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Application of near infrared spectroscopy technology for the detection of fungicide treatment on durum wheat samples

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ABSTRACT

The feasibility of Near Infrared Spectroscopy to detect fungicide treatment on wheat samples was assessed. A total of 213 durum wheat samples from four different trial sites in Andalusia (southern Spain), with different agroclimatic conditions (soil, temperature, rainfall) were selected for been analyzed on VIS+NIR (400 nm–2500 nm) and NIR (1100 nm–2500 nm). Different mathematical pre-treatment on the signal (scatter correction and derivatives) were evaluated for their discrimination accuracies. Using MPLS, the selected models obtained 84% of well classified samples.

1. Introduction

Durum wheat is an important crop in the Mediterranean area. The main uses are in human food products, like bread, pasta and couscous [1].

Andalusia is the leading region producing durum wheat in Spain. It contributes more than 74% of the total national production [2].

Wheat genotypes, agronomics conditions and fertility inputs are the foremost factors determining durum wheat yield and quality characteristics [3]. Nevertheless, an important bounding aspect of durum wheat is the damage caused by diseases. Two of the most important are, above all, leaf rust and septoria leaf spot (incited by *Puccinia triticina* and *Septoria tritici*, respectively). Plant diseases are greatly influenced by environmental factors, including known stresses as deficiencies of essential nutrients and/or toxicities of other mineral elements [4]. Modifications in cultural practices, such as direct sowing, use of Nitrogen fertilizers and irrigation, may contribute to an increase on the disease severity [5]. *Puccinia triticina* is the most common rust of wheat. It has affected wheat for thousands of years. Yield losses in wheat from *Puccinia triticina* infections are usually the result of decreased numbers of kernels per head and lower kernel weights [6].

Methods used to fight fungal diseases and in the development of new fungicides in cereals, are based on etiological and epidemiological knowledge. The presence of a particular fungal disease is related to the degree of susceptibility of the variety, presence of inoculum, plant phenological status and climatological factors, especially those associated with humidity [7].

When infective fungus parts get accumulated on the grain surface; enzymes destroy proteins, starch granules and grain cell walls [8].

Nowadays, consumers are more conscious of eating high quality products free of toxic agents. Increased food scrutiny requires the development of improved and more readily available analytical methods for food products authentication and detection of contaminant [9–12]. Near Infrared Spectroscopy (NIRS) has been used for the determination and quantification of proximate quality parameters on food (protein, fat, sugar) and for the recognition of transgenic foods [13].

The objective of this work was to evaluate NIR technology to detect differences between durum wheat seed samples coming from plants which have been treated with fungicide and those coming from non treated plants, using discrimination models.

2. Materials and methods

2.1. Experimental design

All the durum wheat samples came from trials carried out on randomised complete block designs with four replications. It is the most common design used in field trials. Crop management

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on trials was the standard used by farmers on the area. Experimental plots were 12 m^2 (10 m × 1.20 m).

2.2. Wheat samples

The 213 wheat samples from the 27 durum wheat varieties were provided by the Andalusia Network of Agrarian Experimentation (RAEA), managed by the Andalusian Institute of Agricultural Research and Training (IFAPA). Samples originated from four different trial sites located in Jerez (Cadiz), Camino de Purchil (Granada), Tomejil (Seville) and Santaella (Cordoba), each having quite different agroclimatic conditions and grown 2009–2010 seasons, were used.

Durum wheat seeds were sent to IFAPA Alameda del Obispo, Cordoba, in paper bags containing approximately 500 g, and then they were kept on small 250 g plastic containers. Two samples of every variety were received, one, coming from fungicide treated plants (T) and the other from plants free of it (O), 105 and 108 sample of each respectively.

2.3. Fungicide treatment

Fungicide treatment against leaf diseases, as the leaf rust Roya and Septoria leaf spot (incited by *Puccinia triticina* and *Septoria tritici*, respectively) consisted of one application with a concentrated suspension of 12.5% p/v of epoxiconazol, Lovit (Basf España S.A.).

A dose of 1 L ha^{-1} (the maximum recommended dose) was applied at the phenological phase of flag leaf unfolded. When the crop was in stage 39 of the code of Weber and Bleiholder [14], flag leaf stage: flag leaf fully unrolled, ligule just visible [15–16]. The security time limit of 42 days before harvest was followed.

2.4. Chemical analysis

Traditional reference methods were used to compare the quality parameters of both groups of samples. For total content of crude protein the Kjeldahl method was used (Panreac B.O.E 19-7-1977 and 20-7-1977). Moisture was determined by the Panreac air oven method (B.O.E 19-7-1977 and 20-7-1977). Gluten index in order to determine water insoluble protein the Panreac official method was followed (B.O.E 19-7-1977 and 20-7-1977). Finally, total weight of 1000 wheat kernels was performed with Numigral I.

2.5. NIR spectra

The spectra were recorded on a *Foss NIRSystems* (model 6500 Foss-NIRSystems, Inc., Silver Spring, MD, USA) in reflectance mode, over a wavelength range between 400 and 2500 nm (Visible and NIR region), measured in a 2 nm steps.

Intact grain samples were placed in a cuvette of 16.5 × 3.5 cm, with a quartz window (Sample Cell NR-7080) showed in Fig. 1. Two spectra per sample were obtained. The cell was filled with about 20 g, scanned and finally returned to the container and mixed with the remaining sample (approximately 480 g). The process was repeated again to obtain the second sample spectrum. To avoid packing variations, only one analyst did sample preparation.

Optical density was stored as log (1/R), where R is the reflectance energy recovered by a split detector system with silicon (Si) between 400 nm and 1098 nm and a lead sulfide (PbS) between 1100 nm and 2500 nm. A personal computer with the software ISIscan (v2.81; Infrasoft International LLC. Port Matilda now State College, PA, USA) was used for the operation of the spectrometer, and to store and manage optical data.

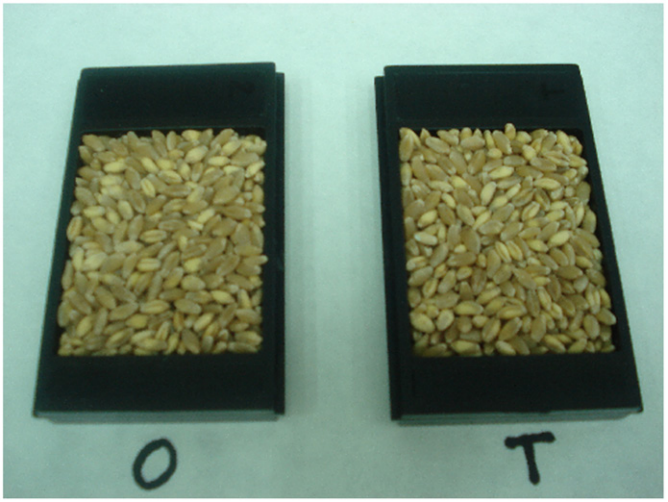

Fig. 1. Display of wheat intact grains in the cuvette. Wheat with and without treatment (T and O).

2.6. Statistical analysis and discriminant equations

2.6.1. Root Mean Squared (RMS)

Filtering of the subsamples spectra was done by calculating the RMS for this sample presentation form.

The following expressions were applied to calculate RMS values:

$$\mathrm{RMSj} = \sqrt{\frac{\sum_{i=1}^{n}(Y_{ij} - \overline{Y}_j^{\,2})}{n}}$$

$$\mathrm{STD} = \sqrt{\frac{\sum_{j=1}^{n}(RMSj)^2}{n-1}}$$

$$\mathrm{STD_{Limit}} = 1.036x\sqrt{\frac{\sum_{k=1}^{k=m} STD_k^2}{m}} = 1.036x\sqrt{\overline{STD}^2}$$

where n is the number of data (absorbance readings), m is the number of samples, Y_{ij} is the absorbance value log (1/R) for subspectrum j at wavelength i (λ_i) and $\overline{Y}$ is the absorbance value log (1/R) for the average spectrum of a sample at wavelength i (λ_i).

The $\mathrm{STD_{Limit}}$ (Standard Deviation Limit) values were used to obtain $\mathrm{RMS_{Limit}}$. Once the spectra of samples that exceeded the cut-off limit were eliminated, other spectra were obtained on the same sample, obtaining new RMS values. If the new RMS value exceeded the limit again, this sample was marked as not suitable to be included in the calibration set [17].

2.6.2. Calibration and validation sets

The sample set used in the study (185 samples after RMS) was split into a calibration set containing 158 samples (80% of the total) and a validation sample set comprising 25 samples (20%). This splitting was carried out in a random way by WinISI software.

WinISI III (v1.50e, Infrasoft International LLC) software was used for spectral data analysis and development of chemometric models.

2.6.3. Principal Component Analysis (PCA)

Prior to classification models, Principal Components Analysis (PCA), an orthogonal transformation that enables a subspace of R^d to be obtained with a minimum loss of information [18] was carried out. Twenty-four Math Pretreatments were applied to spectra to develop the PCA as a result of combinations of derivative (0, 0, 1; 1, 4, 4; 2, 4, 4; 2, 10, 5), scatter correction (SNVD, Standard Normal Variate and Detrend; MSC, Multiplicative Scatter Correction) and spectral range (VIS–NIR; NIR).

The qualitative difference between varieties and removal outliers was done with a standardized Mahalanobis (GH) distance. Distances between each sample and the population center greater than 3 are marked as possible spectral outlier [19]. A sample was eliminated if it appeared as anomalous repeatedly in the different math treatment mentioned.

2.6.4. Partial least squares modified (MPLS)

The discriminant model was built using Modified Partial Least Squared (MPLS), in winISI software; this assigns to each spectrum a value called a "dummy" variable (or discriminant variable). The new variable obtained, acquired a value between 1 (samples in the group with no fungicide treatment) or 2 (with fungicide treatment group). The discriminant variable limit established for group selection was ≥ 1.5 [20]. Which means: samples with a value < 1.5 is included in one group (in this case are O samples) and samples with a high value of 1.5 belong to the other group.

A maximum of twelve PLS terms were selected, if the model selected the 12 PLS terms, the process was repeated with two more terms to avoid overfitting effect. Internal cross validation (with five cross validation groups) was used in order to estimate the final number of PLS terms. Using a cross validation with five groups, on the first pass, the samples of group 1 are used for the validation, and those of the remaining four groups are used for the actual calibration. In pass 2, group 2 is used for the actual calibration; in pass 3, group 3, and so on [21]. The Math treatments used in both cases were the same as applied in PCA analysis.

The criteria used to select the best models were: Coefficient of determination of calibration (R^2), Standard Error of Cross-Validation (SECV) and % of samples correctly classified.

3. Results and discussion

3.1. Prior analysis

When Fungus leave infection is produced early in the season, often prevents the development of the grain. When foliar fungus infection appear, the photosynthesis area decreases which normally means a drop in the amount of protein, starch and kernels size and weight. However, once the grain has been filled, and subsequently infection occurs, its size will not be affected, even the color and appearance.

Fig. 2 represent the mean spectra [log 1/*R*] of *T* and *O* samples. In the 1200–1318 nm, 1440–1880 nm and 1920–2498 nm wavelength regions there were small differences which might be due to different chemical composition inn wheat samples. Burns [22] reported that the 1940 nm peak is related with moisture in flour, while 2180 nm was assigned to protein absorption, 2150 nm area has been used for protein [23] and for starch 2100 nm. Delwiche and Hareland [24] indicated that the region 1130–1190 nm was a stable region for defining a difference that could be used in classifying normal and scab damaged kernels.

3.2. Reference analysis

At first sight there is no evidence of any modification in kernels size or color by fungus infection. In order to find kernels matrix differences, chemical analysis were performed. There were not important differences between both groups in moisture or gluten index, being both groups compensated in a similar percentage. On the other hand, in protein and 1000 wheat kernels weight showed remarkable differences.

Figs. 3 and 4 represent the difference between T and O samples. When the difference appears in the positive part means that the

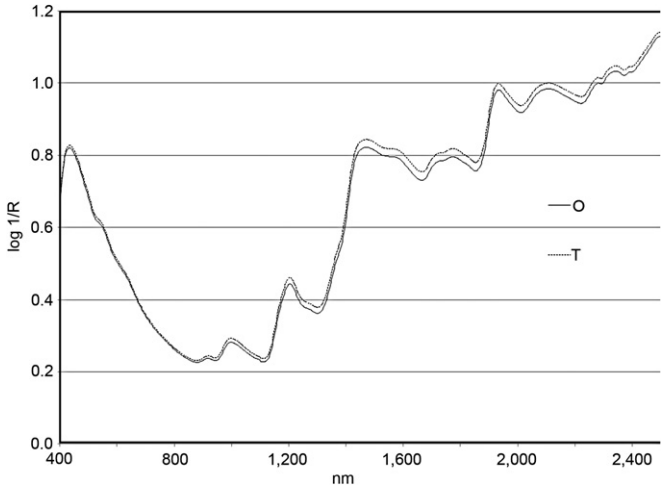

Fig. 2. Average spectra of treated (-) and untreated (- -) samples.

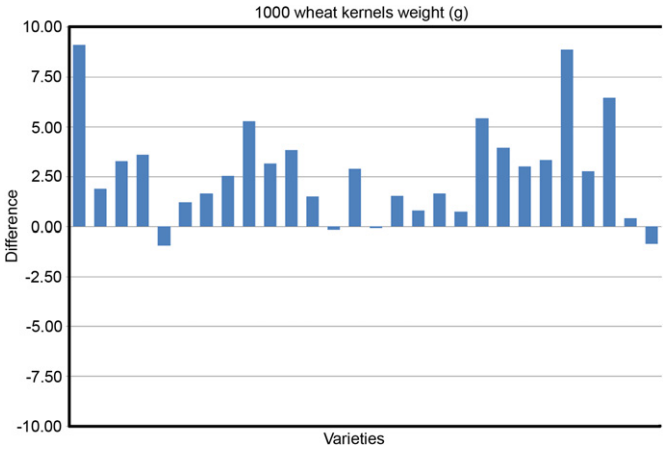

Fig. 3. Difference between T and O samples in weight of 1000 wheat kernels (grams).

measure of T samples is higher than in O samples. As can be seen there is an evident dominance in both parameters in T samples.

Thus, the parameters protein and thousand grain weight (indicators of the quality of the grain) were higher in treated samples (*T*), mainly due to the effect that caused the fungicide that prevents the onset of disease.

3.3. RMS

In order to obtain the RMS cut-off value, the method described in Section 2.5 was followed. An individual value for each sample was calculated setting a maximum value (RMS cut off) of 10000. There was a tiny difference between the limit value of T and O samples with a value of RMS limit of 8000 and 9000 respectively.

Samples which surpassed the RMS cut-off limit were scanned again following the steps detailed in Section 2.5. Finally a total of 12.5% (14 samples) of *T* and 14.7% (16 samples) of *O* were eliminated from whole group.

3.4. PCA

To extract initially spectra information and qualitative differences between all samples, data analysis was carried out applying the limit criteria discussed in Section 2.4. After all the mathematical treatments, only one spectrum was eliminated (T sample). WinISI program picked 9 factors to cover 99.97% of the explained variance.

Before MPLS analysis of the calibration group the accumulative reliabilities of the first 3 PCs were 99.18%, the fourth PC contributed an additional 0.45% of the total variance, the fifth 0.10% and 0.24% the remaining four. This means that the most important information and spectra features were included in the three first PCs.

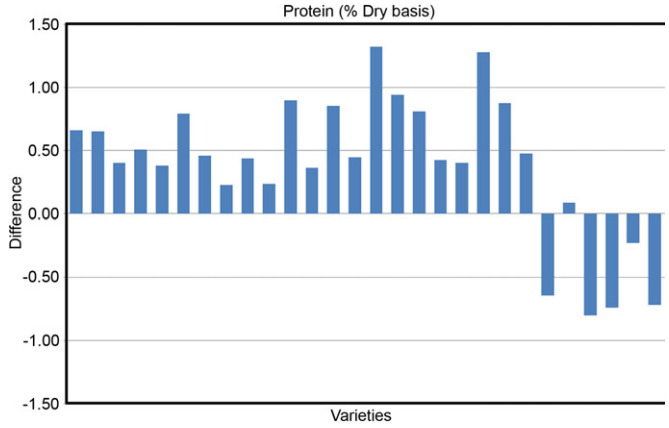

Fig. 4. Difference between T and O samples in wheat % protein (dry basis).

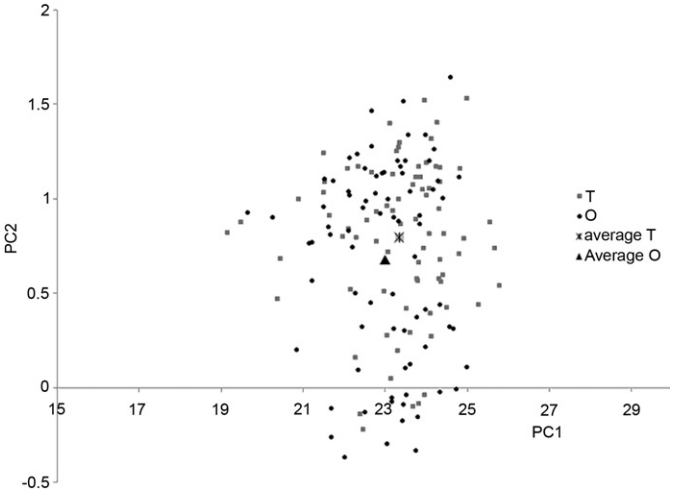

Fig. 5. Principal Component Analysis: first PC versus second PC on T (treated) and 0 (non treated) samples.

Using the PCA scores, there was no separation between T and O groups and their corresponding centroids (Fig. 5).

3.5. MPLS

MPLS discriminant models were developed. The dummy variable was set as a referent values for the O and T group. The specification set was 1 for O samples and 2 for T samples.

A maximum of 12 PLS factors and 5 groups of cross validation were used in all the PLS models. After all cross validation passes and with the individual statistical parameter of each group, the number of factors for the smallest error is established. The number of factors required on the spectral MPLS analysis was 7–8 depending on the mathematical treatment applied.

A blind test with 25 samples (12 T and 13 O) was carried out in order to obtain an external validation. With appearance not difference between both groups of samples (T and O samples), high percent of correctly classified were obtained. Table 1 shows the best results obtained on the reflectance mode in NIR and VIS+NIR regions. The best discriminant model was that obtained using the derivative treatment MSC 2,4,4, which displayed a SECV of 0.34, R^2 of 0.78 and with an external validation of 84% of samples correctly classified. Despite those final values of the models for both regions result very similar, higher R^2 and lower SECV were obtained when the VIS was included.

Lou et al. [25] and Chandra et al. [26] developed discrimination models to assign categories of wheat kernel damage using a color machine and hyperspectral image. Their results were similar to those of the Foss NIR system 6500.

Fig. 6 shows a linear representation of the external validation. Quadrant T (T samples) displays one sample belonging to group O; this sample could present a higher resistance to fungus infection which means less modification of the chemical composition. On the other hand, quadrant O presents three samples misclassified, samples coming from treated plants but appearing in the region on untreated samples. Menniti et al. [27] reported the ineffectiveness of epoxiconazol at controlling some other fungal diseases, which could also be the case in the plants from which these samples come from.

T samples, including as in the group of O, can be due to a failure of treatment which is not 100% effective and therefore has caused disease in some plants. Conversely, the O samples included in the T group may be because some varieties or growing conditions have allowed disease does not develop in some untreated plants, which are included in the treated group. In addition to these justifications must be added the error of the model itself.

Table 1
Parameter values of the best models developed for VIS+NIR and NIR regions.

Spectral range	*Math treatment*			*Classification matrix*				*External validation*
	Scatter	*Derivative*	*PLS factors*	O	T	R^2	*SECV*	(% Correctly classified)
VIS+NIR (400–2500 nm)	None	2, 4, 4	8	***61***	16	0.76	0.35	0.84
				19	***62***			
	SNV+DT	2, 4, 4	7	***65***	9	0.74	0.35	0.84
				15	***69***			
	MSC	2, 4, 4	8	***67***	10	0.78	0.34	0.84
				13	***68***			
NIR (1100–2500 nm)	None	2, 4, 4	8	***67***	14	0.71	0.37	0.80
				13	***64***			
	SNV+DT	2, 4, 4	12	***67***	16	0.84	0.39	0.76
				13	***62***			
	MSC	2, 4, 4	8	***66***	14	0.72	0.36	0.80
				14	***64***			

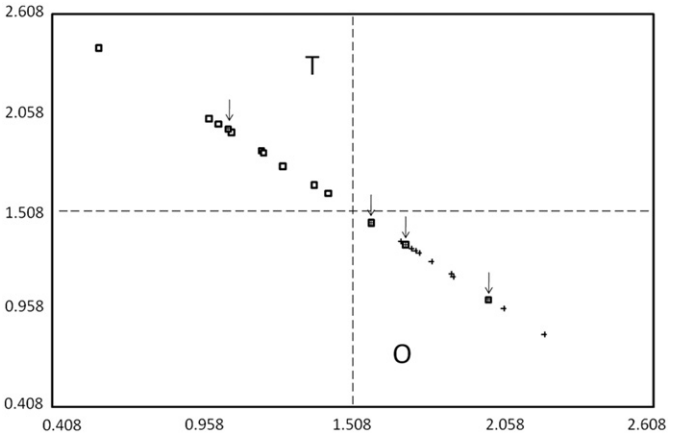

Fig. 6. Samples misclassified on the external calibration group.

4. Conclusions

The results obtained in this study showed that the application of Near Infrared Reflectance Spectroscopy could be a useful and fast method to use for the assessment of fungicide treatment on durum wheat. We developed a model using 158 samples of durum wheat seeds coming from plants with and without fungicide treatment. This model displayed an accuracy of 84%. These results were obtained using samples from only one crop season. Currently we are in the process of obtaining models applied to further cropping seasons to assess the reproducibility of the method.

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