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Differences between conventional and glyphosate tolerant soybeans and moisture effect in their discrimination by near infrared spectroscopy ☆

Lidia Esteve Agelet [a,*], Paul R. Armstrong [b], Jasper G. Tallada [b], Charles R. Hurburgh Jr. [c]

[a] Department of Agricultural and Biosystems Engineering, Iowa State University, United States
[b] Engineering and Wind Erosion Research Unit, USDA-ARS, Center for Grain and Animal Health Research, 1515 College Ave, Manhattan, KS, United States
[c] Department of Agriculture and Biosystems Engineering, Iowa State University, United States

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ABSTRACT

Previous studies showed that Near Infrared Spectroscopy (NIRS) could distinguish between Roundup Ready® (RR) and conventional soybeans at the bulk and single seed sample level, but it was not clear which compounds drove the classification. In this research the varieties used did not show significant differences in major compounds between RR and conventional beans, but moisture content had a big impact on classification accuracies. Four of the five RR samples had slightly higher moistures and had a higher water uptake than their conventional counterparts. This could be linked with differences in their hulls, being either compositional or morphological. Because water absorption occurs in the same region as main compounds in hulls (mainly carbohydrates) and water causes physical changes from swelling, variations in moisture cause a complex interaction resulting in a large impact on discrimination accuracies.

1. Introduction

Genetically modified (GM) organisms have been manipulated to avoid diseases, to enhance resistance to herbicides, and to increase their nutritional value. Not all world markets fully accept GM products however, for a variety of reasons regarding the introduction of new allergens, possible development of antibiotic-resistant bacteria strains and environmental biodiversity issues (Cohen, Chang, Boyer, & Helling, 1973; Bakshi, 2003). Many countries have set regulations for identification, quantification, and appropriate labeling of products containing GM organisms. Roundup® is a popular glyphosate-based herbicide intended to kill a broad variety of plants on contact. Roundup® application in crops used to be only possible at certain developmental stages without direct application (Benbrook, 2009). The development of herbicide resistant crops reduced these restrictions. The patenting and marketing of Roundup® resistant crops, licensed with the name of Roundup Ready®, was initially done by Monsanto in 1996 (Patent EP 546090). Using genetic recombinant DNA technology, genetic material from the bacteria *Agrobacterium tumefacien* was introduced to the crop genoma, conferring the crop a high tolerance to the herbicide. This removed many restrictions on Roundup® use, lowered production costs and increased crop yields (Schenepf, 2003).

Soybeans (*Glycine max* L.) were the first Roundup Ready® (RR) crop to be introduced into markets in 1996. They rapidly displaced conventional soybeans because of advantages for crop management and yields, and currently account for more than half of the soybean fieldcrops around the world (Konduru, Kruse, & Kalaitzandonakes, 2008). RR soybeans are widely accepted in the global markets; they are one of the two currently accepted GM varieties of soybeans in Europe, which has the most restrictive laws regarding GM importation. But despite of their acceptance in many markets, they must be labeled as a GM crop, even if they are present as adventitious contamination in conventional batches whenever their percentage exceeds pre-established thresholds. Current thresholds of adventitious GM contamination in conventional soybeans for feeding purposes range from 0.9% for Europe to 5% for Japan and Taiwan. In the case of Europe, the tolerance limit applies to contamination of recognised GM events; otherwise, the threshold is reduced to 0.5% if the events are proven safe, even if not politically accepted.

Determination of GM contamination levels for large shipments is challenging. Current methods are time consuming, complex, and not suitable for rapid on-site measurements because of laboratory-based analysis. The analyses are divided into protein-based methods and DNA-based methods. Protein-based methods such as Enzyme-Linked Immuno Sorbent Assay (ELISA) use specific antibodies, which require previous knowledge of the GM to be

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* Corresponding author. Address: 1545 Food Science Building, Iowa State University, Ames, IA 50014, United States. Tel.: +1 515 294 8319.
E-mail address: esteve.lidia@gmail.com (L. Esteve Agelet).

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analysed, but are quicker, cheaper, and simpler than DNA-based methods (Konduru et al., 2008). DNA-based methods are laboratory sensitive, expensive and slow; the lowest practical limit of detection of GM DNA material is around 0.1% (Miljuš-Djukić et al., 2010). These methods are destructive meaning that even in the case they could perform faster in an on-line setting, only a small portion of the sample could be analysed. This leads to the added problem of taking representative samples from large shipments, proven to be a function of grain type and the threshold to be analysed (Hübner, Waiblinger, Pietsch, & Brodmann, 2001).

Near infrared spectroscopy cannot be used to analyse trace elements or genetic information, but it can measure changes in structure or concentration of organic compounds that can be the fruit of the phenotypic expression of genes. Several researches reported that no significant differences in concentration of major biochemical compounds (protein, oil, or fiber among others) exist between conventional and their corresponding transgenic counterparts (Harrigan et al., 2010; McCann, Liu, Trujillo, & Dobert, 2005; Taylor, Fuchs, MacDonald, Shariff, & Padgette, 1999) . However, some researchers suggest differences in minor compounds such as length of chain acids (Jimenez, Bernal, Nozal, Toribio, & Bernal, 2009) or other unintended (pleiotrofic) effects from the genetically modification of RR which may be noticeable in specific varieties or specific environmental conditions. For instance, RR crops were found to suffer higher weight loss under water shortage (Gertz, Vencill, & Hill, 1999). Any of those side-effects from introducing the gene of RR resistance may have lead Roussel et al. to use Near Infrared Spectroscopy (NIRS) to discriminate RR and conventional soybeans by NIR transmittance with notable success (Roussel, Hardy, Hurburgh, & Rippke, 2001). Over 3000 bulk soybean samples were scanned from each class (RR and conventional) by transmittance instruments. Non-linear classification methods such as locally weighted principal component regression (LW-PCR) and artificial neural network (ANN) were used to achieve classification accuracies of 93% and 88% respectively, with validation and training sets from a single crop-year and combining two spectrophotometers. Two recent studies were carried out to analyse the discrimination by diffuse reflectance NIRS at single seed level. Lee and Choung (2011) carried out a feasibility study involving 10 samples of conventional soybeans (50 seeds per sample) and 10 herbicide resistant soybeans (50 seeds per sample). Accuracies of 97% were achieved utilising NIR and visible (VIS) radiation with Partial Least Squares Discriminant Analysis (PLS-DA), although major spectral differences between the two classes in the study arise in the VIS region. A more recent study by Agelet, Gowen, Hurburgh, and O'Donell (2012) was conducted involving three reflectance NIR technologies, over 240 samples from several crop-years (over 3000 beans total), and two validation sets: (1) new seeds from samples represented in the training set, and (2) a validation set with seeds from new samples, not included in the training set. Algorithms used for discrimination were those from the first study with bulk samples and transmittance NIR: LW-PCR and ANN (Roussel et al., 2001). Best discrimination accuracies were achieved with LW-PCR when beans for validation belonged to samples represented in the training set (up to 94%), similar to Lee and Choung (2011) results. Results were worse with ANN (lower 80% range). Those results were very similar to those of Roussel et al. for bulk samples (Roussel et al., 2001). When new beans in the validation set were from samples not represented in the training set, classification accuracies dropped to the mid 70% on average. However, a few questions remain about what is detected by NIRS in differentiating RR and conventional varieties. Both Roussel et al. (2001) and Agelet, Gowen, Hurburgh, and O'Donell (2012) suggested the carbohydrate region to be the most influential in the discrimination of large sets of samples. Lee and Choung (2011), on the other hand, found differences in color and several regions related to CO_2H, CCl, and H_2O. Roussel et al. (2001)) also mentioned that both RR and conventional misclassified samples in their research had moisture content higher than 13%.

In this research we took a closer look at the differences between 5 conventional soybean varieties and their RR counterparts, regarding their chemical composition and what is detected by NIRS. We utilised two NIR instruments for the latest task: A Fourier-Transform Near Infrared Transmittance (FT-NIR) (NIRFlex N-500 by Buchi Corporation) and the USDA light-tube, working by diffuse reflectance (Armstrong, 2006; Tallada, Palacios-Rojas, & Armstrong, 2009). We proceeded to study the impact of moisture changes in the discrimination accuracies. Since beans destined to elevators and commodities have variable moisture, any effect that moisture may induce to the discriminative ability of the models should be taken in consideration.

2. Materials and methods

2.1. Samples

Five conventional public soybean varieties from 2007 crop-year (labeled as M97-302, M97-303, M97-304, M97-305, and M97-306 varieties) and their same respective varieties with the Roundup Ready® (RR) gene were used in this study. This made a set of 10 samples. The samples were harvested from a same location (Iowa State University Curtiss farm, Ames, IA), and neither the plant nor the seeds received chemical treatments. Samples belonged to the same crop year in order to reduce phenotype–environment interactions.

One hundred and fifty seeds were selected from each sample and scanned by the two instruments consecutively (1500 scanned seeds total). The initial average moisture of the bulk seeds was measured by with an Infratec 1221 transmittance instrument (Foss North America, Eden Prairie, MN, USA) using a cuvette and the Iowa State moisture calibration. Sample composition predicted with ANN Iowa State calibrations as shown in Table 1. The standard error of prediction (SEP) in an independent validation of the moisture calibration was 0.37%, SEP = 0.52% for protein, SEP = 0.37% for oil, and SEP = 0.08% for fiber.

Additional sets of 150 seeds from each sample were selected and sealed in individual small plastic bags with a wet paper towel on the top, avoiding direct contact with the seeds to avoid spoilage. The paper towels were cut from conventional disposable laboratory cellulose towels of one sheet, measuring 3×3 cm of surface. The sealed bags were kept at 2 °C for around 3 weeks or until their average moisture was over 13%. The moisture on the seeds was monitored and predicted with the Infratec 1221 instrument. During that period of time, the paper towels were replaced when were slightly dump at touch, and seeds were shaken to allow better equilibration of moisture within the samples. After scanning each

Table 1
Bulk composition of the 10 samples used in the study predicted with NIR transmittance and Iowa State calibrations.

Sample	Initial Moisture (%)	Protein[a] (%)	Oil[a] (%)	Fiber[a] (%)
M97-302 RR	8.8	34.9	16.9	5.0
M97-302	8.6	36.1	18.1	4.8
M97-303 RR	8.4	36.0	18.8	4.7
M97-303	8.4	36.4	17.4	4.8
M97-304 RR	8.2	37.9	17.0	4.7
M97-304	8.3	36.2	18.0	4.8
M97-305 RR	9.3	38.0	17.3	4.6
M97-305	8.9	36.3	17.9	4.8
M97-306 RR	9.5	36.2	18.3	4.7
M97-306	8.9	34.6	18.0	4.7

[a] 13% Moisture content basis.