GMO DETECTION USING NIR AND CHEMOMETRICS

J. A. Fernández Pierna, V. Baeten, Ph. Vermeulen, Th. Buhigiro, G. Berben, E. Janssen & P. Dardenne

Walloon Agricultural Research Centre (CRA-W), Quality of Agricultural products Department,
Chaussée de Namur, 24, 5030 Gembloux, Belgium
fernandez@cra.wallonie.be

INTRODUCTION

Several studies indicate that genetically modified (GM) crops can be identified by some physical means. In the present work, the aim is to assess the possibilities and limitations of some physical method as the near-infrared (NIR) spectroscopy and the NIR hyperspectral imaging methodology [1].

The potential of the NIR and NIR hyperspectral techniques for the detection and quantification of GMO is evaluated for on site use, cost effectiveness and non-invasiveness together with chemometric tools in order to discriminate for barley and soybean between GMO and non GMO grains.

HYPERSPECTRAL IMAGING

The instrument used consists of a near-infrared (NIR) camera which combines the advantages of spectroscopic and microscopic methods. [2, 3] This imaging spectrometer gathers spatial and spectral (and therefore chemical) information simultaneously by recording sequential images of a pre-defined sample; each image of the absorbances is collected at a single wavelength. The instrument used at the CRA-W includes a near-infrared imaging system, a power supply and a workstation. It is operated using the MatrixAcquire and ISys Data Analysis software from Malvern Instruments Ltd (Malvern, UK).

Reflectance images were collected in the 900 - 1700 nm window, with an increment of 10 nm. The result is a hyperspectral cube of cells giving per pixel (x, y) and per increment of wavelength (z), the obtained reflectance. The imaging spectrometer gives 240 x 320 pixels (76 800 spectra), and utilises a liquid crystal tuneable filter (LCTF) for wavelength selection. In the configuration used, the sample area analysed is approximately 5 cm², allowing simultaneous analysis of 10-15 grains by image.

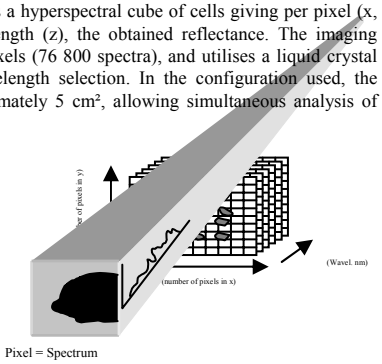


Figure 1 - a) Measuring the samples using the NIR hyperspectral system and b) the hyperspectral cube obtained.

EXPERIMENTAL - SOYBEAN

The first study corresponds to samples collected in the framework of the **KeLDA** (**Ke**rnel **L**ot **D**istribution **A**ssessment) project [4] consisting in a study on the distribution of GMO in large soybean shipments. The samples used here come from three ships and have GM contents (Roundup Ready) ranging between 25 and 100%. Around 300 spectra of bulk soybeans were collected in reflectance mode using a NIR spectrometer Brucker MPA. Data treatment of the spectral data collected was performed by supervised PLS-DA (Partial Least Squares – Discriminant Analysis) to differentiate the different boats. Table 1 shows the classification error (in %) for both cross-validation and external test set for the different models (boat 1 *vs.* the rest, boat 2 *vs.* the rest and boat 3 *vs.* the rest).

Boat	10 PLS
1	8,2
2	2,4
3	6,2

Cross-validation

Boat	10 PLS
1	15,5
2	2,8
3	10,4

External validation

Boat 1	10 PLS
false positive	2
false negative	29

Boat 2	10 PLS
false positive	3,7
false negative	2

Boat 3	10 PLS
false positive	11
false negative	10

Table 1- PLS-DA results (classification error in %) for the soybean samples a) using leave-one-out cross-validation and b) for a external validation set.

The results have shown that a correct discrimination can be performed according to the ship. In order to certificate the results a second study is being performed where some kernels from each ship are selected and measured with NIR and with PCR analyses performed in parallel.

The authors would like to thank Dr. Wendy Harwood from the John Innes Centre, UK for providing part of the barley data set.This work is performed in the framework of the **Co-Extra** FP6 project (GM and non-GM supply chains: their **CO-Ex**istence and **TRA**ceability - FP 6 Priority 5, Project number: 007158). For more info: http://www.coextra.eu

EXPERIMENTAL - BARLEY

The second study consists of barley grains. Barley has been added as for this crop we could analyse transgenic lines and their isogenic non transgenic counterpart (material obtained from the John Innes Centre, UK). In total 22 lines were available with 5 transgenic ones. From each line, 10 grains have been measured twice. In total 440 spectra were collected coming from 2 different origins (200 from UK and 240 from Belgium), several varieties with some of them being transgenic (in total 100 transgenic spectra were available). Figure 2a shows an image of the grains obtained with the NIR camera at a certain wavelength. Figure 2b is a mask performed on the previous figure in order to determine the mean of each grain. This mask is based on the different absorbances of the pixels from the figure. Each grain covers the surface of +/- 3500 pixels. The mean spectrum of each grain is calculated by averaging the spectra of all these pixels.

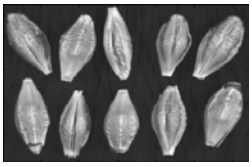
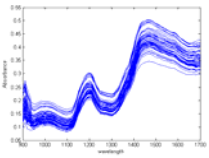


Figure 2 - a) Image of barley grains obtained with the NIR camera and b) mask of the image used to calculate the mean spectrum of each grain.



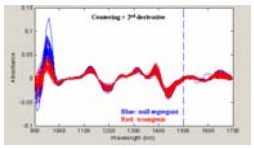


Figure 3 - a) Mean NIR spectra obtained for the barley grains b) corrected spectra.

Data treatment of the spectral data collected was applied using the unsupervised method PCA after mean centering and the second derivative. The results are shown, in the way of score plots, in Figures 4. Figure 4a shows a distinction according to the origin of the samples. The rest of the figures show, for each individual variety, the PCA results differentiating between transgenic and non transgenic samples.

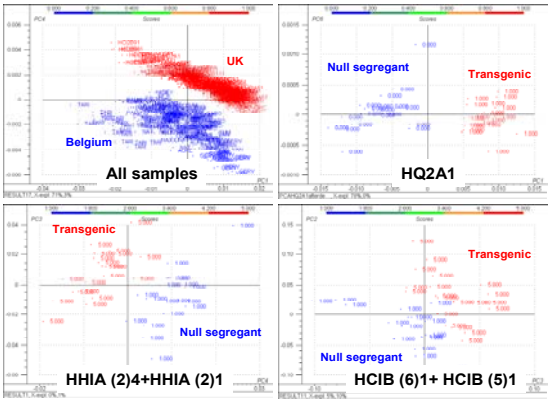


Figure 4 - PCA results; a) differentiating according to the origin, b) c) d) for each variety, differentiating according to the presence or not of transgenic.

CONCLUSION

The different analyses performed during this preliminary study allow us to demonstrate the interest of the use of a NIR or NIR hyperspectral system combined with chemometrics for the kernel study.
Next to a merely qualitative detection, another study is ongoing to determine the quantification potentialities of the method with respect to Roundup Ready content.

REFERENCES

[1] Hurburgh, C. R. and G. R. Rippke (2000). '*Detection of genetically modified grains by near infrared spectroscopy*'. PITTCON 2000 - Science for the 21st century, New Orleans, LA.
[2] Baeten V. and Dardenne P. (2005). '*Applications of NIR spectroscopic imaging to agricultural, food and feed monitoring*', chapter in Spectrochemical analysis using multichannnel infrared detectors (Eds. Levin and Barghava, Blackwell Publishing).
[3] Baeten V., Fernández Pierna JA. and Dardenne P. (2006) '*Hyperspectral imaging techniques: an attractive solution for the analysis of biological and agricultural materials*', chapter in Techniques and Applications of Hyperspectral Image Analysis (Eds. Hans F. Grahn & Paul Geladi, in press).
[4] Paoletti C., Heissenberger A., Mazzara M., Larcher S., Grazioli E., Corbisier P., Hess N., Berben G., Lubeck P.S., De Loose M., Moran G., Henry C., Brera C., Folch I., Ovesna J., Van den Eede G. (2006) '*Kernel lot distribution assessment (KeLDA): a study on the distribution of GMO in large soybean shipments*', Eur. Food Res. Technol.

walloon Agricultural Research Centre