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Research Application Summary

Relationship between causal pathogens for maize ear rots and grain yield in tropical maize in Uganda

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Abstract

Maize ear rots caused by *Aspergillus flavus, Fusarium graminearum* and *Stenocarpella maydis* reduce the quality of maize through production of mycotoxins and carcinogenic compounds that are harmful to both humans and animals when consumed. The objective of this study was to determine the relationship between yield and fungal ear rot infections caused by the three fungal pathogens. Tropical inbred lines with varying resistance to *A. flavus* and both *F. graminearum* and *S .maydis* were mated in a North Carolina II Design. The progeny consisting of single crosses, test crosses and their parental inbred lines were thereafter evaluated using single pathogen inoculation. Regression of the three ear rot mean infection scores on mean yield scores across three test locations for both the test and single cross hybrids revealed low yieldvariations in response to fungal ear rot infections. Further, it suggests that maize grain yield cannot be reliably predicted from any of the three ear rot pathogen infection scores. This also suggests that multiple resistance to the three pathogens is inherited independently of yield performance. Resistance to the three ear rots and yield performance should be directly evaluated and improved by separate and independent breeding strategies.

Key words: *Aspergillus flavus,* ear rots, *Fusarium graminearum,* mycotoxins, *Stenocarpella maydis*

Résumé

Les pourritures du maïs causées par *Aspergillus flavus, Fusarium graminearum* et *Stenocarpella maydis* réduisent la qualité du maïs à travers la production de mycotoxines et de composés cancérigènes qui sont nocifs pour les humains et les animaux lorsqu'ils sont consommés dans les aliments. L'objectif de cette étude était de déterminer la relation entre le rendement et les infections des pourritures causées par les trois agents pathogènes fongiques. Des lignés pures de maïs tropical ayant une résistance variable à *A. flavus* et aux *F. graminearum* et *S. maydis* étaient croisées dans le modèle de croisement du North Carolina II. La progéniture composée de croisements simples, les croissements tests et leurs parents lignées pures a ensuite été évaluée en utilisant une inoculation simple de pathogène. La régression des moyennes des scores des trois types de pourritures avec les scores moyens de rendement sur les trois sites expérimentaux à la fois pour les hybrides simples et ceux issus des croisements tests, a révélé une faible variation du rendement en réponse aux infections fongiques. En outre, il est suggéré que le rendement en grain du maïs ne peut être prédit de façon fiable à partir de scores de l'une des trois des agents

causals des pourritures du maïs. Cela suggère aussi que la résistance multiple aux trois agents pathogènes est héritée indépendamment de la performance en rendement du grain du maïs. La résistance aux trois types de pourritures et la performance en rendement devrait être directement évaluée et améliorée par des stratégies de sélection séparées et indépendantes.

Mots clé : Aspergillus flavus, pourritures des épis, *Fusarium graminearum, mycotoxins, Stenocarpella maydis*

Background

Maize *(Zea mays* L.) is one of the world's important cereal crops (Agona and Jane, 2000). In East Africa, the crop is a major staple food crop for a large proportion of the population as well as an important source of animal feed and export crop. Uganda's maize production in 2014 was 2,750,000 tonnes with an average yield of 2.5 tonnes per hectare (USDA, 2015). Most of the maize is directly eaten as food but also supports the animal feed industry, local brewery industry, where flour is fermented to produce local brews (Agona and Jane, 2000). The importance of maize is centered on the large quantity of carbohydrates, proteins, vitamins and fats, contained in the kernels, making it compare favourably as an energy source with root and tuber crops. Maize is also a good source of starch and ethanol for fuel (Peter *et al*., 2014). Ear rots caused by *A. flavus, F. graminearum* and *S. maydis* are some of the major biotic constraints affecting maize production worldwide (CIMMYT, 2004). Besides reducing maize yield, ear rots reduce the quality of maize through production of mycotoxins and carcinogenic compounds that are harmful to humans and animals when consumed in food and feed, respectively (Martin and Johnson, 1982; Vigier *et al.*, 2001; Pressello *et al.,* 2008). Major control measures for maize ear rots have involved application of sanitary measures, use of seed treatments in addition to use of sophisticated maize storage measures (CIMMYT, 2004). These control measures have not produced satisfactory results; leaving development of resistant varieties as the most economical and effective ear rot control measure.

Literature summary

Stenocarpella maydis and Fusarium species particularly *F. graminearum* and *F. verticillioides* have been identified as the main ear rot causing fungi in Uganda (Bigirwa *et al.*, 2007). Maize ear rots reduce grain yield and quality with implication on food security and health (Michael, 2008; Mwesi *et al*., 2009; Lieketso *et al.,* 2010; Masibonge *et al*., 2015). Indeed Mwesi *et al*. (2009) reported that some of the pathogenic fungi produce mycotoxins in maize grain posing a health risk to humans and livestock. Maize ear rots are a major constraint to maize production globally, and yield loses of up to 70% have been reported in Uganda (Tembo *et al.*, 2010). Resistance to ear rots in maize may be associated with undesirable traits such as small seeds and low yields (Duvick, 2001). Breeding efforts for ear rot resistance should also incorporate breeding and selection for desirable yielding germplasm. This was emphasized by Langa (2012) who reported that when breeding for ear rot resistance, yield was selected by

farmers as a critically important trait. The objective of this study was therefore to determine the relationship between yield and fungal ear rot infections caused by *A. flavus, F. graminearum* and *S. maydis*.

Study description

The study was carried out in three experimental sites in Uganda: Bulindi (1°25 N, 31°21 E; altitude 1140 m) located 210 km North West of Kampala, Namulonge (0°32 N, 32°35 E; altitude 1150 m) located 30 km north of Kampala and Ngetta (2°31 N, 32°93 E, altitude 1128 m) located 327 km north of Kampala. All the three locations experience a bimodal rainfall pattern, with the first season A, from March to August and the 2nd season B, from September to December/January. The germplasm included tropical inbred lines, selected based on their resistance to the fungal ear rot pathogens. Hybrid F_1 crosses consisting of test crosses and single crosses were evaluated for yield, ear rot resistance and agronomic performance. A total of 30 inbred lines, out of which 23 were resistant to *Aspergillus flavu***s** (male lines) were evaluated. Three inbred lines; WL118-10, CML506 and WL429-35 had dual resistance to *F. graminearum* and *S. maydis* (female lines). Line NML 89 was used as a susceptible check for both *F. graminearum* and *S. maydis.* Inbred seed multiplication and pollinations are done concurrently at a seed nursery established at Namulonge in season 2013A. Crosses were made using the North Carolina Design (NCD 2) to develop both the test and the single crosses. Three inbred lines CML 216, CML 395 and CML 442 were included in the study as yield testers. These were crossed with the male lines using the NCD 2 to produce test crosses. Single crosses were also developed by crossing the three dual resistant inbreds; WL118-10, CML506 and WL429-35 with the *A. flavus* resistant line (male lines) using the NCD 2 crossing design.

The three separate experimental trials were each laid out following an alpha lattice design with two replications. The plants were established in two row plots at a spacing of 75cm X 30 cm, each row measured 5 meters long and had 17 plants per row with one seed planted per hill. Both the single and test crosses were made during the first cropping season of 2013 (2013A) at NaCRRI to get F_1s. In the second season (2013B), the parental inbred lines, F_1s including the single and test crosses were thereafter evaluated in the three test locations: Namulonge, Bulindi and Ngetta using an alpha lattice experimental design. The evaluation of the inbred parents, test crosses and single crosses was done in three separate trials established adjacent to each other in each of the three test sites.

Pathogen culture and inoculation was done by isolating the three pathogens with *F. graminearum, S. maydis* and *A. flavus* isolated from infected maize cobs. Inoculum was prepared following the modified procedure of Chambers (1998). Infected maize kernels were first sterilized in 10% commercial bleach (JIK brand) 0.39% sodium hypochlorite (NaCIO) (Reckitt Benkiser East Africa Limited, Nairobi, Kenya) for three minutes and then rinsed thrice in distilled water. The seeds were subsequently

blotted dry on sterilized filter paper and then 2 – 3 seeds plated on 3% potato dextrose (Becton Dickinson, Sparks, MD, USA) agar plates and incubated at 28 – 30°C. The fungal growths on plates were sub-cultured after four days and were ready for transfer to toothpicks after 5 – 7 days.

Toothpicks were initially sterilized by boiling in water three times to wash out tannins and other fungal growth-inhibiting compounds from the wood and air-dried. They were then placed upright in bottles measuring 6 cm in diameter and 11 cm in height, containing 100 – 150 ml of potato dextrose broth (prepared by infusion of 200 g freshly unskinned potato and 20g of dextrose in 1 litre of distilled water) to coat the toothpicks, autoclaved for 30 min and left to cool to room temperature. Each sterile bottle contained approximately 600 – 650 toothpicks. Fungal plugs from pure cultures of either *F. graminearum* or *S. maydi*s were placed in each bottle and allowed to colonize tooth picks for 10 days following procedure described by (Chambers, 1988). After the toothpicks were fully colonized, they were air-dried before using them to inoculate the test genotypes. Inoculation was performed by piercing developing ears through the middle using colonized toothpicks 20 days after mid-silking (R3) stage (Chambers,1988). Care was taken when piercing to only prick the developing kernels and not the underlying cob thus very deep piercing was avoided. Ears were inoculated by single pathogens. Five plants per row selected from the opposite sides of the row were inoculated by either *F. graminearum* or *S. maydis*.

At maturity, the cobs in the inoculated rows were harvested separately, dehusked and assessed for fungal infection. Severity rating were done using a scale of 1 - 5 for *S. maydis* where 1 = 0-25%; 2 = 26- 50%, 3 = 51 - 75%, 4 = 76 - 99% and 5 = 100% (completely rotten) (Kapindu *et al.,* 1999). For *F. graminearum* the scale was 1 = 1 – 3%, 2 = 3 – 10%, 3 = 11 – 25%, 4 = 26 – 50%, 5 = 51 – 75%, and 6 = 76 – 100% (Reid *et al*., 1992). For *A. flavus* the scale of 1 - 5 was used with; 1 = 0-25%; 2 = 26-50%, 3 = 51 - 75%, 4 = 76 - 99% and 5 = 100% (completely rotten).

Figure 1. Singly tooth pick inoculated cobs (*S. maydi*s): intact (left) and unhusked (right)

Average disease scores were computed for each entry and for each pathogen. Cobs in the non inoculated rows were also harvested separately, bulked, counted and the following data also taken; cob aspect (CA), number of ears per plot, cob field weight, number of cobs with natural ear rot infestation and grain texture as described by Betr'an *et al.* (2002) where; 1 = flint; round crown kernel with vitreous appearance, to 5 = dent: kernel dented and having a floury endosperm. The cobs were then hand shelled and bulked into paper bags where they were carefully dried to avoid direct heat on the kernels to facilitate uniform drying prior to evaluation for *A. flavus* resistance in the laboratory.

Dry kernels were soaked in distilled water for 1 minute to enable them gain at least 30% moisture content. This was meant to mimic the field moisture conditions of the kernels on the cob. The kernels were then removed from water, drained and placed singly in bottle caps and put on disposable aluminum foil plates lined with wet cotton wool. A total of 15 kernels were put on each plate. Two plates were prepared for each sample implying that a total of 30 kernels per sample were assayed. For each genotype, two replications (60 kernels) were used. Each of the kernels was then inoculated by applying a 20 µl conidial suspension of *A. flavus* inoculum (with concentration of 1.0 ×106 conidia /ml) to its surface using a micro-pipette.

To enable homogeneous incubating conditions, plates were stacked and incubated at 31 ^{o}C and a relative humidity of 95 - 100% for 7 days. This was made possible by closing the plate lids tightly. The wet cotton wool maintained humid incubation conditions. On the 7th day of incubation, the plate lids were removed and the kernel infection rates recorded by counting the number of infected kernels and noting the number of those severely infected. A kernel was considered severely infected if fungal growth covered over 50% of its surface. Three measurements of kernel infection rate were evaluated; incidence of kernel infection expressed as Percent-of-Kernels-Infected (PKI), Incidence of Severely-Infected Kernels (ISIK), and Percent-Severely-Infected Kernels (PSIK).

A genotype infection score was also computed and this was obtained by averaging scores recorded for the two plates representing each entry. The above measurements were calculated as follows:

$$PKI = \frac{\text{Number of infected kernels}}{\text{Total number of kernels incubated}} \times 100\ \% \quad \text{.....(i)}$$

$$ISIK = \frac{\text{Number of severely infected kernels}}{\text{Total number of incubated kernels}} \times 100\ \% \quad \text{.....(ii)}$$

$$PSIK = \frac{\text{Number of severely infected kernels}}{\text{Total number of infected kernels}} \times 100\% \quad \text{.... (iii)}$$

Data were also recorded for plant growth rate, silking and tussling and crop yield.

In addition data were taken for plant aspect (PA), anthesis (AD) and silking dates (SD) (recorded as from the dates to anthesis and silking, respectively), anthesis silking interval (ASI), ear height (EH), plant height (PH), extent of husk cover (HC). Husk cover was determined using 1 - 5 scale described by Betr'an *et al*. (2002) where 1 = good, tight husk extending beyond the tip of the cob, to 5 = poor, loose short husk with exposed cob tip.

Data analysis and determination of resistance levels

Mean disease scores for the three fungal infections and yield means were computed. The relationship between yield and disease resistance was evaluated by performing a regression analysis of the mean scores for each of the three diseases and yield means across locations. Regression of *A. flavus, F. graminearum* and *S. maydis* mean scores on the yield means was graphically visualised on a scatter plots in EXCEL.

Results

Regression of *A. flavus, F. graminearum* and *S. maydis* on yield values among the test crosses. Among the test crosses, multi-location regression data indicated that 0 - 11% (R^2) of the total variation in yield was accounted for by the ear rot pathogen infestation among the test cross hybrids (Figure 2). Similarly, regression of *A. flavus* kernel infection measurements on yield indicated that *A. flavus* infection accounted for 5 - 10% (R^2) of the total variation in yield (Figure 3).

Regression of *A. flavus, F. graminearum* and *S. maydis* on yield values among the single crosses. Among the test crosses, multi-location regression data showed that 0 - 5% (R^2) of the total variation in yield was accounted for by the ear rot pathogen infection among the single cross hybrids (Figure 4). Likewise, regression of *A. flavus* kernel infection measurements on yield indicated that *A. flavus* infection accounted for 2 - 6% (R^2) of the total variation in yield (Figure 5).

Table 1. Multi-location analysis of variance for regression of *A. flavus, F. graminearum* and *S. maydis* on yield values among the test crosses

Source of variations	df	Asp lab	PKI	Fus	Steno
Regression	1	3.32*	1.64	0.80	0.04
Residual	38	0.71	0.75	0.78	0.80
Total	39	0.78	0.78	0.78	0.78
'b''		-0.10	-2.80	-0.03	0
S.E		0.84	0.87	0.88	0.89

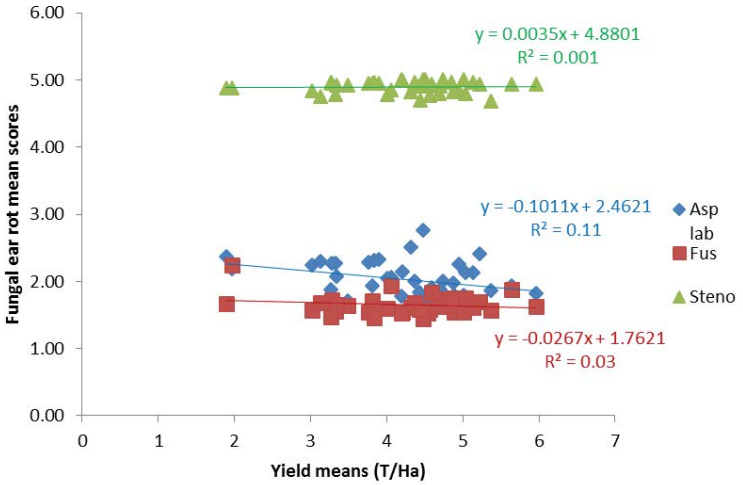

Figure 2. Regression of *A. flavus, F. graminearum* and *S. maydis* mean scores on the yield means among the test crosses (multi-location evaluation).

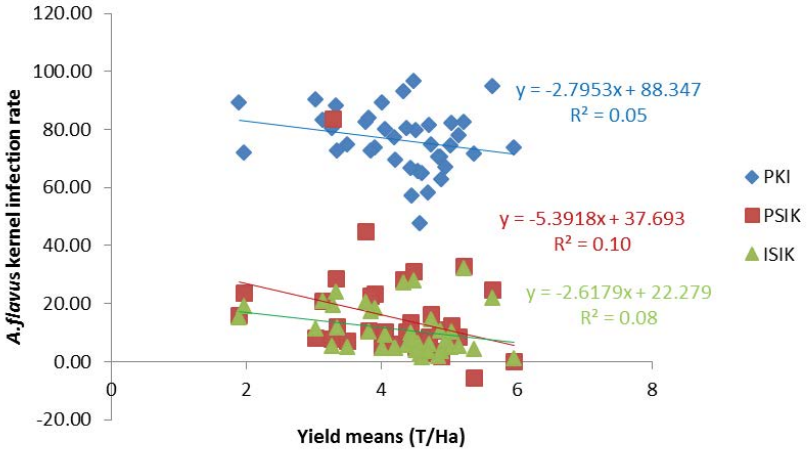

Figure 3. Regression of *A. flavus, F. graminearum* and *S. maydis* mean scores on the yield means among the test crosses (multi-location evaluation).

Table 2. Analysis of variance for regression of *A. flavus, F. graminearum* and *S. maydis* on yield values among the single crosses (multi location)

Source of Variations	df	Asp lab	PKI	Fus	Steno
Regression	1	0.45	0.65	0.35	0.03
Residual	69	0.67	0.67	0.83	0.84
Total	70	0.67	0.67	0.83	0.83
‘b’’		0.09	-2.80	0	0
S.E		0.82	0.82	0.91	0.92

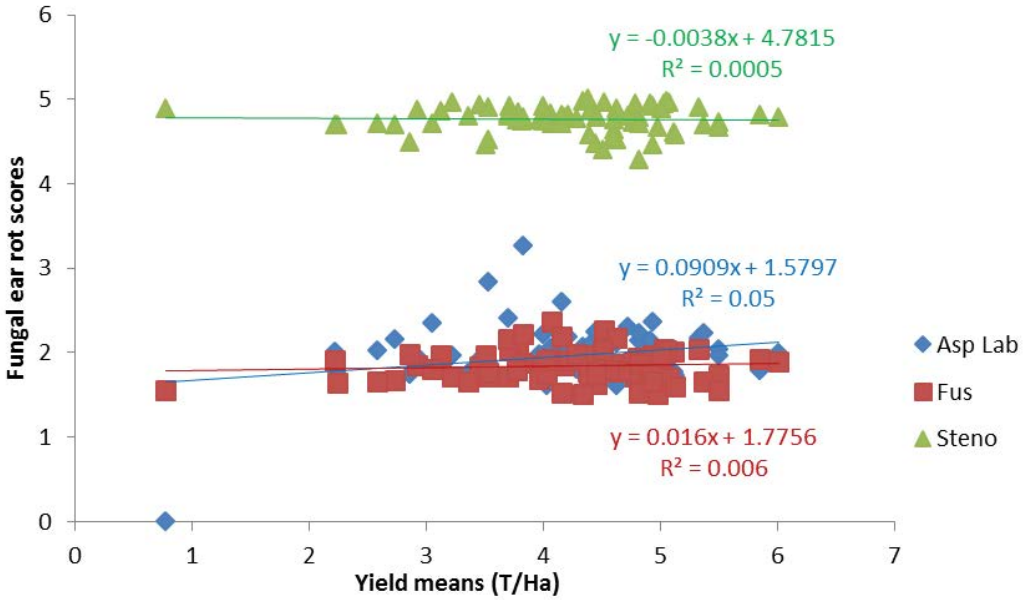

Figure 4. Regression of *A. flavus, F. graminearum* and *S. maydis* mean scores on the yield mean among the single crosses (multi-location evaluation).

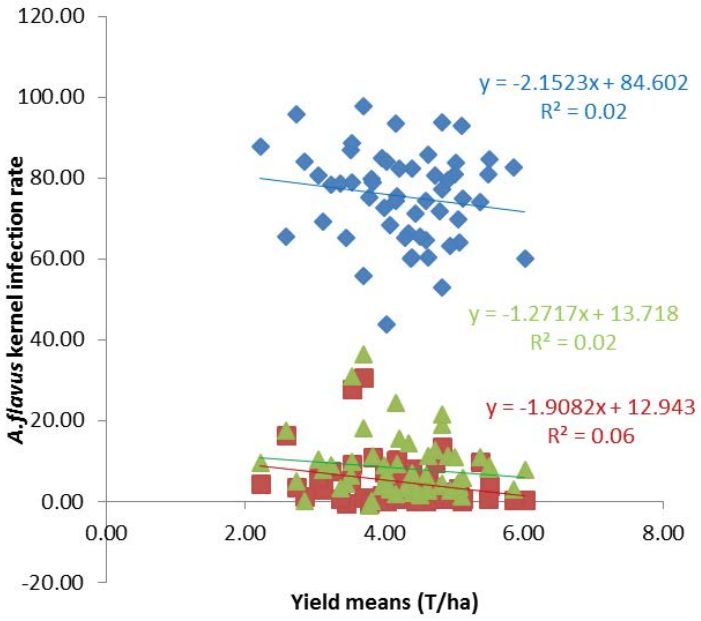

Figure 5. Regression of *A. flavus* kernel infection mean scores on the yield means among the single crosses (multi location)s.

Research application

Resistance breeding is aimed at effectively selecting and producing disease resistant varieties. To ensure effective adoption of the resultant variety, other agronomic traits and special attributes like yield potential need not to be neglected. Regression of the three ear rot mean scores on mean yield scores across locations for both the test and single cross hybrids revealed low relationship (R^2) of yield mean variation being attributed to any of the three disease scores. These low yield variations in response to fungal ear rot infection suggest that maize grain yield cannot be reliably predicted from any of the three ear rot pathogen infection scores. Therefore, results of this study reveal that the three fungal ear rot disease infections had negligible effects on maize grain yield for the hybrids evaluated. This suggests that multiple resistance to the three pathogens is inherited independently of yield performance.

Recommendations

In general, results from this study show that it is difficult to breed high yielding and resistant genotypes to ear rot as defined by Nagy and Cabulea (1996). It can be concluded that resistance to the three ear rots should be directly evaluated and improved by separate and independent breeding strategies. These breeding efforts should be supplemented by other yield and disease resistance enhancing strategies such as fertiliser application, proper crop management practices within breeding programs. Additionally, the lack of significant correlations between the ear rot diseases and yield suggest a possibility of simultaneous recombination of these two important traits in a breeding program.

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