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Testing Methods for Agriculture and Food Safety

Jose C. Jimenez-Lopez[1*] and María C. Hernandez-Soriano[2]
[1]*Purdue University, Department of Biological Sciences, College of Science, West Lafayette, IN*
[2]*North Carolina State University, Department of Soil Science, College of Agriculture and Life Sciences, Raleigh, NC USA*

1. Introduction

Sustainable agricultural production is a critical concern in response to global climate change and population increase (Brown and Funk, 2008; Turner et al., 2009). In addition, recent increased demand for Biofuel crops has created a new market for agricultural products. One potential solution is to increase plant yield by designing plants based on a molecular understanding of gene function and on the regulatory networks involved in stress tolerance, development and growth (Takeda & Matsuoka, 2008). Recent progress in plant genomics, as well as sequencing of the whole genome of new plant species have allowed to isolate important genes, discover new traits and to analyze functions that regulate yields and tolerance to environmental stress.

Recent remarkable innovations in platforms for omics-based research (genomic, proteomic, transcriptomic, metabolomic) and application development provide crucial resources to promote research in model and applied plant species. A combinatorial approach using multiple omics platforms and integration of their outcomes is now an effective strategy for clarifying molecular systems integral to improving plant productivity. Furthermore, support of comparative genomics and proteomis among model and applied plants allow to reveal the biological properties of each species and to accelerate gene discovery and functional analyses of genes. Bioinformatics platforms and their associated databases are also essential for the effective design of approaches making the best use of genomic resources, including resource integration.

Currently, many crop species can be considered important on a global scale for food security in which, have been developed large-scale genomic and genetic resources, i.e. array technology markers, expressed sequence tags or transcript reads, bacterial artificial chromosome libraries, genetic and physical maps, and germplasm stocks with rich genetic diversity, among others. These resources have the potential to accelerate gene discovery and initiate molecular breeding in these crops, thereby enhancing crop productivity to ensure food security in developing countries. In this line, and with all above molecular genetics

* Corresponding Author

tools development, it has been possible that more and more plant crop species has been modified genetically obtaining desirable traits compared with wild plants.

Classical plant breeding uses deliberate interbreeding (crossing) of closely or distantly related individuals to produce new crop varieties or lines with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background. Progeny from the cross would then be crossed with the high-yielding parent to ensure that the progeny were most like the high-yielding parent, (backcrossing). The progeny from that cross would then be tested for desirable traits, i.e. resistance and high-yielding resistant plants to be further developed. Plants may also be crossed with themselves to produce inbred varieties for breeding.

Classical breeding relies largely on homologous recombination between chromosomes to generate genetic diversity (Zhu et al., 2009). The classical plant breeder may also makes use of a number of in vitro techniques such as protoplast fusion, embryo rescue or mutagenesis to generate diversity and produce hybrid plants that would not exist in nature.

Traits that breeders have tried to incorporate into crop plants in the last 100 years include:

1) Increased quality and yield of the crop, 2) Increased tolerance of environmental pressures (salinity, extreme temperature, drought), 3) Resistance to viruses, fungi and bacteria, 4) Increased tolerance to insect pests, and 5) Increased tolerance of herbicides.

Modern plant breeding uses techniques of molecular biology to select, or in the case of genetic modification, to insert, desirable traits into plants (Bhatnagar-Mathur et al., 2008). Genetic modification of plants is achieved by adding a specific gene or genes to a plant, or by knocking out a gene with RNAi, to produce a desirable phenotype. Genetic modification can produce a plant with the desired trait or traits faster than classical breeding because the majority of the plant's genome is not altered. The use of tools such as molecular markers or DNA fingerprinting can map thousands of genes, allowing plant breeders to screen large populations of plants for those that possess the trait of interest. The screening is based on the presence or absence of a certain gene as determined by laboratory procedures, rather than on the visual identification of the expressed trait in the plant. The majority of commercially released transgenic plants are currently limited to plants that have introduced resistance to insect pests and herbicides. Insect resistance is achieved through incorporation of a gene from Bacillus thuringiensis (Bt) that encodes a protein that is toxic to some insects. For example, the cotton bollworm, a common cotton pest, feeds on Bt cotton it will ingest the toxin and die. Herbicides usually work by binding to certain plant enzymes and inhibiting their action. The enzymes that the herbicide inhibits are known as the herbicides target site. Herbicide resistance can be engineered into crops by expressing a version of target site protein that is not inhibited by the herbicide. This is the method used to produce glyphosate resistant crop plants (Pollegioni et al., 2011).

Testing for the presence of agricultural biotechnology products is being performed on many grain and food products. Currently, there is an absence of standardized tests to detect genetic modified (GM) crops, which can result in inaccurate claims and enforcement actions being taken without a means to challenge the results. Development of reliable, validated methods is necessary to avoid negative economic impacts due in invalid test results, as well as to ensure the safety of the consumer under arising of GM product in the markets. We also emphasize the need for such global compatibility of test results in order to facilitate international trade. A quality control of GM-crops product should be implemented through

self, supervisory, and peer review systems, which have to be organizationally independent of the testing staff or organization, performing internal test in accordance with the government requirements for.

Identification of GM product in the markets is another pending task, due that government from different countries are not in agreement about information that labelling product should contain. Authoritative, independent and public acceptable of green (eco-)/GM label scheme that identify products no-GM, in many case more environmentally desirable than other similar GM products with the same function in the market, is urged to be implemented with a general acceptance. Establishing compliance with GM food labeling laws is dependent on the availability of test methods capable of determining the presence and or concentration of GM ingredients in food or bulk consignments of agricultural commodities such as seed and grain.

Thus, current global regulatory requirements for labeling of products derived from plant biotechnology means that test methods, i.e. for the introduced trait(s) have to be developed and validated. Such methods require appropriate reference materials, controls and protocols in order to give accurate and precise identification and quantification of GM products.

2. Genetic modified crops

The term genetically-modified (GM) foods u organisms (GMOs) is most commonly used to refer to crop plants created for human or animal consumption, using molecular biology techniques. These plants have been modified in the laboratory to enhance desired traits such as increased resistance to herbicides or improved nutritional content. The enhancement of desired traits has traditionally been undertaken through breeding, but conventional plant breeding methods can be very time consuming and are often not very accurate.

During the past decade, a large number of genetically modified (GM) crops have been developed using methods of modern biotechnology. These GM or "biotech" crops exhibit unique agronomic traits such as herbicide tolerance or insect resistance, which offer significant benefits to farmers. The development of GM crops is accomplished by using molecular biology methods, essentially by the integration of novel DNA sequences into the plant genome. The new DNA encodes for the expression of the novel protein in the targeted tissue, resulting in the unique agronomic trait. The novel protein and DNA are present in many parts of the plant, in harvested grain, and often in the food fractions prepared from grain.

The production and global trade of genetically modified (GM) grain is increasing. At the same time, companies are required to provide validated diagnostic methods proving the inclusion of GM material, as a condition of the regulatory approval process in some jurisdictions. Numerous governmental agencies and industry organizations are attempting to develop standardization guidelines independently. Global harmonization of these efforts is necessary to ensure a consistent standard. An international coordination of detection methods for plant biotechnology products and the proper development of guidelines for their use are necessary and an awaiting mission.

2.1 Detection methods for GM-Crops

The detection of genetically modified organisms in food or feed is possible by using well-developed genetic and biochemical tools. It can either be qualitative, showing which

genetically modified organism (GMO) is present, or quantitative, measuring in which amount a certain GMO is present. Being able to detect a GMO is an important part of food safety, as without detection methods the traceability of GMOs would rely solely on documentation.

2.1.1 Methods based on DNA: polymerase chain reaction

Methods for GMO should contain three analytical components of tests for detecting the presence of transgenic plant products: (1) detection, to screen for the presence of GM events in food and agricultural products; (2) identification, to reveal how many GM events are present and determine their molecular registers; and (3) quantification, to determine the amount of authorized GM product and compliance with threshold regulation. Analytical procedures for GM plants are directed to detect either the novel gene product or the gene construct itself.

Testing on GMOs in food and feed is routinely done by molecular techniques like DNA microarrays or qPCR, and the test can be based on screening elements (like p35S or terminator Nos) or specific markers for the official GMOs (like Bt11 or GT73).

Technologies involving amplification of gene fragments by the polymerase chain reaction (PCR) have the greatest potential for detecting transgenic plants and foodstuffs derived from them (Ahmed, 2002; Schmidt et al., 2008). PCR is a biochemistry and molecular biology technique for isolating and exponentially amplifying a fragment of DNA, via enzymatic replication, without using a living organism. It enables the detection of specific strands of DNA by making millions of copies of a target genetic sequence. Target sequences may be those of the marker gene, the promoter, the terminator, or the transgenes themselves. Confirmatory tests are essential to ensure authenticity of PCR product. The use of genomic fragments that include the border sequence at the insertion site and the inserted genes (edge or junction fragments) may be a better target for unequivocal identification of GM plant sources (Windels et al., 2001), particularly when detection is based on regulatory sequences in promoters and terminators that could occur in microbial contaminants.

Improving PCR based detection of GMOs is a further goal of different governmental research programme. Research is now underway to develop multiplex PCR methods that can simultaneously detect many different transgenic lines. Another major challenge is the increasing prevalence of transgenic crops with stacked traits. This refers to transgenic cultivars derived from crosses between transgenic parent lines, combining the transgenic traits of both parents.

Whether or not a GMO is present in a sample can be tested by qPCR, but also by multiplex PCR. Multiplex PCR uses multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Furthermore, DNA array technology using chip platforms may also be a useful tool for determining the presence of insertional sequences. The possibility of testing against a large number of oligonucleotides representing various gene sequences will be particularly useful when the specific construct is unknown. To avoid any kind of false positive or false negative testing

outcome, comprehensive controls for every step of the process is mandatory. A CaMV check is important to avoid false positive outcomes based on virus contamination of the sample.

Sometime it is required to quantify GM product that contain food or directly in plants. For that purpose it is frequently used quantitative PCR (qPCR), to measure amounts of transgene DNA or PCR product in a food or feed sample, preferably real-time qRT-PCR (Ref.), with currently the highest level of accuracy. If the targeted genetic sequence is unique to a certain GMO, a positive PCR test proves that the GMO is present in the sample.

In addition, the array-based methods combine multiplex PCR and array technology to screen samples for different potential GMOs (Querci et al., 2009; Dorries et al., 2010) combining different approaches (screening elements, plant-specific markers, and event-specific markers). Advanced PCR technologies, including competitive multiplex PCR and real-time PCR, are useful for quantifying the level of GM plant material in foodstuffs (Matsuoka et al., 2000).

Alternatively, there are methods to detect specific genetic construct, instead the specific genetic product. Since different GMOs may produce the same protein, construct-specific detection can test a sample for several GMOs in one step, but is unable to tell precisely, which of the similar GMOs are present.

Almost all transgenic plants contain a few common building blocks that make unknown GMOs easier to find. Even though detecting a novel gene in a GMO can be like finding a needle in a haystack, the fact that the needles are usually similar makes it much easier. Researchers now compile a set of genetic sequences characteristic of GMOs. After genetic elements characteristic of GMOs are selected, methods and tools are developed for detecting them in test samples. Approaches being considered include microarrays and anchor PCR profiling.

2.1.2 Methods based on protein: immunoassay technology

GM content can be determined by methods that detect either the novel protein or the inserted DNA. Detection of the novel proteins produced by GM crops relies almost exclusively on the application of immunoassay technology (Fantozzi et al., 2007). Commercial immunoassays are available for most of the GM crops on the market today and have been used in a variety of large-scale applications, determining GM content (%GM) ensuring compliance with non-GM labeling requirements, and confirming the presence of high-value commodities.

Immunoassays are based on the reaction of an antigen (Ag), e.g., transgenic protein, with a specific antibody (Ab) to give a product (Ag-Ab complex) that can be measured. There are many different immunoassay formats, and the choice of format is dependent on the target molecule and application. For macromolecules, the most commonly used test formats are enzyme-linked immunosorbent assay (ELISA) that can be used as either a qualitative or a quantitative assay, and lateral flow device (LFD) designed for qualitative yes/no testing.

ELISA based commercial kits are available for serological detection of selected GM gene products. ELISA is a comparatively easy and cost-effective procedure to apply to large numbers of samples, but specificity of antibodies is critical for an accurate test.

Two other test formats used for seed quality testing are Western blot and immunohistochemical staining.

LFDs are used for qualitative or semiquantitative detection of antigens. LFDs for the detection of GM proteins use antibodies in the same sandwich immunoassay format used in ELISA, except that the secondary antibody is labeled with a colored particle such as colloidal gold rather than an enzyme as a means of generating a visible signal.

Furthermore, the Western blot is primarily a qualitative analytical method and is particularly useful in protein characterization because it provides additional information regarding molecular weight. Immunohistochemical staining is used to determine the location of the expressed proteins in the plant.

The key component of immunoassay, antibody, have the atribute that makes it useful as a reagent in a diagnostic kit, being its capacity to bind specifically and with high affinity to the antigen that elicited its production. Polyclonal antibodies are relatively easy and inexpensive to prepare in a relatively short time frame (e.g., 3–4 months); however, the quality of the antibody reagent varies from animal to animal, and it is necessary to prepare large pools of qualified reagent to support long-term commercial production of uniform product. Monoclonal antibodies require greater time (e.g., 6 months) and skill to produce and are more expensive to develop than polyclonal antibodies. In applications where discrimination between very closely related molecules is required, it may be more advantageous to use a highly specific monoclonal antibody reagent. Conversely, in an application designed to detect all the members of a family of closely related molecules it may be more advantageous to use a polyclonal antibody reagent. The selection of one reagent type over another is dependent on the desired performance characteristics of the test method.

Another key component of an immunoassay is the antigen that can be defined as substances that induce a specific immune response resulting in production of antibodies. The interaction between antibody and antigen involves binding of the antigenic epitope to the complementarily determining region (CDR) of the antibody. The strength of binding between the 2 is referred to as the affinity of the bond. In general, the greater the affinity of the bond, the greater the sensitivity (lower limit of detection; LOD) of the test method. Sensitivity of a test method is determined not only by the affinity of the antibody for the antigen, but by factors such as protein expression level, extraction efficiency, and the size of the sample taken for analysis. In addition, an antibody binds only to the antigenic determinant that elicited its production. This specificity enables the development of test methods that require minimal sample preparation. Cross-reactivity can result in false-positive responses over-estimation of antigen concentrations. Cross-reactivity of an antibody to a component of the sample or other GM crop is highly unlikely and almost never a significant issue.

The ideal antigen for immunization would be the actual GM protein as it is expressed in the plant. However, purification of the novel protein from plant tissue can be difficult and may result in undesirable modifications to the target protein. In addition, purification rarely results in 100% pure protein and immunization of animals with such preparations results not only in the production of antibodies to the target protein but to the contaminants as well. Polyclonal antibodies made from these preparations typically exhibit high background

and poor sensitivity. A more common approach to making antibodies to GM proteins is to express and purify the protein of interest from an alternate host such as *E. coli* using genetic engineering techniques. Although the amino acid sequence of these recombinant proteins may be the same as the plant-produced protein, post-translational modification may be subtly different, and purification may result in modifications to the secondary and tertiary structure (e.g., denaturation). As long as antibodies that bind to the plant-produced protein with sufficient sensitivity and specificity can be isolated, then differences in structure between plant-produced and microbial-derived proteins are not an issue.

In certain instances where purified or recombinant antigens are not available or are exceedingly difficult to obtain, or where antibodies to very specific amino acids are desired, short peptides conjugated to carrier proteins may be used to develop antibodies. However, peptide antibodies may be more reactive to denatured forms of the protein and therefore often find better utility in Western blot (De Boer, 2003; Grothaus et al., 2006).

2.1.3 Others methods

To date there is no molecular approach to testing that can distinguish between the presence of a low (or high) percentage of GM events in a bulk sample, and the presence of a mixture of the two or more individual events that comprise the stack. In addition, there are added problems because gene products can become degraded during preparation and cooking. Thus, serology may not be suitable for their detection in some processed food products.

These reasons have lead to use alternative methods, por example to monitor changes in the chemical profile of oils derived from GM Plants by using chromatophaphic methods, i.e. HPLC (Lopez et al., 2009), or near infrared spectroscopy (NIR) for detection of changes in fibre structure (Michelini et al., 2008).

NIR detection is a method that can reveal what kinds of chemicals are present in a sample based on their physical properties. It is not yet known if the differences between GMOs and conventional plants are large enough to detect with NIR imaging. Although the technique would require advanced machinery and data processing tools, a non-chemical approach could have some advantages such as lower costs and enhanced speed and mobility.

Another alternative method to differentiate and quantify GM from non GM seed contained in a seed lot is a statistical approach. The approach is a pooled testing approach and involves the examination of as many as 10-20 pools. However, if the percentage of positive seeds in the sample is higher than a few percent of the seeds, the model may not give clear results.

2.1.4 Reference materials, standards and control for validation and standardization of detection and analysis methods

The request for powerful analytical methods for routine detection of GMOs by accredited laboratories has called attention to international validation and preparation of official and non-commercial guidelines. Among these guidelines are preparations of certified reference material (CRM), sampling, treatment of samples, production of stringent analytical protocols, and extensive ring-trials for determination of the efficacy of selected GMO detection procedures. Any detection method to be implemented in the identification of GM

crop plants and its derived products used in food needs materials to be used for calibration and validation of such detection methods as well as proficiency testing of laboratories. The reference materials should be controlled and regulated by government agencies as general use, in order to ensure a globally harmonized approach and provide them under principles for transfer in order to control the distribution and use of intellectual property (Trapmann et al., 2010).

Reference material is material with sufficiently stable and homogeneous properties and well established to be used for calibration, the assessment of a measurement method or for assigning values to materials. Certified reference material (CRM) is reference material accompanied by a certificate issued by a recognised body indicating the value of one or more properties and their uncertainty. The certified values of these materials have been established during the course of a certification campaign including inter-laboratory studies (which should be available upon request). In the absence of CRM, standards validated by a laboratory can be used.

Reference Materials are required as reference standards in method calibration and must be produced according to international standards and guidelines and may be certified. Reference materials will be made available for all products which are commercially available. These reference materials will be made available globally and on a single GM event detection, and designated by a third-party source. This source will be selected by each company based upon factors such as global presence, operational independence or experience in working with such materials under ISO standards. For compliance with the 1% threshold level of cross-contamination of unmodified foods with GM food products, certified reference materials for precise quantification and method validation are needed. According to commonly accepted rules, the production of reference materials should preferably follow metrological principles and should be traceable to the SI system. Arbitrary definition of measurement units could lead, as a consequence, to difficulties with non-consistent standards and a lack of long-term reproducibility. In the future, efforts should be concentrated on establishing reliable quantification methods accompanied by the production of reference materials with high DNA quality and DNA degraded under controlled conditions (simulating real samples in food production) using very well characterized base materials.

European Union's Joint Research Centre, Institute for Reference Materials and Measurements, Belgium, is currently developing a system for distribution of GMO reference material (http://www.irmm.jrc.be/).

Identification and quantification of gene products in a GM plant must be done with standards that correlate to known concentrations of the antigen (protein) that it is used to produce a dose-response curve. The standard curve and the assay response from the samples are used to determine the antigen concentration. The material used to make the standards should yield a response that correlates to the actual concentration of antigen in the sample type and assay conditions specified by the test procedure. Recombinant proteins, which contain a similar or identical amino acid sequence and immunoreactivity as the GM plant-expressed protein, are often used as ELISA standards. Uniform preparations of actual samples (such as ground corn) having known concentrations of GM proteins may also be used as standards (Trapmann et al., 2002). Protein reference materials are critical for the

validation of externally operated immunochemistry processes. Reference materials can be derived from a number of production sources, and can take on a variety of final forms (stabilized plant extracts to highly pure protein). Three types of certified GMO reference samples for GMO testing are especially needed: 1) DNA-CRM, 2) matrix-CRM for events of major importance, and 3) protein-CRM. An important issue to consider is that the CRMs are stable and non-degraded. Often problems with degradation of CRMs are encountered.

The European Network of GMO Laboratories has prepared a list of wishes concerning CRMs for GMO inspection as follows:

1. For production of GMO-CRMs one variety per transformation event common in USA and EU should be used.
2. GMO and non GMO should be corresponding near isogenic lines.
3. For each EU-approved GMO varieties CRMs are needed (T25, Bt176, Mon810, Bt11, Mon809, Ms1/Rf1, Ms3/Rs8, RR soy, topas 19/2).
4. CRMs for some special cases of US-approved lines like CBH351 CRMs should be available.
5. Powdery reference materials from certified commercial seeds for relative quantification of GMO should be available with a GMO content of 100, 5, 2, 1, 0.1, and 0 %.
6. Plasmids would be helpful for absolute quantification (native and competitors sequence, transgenic GMO, and housekeeping sequences).

Controls are reagents and specifications that validate each method run. Reagent controls may be different from standards. Per example, every ELISA test, qualitative or quantitative, should include known positive and negative controls to ensure assay validity. Typical controls specify limits for background, assay response to a known concentration, quantitative range, and variability between replicates.

Validation of methods is the process of showing that the combined procedures of sample extraction, preparation, and analysis will yield acceptably accurate and reproducible results for a given analysis in a specified matrix. For validation of an analytical method, the testing objective must be defined and performance characteristics must be demonstrated. Performance characteristics include accuracy, extraction efficiency, precision, reproducibility, sensitivity, specificity, and robustness. The use of validated methods is important to assure acceptance of results produced by analytical laboratories.

Each new method should be tested in trials using numerous laboratories in order to demonstrate reproducible, sensitive and specific results. In these trials the same measurements should be assessed on identical materials. The experimental designs of each trial are crucial and several questions should be considered when planning such experiments. Examples of important issues to consider include availability of satisfactory standards, number of laboratories and how they should be recruited. It is also necessary to specify the manner of calculating and expressing test result.

Unfortunally, at this moment, no single validated method has yet been developed which is capable of accurately determining all GM products in a timely and cost effective manner. Testing programs will need to incorporate the best qualities of each technology in developing testing programs. The collaborative efforts of many organizations will be required to facilitate the development of reliable, validated diagnostic tests with broad global acceptance among users and regulators.

According to European Union legislation state laboratories participating in inspection should, whenever possible, use validated analytical methods. This is also the case for all laboratories aiming at accreditation. There are some examples of methods that have been validated or accredited recently are given below:

1. Bt176, Bt11, T25 and MON810 maize using real time quantitative PCR have been accomplished by the BgVV, Federal Institute for Health protection of Consumers and Veterinary Medicine in Germany).
2. A PCR and an ELISA method for Roundup Ready™ soybean and a PCR for Maximizer maize (Bt176) have been validated for commercial testing of grain by the European Union's Joint Research Centre, JRC.
3. An ELISA for MON810 maize has also been validated by AACC (American Association of Cereal Chemists).
4. The Varietal ID PCR methods (based on primers that span unique sequence junctions) have been accredited through the United Kingdom Accreditation System (UKAS).

However, these methods based on a relatively expensive instrumentation, requiring substantial efforts in training and available only to a limited number of participants as e.g. Real-time PCR or Microarrays for validation studies may not be useful at the moment, as methods to be implemented in routine laboratories on European scale. Furthermore, GMO testing laboratories should participate in an internationally recognised external quality control assessment and accreditation scheme. In accordance with this, authorised laboratories (approved for official inspection purposes) must participate regularly in appropriate proficiency testing schemes.

2.2 Uses and concerns about genetically modified organisms

GMOs are used in biological and medical research, production of pharmaceutical drugs, experimental medicine (e.g. gene therapy), and agriculture (e.g. golden rice). The term "genetically modified organism" does not always imply, but can include, targeted insertions of genes from one species into another.

To date the most controversial but also the most widely adopted application of GMO technology is patent-protected food crops which are resistant to commercial herbicides or are able to produce pesticidal proteins from within the plant, or stacked trait seeds, which do both. Transgenic animals are also becoming useful commercially. On February 6, 2009 the U.S. Food and Drug Administration approved the first human biological drug produced from such an animal, a goat. The drug, ATryn, is an anticoagulant which reduces the probability of blood clots during surgery or childbirth. It is extracted from the goat's milk (Niemann & Kues, 2007). Furthermore, transgenic plants have been engineered to possess several desirable traits, such as resistance to pests, herbicides, or harsh environmental conditions, improved product shelf life, and increased nutritional value. Since the first commercial cultivation of genetically modified plants in 1996, they have been modified to be tolerant to the herbicides glufosinate and glyphosate, to be resistant to virus damage as in Ring-spot virus-resistant GM papaya, grown in Hawaii, and to produce the Bt toxin, an insecticide that is non-toxic to mammals (Nasiruddin & Nasim, 2007).

Most GM crops grown today have been modified with "input traits", which provide benefits mainly to farmers. The GM oilseed crops on the market today offer improved oil profiles for

processing or healthier edible oils (Sayanova & Napier, 2011). The GM crops in development offer a wider array of environmental and consumer benefits such as nutritional enhancement, drought and stress tolerance. Other examples include a genetically modified sweet potato, enhanced with protein and other nutrients, while golden rice, developed by the International Rice Research Institute (IRRI), has been discussed as a possible cure for Vitamin A deficiency.

The most common genetically engineered (GE) crops now being grown are transgenic varieties of soybean, canola, cotton, and corn. Varieties of each of these crops have been engineered to have either herbicide tolerance or insect resistance (or in a few cases, both). All of the genetically engineered insect-resistant crop varieties produced so far use specific genes taken from *Bacillus thuringiensis*, a common soil bacterium, to produce proteins that are toxic to certain groups of insects that feed on them. Currently, only Bt corn and Bt cotton varieties are being grown in the U.S., but Bt potatoes were on the market for several years until being discontinued in 2001. In addition, several different genetic modifications have been used to engineer tolerance to herbicides, the most widely adopted GE trait overall. Genetically engineered herbicide tolerant varieties of each of the four major crops listed above have been developed for use with glyphosate or glufosinate herbicides, and some cotton varieties grown in the U.S. have genetically engineered tolerance to bromoxynil or sulfonylurea herbicides. About half of the papaya crop produced in Hawaii is now from genetically engineered virus-resistant varieties, but most of the world-wide papaya crop is not genetically engineered. There is currently some limited production of squash genetically engineered for virus resistance in the U.S.

All together, about 50 different kinds of genetically engineered plants (each developed from a unique "transformation event") have been approved for commercial production in the U.S. These include 12 different crops modified to have six general kinds of traits:

Transgenic trait	**Crops**
Insect resistance	Corn, Cotton, Potato, Tomato
Herbicide tolerance	Corn, Soybean, Cotton, Canola, Sugarbeet, Rice, Flax
Virus resistance	Papaya, Squash, Potato
Altered oil composition	Canola, Soybean
Delayed fruit ripening	Tomato
Male sterility and restorer system (used to facilitate plant breeding)	Chicory, Corn

Table 1. Genetically modified crops.

Not all of the genetically engineered varieties that have received regulatory approval are currently being grown. Some have not yet been marketed (herbicide tolerant sugarbeets and most kinds of GE tomatoes, for example), and some have been commercially grown but were later withdrawn from the market. More details on the transgenic crops listed in the table above and short descriptions of how each of the transgenic traits works are available at http://www.comm.cornell.edu/gmo/traits/traits.html.

There are many perceived risks and benefits associated with the use of transgenic crop plants for agricultural food production (Wolfenbarger & Phifer, 2000). Some of the risks

relate to the use of specific transgenes while others emphasize a broader concern that addresses the entire approach of engineering heterologous genes into plants.

Most concerns about GM foods fall into three categories: 1) *environmental hazards*, including an unintended harm to other organisms, i.e. B.t. corn caused high mortality rates in monarch butterfly caterpillars; reduction of the effectiveness of pesticides; and gene transfer to non-target species, i.e. transfer of the herbicide resistance genes from the crops into the weeds.

2) *Human health risks*, including Allergenicity and toxic effects and 3) *Economic concerns*, since GM food production is a lengthy and costly process, being many new plants GM technologies patented raising the price of seeds.

One of the primary concerns about genetically engineered crop plants is that they will hybridize with wild relatives, permitting the transgene to escape and spread into the environment, which depends on its potential fitness impact. Depending on the nature of the plant and its propensity to cross-pollinate, the flow of transgenes in the field may be an important consideration. Perhaps in some instances, transgenic plants should not be grown in geographic areas where close relatives may be pollinated with transgene containing pollen. It is feared that gene flow to non target plant populations may diminish diversity within plant species. Whether or not genetic diversity is threatened by gene flow when transgenic plants are grown in the vicinity of a native gene pool, the susceptibility of native flora to contamination by transgenes ought to be taken into account.

There are several possible solutions to the problems mentioned above. Genes are exchanged between plants via pollen. Two ways to ensure that non target species will not receive introduced genes from GM plants are to create GM plants that are male sterile (do not produce pollen) or to modify the GM plant so that the pollen does not contain the introduced gene (Warwick et al., 2009). Cross-pollination would not occur, and if harmless insects such as Monarch caterpillars were to eat pollen from GM plants, the caterpillars would survive.

Another possible solution is to create buffer zones around fields of GM crops. Beneficial or harmless insects would have a refuge in the non GM corn, and insect pests could be allowed to destroy the non GM corn and would not develop resistance to Bt pesticides. Gene transfer to weeds and other crops would not occur because the wind blown pollen would not travel beyond the buffer zone (Hüsken & Dietz-Pfeilstetter, 2007).

Potential unanticipated events relating to the safety and acceptability of transgenic plants include the transfer of antibiotic-resistant genes, up regulation of non-target genes by foreign promoter sequences, production of allergenic compounds and proteins, including cross-reactivity between plant-derived food and/or pollen (Jimenez-Lopez et al. 2011) , or gene products with mammalian toxicity. Different strategies have been developed for reducing the probability and impact of gene flow, including physical separation from wild relatives and genetic engineering. Mathematical models and empirical experimental evidence suggest that genetic approaches have the potential to effectively prevent transgenes from incorporating into wild relatives and becoming established in wild populations that are not reproductively isolated from genetically engineered crops. In addition, transgene strategies for controlling plant disease do not raise some of the same concerns that relate to the release of herbicide-tolerant cultivars or insect-protected varieties. The environmental and food safety aspect of each gene construct, however, must be

evaluated on the basis of its genetic background, specific gene product, and the environmental context of the host crop.

Resistance to disease based on a product from a single gene, like resistance to insects, is often overcome by development of new pest strains. Much concern has surrounded the development of plants engineered to produce their own pesticide and the subsequent development of resistant insects. Engineered resistance to insects has focused on the use of the gene for Cry (Bt) toxins, for controlling lepidopterous. Carefully management strategies will be further required to prevent development of resistance breaking pathogens, when disease-protected GM plants are grown commercially. Certainly, the breakdown of resistance as a result of pathogen adaptation, occurs in cultivars developed by classical breeding and can be anticipated if single genes are used for engineering disease-protected GM plants (Wally & Punja, 2010). Moreover, evolution of recombinant pathogens has been raised as a particular concern in strategies using structural viral genes that could recombine with naturally infecting viruses to form new viral forms (Regev et al., 2006).

Finally, the possibility of increased weediness of plants is particularly relevant to the design of plants with herbicide tolerance. Genetically modified plants themselves could become weedy and difficult to control on account of their resistance to a particular herbicide of equal concern is movement of the herbicide-tolerance trait to weedy relatives by pollen transfer. The invasiveness of any species and long-term environmental effects are difficult to project for any modified plant released into the ecosystem. However, it is not well established whether genetic modifications created by molecular techniques pose a greater risk of invasiveness than those created by classical breeding techniques (Jacobsen & Schouter, 2007).

2.2.1 Food safety and side effects of GM-crops

The safety of GM foods has been a controversial issue over the past decade. Despite major concerns, very little independent research has been carried out to establish their long-term safety.

In general terms, the safety assessment of GM foods should investigate: a) toxicity, b) allergenicity, c) specific components thought to have nutritional or toxic properties, d) stability of the inserted gene, e) nutritional effects associated with genetic modification, and f) any unintended effects which could result from the gene insertion (WHO, 2002).

Construction of transgenic crop plants must take into consideration any possible impact on food safety, concretely in the two areas of concern which are allergenicity and the production of gene products that are toxic to mammalian metabolism (Uzogara, 2000; Malarkey, 2003; Dona & Arvanitoyannis, 2009).

Biotechnology companies argue that many of the individual proteins used in GM crops have been consumed over a long period in their natural host with no health effects seen, so simply creating the proteins in a new plant will surely be the same. This assumes both that the new protein in the GM plant is identical to the naturally produced protein, and that no unintended effects have occurred during the genetic modification process that could produce other proteins. This assumption can be seen in action in the USA, where after ten years of commercialisation of GM crops there is still no post-market surveillance for allergic

reactions (Davies, 2005). New research now challenges this assumption, and brings into question the safety of both new and previously approved GM foods.

About 1–2% of adults population in the world and about 5% of children display food allergies. Around 90% of food allergies are induced by peanuts, soybeans, vegetables, fruits, milk, eggs, cereals, nuts, some fish and shellfish. Generally speaking, the allergic reaction is caused not by whole food items, but only by certain components called allergens, which most commonly are proteins, or in fact only segments of proteins (peptides) called allergenic epitopes. Biotechnology allows crop breeders to add new genes to a plant, but also to remove or inactivate a specific gene. This opens the possibility of removing specific allergens so that those people who suffer from a specific food allergy can again eat that GM food. Such "allergen-free" foods have not yet come on the market, but they are being developed in various laboratories. One group in Japan reported several years ago that they had removed the major allergen from a variety of rice. In the US research is being done to remove the main allergen from peanuts and shrimps (Randhawa et al., 2011).

Allergenicity Many children in the US and Europe have developed life-threatening allergies to peanuts and other foods. There is a possibility that introducing a gene into a plant may create a new allergen or cause an allergic reaction in susceptible individuals.

A proposal to incorporate a gene from Brazil nuts into soybeans (a methionine-rich protein) was abandoned because of the fear of causing unexpected allergic reactions (Nordlee et al., 1996). Some people are allergic to proteins that occur naturally in soybeans, and they could have a reaction if they are exposed to either conventional or transgenic soybeans or soy products. Soybeans are one of the eight most common sources of food allergies. Although less common, some people have food allergies associated with corn and they could be affected by either conventional or transgenic corn. No allergic reactions attributable to the proteins present as a result of genetic engineering have been reported in the transgenic soybeans being grown commercially at this time. Reports of an allergenic protein made as a result of genetic engineering in one particular type of transgenic corn could not be confirmed by subsequent testing.

While there isn't any evidence that allergens have been introduced into food crops by genetic engineering, two incidents have received quite a bit of publicity and caused public concern about food allergies resulting from transgenic crops:

The first incident involved soybean plants, and a gene from Brazil nuts to make soybeans that contained higher levels of the amino acid methionine, to improve nutritious chicken feed that would eliminate the need for expensive feed supplements.

The second incident involved reports of allergic reactions in people who may have eaten food containing the insecticidal protein called Cry9C, one of several forms of the Bt insecticide. When food from grocery shelves tested positive for Cry9C, demonstrating an accidental way into the food supply. During this time, the reports surfaced of allergic reactions in people who had eaten corn products that may have been contaminated by Cry9C, but special test developed by the FDA (an enzyme-linked immunosorbent assay, or ELISA test, to detect people's antibodies to the Cry9C protein) did not find any evidence that the reactions in the affected people were associated with hypersensitivity to the Cry9C protein. The test isn't 100% conclusive, though, partly because food allergies may sometimes

occur without detectable levels of antibodies to allergens. Extensive testing of GM foods may be required to avoid the possibility of harm to consumers with food allergies, and labeling of GM foods and food products will acquire new importance.

Before any GMO or derived product can be marketed in the EU, it must pass through an approval system which is intended to assess its safety for humans, animals and the environment. The GMO Panel of the European Food Safety Authority (EFSA), which provides scientific advice and technical support for GM food safety issues, published guidance for applicants seeking authorisation of GM food and/or feed, and a section of the guidance covers current requirements for assessment of allergenicity. The guidelines are based on the recommendations of the Codex Alimentarius Commission's ad hoc Intergovernmental Task Force on Foods Derived from Biotechnology. Codex is an organisation that develops international standards for food standards (Codex Procedural Manual 20th *Ed*. 2011, WHO/FAO).

Toxicity of transgene products in new and another major concern about GMOs, regardless of the source of the gene sequence. There is a growing concern that introducing foreign genes into food plants may have an unexpected and negative impact on human health. Various food plants produce compounds that would be toxic at high levels and enhancement of their production above normal levels in transgenic plants could be detrimental to human health. Up regulation of glycoalkaloids in potato by genetic manipulation, for example, would be of concern whether the increased levels resulted from classical breeding or genetic engineering (Friedman & McDonald, 1997). The gene introduced into the potatoes was a snowdrop flower lectin, a substance known to be toxic to mammals. The scientists who created this variety of potato chose to use the lectin gene simply to test the methodology, and these potatoes were never intended for human or animal consumption.

Side effects of gene products expressed in transgenic plants are also not to be ignored. The possible effect of Bt endotoxin in pollen ingested by monarch butterflies has received public attention, although further field research demonstrated low impact on lepidopterans that could be at high potential risk (Losey et al., 1999). In additinon, concerns about secondary effects on non-target insects must be balanced by the impact of traditional pesticides that would have been used if Bt transgenics were not grown.

Other example of possible secondary effects can be envisioned, such as the effect of antifungal or antibacterial gene products on degradation of crop residues in the field. Decrease in plant decomposition could affect soil fertility and, in some cases, antimicrobial gene products could lower the diversity of soil microorganism communities (Wolfenbarger & Phifer, 2000).

Whether or not these effects are important for the environment or ecology of particular macro- or micro-organisms is something that needs to be evaluated within the scope of establishing the safety of any particular transgenic plant species.

2.2.2 Controversy about the safety to eat GM crops

The primary concern many people have about genetically engineered (GE) crops is the safety of food made from them. It is unlikely that eating DNA poses any significant risk to

human or animal health, and there is no evidence to suggest that there is any additional risk from the transgenes present in genetically engineered plants. Although there continues to be quite a bit of controversy over this issue, no evidence has been found that foods made with the genetically engineered crops now on the market are any less safe to eat than foods made with the same kinds of conventional crops.

The overall goal about GE crops is not to establish an absolute level of safety, but rather the relative safety of the new product so that there is a reasonable certainty that no harm will result from intended uses under the anticipated conditions of production, processing and consumption. Most of the DNA we eat is degraded in the digestive system, but some experiments have shown that small amounts of it can be found in some cells in the body. It is thought to be unlikely that this DNA would be incorporated into the DNA of those cells, but even if it was, the chance of any undesirable effect on the whole organism is thought to be very low. Normal diets for humans and other animals contain large amounts of DNA. This DNA comes not only from the cells of the various kinds of plants or animals constituting the food, but also from any contaminating microorganisms or viruses that may be present in or on the food. We have been exposed to this variety of DNA throughout our entire history. It seems that we are well adapted to handling exposure to DNA, and there is no obvious reason that the DNA from other organisms introduced into crops by genetic engineering would have any additional effect.

Some critics of GE crops point out that a lack of evidence for harmful effects does not mean they do not exist, but just as likely could mean that we have not done the proper studies to document them. Some reject the idea that we face the same kinds of risks from GE crops as from conventionally developed crops, believing the genetic engineering process itself introduces unique risks. Genetically engineered crop varieties are being subjected to far greater scientific scrutiny than that ordinarily given to conventional varieties, even though many scientists have argued that there is no strict distinction between the food safety risks posed by genetically engineered plants and those developed using conventional breeding practices.

Safety assessments of foods developed using genetic engineering include the following considerations:

1. Evaluation of the methods used to develop the crop, including the molecular biological data which characterizes the genetic change,
2. The evaluation for the expected phenotype,
3. The general chemical composition of the novel food compared to conventional counterparts,
4. The nutritional content compared to conventional counterparts,
5. The potential for introducing new toxins, and
6. The potential for causing allergic reactions.

A major concern often expressed about GE food safety is the risk for unintentional, potentially harmful changes that may escape detection in the evaluation process. It is true that the number of factors that are examined for change is small compared to the total number of components produced by plants. Also, more extensive comparisons of plant chemical compositions would be difficult because complete data describing the composition of conventional crop plants, including knowledge of variability among different cultivars or

that due to environmental influences, is lacking. The random nature of transgene insertion when making GE plants, it is argued, may cause disruption of important genes, causing significant effects but little obvious change to the plant's phenotype.

Antibiotic resistance genes are frequently used at several stages in the creation of GE plants as convenient "selectable markers". Bacteria or plant cells without a gene for resistance to the antibiotics used can be killed when the antibiotic is applied to them. So when scientists link the gene for the desired trait being introduced into a plant with an antibiotic resistance gene, they can separate cells carrying the desired gene from those that don't by exposing them to the antibiotic. The antibiotic resistance genes end up in the genetically engineered plants as excess baggage whose function is no longer required after the process of making them is complete. Concern has been raised about the possibility that antibiotic resistance genes used to make transgenic plants could be transferred to microorganisms that inhabit the digestive tracts of humans or other animals that eat them, and therefore might contribute to the already serious problem of antibiotic resistant pathogens. Transfer of DNA from one microbe to another (horizontal gene transfer) is known to occur in nature and has been observed in some laboratory experiments under specific conditions, but the likelihood of DNA being transferred from plant material in the digestive system to microbes has not yet been experimentally determined. It is thought that for such a transfer to be possible, it would have to come from consumption of fresh food since most processing would degrade the plant's DNA. Also, there is evidence that most DNA is rapidly degraded by the digestive system. Overall, the risk of antibiotic resistance genes from transgenic plants ending up in microorganisms appears to be low.

A second concern about the use of some antibiotic resistance genes is that they could reduce the effectiveness of antibiotics taken at the same time transgenic food carrying the resistance gene for that antibiotic was consumed. In cases where this has been identified as a risk based on the mechanism of resistance, studies have suggested the chance of this happening was probably very low due to rapid digestion of the inactivating enzymes produced by the transgenic resistance gene. Most transgenic plants do not carry resistance genes for antibiotics commonly used to treat infections in humans. Scientists are developing and using different selectable markers, and are also experimenting with methods for removing the antibiotic resistance genes before the plants are released for commercial use.

2.2.3 Advantages of transgenic plants

Despite the many concerns transgenic plants raise, they do have immense potential for benefit to society (Peterson et al., 2000).

Positive effects may include soil conservation, as new cultural practices permit low till methods and consequential maintenance of soil structure and decreased erosion.

Transgenic plants with stable resistance to disease will restrict crop losses and permit increased yield. Losses of food during postharvest storage can be decreased. There are also direct benefices to decreased use of pesticides and savings in resources and energy to manufacture and apply chemicals. Genetically modified plants may one day allow us to grow profitable crops without the need for environmentally unfriendly disease control plans. Globalization of the agricultural industry inevitably results in globalization of plant diseases. Various diseases, such as blight on potato, appear to be spreading worldwide,

karnal bunt on wheat is on the increase in Asia and parts of North America, mosaic is increasing on cassava in Africa, and leaf blight continues to spread on rice in Japan and India (Moffat, 2001). Disease protected transgenic plants may yet demonstrate to be an important alternative against plant pathogens. Molecular biology has the potential to contribute significantly to a better society in which the environment is respected and an adequate food supply is provided.

Furthermore, the world population has topped 6 billion people and is predicted to double in the next 50 years. Ensuring an adequate food supply for this booming population is going to be a major challenge in the years to come. GM foods promise to meet this need in a number of ways: 1) Pest resistance Crop losses from insect pests can be staggering, resulting in devastating financial loss for farmers and starvation in developing countries, beside health hazards risks of chemical treatments, and contaminations of water and the environment; 2) Herbicide tolerance, avoiding utilization of chemicals to kill weed. Crop plants genetically-engineered to be resistant to one very powerful herbicide could help prevent environmental damage by reducing the amount of herbicides needed; 3) Disease resistance caused by viruses, fungi and bacteria; 4) Cold tolerance, avoiding destruction of sensitive seedlings; 5) Drought tolerance/salinity tolerance; 6) Nutrition. Some crops do not contain necessary nutrients to prevent malnutrition. GM crops are directed toward increase minerals and vitamine, i.e. β-carotene; 7) Pharmaceuticals production, such as edible vaccines in tomatoes and potatoes, which will be much easier to ship, store and administer than traditional injectable vaccines; 8) Phytoremediation. Soil and groundwater pollution continues to be a problem in all parts of the world. Plants have been genetically engineered to clean up heavy metal pollution from contaminated soil.

3. Unification, development and implementation of official standard technologies

The coexistence of GM plants with conventional and organic crops has raised significant concern in many European countries. Due to relatively high demand from European consumers for the freedom of choice between GM and non-GM foods, EU regulations require measures to avoid mixing of foods and feed produced from GM crops and conventional or organic crops. European research programs are investigating appropriate methods and tools to keep both GM and non GM crops isolated, i.e. isolation distance and pollen barriers, which are usually not used in North America because they are very costly and there are no safety-related reasons to employ them (Ramessar et al., 2010).

Certain global regulatory bodies require development of DNA detection methods that allow for unique identification of commercial transgenics, harmonised guidelines for the validation and use of these methods are not yet in place. As a result, numerous governmental agencies, global standards organizations, and industry organisations are attempting to develop their own independent standardisation guidelines for testing methodologies.

In the European Union, the (JRC) is playing a leading role in ensuring a harmonised approach between EU Member States, industry and stakeholders. It now hosts six European Union Reference Laboratories (EU-RLs) on food and feed safety in support of EU Member States' National Reference Laboratories (NRLs) in the respective fields. It is the National

Food Authorities who are responsible for the appropriate implementation of legislation. The latter is in place both to ensure the safety and quality of food products including animal feed and to ensure public health.

In order to ensure public health, potentially hazardous residues and contaminants are put under vigorous scrutiny and strict authorisation procedures for new additives and crops for feed and food production are in place. The aim of EU-RLs is to guarantee uniform detection, quantification and authorisation procedures. The activities of EU-RLs cover all the areas of feed and food law and animal health. In particular, those areas where there is a need for precise analytical and diagnostic results. The main objective of the EU-RLs is to contribute to a high quality and uniformity of results obtained in the various official food and feed control laboratories throughout the European Union.

Two JRC EU-RLs support authorisation for additives for feed production and of crops to be used in food and feed that have been genetically modified i.e. containing genetically modified organisms (GMOs). This work is carried out in close collaboration with the European Food Safety Authority (EFSA), the latter being responsible for risk assessment of such new substances and crops.

The main responsibilities of the EU-RLs for feed and food are to: 1) provide National Reference Laboratories (NRLs) with details of analytical methods, including reference methods, 2) organise comparative (proficiency) testing amongst the NRLs, 3) conduct training courses for the benefit of staff from the NRLs and of experts from developing countries, and 4) provide scientific and technical assistance to the European Commission, especially in cases when Member States contest the results of analyses.

The work of the EU-RLs contributes to increasing European and worldwide standardisation of analytical methods. This helps to ensure that the quality of analytical data obtained in various laboratories are increasingly comparable. Methods are developed by EU-RLs and then validated through collaborative trial testing in collaboration with the NRLs and other expert laboratories in the respective field. Proficiency tests are also organised by the EU-RLs (for NRLs) and by the NRLs (for national official laboratories) to ensure the quality of data obtained in the various laboratories that are also required for European and other international monitoring databases for exposure and risk assessment.

In this way, EU-RLs are working towards the best interests of the consumer. They are helping to build confidence in the results obtained by food control laboratories and to ensure that products purchased are in compliance with legislation and have the highest food hygiene standards.

EU-RLs also represent a unique platform for information exchange on analytical methodology and quality assurance tools for control laboratories. Together with the network of NRLs, they provide a pool of knowledge and facilities that makes them best placed to handle emerging issues.

The JRC is currently managing six EU-RLs. These are located in the Institute for Reference Materials and Measurements (IRMM) in Belgium and the Institute for Health and Consumer Protection (IHCP) in Italy. It is worth noting that the JRC-IRMM also chairs a board of expert laboratories which acts as EU-RL on behalf of the European Commission's Directorate General Agriculture. Its purpose is to harmonise analytical methodologies for

the determination of water content in poultry to ensure the quality and to prevent fraud: 1) EU-RL for GMOs in food and feed, 2) EU-RL for feed additives, 3) EU-RL for food contact materials, 4) EU-RL for heavy metals in feed and food, 5) EU-RL for mycotoxins in food and feed, and 6) EU-RL for polycyclic aromatic hydrocarbons.

Regulation of genetically engineered crops by US government is made because guidance of the first the federal government adopted a "Coordinated Framework for Regulation of Biotechnology". Under this system, three federal agencies have regulatory authority over genetically engineered (GE) crops. Each agency has a different role to ensure safety under specific legislation. These agencies and their regulatory responsibilities are:

1) *The U.S. Department of Agriculture* (USDA), through the Animal and Plant Health Inspection Service (APHIS), is responsible for assuring that any organism, including genetically engineered organisms, will not become pests that can cause harm if they are released into the environment. APHIS has used their authority to grant permission and set the rules for field testing of genetically engineered crops. These crops cannot be commercialized until they are granted "non regulated" status by APHIS upon satisfactory review of the field testing data.

2) *The Food and Drug Administration* (FDA) is responsible for ensuring the safety of most food (except for meat, poultry and some egg products, which are regulated by the U.S. Department of Agriculture), including food from genetically engineered crops. If the allergen, nutrient and toxin content of new GE foods fall within the normal range found in the same kind of conventional food, the FDA does not regulate the GE food any differently. So far, all genetically modified foods in the U.S. marketplace have gone through a voluntary review process where the FDA determines whether they are "not substantially different" from the same conventional foods by consulting with developers of new GE foods to identify potential sources of differences, then reviewing a formal summary of data provided by the developer. Recently, the FDA has announced a new rule that would make pre-market consultation mandatory. The FDA has the authority to order foods to be pulled from the market at any time if are found to be unsafe, or to require labeling of any food that has different amounts of allergens, nutrients, or toxins than a consumer would expect to find in that kind of food.

3) *The Environmental Protection Agency* (EPA) evaluates the safety of any pesticides that are produced by genetically engineered plants. The EPA calls novel DNA and proteins genetically engineered into plants to protect them against pests "plant incorporated protectants" (PIPs) and regulates them the same way they regulate other pesticides.

Under the Coordinated Framework, some kinds of genetically engineered crops might not be subject to the oversight of all three agencies. For example, an ornamental flower like petunias engineered to have longer lasting blooms may only have to meet the requirements of APHIS, but a food crop like soybeans engineered to produce an insecticidal compound would be subject to the rules of all three agencies. Additional regulations are imposed by some states. Also, the National Institutes of Health has developed safety procedures for research with recombinant DNA. Most institutions developing genetically engineered crops follow the NIH guidelines, and they are required for federally funded research.

4. Labeling of genetically engineered foods

GMO labelling was introduced to give consumers the freedom to choose between GMOs and conventional products. Essentially, if a foodstuff is produced using genetic engineering, this must be indicated on its label. The target of most labeling efforts is food products that were genetically engineered, that is, they contain genes artificially inserted from another organism.

Whether or not to require mandatory labeling of genetically engineered (GE) foods is a major issue in the debate over the risks and benefits of food crops produced using biotechnology. The issue is complex because 1) many arguments put forth in the debate are based on disagreements about the adequacy of our scientific understanding of the consequences of genetic engineering; and (2) significant changes to our current food marketing and manufacturing system, with potentially large economic impacts, would be required to implement mandatory labeling.

Actual labelling practice, however, is far more complicated, and must be planned and regulated with issues such as feasibility, legal responsibilities, coherence and standardisation in mind.

While some groups advocate the complete prohibition of GMOs, others call for mandatory labeling of genetically modified food or other products. Other controversies include the definition of patent and property pertaining to products of genetic engineering. According to the documentary Food, Inc. efforts to introduce labeling of GMOs has repeatedly met resistance from lobbyists and politicians affiliated with companies developing GM crops.

Governments around the world are hard at work to establish a regulatory process to monitor the effects of and approve new varieties of GM plants. Yet depending on the political, social and economic climate within a region or country, different governments are responding in different ways.

Agribusiness industries believe that labeling should be voluntary and influenced by the demands of the free market. If consumers show preference for labeled foods over non-labeled foods, then industry will have the incentive to regulate itself or risk alienating the customer. Consumer interest groups, on the other hand, are demanding mandatory labeling.

Central to the arguments for mandatory labeling is that consumers have the right to know what they are eating. This is especially true for some products made with biotechnology where health and environmental concerns have not been satisfactorily resolved. Historically industry has proven itself to be unreliable at self-compliance with existing safety regulations. Some people do not wish to use genetically engineered products for religious or ethical reasons. Labeling is the only way consumers can make informed choices, whatever their reasons may be.

Major arguments against mandatory labeling have addressed the practical concerns about the expense and complex logistics that would be required to ensure GE and conventional foods are kept separate or to test all foods for GE content. It is argued that such measures are unnecessary since no significant differences have been found between today's GE foods and conventional foods. Enacting mandatory labeling will also require resolving certain other questions. Major issues include defining exactly what kinds of technologies would be

covered, deciding on tolerance levels for genetically engineered content or ingredients before labeling would be required, and choosing a method for verifying that products are properly labeled.

In Europe, anti-GM food protestors have been especially active. In the last few years Europe has experienced two major foods scares: bovine spongiform encephalopathy (mad cow disease) in Great Britain and dioxin-tainted foods originating from Belgium. These food scares have undermined consumer confidence about the European food supply, and citizens are disinclined to trust government information about GM foods, establishing a mandatory food labeling of GM foods in stores, with a 1% threshold for contamination of unmodified foods with GM food products, a commonly proposed threshold. In other words, if any ingredient of a product exceeds one percent GM content, the product needs labeling. One percent is the labeling threshold decided upon by Australia and New Zealand. The European Union has decided on a level of 0.9 percent, while Japan has specified a five percent threshold. Thresholds as low as 0.01 percent (the approximate limit of detection) have been recommended (Davison, 2010).

The shift of global agriculture towards biotech varieties, however, has not been supported by all elements of society. In response to these differing levels of acceptance of the use of this technology, several countries have adopted regulations requiring that foods prepared from GM ingredients be labeled as such. However, labeling of foods is necessary only when the concentration of GM material in a food ingredient measures above a specified threshold concentration (%GM). The adoption and implementation of such laws can have significant consequences to global commerce in agriculture, food, and feed. Meeting these global market requirements for GM compliances is further complicated by the fact that each country has different regulations, including different GM ingredient thresholds for labeling and different methods of testing.

In the United States, the regulatory process is confused because there are three different government agencies that have jurisdiction over GM foods. The EPA evaluates GM plants for environmental safety, the USDA evaluates whether the plant is safe to grow, and the FDA evaluates whether the plant is safe to eat. The EPA is responsible for regulating substances such as pesticides or toxins that may cause harm to the environment. GM crops such as B.t. pesticide-laced corn or herbicide-tolerant crops but not foods modified for their nutritional value fall under the purview of the EPA. The USDA is responsible for GM crops that do not fall under the umbrella of the EPA such as drought-tolerant or disease-tolerant crops, crops grown for animal feeds, or whole fruits, vegetables and grains for human consumption. The FDA historically has been concerned with pharmaceuticals, cosmetics and food products and additives, not whole foods. Under current guidelines, a genetically-modified ear of corn sold at a produce stand is not regulated by the FDA because it is a whole food, but a box of cornflakes is regulated because it is a food product. The FDA's stance is that GM foods are substantially equivalent to unmodified, "natural" foods, and therefore not subject to FDA regulation.

Independently of the government organism, there should be regulated the verification claims to know if a food is or is not genetically engineered. There are two ways this can be done: 1) Content-based verification requires testing foods for the physical presence of foreign DNA or protein. A current application of this type of procedure is the analysis and

labeling of vitamin content of foods. As the number of transgenes in commercialized crops increases, the techniques for detecting an array of different transgenes have become more sophisticated (Shrestha et al., 2008). 2) Process-based verification entails detailed record-keeping of seed source, field location, harvest, transport, and storage (Sundstrom et al., 2002).

There are many questions that must be answered whether labeling of GM foods becomes mandatory:

First, it is concern about whether consumers are willing to absorb the cost of labeling. Accurate labeling requires an extensive identity preservation system from farmer to elevator to grain processor to food manufacturer to retailer (Maltsbarger & Kalaitzandonakes, 2000). If the food production industry is required to label GM foods, factories will need to construct two separate processing streams and monitor the production lines accordingly. Farmers must be able to keep GM crops and non GM crops from mixing during planting, harvesting and shipping. It is almost assured that industry will pass along these additional costs to consumers in the form of higher prices. Either testing or detailed recordkeeping needs to be done at various steps along the food supply chain. Estimates of the costs of mandatory labeling vary from a few dollars per person per year to 10 percent of a consumer's food bill (Gruere & Rao, 2007). Consumer willingness to pay for GE labeling information varies widely according to a number of surveys, but it is generally low in North America. Another potential economic impact for certain food manufacturers is that some consumers may avoid foods labelled as containing GE ingredients.

Secondly, the acceptable limits of GM contamination in non GM products. The EC has determined that 1% is an acceptable limit of cross-contamination, yet many consumer interest groups argue that only 0% is acceptable. In addition, it is necessary to know who is going to monitor these companies for compliance and what could be the penalty if they fail.

Third, concerns the level of traceability of GM food cross-contamination. Scientists agree that current technology is unable to detect minute quantities of contamination, so ensuring 0% contamination using existing methodologies is not guaranteed. Yet researchers disagree on what level of contamination really is detectable, especially in highly processed food products such as vegetable oils or breakfast cereals where the vegetables used to make these products have been pooled from many different sources. A 1% threshold may already be below current levels of traceability.

Finally, who should be responsible for educating the public about GM food labels. Food labels must be designed to contain clearly and accurate information about the product in simple language that everyone can understand. This may be the greatest challenge faced be a new food labeling policy: how to educate and inform the public without damaging the public trust and causing alarm or fear of GM food products.

Under current policy, the U.S. Food and Drug Administration do not automatically require all genetically engineered food to be labeled. Conventional and genetically engineered (GE) foods are all subject to the same labeling requirements, and both may require special labeling if particular food products have some property that is significantly different than what consumers might reasonably expect to find in that kind of food. Therefore, particular genetically engineered foods are subject to special labeling requirements if the FDA concludes they have significantly different properties including:

1. A different nutritional property from the same kind of conventional food,
2. A new allergen consumers would not expect to be in that kind of food (a hypothetical example would be an allergenic peanut protein in GE corn or some other crop),
3. a toxicant in excess of acceptable limits.

Examples of genetically engineered foods that require special labeling are those that contain vegetable oil made from varieties of GE soybeans and canola where the fatty acid composition of the oils extracted from the seeds of these crops was altered. Since the oils from these varieties have different nutritional properties than conventional soy and canola oils, foods made with them must be labeled to clearly indicate how they are different. You might see "high laurate canola" or "high oleic soybean" on food labels if these products were used. The FDA does not require them to be labeled as "genetically engineered", but that information could also be included on the label.

So far, no approved, commercially grown genetically engineered food crops have known properties that would require foods made from them to be labeled because they contain a new allergen or excess levels of toxic substances.

Federal legislation has been proposed that would require mandatory labeling of genetically engineered foods and similar initiatives at the state or local level have been considered or are currently pending.

5. Conclusions

The present atmosphere surrounding genetically engineered crops has led to a situation where food safety assessment is not just about science, but also about concerns, and standards about how to assure “safety.” The detection, identification and quantification of the GMO content in food or feed products are a great challenge. The existing analytical methods for GMO testing leave the inspection authorities with many choices and compromises.

Methods, which can guarantee absence of non approved GMOs in seed samples even at the suggested 0.1% level of GM contamination does not exist at present. However, PCR and immunoassay based technologies are often used for the detection of products of agricultural biotechnology. They are valuable and reliable tools for the detection of GM products in seed production and very early in the food and feed supply chain. Concretely, when operated within specifications, immunoassays have been proven, in most cases, to be fast, reliable, and economic test methods.

It is critical that such methods are reliable and give the consistent results in laboratories across the world. This includes the need for a proper validation of the methods. The choice of the appropriate reference material will impact the reliability and accuracy of the analytical results, and numerous biological and analytical factors need to be taken into account when reporting results. Furthermore, as scientific opportunities advance, agreement on reasonable standards of safety for developing countries will be critical, and exchange of data as well, which will help ensure that data requirements are manageable across the developing world.

We don’t know yet all the potential risks that the GMO could have, by long term accumulation, upon the environment. New strategies, therefore, are required to face the

continuing challenge of disease spread to new environments and emergence of resistance-breaking strains of microbial plant pathogens. Disease-protected transgenic plants may yet prove to be an important arsenal in the battle against plant pathogens. With a judicious approach and careful development of new innovations, molecular biology has the potential to contribute significantly to a better society in which the environment is respected and an adequate food supply is provided. Genetically-modified foods have the potential to solve many of the world's hunger and malnutrition problems, and to help protect and preserve the environment by increasing yield and reducing reliance upon chemical pesticides and herbicides. Yet there are many challenges ahead for governments, especially in the areas of safety testing, regulation, international policy and food labeling.

The achievements of the genetic engineering have nowadays considerable benefits, but now we don't know the price we, or the future generations, will have to pay for these benefits. The long term risks of the GMO are not entirely known today, and long-term studies are clearly necessary. We must proceed with caution to avoid causing unintended harm to human health and the environment as a result of our enthusiasm for this powerful technology.

Globalization of the agricultural industry inevitably results in globalization of markets, so competency in assuring food safety for GM crops is essential. This competency will enable countries to conduct independent research when necessary. Building such capacity also creates sufficient infrastructure to allow scientifically defensible decisions in the face of food safety questions colored by each country's perceptions and circumstances. It is obvious that international collaboration is needed to ensure that the methods offered by the different companies hold promises, which can be done by elaboration of:

1. Further research to understand the appropriateness of DNA and protein based methodologies,
2. Compatibility between methods,
3. Appropriate protocols for validation studies and for proficiency testing,
4. Make appropriate reference materials readily and globally available.

In the future, the number of different GMOs is expected to grow, and research is going in the direction to develop GM plants with inducible promoters that activate specific traits when needed. It can be expected that detection of GMOs will become more complicated in the near future, being one of the mayor challenges for the future will be to develop analytical identification methods that facilitate screening for all the promoters used worldwide.

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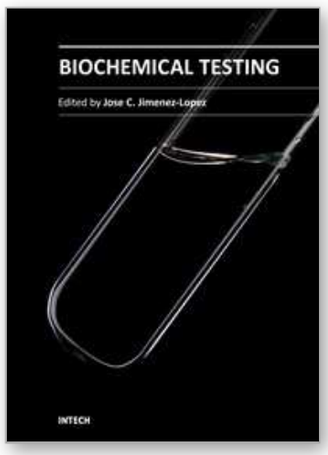
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Biochemical testing necessitates the determination of different parameters, and the identification of the main biological chemical compounds, by using molecular and biochemical tools. The purpose of this book is to introduce a variety of methods and tools to isolate and identify unknown bacteria through biochemical and molecular differences, based on characteristic gene sequences. Furthermore, molecular tools involving DNA sequencing, and biochemical tools based in enzymatic reactions and proteins reactivity, will serve to identify genetically modified organisms in agriculture, as well as for food preservation and healthcare, and improvement through natural products utilization, vaccination and prophylactic treatments, and drugs testing in medical trials.

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InTech Europe
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