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Case study :

Seed testing of GMOs (Food crops)

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A plant, such as cotton or soyabean, is considered genetically modified when genetic material from outside of that organism is inserted into DNA sequence. Plants grown from seed harvested from genetically modified plants will also contain the genetic modification. To date, the most common genetic modifications in crops confer an herbicide or an insecticide resistance to the plant. This resistance is achieved through production of a novel protein encoded by the inserted DNA sequence. Detection methods for genetically modified organisms (GMOs) are necessary for many applications, from seed purity assessment to compliance of food labeling in several countries. Numerous analytical methods are currently used or under development to support these needs. The currently used methods are bioassays and protein- and DNA-based detection protocols. The most frequently used approach in the field of genetically modified organism (GMO) quantification in food or feed samples is based on the 5'-3'-exonuclease activity of Taq DNA polymerase on specific degradation probes. To avoid discrepancy of results between such largely different methods and, for instance, the potential resulting legal actions, compatibility of the methods is urgently needed. Performance criteria of methods allow evaluation against a common standard. The more-common performance criteria for detection methods are precision, accuracy, sensitivity, and specificity, which together specifically address other terms used to describe the performance of a method, such as applicability, selectivity, calibration, trueness, precision, recovery, operating range, limit of quantitation, limit of detection, and ruggedness.

Key words : GMOs, Bioassays and Protein, DNA.

INTRODUCTION

In plants that are genetically modified for commercial agricultural purposes, the recombinant sections of DNA that are artificially inserted into the natural plant genome have some common genetic elements. Each inserted DNA sequence consists of at least a promoter, a protein-coding site (the structural gene) and a terminator. The promoter is a sequence of DNA that acts like an “on switch” for the transcription of DNA into mRNA, the first step in the activation of the cells like protein producing tools. The terminator marks the end point for this transcription procedure. The structural gene determines the particular protein that is to be made.

Genetically modified (GM) crops are increasingly being introduced into the world’s food supply. Concerns raised by consumers and regulatory agencies in various countries have highlighted the need for reliable and accurate testing for the presence and the amount of genetically modified components.

Techniques for GMO testing :
ELISA (Enzyme linked immunosorbent assay):
Is designed to detect the presence of the novel protein encoded by the inserted DNA sequence. A number of variations of Elisa have been developed, allowing qualitative detection or quantitative measurement of either antigen or antibody the presence of antibody. Each type of Elisa can be used qualitatively to detect the presence of antibody or antigen. Alternatively, a standard curve based on known concentration of antibody or antigen is prepared, from which the unknown concentration of sample can be determined. Protein strip tests and ELISA tests are preferred for these types of applications because they allow relatively rapid turnaround times, and they require a relatively small investment in equipment and personnel.

ELISA Tests for Specific Events Currently Available for following :
Qualitative :

Soybean RR (also soymeal, full fat flour, defatted flakes), Corn RR (NK603), Cotton RR
Cry1Ab - Corn (Mon809, Mon810, Bt11, E176)
T25 - Corn (PAT, BAR, Liberty Link)
Cry3Bb - Corn (Mon863)
Cry1Ac - Cotton (Bollgard I)
Cry2A - Cotton (Bollgard II)

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Quantitative :

Cry1Ab - Corn (Mon809, Mon810, Bt11, E176) (~0.15% detection level)
Cry9C - Corn (CBH351, Starlink) (~0.04% detection level) Cry1F - Corn (Herculex)
CP4 (EPSPS) - Soybean RR (~0.05% detection level) Antibody Strip Tests for Specific Events

Qualitative :

Cry3Bb - Corn (Mon863)
Cry9C - Corn (CBH351, Starlink)
Cry1F - Corn (Herculex)
Cry1Ac - Cotton (Bollgard I)
Cry2A - Cotton (Bollgard II)
CP4 (EPSPS) - Soybean, RR, Corn RR, Cotton RR

Other ELISA Tests :

Qualitative Aflotoxin Assay
Qualitative Fumonisn Assay
Qualitative Vomitoxin Assay
Qualitative Ochratoxin Assay
Qualitative Zearalenone Assay

PCR (Polymerase chain reaction):

PCR is a method that selectively generates copies of a defined section of original DNA molecules to detectable levels. This in vitro reaction that multiplies a specific DNA region is performed by a DNA copying enzyme (DNA-polymerase). It requires the presence of a template for the DNA sequence in question. To construct the copies of the targeted DNA, the process uses nucleotide building blocks supplied by the reaction mixture. Moreover, the DNA polymerase requires starting points for its activity.

To analyze an agricultural or food product by PCR testing, first an aqueous solution of the DNA present in the sample must be obtained. Therefore the DNA is extracted from the sample and purified to a degree that makes it suitable to serve as a template in the subsequent PCR reaction. The sample DNA is combined with the other components of PCR reaction and placed into a thermocycler. Initially, double- -standard DNA molecule is separated into two complementary, single strands by heating. Afterwards, at a lower temperature, the primers recognize their specific target DNA sequence on each single strand of the DNA. Only if the sample DNA contains the DNA sequences in question, targeted by the primers, will the primers anneal to the single-stranded templates and there by mark the starting points for the synthesis of the complementary strands.

At the end of the first PCR cycle, two complete double stranded DNA molecules results from each original double stranded DNA molecule present at the beginning of the PCR. In each successive cycle, these newly formed double stranded DNA molecules serve as templates as well, and each will yield two copies. As the number of molecules theoretically doubles with each cycle, multiple repetitions of the PCR reaction can then be visualized by agarose gel electrophoresis.

Since the increase of target DNA molecules during the PCR process theoretically is exponential until the supply of the reaction's components is exhausted, the number of PCR cycle can be crucial for the sensitivity of the analysis. Especially if only very few target DNA molecules are present in the beginning of the reaction, ten cycles more or less easily be critical to obtain a detectable amount of PCR products.

Quantitative Method :

Quantitative Real time PCR is used to determine the exact percentage of genetically modified DNA in a sample. Quantitive PCR tests a sample for the presence or absence of particular DNA sequence indicative for the presence of genetically modified materials.

Detection Limit :

Theoretically, a PCR analysis can detect a single molecule of DNA. In the routine testing in the various seed testing laboratories, there is a proof for each individual sample DNA that detection limit is ten copies of target DNA or less. However, a specific detection limit, expressed as a percentage, can only be determined by counting the number of copies of DNA molecules present using a real time quantitative PCR analysis. Daily routine testing in various laboratories, using real time PCR, has shown that amounts less than 0.1% of GM soy can be reliably detected in soybeans. In some matrices a detection limit in the range of 0.01% can be achieved. The detection limit (DL) of a quantitative PCR, given as a percentage of GM material, depends upon the amount of DNA molecules from the plant species present in the sample. It is axiomatic to say that the DL, given as a percentage, cannot be started be stated unless a quantitative PCR is performed on that particular sample since the number of plant species DNA molecules extracted from the sample is not known until the completion of a quantitative analysis.

Quantification limit (QL) :

Similarly, the amount of DNA present is a factor in determining the limit of quantification for each real time PCR analysis. The QL for a sample is the lowest level at which reproducible results can be calculated.

Conclusion:

A modification specific test can detect and identify a particular GM construct through the use of primers that target a unique to this specific genetic modification. When selecting a method of detection, it is also important to consider its applicability, its field of application, and its limitations, by including factors such as its ability to detect the target analyte in a given matrix, the duration of the analyses, its cost effectiveness, and the necessary samples sizes for testing. Thus the current GMO detection methods should be evaluated against a common set of performance criteria. However, they do have some disadvantages that tend to limit their use. For example, ELISA or strip assays are limited to protein of specific events which often times are not readily available. Thus, strip tests and ELISA tests are not useful for detecting "any GMO" in a commodity or product. The trend in biotechnology is a key in pursuing modern methods in seed testing to ensure that the government can maintain the quality of seeds to improve agricultural production in the country.

REFERENCES

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