Genetic Enhancement of Groundnut for Resistance to Aflatoxin Contamination

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Problem

- Naturally occurring fungal metabolites
- Produced by *Aspergillus flavus and A. parasiticus*
- Pre-harvest and post-harvest occurrence
- Primarily associated with groundnuts, corn tree nuts, cotton seed, dairy products
- Stable to heat and processing procedures
- Associated with human liver cancer and aflatoxicosis, in livestock, domestic animals and humans

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Economic impact of aflatoxin

- Related directly to crop and livestock losses as well as indirectly from cost of regulatory programs designed to reduce risks to animal and human health
- The FAO estimates that 25% of the world's food crops are affected by mycotoxins, of which aflatoxins are the most notorious
- Losses to livestock and poultry producers from aflatoxin-contaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency

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Aflatoxin contamination in groundnut

- Groundnut cultivated in 22 m ha is over 100 countries, production 38.61 million t
  - Asia produces 26.2 m t (68%)
  - Africa produces 9.4 m t (9%)
- Two thirds of the total production comes from rainfed crop, which suffers drought which predisposes crop to aflatoxin contamination
- The problem of aflatoxin contamination in groundnut first recognized in 1960-outbreaks of Turkey-X disease in the UK
- Use of resistant cultivars to contain problem of aflatoxin in groundnut (Mixon and Rogers 1973)

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Aflatoxin contamination in groundnut

Causes

- Moisture and heat stress during pod development, damage to the pods by insect pests and nematodes, and other injury during cultural operations facilitate pre-harvest seed infection
- Postharvest aflatoxin contamination can be minimized by appropriate drying, curing and storage practices
- Postharvest practices fail, if pre-harvest infection occurs
- In breeding for resistance to pre-harvest aflatoxin contamination (seed infection and aflatoxin production) is a key issue

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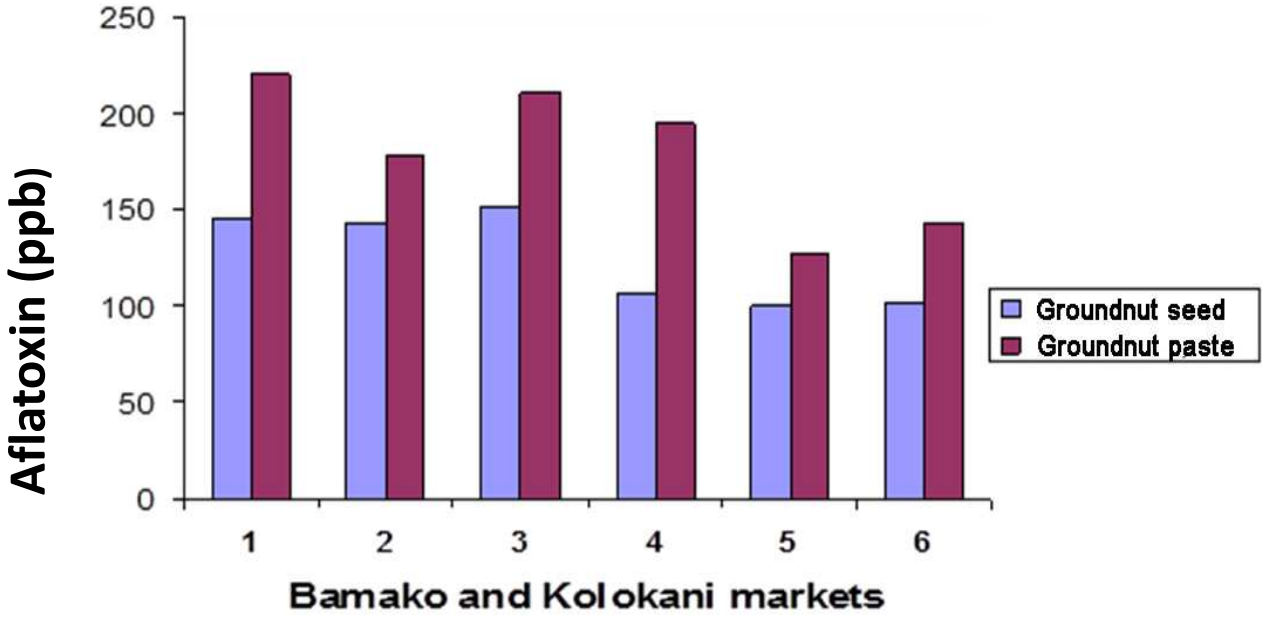
Importance of aflatoxin in groundnut

- In Malawi aflatoxin contamination in groundnut ranged from 0.0 ppb to 3871 ppb
- In Mali, groundnut cake had much higher levels of aflatoxin in market samples



Aflatoxin content in groundnut paste and seed from Bamako and Kolokani markets, 2008-2009.

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Opportunities
Genetic enhancement for resistance to aflatoxin contamination in groundnut

Three resistance barriers

Host and aflatoxin-producing fungi interactions

1. **Pod wall**: physical barrier and resistance is attributed to pod shell structure (Zambettakis 1975)
2. **Seed coat**: Moisture and heat stress can cause microscopic fissures in seed coat
3. **Cotyledons**: provide sustenance to pathogen and aflatoxin is produced

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Inheritance of aflatoxin resistance

- Inheritance of resistance to pre-harvest seed infection, IVSC, or aflatoxin production- less studies
- Low to moderate broad sense heritability and combining ability of resistance sources (Utomo et al 1990, Upadhyaya et al 1997)
- No significant relationships among three resistance mechanisms
- Three components of resistance inherited independently; different genes governed them (Upadhyaya et al 2002)
- Predominantly non additive genetic variance for aflatoxin production, ineffective selection in early generations (Xu et al 2004)

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Breeding for resistance to aflatoxin contamination

Resistant sources of germplasm

- Sources of all the three types of resistance (pre-harvest seed infection, IVSC, and aflatoxin production by *A. flavus*) have been reported in cultivated peanut

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Screening of germplasm

- Over 2500 groundnut lines have been screened for their resistance to *A. flavus* seed infection in a sick plot under imposed drought conditions at ICRISAT Center Patancheru, India
- Screened groundnut mini core collection (184 accessions)
- Screened 35 accessions belonging to 24 species of six sections

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Resistant sources identified

- 21 accessions identified as resistant (≤ 2% seed infection) at ICRISAT and 6 at Dharwad, India
- 6 accessions identified as resistant in India and Senegal
- 29 accessions identified as resistant in Niger
- 4 resistant accessions from mini core collection in China
- 7 accessions for both *in vitro* seed colonization and *A. flavus* seed infection
- 10 accessions for pre-harvest seed infection and aflatoxin production
- ICGs 7633, 4749, 1326, 3263, 9407, 10094, 1859, 9610 showed high levels of resistance across tests, locations
- 9 accessions of 5 wild *Arachis* species

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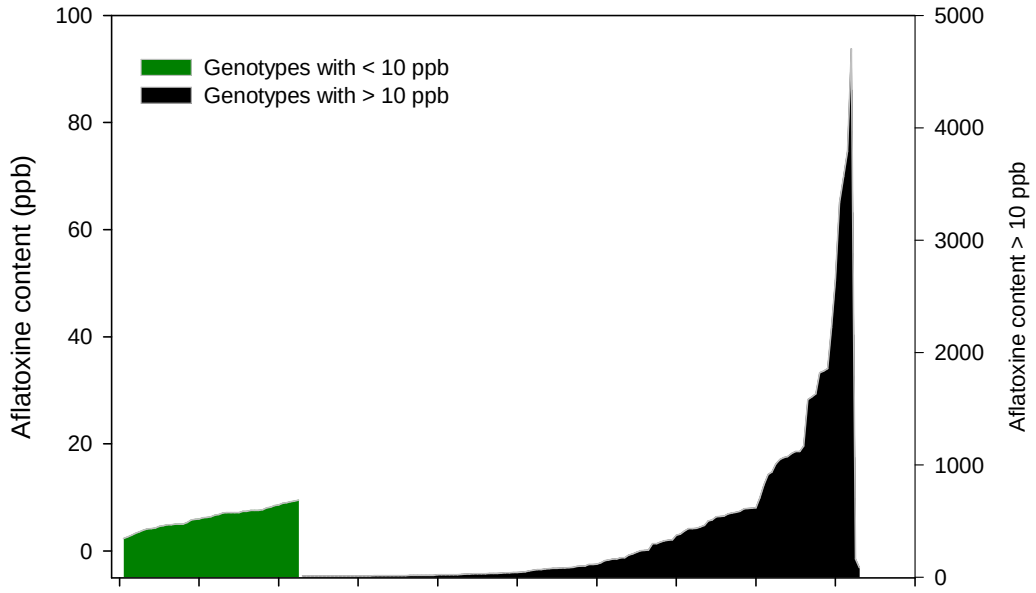
Aflatoxin contamination - higher levels of resistance

Screened 180 groundnut mini core collection at Sadoré, Niger (08/09)



- Contamination varied from 2 to 4700 ppb
- Highly resistant genotypes were identified: ICGs 5195, 6646, 14630 showed <3 ppb

Groundnut germplasm resistant to aflatoxin contamination

Genotype	Aflatoxin (ppb)
ICG 10609	0.4
ICG 11682	0.4
ICG 5158	0.5
ICG 10615	0.6
ICG 6760	0.6
ICG 23	0.7
ICG 9610	0.7
ICG 2925	0.8
ICG 10094	0.9
ICG 11480	0.9
ICG 7	1
ICG 1323	1
ICG 5195	1
Susceptible Checks	
Fleur 11	171.4
JL24	304.6

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Progress in resistance breeding at ICRISAT

- Elite breeding lines ICGVs 88145, 89104, 91278, 91283 and 91284 were developed and released as improved germplasm (Rao et al 1995, Upadhyaya et al 2001)
- ICGVs 87084, 87094 and 87110 were resistant to pre-harvest seed infection in Niger, Senegal and Burkina Faso
- ICGVs 95440, 95422, 94435, 94434, 94433, 95435 and UF 71315 showed high level of resistance to *A. flavus* seed infection (Zhou et al 2002)

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Groundnut breeding lines resistant to aflatoxin contamination

Genotype	Aflatoxin (ppb)
ICGV 89092	0.2
ICGV 91289	0.4
ICGV 00362	0.7
ICGV 86168	0.7
ICGV 02313	0.9
ICGV 91283	1.2
ICGV 06423	1.5
ICGV 99240	1.8
ICGV 07220	2.1
ICGV 91324	2.1
ICGV 01258	2.5
ICGV 91278	2.7
ICGV 93305	2.8
ICGV 91317	3.4
ICGV 89106	3.9
ICGV 91304	3.9
ICGV 89115	4.1

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Progress in resistance breeding

Performance (2 seasons) of some of the newly developed aflatoxin tolerant Spanish breeding lines at ICRISAT Center, Patancheru, India

Genotype	Pod yield (kg ha-1)[a]		IVSC (%)[b]		Natural aflatoxin production (µg kg^{-1})[b]	
	R 2005	PR 2005/06	R 2005	PR 2005/06	R 2005	PR 2005/06
ICGV 03328	2095	2790	0.0	1.8	1.9	0.5
ICGV 03331	2136	3532	0.0	1.3	2.8	1.5
ICGV 03332	1808	3042	0.0	2.3	4.8	0.7
ICGV 03346	1786	3485	0.0	2.0	4.9	1.1
J 11 (Resistant control)	1435	2424	0.0	8.9	18.9	9.8
JL 24 (Susceptible control)	1603	3137	0.0	9.6	25.6	222.0
SE(±)	113.1	292.2	0.65	1.48	1.69	215.7
CV (%)	8.1	13.1	343.7	113.0	101.2	515.6

[a] = recorded in yield trials in normal fields, [b] = recorded in screening trials in an *A. flavus* sick plot, R=Rainy season and PR=Post-rainy season

On-farm performance of aflatoxin resistant advanced breeding lines in India

14 advanced breeding lines and TMV 2, evaluated in on-farm trials in Anantapur and Chittoor districts, India during 2003-2006

- Tested lines produced 12-45% higher pod and haulm yields, than TMV 2 with 0-7 µg kg^{-1} compared to 0-150 µg kg^{-1} in TMV 2
- ICGVs 91341, 93305, 94379, 94434 selected by farmers in Chittoor
- ICGV 91278, 91328, 94379, 94434 selected by farmers in Anantapur
- These materials have good tolerance to drought, high pod and haulm yields and low aflatoxin risks

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Progress in resistance breeding

- Available genetic resistance was transferred to superior agronomic background
- Several advanced lines released in India, China and USA
- Aflatoxin + short-duration + drought tolerance is now focus at ICRISAT
- Research efforts to identify molecular markers for selection of resistance to aflatoxin

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International Aflatoxin Nursery

- To identify stable sources with high levels of combined resistance to seed infection, IVSC, and aflatoxin production, extensive evaluation in diverse growing environments
- International Aflatoxin nursery constituted with 116 lines, 79 form *vulgaris* and 37 *hypogaea*; 33 from USA, 4 from Niger, 79 from ICRISAT
- Evaluated in 2011/12 and 2012/13 post rainy seasons
- 30 lines (6 USA, 2 Niger, 22 ICRISAT) had aflatoxin 0.0 µg kg^{-1} seed and 0% infection in 2011-12 PR season

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Partners in aflatoxin Research

- International research institutes
- National Research Programs
- Advanced Research Institutes, health institutions
- Farmers and farmers' organizations (e.g. NASFAM in Malawi)
- NGOs

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Why invest in breeding for afaltoxin resistance?

- Resistant cultivars an integral part of overall management of aflatoxin contamination particularly in developing countries where two thirds of groundnut is cultivated under rainfed conditions
- Good progress made in developing resistant breeding lines though not yet adequate
- Cultivated and wild *Arachis* germplasm needs to be pursued more vigorously and screening methods improved
- Recent advancesthrough modification of aflatoxin biosynthesis pathway or use of genes with antifungal properties may lead to development of transgenic groundnut events with high and stable levels of resistance to fungal infection and aflatoxin production

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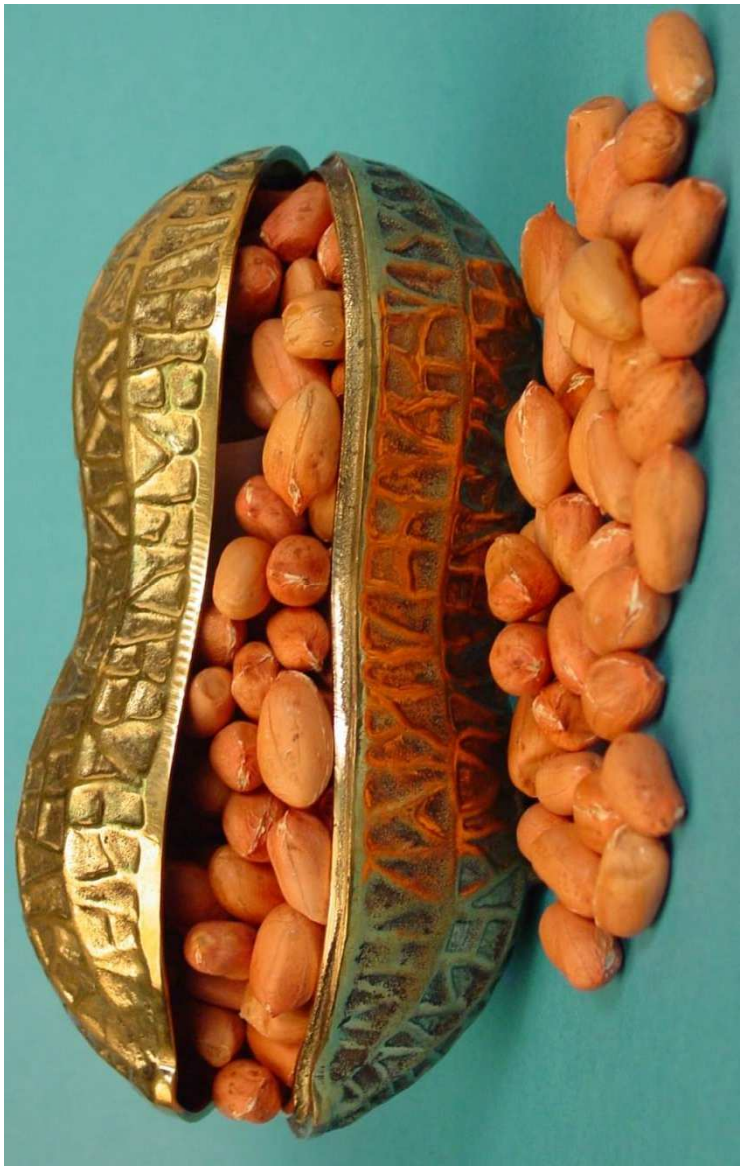
Thank You



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