Detection of fungal contaminated maize kernels by NIR hyperspectral imaging and multivariate data analysis

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Introduction

Fusarium verticillioides, a pathogen of maize (*Zea mays* L.), is a concern primarily because it produces secondary metabolites toxic to animals and humans.[1-3] *Fusarium verticillioides* is an endophyte[4-8] and is capable of inhabiting plants without visible disease symptoms.[9] Thus, if the fungal contamination of maize plants and kernels is not detected early, contaminants in the form of mycotoxins can enter the food chain. Many methods have been utilised to determine fungal contamination and the presence of fungi on cereals. Conventional methods include microbiological techniques (diagnostic media and microscopy) or immunological methods for detection of the toxin. However these techniques are often time-consuming, labour intensive, produce harmful by-products and are usually expensive. The aim of this work was to detect fungal contamination of maize kernels prior to the appearance of visual symptoms and to monitor chemical changes associated with fungal activity using near infrared (NIR) hyperspectral imaging and multivariate data analysis techniques.

Materials and Methods

Samples

White maize kernels of intermediate hardness were used for the study. To completely remove both surface and internally borne fungi, the kernels were surfaced sterilised by first rinsing in a 70% ethanol solution, then a 1% NaOCl solution followed by rinsing with sterile distilled water (dH_2O). Thereafter, kernels were imbibed in sterile dH_2O for 4 hrs, soaked in a water bath at 60°C for 5 min[10] and left to dry in a laminar flow for 1 h. A subset was taken for use as controls. Fungal spore suspensions were prepared from petri dishes cultured with *Fusarium verticillioides* (MRC 0826) kindly supplied by the Department of Plant Pathology, Stellenbosch University. Prior to spore preparation, *F. verticillioides* (MRC 0826) was cultured on potato dextrose agar and incubated at 28°C. After 7 days, autoclaved water with Tween 20 (3 drops L^{-1}) was used to wash the spores from the agar. The spore suspension was poured through two layers of sterile cheesecloth to remove mycelium and thereafter the suspension was adjusted to 10^6 spores mL^{-1} using a haemocytometer. Finally, the kernels were inoculated by being dipped into the spore suspension for 30 s and subsequently being left to dry. Afterwards, kernels were placed in petri dishes (20 kernels per plate) and incubated at 28°C for five days.

Near infrared spectroscopy

Hyperspectral images were acquired with a SisuCHEMA short wave infrared (SWIR) camera (Specim, Spectral Imaging Ltd, Oulu, Finland). The camera comprised an imaging spectrograph coupled to a 2-D array mercury-cadmium-telluride (HgCdTe) detector. Images were acquired within the spectral range of 900–2500 nm at 10 nm resolution, spectral sampling per pixel of 6.3 nm and a field of view of 100 mm × 100 mm. Images of the entire petri dish were collected at predetermined time intervals after inoculation; contamination was prevented by not removing the lid. In total there were nine time points, T0–T8, corresponding to 0, 17, 20, 23, 26, 40, 43, 69 and 90 h post-inoculation. The time intervals were supposed to be evenly spread but technical issues made this impossible. White and dark references were captured prior to each sample image and were subsequently used for image correction and calibration. For the white reference, a 100% standard (Spectralon, Labsphere, North Sutton, NH, USA) was used while for the dark reference the shutter was closed. Images were analysed using Evince hyperspectral image analysis software package (v.2.5.0; UmBio AB, Umeå, Sweden) and MATLAB (v.7.10; The MathWorks, Natick, MA, USA). The image calibration and correction to absorbance was done automatically in the Evince software package. The individual images for each treatment (including the control) were merged in sequence of collection time to form a mosaic image (684 × 1600 pixels × 242 wavelengths). Sample T5 was later removed from the mosaic due to problems arising from instrument malfunction following irregularities in power supply. A principal components analysis (PCA) model with six components was calculated on each of the mosaic images. Using

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the brushing technique,[11-14] all irrelevant pixels were removed and PCA was recalculated on the cleaned images. PCA score plots, score images and loading line plots were used to locate and identify regions of interest.

Results and Discussion

When plotting principal component four (PC4) against PC5 (0.49% & 0.34% explained variance) three distinct clusters were apparent in the score plot (Figure 1). In the score image of PC4 (Figure 2) there is a distinct decrease in score values from control kernels to T8. This is illustrated by the change in colour from red in the control samples to blue in T8. By assigning each cluster to a class in the score plot and projecting it onto the score image, classification plots were constructed (Figures 3 & 4). The green cluster in the classification plot is associated with the control samples in the classification image; the black cluster is associated with T0 and T1, while the red cluster is associated with the rest of the time intervals. In the classification plot (Figure 3) there is a change in degree of infection in the direction of positive to negative PC4 score values. There is a change from the control samples (non-infected), the green cluster, to T0 and T1 (black) and then to those that have been infected for longer (red).

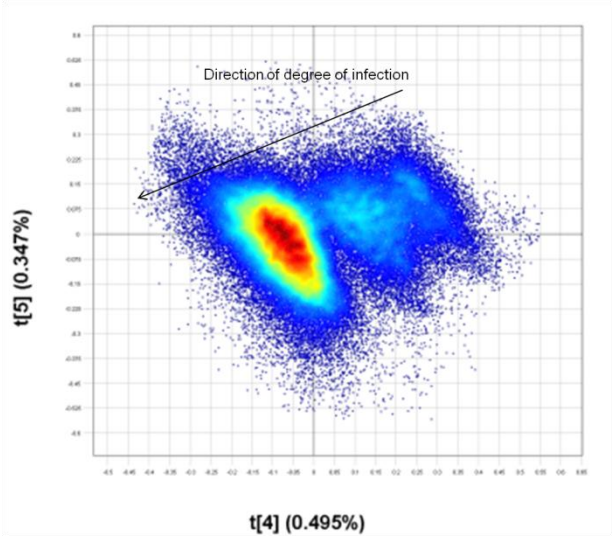

Figure 1. PCA score plot of PC4 vs. PC5 (0.49% and 0.34% explained variance) showing three clusters.

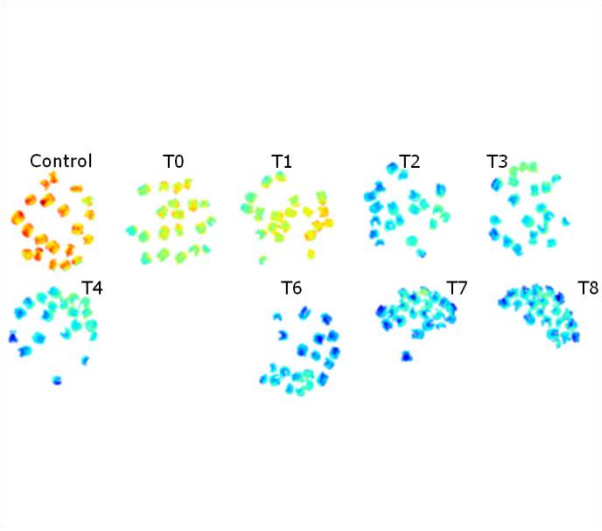

Figure 2. Score image of PC4 showing decrease in score values from control to T8.

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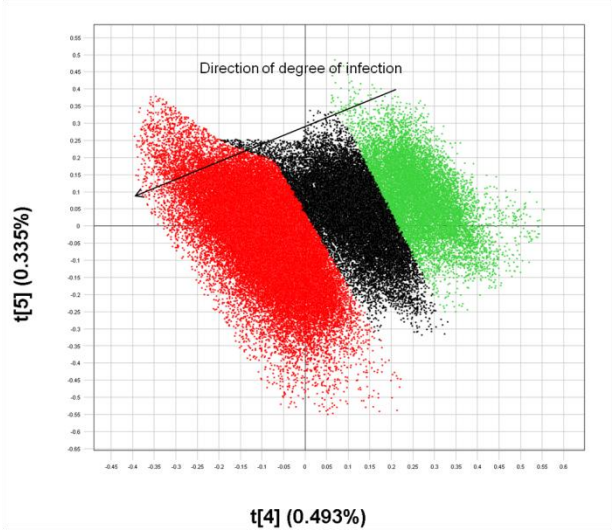

Figure 3. Classification plot of PC4 vs. PC5 with classes and degree of infection in the direction of PC4.

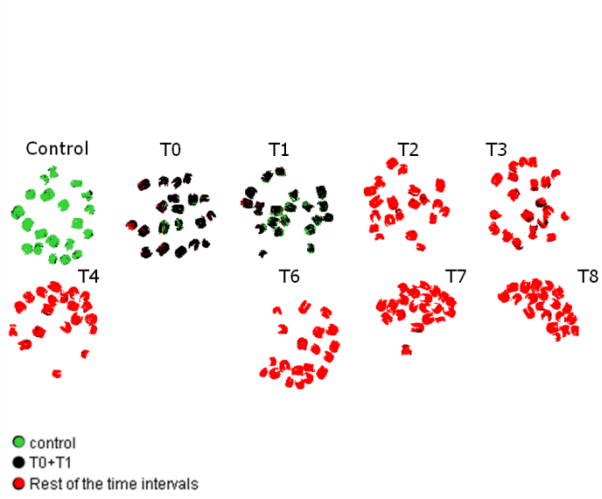

Figure 4. Classes projected onto the score image showing the control (green), T0 and T1 (black), and the other time intervals up to 90 hours post-inoculation (red).

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The loading line plot for PC4 (Figure 5) showed two prominent positive peaks at 1405 and 1900 nm. The peak at 1405 nm, an O-H stretch first overtone attributed to an ROH structure, is most likely associated with starch. The peak at 1900 nm, an O-H stretch + 2 × C-O stretch combination mode, is also attributed to starch. It is clear that the main cause of variation is related to starch, most likely attributed to a decrease in levels of stored food reserves in the kernels as incubation time increases. It is known that *Fusarium* infection depletes food reserves for fungal growth.[15]

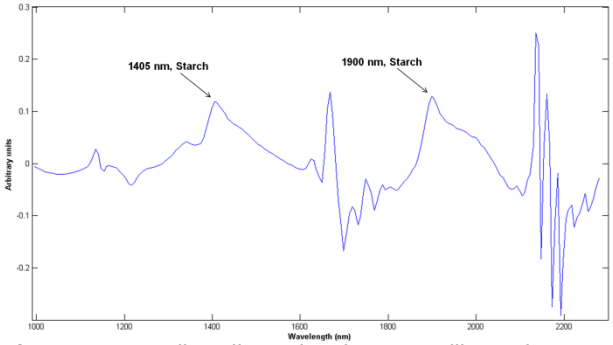

Figure 5. Loading line plot for PC4 illustrating two prominent positive peaks: 1405 nm, an O-H stretch first overtone attributed to an ROH structure most likely associated with starch; 1900 nm, an O-H stretch + 2 x C-O stretch combination mode associated with starch.

Conclusion

Using NIR hyperspectral imaging it was possible to monitor changes in fungal infected maize kernels over time. PCA models, without pre-processing, showed three distinct clusters making it possible to discriminate between the control, T0 and T1, and the rest of the time points. Variables identified as central to this study were 1405 nm, an O-H stretch first overtone attributed to an ROH structure, most likely starch, and 1900 nm, an O-H stretch + 2 × C-O stretch combination mode also associated with starch. More systematic future experiments are needed to confirm this but it can already be concluded that early detection of infection is possible.

Acknowledgments

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