1. Introduction

• The quantification of fungal biomass is crucial in understanding the interactions between host plant susceptibility or resistance to a fungal pathogen as well as identifying competition between individual fungal species during disease progression [1-2].
• qPCR is used to detect and quantify the fungal biomass in various plant host tissues [1–2].
• High level of sensitivity of qPCR enables the measurement of very low infection titres which could correspond to the amount of fungal spores present at the time of infection or during latent, non-infectious infections.
• The use of aflatoxic  A. flavus strains for biocontrol is directly connected with methods for detecting aflatoxins as it confirms that the biocontrol agent reduces aflatoxin contamination.
• The levels of aflatoxin in foods and feeds are strictly regulated [3,4]. This requires rapid, sensitive, quantitative and reliable methods for aflatoxin detection at various stages of the food chain [4,5].
• Bioanalytical techniques TLC, HPLC & LC-MS/MS is widely used in aflatoxin detection and is considered the gold standard for aflatoxin detection [4,5].

2. Objectives

1. To test and develop a qPCR assay to quantify the amount of A. flavus biomass in infected maize tissues.
2. To use the qPCR assay to investigate the aflatoxic maize lines KDV1 and KDV4 grown in different regions of Kenya, contribute to increasing or limiting the biomass of KSM014, an aflatoxic strain.
3. To evaluate the selected methods ability to differentiate symptomatic and aflagtal A. flavus.
4. To investigate the in-vivo bioassay of maize kernels colonized by aflagtal A. flavus strain KSM014, (NCBI accession MG685139) as a biocontrol agent to minimize aflatoxin contamination by the toxigenic A. flavus strain KSM014 (NCBI accession MG685138).
5. To determine the aflatoxic maize infected by KSM014 and KSM014 infected maize by KSM014.

3. Materials & Methodology

5. Conclusion

• The qPCR assay was developed as a suitable method to quantify the A. flavus biomass in maize infected maize plants compared to the ET0-gene and both were specific for A. flavus.
• The MEP primers were specific for maize and had no cross reaction with A. flavus DNA.
• The specificity of the qPCR assay for A. flavus biomass quantification makes it potentially useful tool for screening of A. flavus maize lines for resistance to A. flavus and associated breeding strategies, identifying potential symptomatic infections and help to understand the mechanism of maize defense responses to A. flavus infection.

We found that maize line, which is cultivated in Kenya region, is environment it’s more susceptible to A. flavus infection than ET0 maize line grown in other regions of Kenya. This, susceptibility may be one of the possible reasons for the frequent cases of aflatoxicosis in maize in Hnil and Kumi, Homa Bay and recently at Nandi, Kenya.

We showed that a biocontrol strategy using the aflagtal A. flavus isolate KSM0212, was able to inhibi aflatoxin production by the aflagtal A. flavus isolate KSM014 after co-infection of maize kernels at 50:50 ratios. These findings might be important for future development of a biocontrol system appropriate for aflatoxin mitigation.

4. Results & Discussion

1. The qPCR assay analysis showing fungal load of A. flavus KSM014 in the root and shoot tissue of AFB1 infected maize lines respectively. Fungal biomass was measured in infected maize plants at 10, 20 and 28 days post inoculation (dpi) (Fig. 1).
   • The fungal load of A. flavus KSM014 in the root of AFB1 infected maize lines within 14 days were the A. flavus KSM014 gene was used for fungal quantification against AFB1 infected maize lines within 14 days.
   • The threshold cycle (Ct) values were plotted against the logarithm of the starting fungal biomass.
   • The fungal load of A. flavus KSM014 in the shoot of AFB1 infected maize lines within 14 days.
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2. The qPCR assay analysis showing fungal load of A. flavus KSM014 in the shoot of AFB1 infected maize lines within 14 days at the fungal load of A. flavus KSM014 gene was used for fungal quantification against AFB1 infected maize lines within 14 days.
   • Test residues (P < 0.05). Aflatoxin indicates significant and higher fungal load levels on maize lines (Fig. 2).

3. In vitro biocontrol trials to examine maize kernels colonized by aflagtal A. flavus strain KSM014, (NCBI accession MG685139) as a biocontrol agent to minimize aflatoxin contamination by the toxigenic A. flavus strain KSM014 (NCBI accession MG685138).

3. Materials & Methodology

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1. Cultures of fungi: Obtained from [4]. Five A. flavus isolates: KSM012, KSM014, HK02, H826, H827 were grown on both aflagtal maize (ATCC & NaOCl) media in non-inoculated medium (YPD) for 72 ± 30 °C (Fig. 3).
2. Maize cultivars: KDV1 and KDV4 were purchased from KALRO, Nairobi, Kenya.
3. Seed sterilization and A. flavus infection: 20 seeds were sterilized with 100% EtOH, NaOCl & H2O2 and were inoculated (in triplicate) with 20 ml conidia suspensions (1 x 10⁶ conidia ml⁻¹).
4. Primers and primers selection: A. flavus isolate KSM014 was used to select fungus-specific qPCR primers.
5. PCR amplification: Cycling conditions: 1 cycle at 94°C for 3 min, 35 cycles at 94°C for 30 s, at 60°C for 45 s, at 72°C for 30 s and 90°C.
6. A. flavus standard, standards curves and fungal quantification: 200 ng AF1, AF2, AF21, AF20, AF21 ng and PG1 ngAF1 were used. The threshold cycle (Ct) values were plotted against the logarithm of the starting quantity of the template. Efficiencies of amplifications were generated from the slopes of the standard curves (slope = 2).
7. Linear regression curves were drawn, and the qPCR efficiency was calculated as E = 10⁻¹/(slope-1).

The amount of target DNA in an unknown sample was extrapolated from the respective standard curve. Metabolite extraction: TRL, HPLC and optimization
6. qPCR analysis of maize bioassay and maize bioassay line.
7. Statistical analysis: GraphPad Prism ANOVA, TMT& Post-test for linear trend analysis

8. TACN – co-infected with both alf and aflag isolates of A. flavus

Table 1: Specific primers used for total fungal and strain-quantification

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<tr>
<th>Primers</th>
<th>Membrane (M)</th>
<th>Membrane (MP)</th>
<th>Membrane (MIC)</th>
<th>Membrane (MIN)</th>
<th>Membrane (B)</th>
<th>Membrane (B-ET0)</th>
<th>Membrane (B-KSM014)</th>
<th>Membrane (B-KSM014)</th>
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<td>100</td>
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Standard deviations of mean differences in aflatoxin levels were calculated as a measure of variability in efficacy. The efficiency (E) of each isolate was calculated as: E = 10⁻¹/(slope-1).

References