-8 line was to three months storaged of pod and similar with chek variety of J-11. The good level resistance in the commercial variety of Jerapah and line of J/91283-99-C-90-8 could be useful in minimazing aflatoxin contamination, especially for human consumtion.

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