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Assessment of Groundnut Varietal Tolerant to Aflatoxin Contamination in Indonesia

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Abstract

Aflatoxin is secondary metabolite produced by *Aspergillus flavus* and *A. paraciticus* that grow on the seed coat (*testa*) of groundnut. This toxin is a serious food safety issue throughout the world. The availability of resistant genotype to *A. flavus* infection and/or aflatoxin contamination urgently needed. The experiment found one genotype had aflatoxin contamination under the safe level (≤ 10 ppb), with <15% of seed number infected by *A. flavus*. Recently, the biggest peanut industry, where the main production is roasted-peanut (in shell) produced from fresh pods, grows and develops that variety.

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Keywords: aflatoxin, Aspergillus flavus, *groundnut, varietal tolerance*

Introduction

Aflatoxin contamination, especially the most potent toxic aflatoxin B_1 (mentioned as "aflatoxin" in the rest of this paper), in groundnut seeds is a serious food safety issue throughout the world. The toxin is carcinogenic, teratogenic, mutagenic, immune-suppressive [1] and therefore it hazards both human and poultry health. The aflatoxin-producing fungi, *A. flavus* and *A. parasiticus* [2], can invade groundnut seeds when groundnut pods are still standing in the field (pre-harvest infection), during curing and drying, and in storage and transportation (post-harvest infection) [3]. Pre-harvest infection occur when environmental conditions during crop maturation are unfavorable for crop growth because of elevated temperature (up to 35°C) and prolonged moisture deficit [4]. In semi-arid environment, dry condition during end of growing season when the crop experienced to drought is conducive to pre-harvest contamination [1, 5] whereas postharvest contamination is more prevalent in wet and humid areas. Adopting some cultural practices that create *geocarphosphere* and *rhizosphere* with abundant water supply and free from insect and fungal invasion during late generative growth phase, especially in the last 4-6 weeks of growing season, could minimize pre-harvest contamination. Whilst post harvest management is

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basically undertaken to shorten the length of pods processing by introducing machineries such as pod thresher, dryer, and Sheller/de-hulling [6] and improve the technology of handling (appropriate drying, pre-cleaning, and sorting), storage structure (improving storage practices and facilities), and transportation. In addition, the use of field-aflatoxin detection kit; regulations relating food, animal feed ingredients; education and extension activities on prevention of aflatoxin contamination are some other approaches where people in developing countries would successfully adopt [7]. However, those practices/approaches have not been widely and precisely adopted by small farmers in developing countries who contribute about 90% to the world groundnut production [FAO 2006 *in* 8], where most of them manage a limited capital for groundnut crop. One of cost-effective strategy for reducing aflatoxin contamination is the use of resistant cultivar [3]. The cultivar resistant to seed infection by *A. flavus* or *A. parasiticus* or to aflatoxin production would be of great value to farmers in both developed and developing countries. Therefore, breeding for groundnut resistance to aflatoxin-producing fungi and/or aflatoxin production can play a significant role in preventing aflatoxin contamination, especially for pre-harvest aflatoxin contamination under the condition when irrigation is unavailable [1]. The availability of improved breeding lines coupled with pre and post harvest aflatoxin management practices is the comprehensive tool for reducing aflatoxin contamination of groundnut kernels harvested from farmers [9].

Research on aflatoxin contamination have been starting since 1960s and have documented the importance of drought stress, high soil temperature, and pod damage as the risk factors for increasing aflatoxin production. Later efforts were the works focused on the development of screening techniques and the identification of sources of resistance to *.A flavus* and/or aflatoxin contamination as foundation of conventional resistance breeding program to develop groundnut breeding lines that have high pod yield and low aflatoxin contamination. The recent research efforts focused on the use of molecular genetics approaches to reduce aflatoxin contamination [10]. The purpose of the experiment was to examine the resistance of genetic resources from Indonesia to aflatoxin contamination under end of season drought stress during dry season in Indonesia.

Materials and Methods

The experiment took place at Muneng Research Station, East Java, Indonesia during the dry season. The experiment applied a Randomized Block design with 10 genotypes treatment and three (3) replicates, which resulted in 30 plots. Those ten Indonesia genotypes consisted of two local varieties (Local Pati, Local Blitar), three drought tolerant lines (GH 27, GH 51 and GH 57), one foliar disease tolerant line (GH 15) and four national-improved varieties (Sima, Turangga, Komodo and Tuban). Chemical fertilizers of 23 kg N + 45 kg P_2O_5+22.5 kg K_2O/ha were applied in the furrows at planting time. Dolomite at the rate of 500 kg/ha was broadcasted at flowering stage to ensure the success of pod filling. The plot size was 12.5 m x 5 m where we can obtain at least 5 kg of fresh pods. The trial was under dry condition but irrigation water was available to ensure the success of crop establishment, good vegetative and generative growth. Irrigation was applied at planting time, 19, 36, 52, and 66 days after sowing (DAS) with amount of 135, 147, 181 and 285 m^3 respectively. Irrigation stopped at 66 DAS and, thereafter, the crops were under dry condition (around 30 days) until harvesting time (95 DAS). Unfortunately, there was 37 mm rain (one rainy day) at two days before harvest. A number of measurements was undertaken *i.e.* pod-zone soil temperatures every 4 days; pod moisture content at 50, 60, 70 and 80 DAS and harvesting time (gravimetric method); seed moisture content (gravimetric method) just before ELISA; number of seeds infected by *A. flavus* using AFPA (*Aspergillus Flavus and Paraciticus Agar*) media (100 kernels obtained from each genotype x 3 replicates); weight of three kernel categories (**sound mature kernels-SMK**: intact, mature, clean/no fungal infection; **shriveled kernels**: intact, immature, >50% seed surface was shriveled, clean/no fungal infection; and **damaged kernels**: discolor because of fungal infection, cracked, rotten) done on 2.5 kg of sun-dried pods sample; and aflatoxin content using ELISA (Enzyme Linked-Immunosorbent Assay) method developed by Alice and Kennedy [11]. Aflatoxin contamination was then grouped based on its safety for human consumption based on SNI [12](9), which stated that the maximum level of aflatoxin in peanut and