Genetic Enhancement for Resistance to Aflatoxin Contamination in Groundnut

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Groundnut is an important oilseed crop cultivated in 96 countries worldwide on 23.8 million ha with an annual production of 30.97 million t. It is an important cash crop in several countries of Asia, which accounts for 57.13% of world area and Africa, which accounts for 37.24% area.

Aflatoxin contamination of groundnut is a widespread serious problem in most groundnut-producing countries where the crop is grown under rainfed conditions. The aflatoxin contamination does not affect crop productivity but it makes produce unfit for consumption as toxins are injurious to health. The marketability of contaminated produce, particularly in international trade is diminished to nil due to stringent standards of permissible limits on aflatoxin contamination set by the importing countries. The aflatoxin-producing fungus, *Aspergillus flavus* and *A. parasiticus,* can invade groundnut seed in the field before harvest, during postharvest drying and curing, and in storage and transportation. The semi-arid tropical environment is conducive to preharvest contamination when the crop experiences drought before harvest, whereas in the wet and humid areas, postharvest contamination is more prevalent. Research on aflatoxin contamination is not regularly carried out by all the groundnut-producing countries because of the complex nature of the problem and lack of qualified personnel and appropriate infrastructure. Nevertheless, some countries have been regularly monitoring groundnut and its products for aflatoxin at different stages (farm, markets, and storage). Aflatoxin contamination can be minimized by adopting certain cultural, produce handling, and storage practices. However, these practices are not widely adopted particularly by the small farmers in the developing countries, which contribute about 60% to the world groundnut production.

One of the possible means of reducing aflatoxin contamination of groundnut is the use of cultivars resistant to seed invasion by aflatoxin-producing fungi or to aflatoxin production. These cultivars will be of great value to the farmers in both developed and developing countries as there

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is no cost input. Therefore, breeding for resistance to *A. flavus* and *A. parasiticus* and/or aflatoxin production can play a significant role in preventing aflatoxin contamination in groundnut and consequently associated economic losses and health hazards.

The alleviation of aflatoxin contamination through genetic manipulation has been attempted since mid-1970s. We have achieved significant progress; however, these efforts have not resulted in complete eradication of aflatoxin contamination. In this paper we have briefly discussed the status of research on finding a genetic solution to this problem.

Types of resistance

In groundnut, based on the site at which it is tested or cultivated, resistance to aflatoxin-producing fungi may be of three types: resistance to pod infection (pod wall); resistance to seed invasion and colonization (seed coat); and resistance to aflatoxin production (cotyledons). The fungi have to penetrate the pod wall and the seed coat to reach the cotyledons from which they derive their sustenance. Resistance to pod infection is attributed to pod-shell structure, while resistance to seed invasion and colonization is mostly physical, and has been correlated with thickness, density of palisade cell layers, absence of fissures and cavities, and presence of wax layers. There are conflicting reports regarding the role of fungistatic phenolic compounds in imparting resistance to seed colonization.

Sources of all the three types of resistance have been reported (Mehan 1989). These include Shulamit and Darou IV for resistance to pod infection, PI 337394 F, PI 337409, GFA 1, GFA 2, UF 71513, Ah 7223, J 11, Var 27, U 4-47-7, Faizpur, and Monir 240-30 for resistance to in vitro seed colonization by *A. flavus* (IVSCAF); and U 4-7-5 and VRR 245 for resistance to aflatoxin production. The importance of preharvest aflatoxin contamination was realized only in the late 1980s, and some of the IVSCAF-resistant genotypes (PI 337394 F, PI 337409, GFA 1, GFA 2, J 11, UF 71513, and Ah 7223) were reported to have considerably lower natural seed infection by *A. flavus* than various IVSCAF-susceptible genotypes (Mehan 1989).

The value of a resistant source depends upon the level and stability of its resistance. Resistance to pod infection has been reported to be highly variable and of a low level. Similarly, IVSCAF-resistance is not absolute and even the best sources show up to 15% seed colonization; only a few lines

(J 11, PI 337394 F, and PI 337409) have shown stable resistance. For aflatoxin contamination, resistance levels are not very high (Anderson et al. 1995). Highly significant genotype x environment interaction effects have been observed for aflatoxin contamination.

Relationships between types of resistance

There are conflicting reports on the relationship between IVSCAF-resistance and resistance to natural seed infection, and aflatoxin contamination in the field. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India and in USA, though a significant reduction in the levels of seed infection by *A. flavus* under field conditions in the IVSCAF-resistant genotypes in comparison to the IVSCAF-susceptible genotypes was observed, the correlation was not perfect. In the breeding lines developed and evaluated, very low correlation (-0.07) was observed between IVSCAF and seed infection in the field, indicating two independent genetic mechanisms (Utomo et al. 1990, Upadhyaya et al. 1997). The high correlation observed in an earlier study (Mehan et al. 1987) might have been due to the inclusion of some selected germplasm lines; whereas the absence of correlation observed in breeding lines developed at ICRISAT Center (IC), Patancheru might have resulted from the recombination of genes controlling these mechanisms. Studies conducted, in the 1980s, in USA and at IC showed low levels of aflatoxin contamination in IVSCAF-resistant genotypes. However, the genotypes which were earlier reported to be resistant to IVSCAF or preharvest aflatoxin contamination contained high levels of aflatoxin, and when subjected to an extended period of heat and drought stress in USA, none of them was more resistant than the susceptible cultivar Florunner (Anderson et al. 1995). Highly significant genotype x environment interaction effects for aflatoxin contamination were observed in this study. The exact information on the relationship between different resistance mechanisms, their interactions, and possible contributions in reducing aflatoxin contamination have not been clearly established. Knowledge of these aspects is very crucial in developing strategies to reduce aflatoxin contamination.

Genetics of resistance

There are only few published reports on inheritance of resistance to seed infection, IVSCAF, and aflatoxin production, which give estimates of broad

sense heritability and combining ability. The high estimate (79%) of broad sense heritability for seed colonization was reported from USA in a cross involving PI 337409 (resistant) and PI 331326 (susceptible). The heritability estimates in later studies in USA were 55% in the cross involving AR 4 (resistant) and NC 7 (susceptible), and 63% in a cross between GFA 2 (resistant) and NC 7 (susceptible). At IC, the values were 60% in a cross involving J 11 (resistant) and OG 43-4-1 (susceptible) and 59% in a cross between two resistant parents, J 11 and Ah 7223.

The heritability estimates for resistance to seed infection have been reported to be low in USA: 27% in AR 4 x NC 7 and 33% in GFA 2 x NC 7 (Utomo et al. 1990). However, in our study the estimates were moderate to high (56-87%) (Upadhyaya et al. 1997). For resistance to aflatoxin production, the heritability estimates were reported as 20% in AR 4 x NC 7 and 47% in GFA 2 x NC 7. A report from USA stated that there is no significant correlation among the three types of resistance, indicating that they are controlled by different genes (Utomo et al. 1990).

A study on combining ability of IVSCAF-resistance using lines x tester analysis at IC indicated UF 71513 to be a good general combiner and Var 27 to be a poor combiner for resistance to IVSCAF. J 11 had non-significant general combining ability effect. In a diallel study, significant reciprocal effects were noticed in some crosses indicating maternal influence on testa structure (Rao et al. 1989).

The genetics of resistance mechanisms has not been clearly established. The allelic relationship among various sources for each resistance trait needs to be elucidated to enable breeders to pyramid the non-allelic genes for each resistance mechanism.

Genetic enhancement for resistance

Breeding efforts for resistance to pod infection have not received any attention. Further, it was assumed that if shell thickness was related to resistance, then resistance breeding would result in low shelling percentages or difficulty in shelling groundnut. In the past, seed colonization resistance received maximum attention due to the ease of screening procedures. Of late, natural seed infection and aflatoxin production have received increasing attention, although screening for resistance to aflatoxin production is expensive. A much cheaper enzyme-linked immunosorbent assay (ELISA)-based methodology has been developed at ICRISAT (Reddy et al. 1988).

Research on breeding for resistance to aflatoxin contamination is in progress in India, Senegal, Thailand, and USA. The groups at Tifton (USA) and IC (India) have successfully transferred IVSCAF-resistance to different genetic backgrounds. The group at Tifton produced six breeding lines GFA 1, GFA 2, AR-1, AR-2, AR-3, and AR-4 (Mixon 1983a, 1983b). GFA 1 and GFA 2 (both runner market types), whose yields were equal to or better than that of Florunner, had equal or less than average seed colonization than the resistant control genotype (PI 337409). The yield potentials of AR-U-2, AR-U-3, and AR-U-4 are too low for their practical use as commercial cultivars.

In India, resistance breeding activities are mainly conducted at IC and the National Research Center for Groundnut (NRCG) at Junagadh. At IC, research on breeding for resistance to aflatoxin contamination started in 1976. Several hundred breeding lines have since been tested for yield and IVSCAF-resistance, and many lines with IVSCAF-resistance and high yield have been identified. Four hundred and seventy-two lines were evaluated for preharvest seed infection and yield. Some of these have seed infection and colonization equal to or less than the best resistant control cultivar J 11, and high-yield potential across seasons/years and locations. Of these, ICGV 88145 and ICGV 89104 have been released as improved germplasm lines (Rao et al. 1995). Recently, we have identified and released three more lines, ICGVs 91278, 91283, and 91284 as improved germplasm (Upadhyaya et al. 2001). These lines had seed infection and colonization equal to or less than J 11 and high yield across seasons and locations. These lines have also been evaluated for yield and other agronomic traits in national programs in Thailand and Vietnam, where they performed very well (Upadhyaya et al. 1997). Three lines (ICGVs 87084, 87094, and 87110), bred at IC for resistance to seed infection were also found to be resistant in Niger, Senegal, and Burkina Faso in West Africa (Waliyar et al. 1994).

In Thailand and Senegal, PI 337394 F, PI 337409, UP 71513, and J 11 are commonly used as resistant donors. The lines AR-1, AR-2, AR-3, and AR-4 are also being used in Thailand as sources of resistance; 55-437 has been used in Senegal.

In the breeding scheme at IC, the selection for resistance traits is delayed until later generations. However, it would be desirable to screen segregating generations and select only resistant plants/progenies. This would require modification of screening techniques currently being used to make them more suitable at the single plant level.

Future prospects of breeding for aflatoxin resistance

Although researchers have not been able to locate germplasm lines which show complete resistance to fungi at the pod-wall, seed-coat, and cotyledon levels, it was expected that the levels of resistance could be improved further by pyramiding resistance genes from different and diverse sources. It was also thought that by combining the three different kinds of resistance in one genetic background, the problem of aflatoxin contamination could be overcome to a large extent. Unfortunately, the progress made so far in conventional breeding has not been able to meet these expectations. The recourse to biotechnology, through modification of the aflatoxin biosynthesis pathway or the use of variants of hydrolytic enzymes (chitinases and glucanases) to provide transgenic protection to groundnut against infection by aflatoxin-producing fungi may help in obtaining groundnuts free from aflatoxin. Genetic resistance alone may not be enough to eliminate the problem of aflatoxin contamination in groundnut. It will have to be complimented with good crop husbandry and postharvest practices.

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