

Application of morphometric image analysis system to evaluate the incidence of fusarium head blight wheat infected kernels

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Abstract: *Fusarium Head Blight (FHB) is a disease of complex aetiology affecting winter cereals worldwide. The disease has a great impact on yield, but mostly on health, due to the ability of several Fusarium species involved in the diseases to produce mycotoxins dangerous to human and cattle. Grading of cereals for industry by means of visual estimation of disease incidence is not always accurate in predicting mycotoxin levels in wheat samples. Rapid and accurate analyses of large wheat samples by means of non-destructive methods are needed. Among several techniques available, image-analyses, taking into account the entire external kernels shapes, have been developed. Preliminary experiments using durum wheat kernels artificially infected with Fusarium graminearum and F. culmorum have been performed in Italy. Samples were acquired with a high resolution scanner. After an automated thresholding procedure performed by an edge detection Sobel filtering, 90 points (x, y) equally angularly spaced (one point every 4°) from the centroid were digitized along the outline. Elliptic Fourier analysis was performed to extract shape data to be analyzed via partial least squares discriminant analysis. Three classes of infection were considered: healthy, shrivelled and chalky. F. culmorum infection is better distinguishable than F. graminearum. Percentages of correct classification resulted to be 68.4% for the F. culmorum infection and 56.9% for the F. graminearum.*

1 Introduction

Fusarium head blight (FHB), also known as scab, is a fungal disease that occurs in several winter cereals worldwide. The disease has a great impact on yield, but mostly on health, due to the ability of several *Fusarium* species involved in the diseases to produce mycotoxins, particularly deoxynivalenol (DON), dangerous to human and cattle. These pathogens cause yield and quality losses due to sterility of the florets and formation of fusarium damaged kernels (FDK) typically shrivelled, light in weight and white or pink in colour (BUSHNELL *et al.* 2003). The relationships between FDK, fungal biomass

and DON content have been extensively studied, but the results are sometimes conflicting (PAUL *et al.* 2005). Most of these methods are destructive, time consuming, expensive and laborious. The proposed Optoelectronic non-contact systems are based on near infrared spectroscopy (Nir) (DOWELL *et al.* 1999). The aim of this work is to suggest a quantitative, non-destructive, cost-effective and rapid image analysis method, based on i) simple low-cost greyscale CMOS video camera for profile acquisition; ii) Elliptic Fourier analysis (EFA) of the kernel outlines; iii) multivariate classification techniques (i.e. PLSDA), to discriminate three different classes of FHB wheat infected kernels in order to correlate estimated and actual FDK.

2 Materials and methods

Data collection

Fusarium infected wheat kernels were obtained by artificially inoculating durum wheat plants, of highly FHB susceptible cultivars Simeto, grown at the CRA-PAV farm (Rome, Italy). At flowering, plants were sprayed with a suspensions of *Fusarium culmorum* (ER-1480) or *F. graminearum* (ER-1481) at the concentration of 1×10^5 conidia ml⁻¹ and then covered with plastic sheets for two days after inoculation. At harvest, kernels inoculated were visually grouped in three classes of FHB infection on the basis of their shape (healthy, shrivelled and chalky kernels covered by white mycelium). A total number of 613 kernels, randomly disposed on the plane of a scanner, were acquired with a high resolution scanner (600 d.p.i. 24 bit color). An automated thresholding procedure was performed by an edge detection Sobel filtering in order to detect the edges. Consequently 90 points (x, y) equally angularly spaced from the centroid (one point every 4°), were automatically digitized along the outlines using the software Matlab (rel. 7.1; PLSToolbox Eigenvector b 4.0) (**Figure 1**).

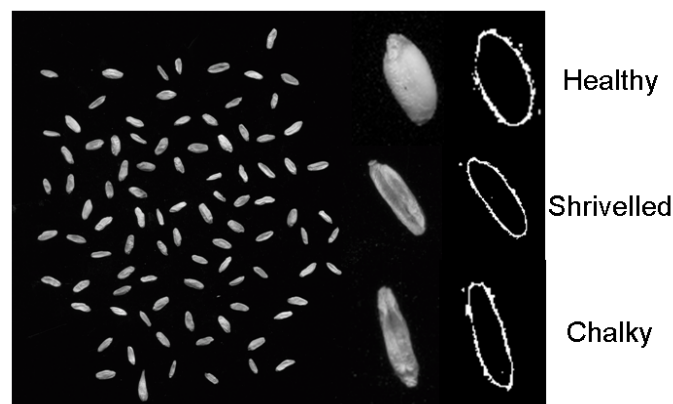


Figure 1: On the left side: samples randomly disposed on the plane of a scanner. On the right side from the top to the bottom: kernels at the three classes of infection (healthy, shrivelled and chalky) before and then the automated outlines acquisition.

Data processing

The overall shape of the artificially infected kernels was studied by EFA on the outline coordinates previously extracted. EFA is based on the separate Fourier decompositions of the incremental changes of the x - and y -coordinates as functions of the cumulative chordal length of an outline polygon. The kernels shape closed contour is expressed in terms of harmonically related trigonometric curves. For each harmonic equation, two Fourier coefficients are computed for both the x - and y -projections thus, the total number of coefficients is $4n$, where n is the number of harmonics fitted to the outline (CRAMPTON 1995). The total number of harmonics for any outline is equal to half of the total number of outline coordinates (the 'Nyquist frequency'). The Fourier series was truncated at the value of k at which the average cumulative power is 99.999% of the average total power (MENESATTI *et al.* 2008). The harmonic coefficients, automatically extracted with Matlab, describe the size, shape and orientation of each harmonic ellipse form the input to multivariate statistics. The classification procedure of the three different classes of infection (healthy, shrivelled and chalky), and affected by the two pathogens, was performed in Matlab on the shape variables (X-block; EFA coefficients) and the shape data were analyzed *via* partial least squares discriminant analysis (PLSDA) (COSTA *et al.* 2009, MENESATTI *et al.* 2008). The X-block values were pre-processed with different Matlab procedures. For the analysis each group was subdivided in: i) 75% of specimens for the class modelling and validation; ii) 25% of specimens for the independent test. PLSDA (SABATIER *et al.* 2003) is a partial least squares analysis (PLS) regression where the response variable is categorical (Y-block; infection status) expressing the class membership of the statistical units.

3 Results and discussion

For the kernels lateral profile the correct number of harmonics (13) was calculated on the whole samplings ('Nyquist frequency' = 45). The PLSDA model performed on EFA coefficients for kernels affected by *Fusarium culmorum* is based on 16 Latent Variables (LV), presents a preprocessing for the X-block as absolute value and a Root Mean Square Error of Calibration (RMSEC) of 0.294. The total percentage of correct classification is 68.4%. **Table 1** shows the results relative to the independent test (25%) of the classification procedure performed by PLSDA. In this case the intermediate class (shrivelled) is poorly represented and badly classified (21.1%).

Table 1: Confusion matrix relative to the independent test (25%) of the classification procedure of the three different classes of infection (healthy, shrivelled and chalky) affected by *Fusarium culmorum*. The mean (%) represents the percentage of cases correctly allocated. The percentage of cases correctly allocated derived by relating the number of cases correctly allocated to the class (in bold) to the total number of cases of that class (Total).

Kernels affected by <i>Fusarium culmorum</i>					
	Chalky	Shrivelled	Healthy	Total	Mean (%)
Chalky	21	0	2	23	91.3%
Shrivelled	5	4	10	19	21.1%
Healthy	2	1	39	42	92.9%

The PLSDA model performed on EFA coefficients for kernels affected by *F. graminearum* is based on 7 LV, presents a “gls weighting” preprocessing for the X-block and a RMSEC of 0.401. The total percentage of correct classification is 56.9%. **Table 2** shows the results relative to the independent test (25%) of the classification procedure performed by PLSDA. The “healthy” class is the best classified (65.6%). The results suggest that *F. culmorum* infection is better distinguishable than *F. graminearum*, probably because this specie is more aggressive than *F. graminearum* and is able to produce stronger damages on kernels.

Table 2: Confusion matrix relative to the independent test (25%) of the classification procedure of the three different classes of infection (healthy, shrivelled and chalky) affected by *Fusarium graminearum*. The mean (%) represents the percentage of cases correctly allocated. The percentage of cases correctly allocated may be derived by relating the number of cases correctly allocated to the class (in bold) to the total number of cases of that class (Total).

Kernels affected by <i>Fusarium graminearum</i>					
	Chalky	Shrivelled	Healthy	Total	Mean (%)
Chalky	6	3	3	12	50%
Shrivelled	6	11	3	20	55%
Healthy	1	10	21	32	65.6%

4 Conclusions

This work was developed to verify the technical and methodological feasibility of the proposed method in order to evaluate the infective incidence of FHB. The shape analysis appeared an important technique to discriminate different classes of FHB wheat infected kernels. The results obtained will be validated on a higher numbers of both, artificially and naturally FHB infected wheat seed lots. Image analysis allows a higher speed of analysis, an increase of the evaluated seeds number and the repeatability of the measure and overpassing the subjectivity of the expert view.

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