"Testing for Biotechnology-Enhanced Grains and Oilseeds"

Remarks by David R. Shipman, Deputy Administrator, Grain Inspection, Packers and Stockyards Administration, before the World Agricultural Outlook Forum, February 24, 2000.

Introduction

Having the ability to distinguish biotech crops, and even specific types of biotech crops from conventional crops, is becoming more important for a variety of reasons.

- Certain food manufacturers want to buy conventional crops separate from biotech crops.
- Some countries have announced regulations that will require the labeling of biotech crops and products.
- The country-by-country regulatory approval process has resulted in certain varieties being approved in some countries but not in others.
- Buyers and sellers will need to confirm the presence of enhanced value traits in future generations of biotech crops if they want to benefit from the enhanced value.

Regardless of the motives for segregating biotech from conventional or one quality trait from another, verification testing will be necessary. Unfortunately, testing for biotech crops is substantially different from traditional grain quality testing. The technology for detecting biotech crops is rapidly evolving and quite variable at this time. You either test for the presence of the actual genetic material (DNA) inserted into the crop or you test for the unique protein expressed by the inserted DNA.

Sampling

Regardless of the method used, a representative sample is essential. As with most grain quality tests, sampling is a significant source of variance. The grain industry typically relies on "systematic sampling" whereby a diverter-type (D/T) sampler diverts a small slice of the grain stream on a pre-set time (e.g. 20 seconds). The small slices are combined to produce a sample representative of the entire lot (truck, railcar, barge, etc.). That is, every kernel in the lot has a chance of ending up in the final sample.

Once the original sample is collected, a subsample is obtained using a divider, such as the Borner divider. This subsample must than be ground and mixed to ensure you have a homogenous mixture.

Types of Testing Available

Biotech crops differ from their conventional counterparts by the addition of one or more new genes (DNA sequences) into the plant genome. Each gene tells the plant to produce a new protein that confers a new trait. Biotech detection methods test either for the new genetic sequences (the DNA) or the new proteins. In general, separate tests must be developed and optimized for each bioengineered variety. While protocols can be designed to test one product for the presence of several biotech traits, there are no universal tests encompassing every biotech product. In choosing the appropriate biotech detection method, it is critical to understand the information needed; both DNA and protein tests have suitable applications.

Conventional DNA

Specific DNA sequences in plant material can be detected using a technique called polymerase chain reaction (PCR). PCR requires specific DNA sequence information, which may be proprietary. The DNA is extracted from the ground sample. Then, primers are used to select only the DNA unique to the inserted genes and then amplify or multiply the unique sequence in a cyclic reaction. In conventional PCR, these fragments of multiplied DNA are than separated on an agarose gel. The size and intensity of the DNA band(s) indicates the presence and relative level of targeted DNA within the sample. This conventional PCR technique is very sensitive, and can detect <0.1% biotech material in a sample (i.e, 1 bean in 1,000).

Let's take a look at the performance of the conventional PCR testing in a controlled study conducted by the European Union ("IUPAC Collaborative Trial Study of a Method to Detect Genetically Modified Soy Beans and Maize in Dried Powder," Lipp et al., published in the Journal of AOAC International, Volume 82, Number 4, 1999). The study used conventional PCR to detect the 35s promoter and the NOS terminator, 2 genetic elements important for the expression of the inserted genes and present in nearly all biotech crops. The study involved 29 laboratories in 13 countries.

Analysis of the 35S Promoter in Soybeans

% GMO	No. of samples Negative	No. of samples Positive	Correctly Classified Negative %	Correctly Classified Positive %
0.0	94	2	97.9	0
0.1	5	93	0	94.9
0.5	0	105	0	100
2.0	0	101	0	100
Overall	99	301	97.9	98.4

Source: Lipp Et Al: Journal of AOAC International Vol. 82, No. 4, 1999

Analysis of the NOS Terminator in Soybeans

% GMO	No. of samples Negative	No. of samples Positive	Correctly Classified Negative %	Correctly Classified Positive %
0.0	96	0	100	0
0.1	7	91	0	92.8
0.5	3	102	0	97.1
2.0	0	101	0	100
Overall	106	294	100	96.7

Source: Lipp Et Al: Journal of AOAC International Vol. 82, No. 4, 1999

Newer PCR technologies have increased the sensitivity and reliability of PCR quantification beyond conventional methods. The newest innovation is referred to as real-time PCR. One important feature of this new technology is that the DNA amplification and analysis occur in the same reaction vessel, simplifying detection. Amplification of target DNA is measured via fluorescence during the PCR reaction. This versatile technology can produce accurate quantitative results as low as 0.01% biotech material. Real-time PCR is also more rapid (1 –2 days) than conventional PCR (2 – 3 days), significantly decreasing the time needed for confirmation of positive results.

PCR tests, however, have their limitations. PCR is susceptible to errors due to contaminants, DNA breakdown, or improper implementation, and testing must be performed under rigorous laboratory conditions with appropriate controls. Personnel performing PCR assays must be highly skilled. Equipment for PCR testing is also quite costly, ranging from $20-30,000 for conventional PCR to $60-100,000 for real-time PCR. For these reasons, PCR is not easily adaptable for rapid on-site testing at elevators or processing plants. Currently, PCR testing of crops and processed products for the presence of biotech products is offered commercially at a cost of $200-450 per test; the tests take 2-3 days to perform.

Protein Detection Methods

Proteins in GMO material may be detected using an ELISA (enzyme-linked immunosorbent assay) test. ELISA tests analyze for a specific antibody reaction that marks the presence of the expected protein. Two types of ELISA kits are available: microwells and lateral flow or strip tests. Microwell kits are easy to use and can provide a semi-quantitative determination of GMO content. Results are obtained in 2-4 hours at a cost of approximately $2-20 per test. Lateral flow tests give qualitative (yes/no) results for GMO material in less than 20 minutes, at a cost of $1-5 per test.

The level of detection for these ELISA tests varies for each product tested (due to differing levels of foreign protein in each product) and for each kit (due to the quality of the antibody). Current ELISA test kit manufacturers claim that the dipstick tests will reliably detected 0.1% GMO for Roundup Ready soybeans and 2% GMO for *Bt* corn. Caution must be exercised in interpreting ELISA test results, however. Quantification is difficult, as protein composition and expression levels for a given trait can vary widely.

One example is *Bt* insect resistance. Three different *Bt* proteins are currently used in corn varieties to confer resistance to the European Corn Borer: Cry1Ab, Cry1Ac, and Cry9c. The amount of protein expressed by variety may differ and not all varieties incorporating Cry1Ab, for example, produce the same levels of *Bt* protein. One variety has been specifically engineered to produce the *Bt* toxin only in the green leafy parts of the plant, where the insects attack. An ELISA test on the seed would yield a negative result. PCR testing would need to be performed to detect this particular genetic modification. The key to successfully employing an ELISA test, or any biotech crop detection method, is to choose an appropriate application.

ELISAs are well suited for testing at the first point-of-sale, enabling verification of identity on the spot by non-technical personnel. ELISAs are being marketed to seed developers, elevators, and processors both in the U.S. and abroad. Used as a risk management tool in conjunction with a segregation system, ELISAs can be highly effective when applied properly. Likewise, PCR testing is valuable for confirmation of sample identity and composition to facilitate final sale. When performed by a credible laboratory, PCR can be accurate, quantitative, and specific.

GIPSA's Role

To improve the reliability of biotech testing and subsequently market confidence in results, USDA through the Grain Inspection, Packers and Stockyards Administration (GIPSA) has initiated steps to establish sampling protocols, and to evaluate and validate the various testing methods being used in the commercial market.

As we speak today, we are holding a workshop in Kansas City, MO to review sampling protocols and to review the PCR and ELISA testing methods used by government and commercial laboratories. We are also in the process of establishing a biotech reference laboratory at the GIPSA Technical Center in Kansas City. The laboratory will have the capability to verify PCR testing as well as ELISA and lateral flow tests. The ultimate goal is to provide the market with reliable biotech detection methods that will facilitate information exchange, and benefit American agriculture by increasing overall market efficiency, decreasing transaction costs, and minimizing buyers' and sellers' risks.

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