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Research Application Summary

Characterization of a diverse set of maize germplasm for resistance to infection by *Aspergillus flavus* and accumulation of aflatoxin

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Abstract

Sources of resistance to *A. flavus* have been identified elsewhere but no such materials are currently available for use by the maize improvement program in Uganda. The goal of this study was to initiate breeding for resistance to the fungus by identifying maize inbred lines, testers and hybrids that show high resistance to *Aspergillus flavus* and field accumulation of aflatoxin through: (1) screening inbred lines, testers and hybrids for resistance, (2) determining general and specific combining ability associated with resistance and determining the relationship between traits associated with resistance. Results indicated a highly significant variation ($P<0.001$) in resistance among inbred lines and testers. Testcrosses also showed significant variability ($P<0.05$). Inbred lines showed a significant GCA for kernel infection rate while testers showed significant GCA for severity of infection ($P<0.05$). SCA for both kernel infection rate and severity was non significant.

Key words: Aflatoxin, *Aspergillus flavus*, general combining ability, kernel infection rate, specific combining ability

Résumé

Les sources de résistance à l' *A. flavus* ont été identifiées ailleurs mais aucun matériau n'est actuellement disponible à l'usage par le programme d'amélioration de maïs en Ouganda. Le but de cette étude était de lancer le programme de lutte contre le mycète en identifiant les souches pures de mais, les échantillons-test et les hybrides de maïs qui montrent de haute résistance à l'accumulation d' *Aspergillus flavus* et de champ de l'aflatoxine à travers : (1) l'examen des souches pures, des échantillons-test et des hybrides pour la résistance, (2) la détermination de la capacité de combinaison générale et spécifique associée à la résistance et la determination du rapport entre les traits liés à la résistance. Les résultats ont montré une variation fortement significative ($P<0.001$) de résistance parmi les souches pures et les échantillons-test. Les techniques de croisement ont montré aussi une variabilité significative ($P<0.05$). Les souches pures

ont montré un GCA significatif pour le taux d'infection de grain tandis que les échantillons-test montraient un GCA significatif pour la sévérité de l'infection ($P<0.05$). SCA pour le taux d'infection et la sévérité de grain était non significatif.

Mots clés: Aflatoxine, *Aspergillus flavus*,capacité de combinaison générale, taux d'infection de grain, capacité de combinaison spécifique

Background

Aflatoxin is a naturally occurring toxin and a very potent carcinogen produced by *Aspergillus flavus* Link: Fr. Maize (*Zea mays* L.) grains that have been contaminated with aflatoxins frequently have a low market value. In Uganda, aflatoxin contamination increasingly poses a serious economic and health threat to maize farmers and consumers respectively. Research has indicated that infection by *A.flavus* and subsequent aflatoxin contamination occurs both before and after harvest (Gorman and Kang, 1990). It is therefore important to develop efficient and cost effective control measures for both levels. This enlists resistance breeding as the most desirable approach to alleviate the problem (Brown *et al.,* 1998). To develop resistant maize varieties for Uganda, there is need to identify local germplasm with resistance, and develop parental lines that combine resistance with superior agronomic qualities. There is also need to understand the mode of inheritance in order to facilitate effective transfer of resistance.

Literature Summary

Screening maize for resistance is a more difficult task than screening most diseases because it is hindered by lack of: (i) a resistant control (ii), inoculation methods yielding infection or aflatoxin enough to differentiate between genotypes (as natural infection is undependable), repeatability across different locations and years, and (iv) rapid and inexpensive assays of fungal infection and aflatoxin levels. A number of screening methods have been used but, simple and inexpensive laboratory Kernel screening Assay (KSA) has been developed (Brown *et al.,* 1998).

Study Description

In cropping season 2009 A, forty inbred lines and four testers were screened for resistance. These materials were planted in an alpha lattice design with 2 replications and each resultant cob was inoculated with 5 ml of a suspension containing 1×10^6 fungal spores per ml. Harvesting was done by plot and samples were taken to the laboratory for analysis of kernel infection rates and aflatoxin accumulation. Aflatoxin concentration in the

kernels was measured using "VICAM-aflatest" test kit following manufactures recommendations. Kernel infection was assayed using both culture media method and media free and isolated- kernel incubation (MIKI) method. Kernel infection was expressed as percent-kernel-infection (PKI) and percentage severity while aflatoxin accumulation was expressed in parts per billion (PPB).

Alongside inbred and tester screening, 121 testcrosses were generated in a line by tester nursery. In cropping season 2009B, the test crosses were evaluated for agronomic traits, ear rots and foliar diseases in three locations namely: Namulonge (central Uganda, hot and humid), Serere (north-eastern Uganda, hot and dry) and Bulegeni (eastern Uganda, cool and humid). Screening for resistance to *A. flavus* was done from Namulonge alone.

Data were subjected to analysis of variance using Restricted Maximum Likelihood (ReML) Linear Mixed Models in GENSTAT 12th edition. Means were separated using Fishers Protected Least Significant difference (FPLSD) at 5% significance level. Correlations between cob characteristics, PKI and aflatoxin level were also determined. To reveal component genetic effects, Analysis for combining ability was conducted for kernel infection and major agronomic traits.

Results

Evaluation of kernel infection rate by media plating indicated a significant and highly significant variation among inbred lines and testers as determined by the PKI and MIKI methods, respectively. The use of MIKI reduced the coefficient of variability by 39.5%. Eleven (11) inbred: had a PKI less than 40% and Aflatoxin concentration less than 20ppb. Testers also showed a highly significant variability ($P<0.001$) with Tester A (CML312 × CML442) being the most tolerant to infection.

In the analysis of combining ability for kernel infection, only inbred lines showed a significant GCA for PKI ($P<0.05$) while testers showed a significant GCA for percent severity. SCA was non significant for kernel infection rate.

There was a significant relationship ($P<0.05$) between PKI, aflatoxin concentrations, ear rot infections and husk coverage. Grain texture showed no relationship with kernel infection rate.

Recommendation

"MIKI" method for evaluating kernel infection rate is more reliable due to the lower coefficient of variability (CV).

Parents with generally low kernel infection rate, low aflatoxin accumulation and significant negative GCA effects could be exploited for developing resistant lines in the maize breeding program and to produce resistant hybrids.

There is need to develop resistant testers and checks for *Aspergillus flavus* In addition to these attributes parents with significantly high positive GCA effects for yield and other agronomic traits are more desirable in developing superior hybrids.

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References

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