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Detection of *Fusarium* head blight contamination in wheat kernels by multivariate imaging

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ABSTRACT

The objective of this study was to evaluate the safety quality of wheat kernels and more specifically the infection of wheat by *Fusarium culmorum*. We have developed a new non-destructive methodology based on multispectral imaging and chemometrics to detect wheat contamination. This method has been applied to evaluate the tolerance to *Fusarium* Head Blight (FHB) on six accessions of *durum* wheat. Two of the accessions are parental lines, one susceptible and one resistant to *Fusarium*, and the other four are their offspring lines.

Multispectral images of dorsal and ventral sides of all kernels were acquired with an in-house imaging system and processed with chemometrical tools, including principal component analysis (PCA) and multiple linear regression (MLR). According to the quantitative PCR (qPCR) results, PCA was able to detect infested kernels and to identify affected areas with kernels. Moreover, regression analysis of the images allowed the degree of contamination for each pixel to be estimated, and thus mapping of contamination for each kernel.

1. Introduction

Fusarium head blight (FHB) mainly affects wheat kernels such as bread and durum wheat, and its prevalence has been increasing for two decades (Gilchrist & Dubin, 2002). Crop rotation with maize or other cereals, and reduced tillage could be the main reasons for the increased occurrence of toxigenic *Fusaria*. The economic impact of *Fusarium* damage is of great importance throughout the world (Windels, 2000). FHB is caused by several *Fusarium* species; wheat kernels are mostly infected by 2 species *Fusarium graminearum* and *Fusarium culmorum*. After harvesting, the presence of areas with diffuse pink or white coloration characterize contaminated grains (Bar-L'Helgouac'h, 2001). Contamination can occur at different stages of grain filling, with the severity decreasing more or less with grain size, thus correlating with the date of inoculation and fungal pressure. Consequently, FHB reduces both grain grade and yield by as much as 50% (Gilchrist & Dubin, 2002). Grain quality is also compromised by *F. culmorum*, due to increased toxic fungal metabolites known as mycotoxins. These are mostly deoxynivalenol (DON), a type B trichothecene, and zearalenone (ZEA) (Visconti & Pascale, 2010).

In order to protect consumers from the risks related to these mycotoxins, the European Commission has set a maximum concentration of 100 μg/kg for ZEA, and 1250 μg/kg and 1750 μg/kg for DON in unprocessed bread and durum wheat respectively for human consumption (European Commission, 2007). The application of these kinds of regulations has increased the number of controls in the cereal industry including animal feeding processes in Europe since infected kernels can cause diseases in animals too (Atoui, El Khoury, Kallassy, & Lebrihi, 2012).

Many analytical methods have been developed for assessing the presence of *Fusarium* spp. and DON levels in harvested grains. Gas- and liquid-chromatography has been widely used to quantify DON and its derivatives (Simsek, Burgess, Whitney, Gu, & Qian, 2012). Analysis of DON in cereals has also been carried out by Enzyme-Linked ImmunoSorbent Assay (ELISA) directly after sample extraction (Abouzied, Azcona, Braselton, & Pestka, 1991; Maragos, Busman, & Sugita-Konishi, 2006). Recently, detection and

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quantification of *F.usarium* spp. have been successfully performed by polymerase chain reaction (PCR) in maize samples (Atoui et al., 2012) and in wheat samples (Ben Amar, Oueslati, Ghorbel, & Mliki, 2012; Hamada, Yin, & Ma, 2012; Pinson-Gadais, Monmarson, Gregoire, Barreau, & Richard-Forget, 2007). This method is highly specific, very sensitive, and fast to implement in comparison to microbiological and chemical methods that are tedious and expensive for evaluating fungal contamination. Quantitative real time PCR allows species or toxin-specific primers to be used, and is applicable to individual kernels at a terminal development stage, thus providing accurate and efficient quantification of contamination by *Fusarium* (Rios, Pinson-Gadais, Abecassis, Zakhia-Rozis, & Lullien-Pellerin, 2009; Terzi et al. 2007; Burlakoti et al., 2007). Furthermore, many studies have shown a positive correlation between *Fusarium* DNA levels and toxin levels (Demeke et al., 2010; Fredlund et al., 2008; Kulik, Jestoi, & Okorski, 2011; Lindlab et al., 2013). Real time PCR is an effective tool for predicting mycotoxin levels in grain lots. Using an alternative method, Eifler et al. (2011) proposed measuring the volatile compounds produced by *Fusarium* species (detected by electronic nose) to identify infected wheat kernels. Although these chemical and molecular techniques are relevant and accurate, they are time consuming and expensive.

In order to rapidly detect head scab in wheat kernels some authors have proposed using Near Infrared spectroscopy either in reflection (Dowell, Ram, & Seitz, 1999; Dvořáček, Prohasková, Chrpová, & Štočková, 2012) or in transmission mode with relatively accurate predictive values and calibration errors (De Girolamo, Lippolis, Nordkvist, & Visconti, 2009). Another spectroscopic technique, diffuse reflectance UV–visible, has also been applied to measure DON levels in scab-damaged wheat (Siuda, Balcerowska, Kupcewicz, & Lenc, 2008). An exhaustive review of the advantages and disadvantages of these methods applied to the analysis of trichothecene has been published by Lattanzio, Pascale, and Visconti (2009). This review emphasized that these accurate screening techniques were complementary.

Coupling spectroscopy with imaging analysis provides information allowing localizing a target product at the surface of the sample. Digital image analysis offers a potential increased analytical throughput, and these imaging systems are being used more frequently in automated inspection quality control. Wiwart, Suchowilska, Lajszner, and Graban (2012) acquired grayscale images of wheat and spelt using a scanner in which they extracted shape and color parameters to discriminate these cereals. In their review, Buschmann, Langsdorf, and Lichtenthaler (2000) presented the possibility of using multispectral fluorescence imaging to detect plant diseases. By acquiring multiple images at selected wavelengths in the UV–Visible–NIR ranges for one sample, multispectral imaging was shown to be particularly relevant in plant sciences (characterization and health status).

Chlorophyll is a widely studied compound, and has been quantitated by multispectral imaging in order to detect plant reactions to the tobacco mosaic virus (Chaerle, Lenk, Hagenbeek, Buschmann, & Van Der Staeten, 2007). In another article (Lenk et al., 2007), the same authors demonstrated the potential of LEDs as a light source for an imaging system, and pointed out that blue and UV lights are well-adapted to the detection of chlorophyll fluorescence. In a recent study, Olesen, Cartensen, and Boelt (2011) combined multispectral imaging with a classification algorithm to separate uninfected spinach seed from seeds infected by several fungi including *Cladosporium* spp and *Fusarium* spp.

Hyperspectral imaging (HSI) - imaging with a spectral dimension (visible or infrared) - is particularly well-suited to the analysis of the outer part of grains, and was used to characterize grain viability (McGoverin et al., 2011), and to detect glassy and floury endosperm, which was not possible with existing methods (Williams, Geladi, Fox, & Manley, 2009). This technique has been successfully used for the detection of fungal contamination in maize kernels by *Fusarium verticillioides* (Williams, Geladi, Britz, & Manley, 2012). Using Chemometrics such as Principal Component Analysis (PCA) or Partial Least Squares (PLS) regression, the authors showed that it was possible to characterize the changes in fungal infected maize kernels with or without a pre-sterilization treatment, and to predict the degree of infection. In the same way, Del Fiore et al. (2010) applied Visible HSI to detect fungi on maize kernels and demonstrated that imaging can be faster and more accurate than traditional methods. Analysis of *Fusarium* damage in wheat kernels was also performed by HSI and Linear Discriminant Analysis (LDA). Shahin and Symons (2011) successfully detected varying degrees of *Fusarium* damage, using hyperspectral imaging over 450–950 nm, and predicted *Fusarium* damage with accuracy of 86%. Delwiche, Kim, and Dong (2011) compared and combined two spectral ranges (Visible 400–1000 nm and NIR 1000–1700 nm) to explore damage in wheat kernels. They were able to identify *Fusarium*-damaged kernels with accuracy higher than 92%, and concluded that NIR was slightly better and more informative. Nevertheless, as their classification was based on visual grain inspection, recognition of contaminated grains without visible symptoms from healthy grains remains the main limitation of these findings. Similar findings have been published by Polder, Van Der Heijden, Waalwijk, and Young (2005), on single wheat contaminated by *F. culmorum*. The results of this study showed that the spectral data from the shortwave infrared (SWIR) region performed better than the VIS region to detect *F. culmorum*, especially for highly contaminated wheat kernels (Q^2 PLS 0.80 versus 0.69). The importance of the SWIR region (>1100 nm) was also underlined by Wang et al. (2014), as they were able to detect a range of Aflatoxin-B1 concentrations when applied to maize kernel surfaces. Thus, hyperspectral imaging provides a non-destructive method to detect non healthy grains, fungal load on grain at maturity, as well as mycotoxin concentration. Recently, Bauriegel, Giebel, and Herppich (2011) compared hyperspectral imaging (wavelength range: 400 nm to 1000 nm) with chlorophyll fluorescence imaging to early detect FHB in winter wheat ears, using a post image processing called Spectral Angle Mapper. This method compares the experimental image spectra to a reference spectrum, and determines the similarity between two spectra by calculating a vector angle between them.

Because hyperspectral cameras, such as an SWIR camera, are prohibitively expensive and not suited to high-throughput routine testing, the development of more simple and affordable devices is warranted. In our laboratory, we have designed and developed a simple imaging system that couples a CDD camera with LEDS illumination, which have been previously described (Chevallier, Bertrand, Kohler, & Courcoux, 2006; Jaillais, Perrin, Mangavel, & Bertrand, 2011). We then assessed whether such a system could be used to detect FHB on single wheat kernels, and allow the level of kernel contamination to be measured. The results obtained by imaging were calibrated and validated against real time PCR measurements of the same samples.

2. Material and methods

2.1. Raw plant material

The plant material was composed of two durum wheat accessions and their four offspring lines segregating for their *Fusarium* spp. resistance. The susceptible parent was a French registered cultivar (“*Silur*”), and the resistant (“*Dic2*”), an accession of *T turgidum dicoccum*. Parental genotypes and the four offspring lines (two susceptible and two resistant) were grown under field