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Breeding for aflatoxin resistance in groundnut: A review

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Abstract
Aflatoxin contamination is the most important disease of groundnut affecting human health and international trading. Easiest method to control the aflatoxin is to develop resistant cultivar. Development of improved varieties resistance to AFs is difficult due to unavailability of high level of resistance lines, unavailability of proper screening technique, the genetics of resistance against AFs not properly understood, no correlation between various resistance types, high G x E interaction etc. Indirect selection through various trait like drought tolerance, phenol content etc. can use for selection of AFs resistance cultivar because they have correlate with it. Non-conventional methods (like transgenic method, molecular markers, protein markers for screening etc.) can help to understand resistance mechanism and development of resistant cultivar.

Keywords: Groundnut, aflatoxin, resistance, cultivar

Introduction
The groundnut (*Arachis hypogaea* L.) is one of the commercially most important oilseed crop of *Fabaceae* family having chromosome number 2n=4x=40. It is believed to be originated from South America and Brazil and currently ccultivated in about 100 countries of the world located in between 40ºN and 40ºS latitudes. Main groundnut growing countries are China, India, Brazil, South Africa, and South-East Asia. It is one of the principal economic crops of the world, ranking 13th among food crops (Anonymous (2012) [1]. It is also first ranking oilseed crop of India. Groundnut is highly nutritious and used as a edible oil, consumed as raw seed or roasted and several other ways.

Quality of Groundnut and its products are adversely affected by fungal and viral disease. Among that aflatoxin is most important contaminant which is produced through the infection of fungi of *Aspergillus* group. The problem of aflatoxin contamination in groundnutnut was first recognized in 1960 after outbreaks of Turkey-X disease in the United Kingdom. Aflatoxin is the group of toxin known as G1, G2, B1, B2, M1, and M2 that produced by plant pathogen (Amaike and Keller, 2011) [16]. These toxins occur naturally and have been found in a wide range of commodities, including groundnuts used for animal and human consumption (Williams *et al*. 2004) [6]. Aflatoxins are toxic, mutagenic, and carcinogenic compounds (Chen *et al*. 2013) [26]. Depending on their levels, toxins can severely affect the liver and induce immune-suppressing effects (Williams *et al*. 2004) [6].

Economic Importance of Aflatoxin: On an average 25% foods contaminated with mycotoxin among which Aflatoxin is major. India is an important exporter of groundnut and its products. Over a decade export of groundnut and it's production reduce from 550 metric tons to 265 metric tons. In India according to ICMR (lucknow) maximum aflatoxin permissible level is 30 ppb (Basu, 2002) [3].

Why Resistance Breeding: Fungi infect groundnut at every stage (field, storage, transportation) so management of it is difficult. If we gate once resistance cultivar then it is easy to control it because no need to manage it at every stage. Genetic variation and reliable and efficient screening techniquesare the two major requirements for developing resistant variety of any crop. Rao and Tulpule (1967) [14] were the first to report varietal differences in resistance to aflatoxin production in groundnutnut. In their laboratory study the introduced genotype US 26 (PI246388) did not support aflatoxin production when colonized by aflatoxin producing strain of *A. flavus*.

However, other researchers could not support this finding. Then after Mixon and Rogers (1973) [9] reported resistant against aflatoxin in two valencia genotype PI 337394F and PI 337409. Genetic resistance to *A. flavus* invasion and aflatoxin production is one of the cheapest and the most viable alternative approaches to combat the aflatoxin problem in groundnut.

Different types of Resistance (Upadhyaya *et al.*, 2002) [21] 1. Resistance to pod infection (pod wall), 2. Resistance to aflatoxin production (cotyledons) and 3. Resistance to seed invasion and colonization (seed coat).

Different screening Techniques for Resistance to Aflatoxin are; (A) Screening by *in vitro* seed colonization (IVSC) given by Mixon and Rogers, 1973 [9]. (B) Field screening for pre-harvest aflatoxin contamination (PAC) (Holbrook *et al*., 1994) [5]. (C) Screening through Aflatoxin detection in genotypes (ELISA, Thin-Layer Chromatography, High Performance Thin-Layer Chromatography Methods, High Performance Liquid Chromatography methods (HPLC) etc.).

Components in Seeds Related to Resistance

(A) Structure of Seed Coat: Outer most layer of seed and check entry of pathogen. Resistance attributed by thickness and/or permeability of coat (LaPrade *et al.*, 1973) [7]. Resistance genotype had smaller hilum, compact pallisade and thick waxy layer surface, tannins layer on coat (Taber *et al.*, 1973) [19].

(B) Polyphenol compound: (Sander and Mixon (1978) [17] Antibiotic phenol like tannins in testa function as inhibitors to *A. flavus.*

(C) Antifungal protein: Plant seed contain number of protein. Certain protein also protect seed from fungal infection. This protein are enzyme inhibitor, ribosome inactivating protein, zeamatin, 2S storage protein *etc*.

Screening of Genotypes

Vasudeva *et al.* (1989) [23] at ICRISAT carried out an experiment for *Aspergillus flavus* seed colonization (%) of selected groundnut breeding lines in multilocational testing in India during 1983-1986. They studied 8 genotype with UF 71533 and J 11 as a resistance control and JL 24 and Kadiri 3 as a susceptible control, in the study they found ICGV 86171 gave lower seed colonization in all environment while ICGV 86168 gave lower colonization only in one environment (rainy season 1986). With this they reported that ICGV 86171gave more stable resistance performance in all environment with respect to resistant control. This will use as a resistant source for aflatoxin resistance breeding. Waliyar *et al.* (1994) [24] studied the percentage of groundnut seed contamination by *A. flavus* during rainy season, they evaluate different lines from which 55-437 gave lowest colonization in 1989 and U4-47-7 gave lowest in 1990, while VAR 27 gave highest colonization in both season, with the study they observed 55-437, J 11, ICGV 87107, UF 71513-1, Ah 7223 and U4-47-7 were resistance to AFs as compare to other lines and use as a resistance source because they gave lower seed colonization in both season and VAR 27 was susceptible to AFs because it gave higher seed colonization in both season as compare to other. Waliyar *et al.* (1994) [24] also studied present seed infection and AFs content of groundnut entries at maradi during rainy season in 1989 and 1990 and reported that VAR 27 gave higher seed colonization in both season was gave lowest aflatoxin after storage, while those gave lower colonization as compare to VAR 27 were gave higher aflatoxin. And based on that concluded that VAR 27 is susceptible to seed colonization but resistance to aflatoxin production and will be used for pyramiding different types of resistance mechanism in one genetic background. Nahdi (1996) [10] take an experiment to know Pre-harvest seed infection by *A. flavus* and AFs contamination in groundnut genotypes grown under irrigation and drought stress post-rainy season. He found that PI 337394F and J 11 were gave lower infection of *A. flavus* and aflatoxin contamination as compared to others and *A. flavus* infection and aflatoxin contamination were higher in non-irrigated condition as compared to full irrigated condition, on that basis he reported that PI 337394F and J 11 are resistance to *A. flavus* and aflatoxin contamination and use as a resistance sources for future breeding programme. He also concluded that *A. flavus* and aflatoxin contamination higher in drought condition as compare to full irrigated condition. Reddy *et al.* (2003) [15] evaluate aflatoxin (B1) levels in different varieties of groundnut by collecting samples of different varieties from market and found that GG-2 had lowest aflatoxin level as compare to all others and TMV-2 had highest mean aflatoxin. Based on that they reported that the variety GG-2 is resistance to aflatoxin and use as a source for resistance. Nigam *et al.* (2009) [11] evaluated 4 newly developed groundnut breeding lines for IVSC and natural AFs production with J11 and JL24 as a check, they found that all 4 lines were sawn higher resistance for both parameter as compare to J11 (resistance check). Girdthai *et al.* (2010) [4] studied *Aspergillus flavus* colonization and aflatoxin contamination of 11 peanut genotypes grown under different water regimes in 2004/2005 and 2005/2006 dry seasons. They stated that Tifton-8 which is drought tolerance genotype was show lower *A, flavus* infection as well as aflatoxin production in both season and also found seed colonization and PAC under drought were higher than those under fully irrigated conditions and drought tolerance genotype have resistancy against seed colonization by *A. flavus* and pre-harvest aflatoxin contamination.

Genetics of Aflatoxin Resistance

Very few studies have been done on inheritance of resistance to seed infection, IVSCAF (In Vitro Seed Colonization of *A. flavus*) and aflatoxin production. There are lack of significant relationships among the three resistance mechanisms so different resistance mechanism governed by different genes (Utomo *et al*. (1990) [22] thus three components of resistance are inherited independently (Upadhyaya *et al*. (2002) [21].

Vasudeva *et al*. (1989) [23] studied general combining ability (GCA) effects for seven parental lines for seed-coat resistance in a line tester design and observed that UF 715 13 and Ah 7223 had shown significant negative gca effects and VAR 27 had significant positive gca effect. Based on the study they concluded their experiment that UF 715 13 and Ah 7223 are good combiner for seed coat resistance because of having significant negative gca while VAR 27 is poor combiner for seed coat resistance. Xue *et al.* (2004) [25] take an experiment on variance component and heritability for aflatoxin production in F_2 population produced from the cross between PI 290626 and Gregory. They estimates variance component and heritability in two models *i.e.* A x D model and epistatic A x A model. Additive form of genetic variance in segregating population of cross between PI 290626 and Gregory was negative in both model. Due to additive variance is negative narrow sense heritability is zero. Finally they conclude that at early generation selection for aflatoxin within population is ineffective because of zero narrow sense heritability. Vasudeva *et al*. (1989) [23] studied stability

parameters of eight breeding lines obtained from four Indian locations in rainy season with UF 71533 and J 11 as a resistance control and JL 24 and Kadiri 3 as a susceptible control. In study they found ICGV 86171 gave lowest seed colonization but it's regression coefficient was not near to one, while ICGV 86177 had regression coefficient near to one. Thus, ICGV 86177 is good source for resistance to seed colonization as compare to ICGV 86171 due to it is more stable than later one. Latha *et al*. (2007) [8] evaluated genotypes for total phenols in leaves and kernels and aflatoxin content at harvest in groundnut under end-of-season drought condition. They reported J 11 had highest phenol in leaves and kernel and lowest aflatoxin at harvest time while ICGV 95322 had lowest phenol in leaves and kernel and highest aflatoxin at harvest time. Thus, they found negative relationship between phenol and aflatoxin contamination, if phenol content increase aflatoxin contamination decrease and *vise-versa.*

Shou *et al*. (2010) [18] in China conduct an experiment by developing RILs with high oil content and resistance to aflatoxin. They developed RILs by crossing Zhonghua 5 and Yuanza 9102. From these study they found transgrasive segregation for oil and aflatoxin contamination. They produce 117 RILs among that select 18 with high oil content and low aflatoxin contamination. This 18 RILs will use for future as a source of high oil with aflatoxin resistance breeding. Prasad *et al.* (2010) [13] at ICRISAT studied correlation between chitinase activity and % *A. flavus* infection. They transfer rice chitinase gene from rice to JL 24 cultivar which gaves chitinase activity was higher 2 to 14 fold in transgenic cultivar as compare to non-transgenic and *A. flavus* infection was lower 0 to 40 % in transgenic cultivar as compare to non-transgenic. Finally they conclude that there is negative relationship between chitinase and *A. flavus* infection, as chitinase activity increase *A. flavus* infection decrease and *vice- versa.*

Arunyanark *et al.* (2010) [2] conduct experiment for estimates of heritability with standard error for seed infection and aflatoxin contamination under drought condition of 4 crosses. They reported that heritability for seed infection by *A. flavus* and aflatoxin contamination were low to moderate for all the crosses so selection for this trait is ineffective. Same author also studied genotypic and phenotypic correlation between seed infection and aflatoxin contamination with HI at final harvest, SLA, SCMR at 67 DAS of 4 crosses. They found significant relation between all trait with seed infection and aflatoxin contamination, among them HI and SCMR had negative correlation and SLA had positive correlation. Finally they conclude that all this will help to indirect selection for seed infection and aflatoxin contamination. SLA and SCMR has low G x E interaction as compared to HI so, they will more important for indirect selection as compare to HI. Arunyanark *et al.* (2010) [2] studied genotypic and phenotypic correlation between seed infection and aflatoxin contamination with biomass, pod yield and draught tolerance index (DTI) for biomass (BIO) and pod yield (PY) of 4 different crosses. They reported most of them were negatively correlate with seed infection and aflatoxin contamination and not all character had consistent significant correlation in all cross except pod yield. So, high pod yield under drought condition will help to selection resistance cultivar against aflatoxin. Girdthai *et al.* (2010) [4] conduct experiment to study correlation between *A. flavus* colonization (%) and aflatoxin contamination (ppb) and surrogate trait for drought tolerance in 11 peanut genotypes under terminal drought. They reported, among all traits only SLA, ChlD, RWC were give consistent significant correlation and concluded that this three will help to indirect selection for seed infection and aflatoxin contamination.

Protein as a marker for screening aflatoxin resistant germplasm

Tong *et al.* (2010) [20] studied to identify proteins associated with resistance against *A. flavus* infection under drought stress condition. They extract protein of susceptible and resistance line and run on 2 d gel electrophoresis to compare it. They analyze differently expressed protein through MALDI-TOF mass spectrometry analysis. They compare this protein with NCBI protein data base. Among these proteins they found two proteins were match with *Arachis hypogaea* protein. They concluded that this protein will help to indirect selection for aflatoxin contamination and also help to understand genetics and inheritance of aflatoxin resistance.

Transgenic approach for AFs resistance

Niu *et al.* (2009) [12] in USA produced transgenic in cultivar Georgia green. They transfer chloroperoxidase gene (*cpo-p gene*) (from *Pseudomonas pyrrocinia*) and *hygromycin* phosphotransferase *(hph)*. They conform transformation by Southern, Northern and Western blot analysis. In southern analysis DNA fragment of transformed gene were hybridized with radio labeled probe and conform their transformation. Same thing done with RNA and protein in northern and western blot analysis respectively. They found significant lower seed colonization in extract of transformed cultivar and transformed cultivar cotyledon as compare to non-transformed. They reported their study that transgenic approach will help to control aflatoxin contamination and also helpful to study inheritance of resistance mechanism for aflatoxin.

Conclusion

Aflatoxin contamination is the most important disease of groundnut and very difficult to control. It can be easily control by developing resistant cultivar but, it is difficult due to unavailability of high level of resistance lines, unavailability of proper screening technique, the genetics of resistance against AFs not properly understood, no correlation between various resistance types, high G x E interaction etc. We can use indirect selection criteria like drought tolerance, phenol content etc. which can use for selection of AFs resistance cultivar because they have correlate with it. Further we can also use various non-conventional methods (like transgenic method, molecular markers, protein markers for screening etc.) to understand resistance mechanism and development of resistant cultivar.

References

1. Anonymous. Directorate of Economics and Statistics, Department of Agriculture and Cooperation. Ministry of agriculture, government of India, 2012.
2. Arunyanark A, Jogloya S, Wongkaewb S, Akkasaenga C, Vorasoota N, Kesmala T *et al*. Heritability of aflatoxin resistance traits and correlation with drought tolerance traits in peanut Field Crops Res. 2010; 117:258-264.
3. Basu AS, Mcdonald C, Gibbons RW. Seed colonization and aflatoxin contamination in groundnut contaminated with *A. flavus*. Oleagineux. 2002; 37:185-193.
4. Girdthai T, Jogloy S, Vorasoot N, Akkasaeng C, Wongkaew S, 1N Holbrook CC *et al*. Association

between phesiological traits and aflatoxin contamination in peanut. Plant Breeding. 2010; 129:693-699.
5. Holbrook CC, Kvien CK, Ruker KS, Wilson DM, Hook JE, Matheron ME. Preharvest aflatoxin contamination in drought-tolerant and drought-intolerant peanut genotypes. Peanut Sci. 1994; 27:45-48.
6. Williams JH, Phillips TD, Jolly P, Styles JK, Jolly CM, Aggarwal D. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions, American Journal of Clinical Nutrition. 2004; 80(5):1106-1122.
7. LaPrade JC, Bartz JA, Norden AJ, DeMuynk TJ. Correlation of peanut seed-coat surface wax accumulations with tolerance to colonization by *Aspergillus flavus*. Proc. Amer. Peanut Res. and Educ. Assoc. 1973; 5:89-94.
8. Latha P, Sudhakar P, Sreenivasulu Y, Naidu PH, Reddy PV. Relationshp between total phenol and aflatoxin production of peanut genotypes under drought conditions. Acta Physiol Plant. 2007; 29:563-566.
9. Mixon AC, Rogers KM. Peanut accessions resistant to seed infection by *Aspergillus flavus*. Agron. J. 1973; 65:560-562.
10. Nahdi. Screening groundnut genotypes for resistance against A. flavus infection and aflatoxin contamination. Peanut sci. 1996; 21:89-95.
11. Nigam SN, Waliyar F, Aruna R, Reddy SV, Lava Kumar P, Craufurd PQ *et al*. Breeding Peanut resistance to aflatoxin. Peanut Science. 2009; 36:42-49.
12. Niu C, Akasaka-Kennedy Y, Faustinelli P, Joshi M, Rajasekaran K, Yang H *et al*. Antifungal Activity in Transgenic Peanut (*Arachis hypogaea* L.) Conferred by a Nonheme Chloroperoxidase Gene. Peanut Science. 2009; 36:126-132.
13. Prasad K, Pooja Bhatnagar-Mathur, Farid Waliyar, Kiran KS. Overexpression of a chitinase gene in transgenic peanut confers enhanced resistance to major soil borne and foliar fungal pathogens. J of Pl. Biochem and Biotech. 2010; 37:121-124.
14. Rao KS, Tulpule PG. Varietal differences of groundnut in the production of aflatoxin. Nature (London). 1967; 214:738-739.
15. Reddy EC, Sudhakar C, Eswara Reddy. Aflatoxin contamination in groundnut induced by *Aspergillus flavu*s type fungi. Inter. J Applied Bio and Pharma. Bio. 2003; 2:180-192.
16. Amaike S, Keller NP. *Aspergillus flavus*, Annual Review of Phytopathology. 2011; 49(1):107-133.
17. Sander TH, Mixon AC. Relation of stress on *A. flavus* invation and aflatoxin production. Peanut sci. 1978; 12:90-93.
18. Shou, Liao Bo, LEI Yong, Li Dong, Wang Sheng-Yu, Huang Jia-Quan *et al*. Novel Germplasm with High Oil Content and Resistance to *Aspergillus flavus* and Bacterial Wilt Developed from Peanut Recombinant Inbred Lines. Acta Agron Sin. 2010; 36:1296-1301.
19. Taber RA, Pettit RE, Benedict CR. Comparison of *A. flavus* tolerance and susceptible lines. Am. Peanut Res. Edu. Assoc. 1973; 5:206.
20. Tong Wang, Erhua Zhang, Xiaoping Chen, Ling Li1, Xuanqiang Liang. Identification of seed proteins associated with resistance to pre-harvested aflatoxin contamination in peanut (*Arachis hypogaea* L). BMC Plant Biology. 2010; 10:267.
21. Upadhyaya HD, Nigam SN, Mehan VK, Reddy AGS, Yellaiah N. Registration of *Aspergillus flavus* seed infection resistant peanut germplasm ICGV 91278, ICGV 91283, and ICGV 91284. Crop Sci. 2002; 41:599-600.
22. Utomo SD, Anderson WF, Wynne JC, Beute MK, Hagler WM, Payne GA. Estimates of heritability and correlation among three mechanisms of resistance to *Aspergillus parasiticus* in peanut. Proc. Amer. Peanut Res. and Educ. Soc. 1990; 22:26. (abstr.).
23. Vasudeva Rao MJ, Nigam SN, Mehan VK, McDonald D. *Aspergillus flavus* Resistance Breeding in Groundnut: Progress made at ICRISAT Center: Aflatoxin contamination of groundnut: proceeding of the International Workshop, ICRISAT Center. 1989-1987.
24. Waliyar F, Ba A, Hassan H, Bonkoungou S, Bose JP. Sources of resistance to aflatoxin contamination in groundnut genotypes in West Africa. Plant disease. 1994; 78:704-708.
25. Xue. Evaluation of Peanut (*Arachis Hypogaea* L.) Germplasm for Resistance to Aflatoxin Production by *Aspergillus flavus* Link Ex Fries. Ph. D Thesis (Unpublished). North Carolina State University, USA, 2004.
26. Chen YC, Liao CD, Lin HY, Chiueh LC, Shih DYC. Survey of aflatoxin contamination in peanut proucts in Taiwan from 1997 to 2011, Journal of Food and Drug Analysis. 2013; 21(3):247-252.