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Genetically Modified Foods' Safety Analysis by using Molecular Profiling Techniques

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ABSTRACT

There are numerous ways for the identification of accidental changes in GM Foods' composition, which may arise due to the genetic changes, such as comparative analysis of (chemical) GM and non GM foods. These ways of identification of alterations commonly include DNA/RNA microarrays, metabolomics and protein profiling methods. These profiling methods are very useful but still studies on sensitivity, specificity and substantiation are needed. Furthermore, bioinformatics studies may be quite useful for the successful application of these profiling methods to analyze the safety of genetically modified (GM) foods. These bioinformatic methods employ the comparison of different linked databases which may cover all the information significant for profiling. These methods are also significant in identifying the changes at various stages of development under diverse environmental conditions.

Keywords: Food, Proteomics, Metabolomics, Genomics, Nucleic acid

INTRODUCTION

In the composition of genetically modified (GM) foods, some unwanted modifications do exist. These genetic modifications are quite helpful in designing procedures for safety assessment of GM foods. Gene knock-out and knock-in approaches into the DNA of the host may cause unwanted changes in metabolic pathways in addition to the anticipated effects. These alterations in the metabolic pathways can cause changes in the concentrations of nutrients, secondary metabolites and production of new toxins. It is considered that unwanted effects are not only related to GM foods but can cause negative effects at molecular level in plant breeding(Joyce *et al.,* 2003). Different strategies for food safety assessment of GM foods have been proposed earlier and these strategies have been accepted worldwide (Kuiper, 2004;Lehrer & Bannon, 2005;Cressman & Ladics, 2009). The idea of significant similarities was designed as a comparative vector to recognize resemblances and alterations between GM and non-GM foods. These resemblances and alterations can help in the evaluation of their impact on the health of animals and humans. In a recent study on the complications related to safety assessment of GM foods some methods have been described to detect unwanted changes which may be produced in GM organisms due the change in their genome (Bradford *et al*., 2005; Conesa *et al.,* 2007; Kuiper, 2004). The present review focuses on "Safety Evaluation of GM Foods" that mainly concentrate on formulation of novel procedures for the safety assessment of genetically modified (GM) food crops and procedures for recognizing unwanted effects in their genetic structure.

RECOGNITION OF UNWANTED EFFECTS – PROCEDURES

Different procedures can be implemented to find out the unwanted effects in GM foods that arise by the genetic alterations (Figure 2). By analyzing the flanking regions of a transgene we can speculate the unwanted effect which is the most common way to find out point of insertion of that transgene. Therefore, the insertions can be analyzed endogenously or exogenously which is not an accurate procedure to predict modifications in genomes and gene expression regulation. In order to predict phenotypic alterations in modified organisms, sequencing of the point of insertion can play a crucial role. Phenotypic variations in genetically modified organisms can be recognized by analyzing some important factors such as disease resistance, chemical composition, growth and yield of the product (Kim *et al.,* 2008).

In order to identify the changes in parental species of genetically modified (GM) organisms

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usually a targeted methodology is adopted. Targeted approach includes the analysis of a single known compound such as micro-nutrients, macro-nutrients and anti-nutrients or toxins. These anti-nutrients may affect the digestion of macro-nutrients and they can stop the gastrointestinal activity of the essential (vital) elements. This assessment of chemical contents exemplifies a significant safety analysis of GM foods as described in case of tubers which are made as virus resistant and insect resistant plants through this technology (Kim *et al.,* 2008). This assessment is a better tool to get the illustrations of the major (carbohydrates, proteins and fats) and minor components (vitamins, minerals and toxins – glycol alkaloids) and also of the proteins structure. The purpose of this analysis was to describe that normal and conventional varieties of potatoes are similar to GM potatoes in their chemical composition. Only two cases have been reported in the literature in which statistically important changes between GM lines and parental lines went far away from the proposed effects of the genetic alterations. These two cases were of GM potato and GM rice varieties respectively. In these cases, soybean glycinin, high amount of glycoalkaloids and vitamin B were observed in GM potatoes and GM rice respectively (Jelenić, 2005; Yabor *et al.,* 2010).

This is a targeted analysis approach but it has a drawback of analyzing only a specific range of compounds. Moreover, it is not possible to detect anti-nutrients and unspecified toxins separately using this approach. These profiling procedures are suggested as methodsto characterize alterations in contents of the genetically modified (GM) plants in order to upturn the possibilities of recognizing unwanted outcomes(Lehrer & Bannon, 2005;Roig & Arnáiz, 2000). It may have a certain significance for genetically modified foods (GM) with enhanced nutritive/health conserving characteristics which can be attained by inserting “multiple genes”. It is a non-targeted methodology which comprises of micro-array technology of DNA or RNA, coupled with some analytical techniques of proteomics. At different cellular integration levels, it also allows impartial profiling of viable alterations in metabolism and physiology of genetically modified organisms. Recently, a review has been published which discussed the ability of profiling procedures in order to study physiology and molecular genetics of plants (Kell *et al.,* 2005).

PROFILING TECHNIQUES

Proteomics

It is the branch of biotechnology which deals with the study of all the proteins produced by the genes which exist in cells, organisms or tissues in definite conditions. It is possible to perform analysis after the transcript profiling using this well-established technique. The method which is currently being in-use involves (2DGE) two-dimensional gel electrophoresis through which protein spots are removed from gel, breaking down into extracts by using definite proteases. The protein assessment is being done using (MS) mass-spectrometry and for consequent recognition of the fragments, some computer utilizing databases are being used (Rahim, 2008).

To perform relative analysis of the protein configurations by utilizing various techniques of proteomics would be an important tool for food safety analysis. This technique analyses the changes in post-translational modifications and identifies concentrations of proteins that arise due to genetic modifications or environmental factors (Cánovas *et al.,* 2004; Xie *et al.,* 2007). Quantification of the proteins is one of the major problems faced because 2DGE technique helps only in the detection of those proteins which are highly expressed (Maurya *et al.,* 2007)due to the little vital scale of quantification. Some of the alternatives are also in use such as isotope-coded affinity tags based quantification (Pawlik *et al.,* 2006) and multi-dimensional liquid-chromatography techniques which can be combined to assist mass-spectrometry procedures(Clarke & Naylor; 2002, Tonack *et al.,* 2009; Frank *etal*., 2007).The proteomic techniques and their applicability are under studies these days by European multi-disciplinary projects for the safety analysis of GM foods (Kuiper, 2004;Kok *et al.,* 2008). GM varieties of potatoes and tomatoes are being tested by protein profiling using two-dimensional gel electrophoresis (2DGE). The ability of polypeptide fractionation and the quantitative analysis of fractionated peptides by the application of isotope coded affinity tags and ability of poly-peptide fractionation are under examination. The recognition of changes related to the genetic alteration might be very tough because big number of proteins are not linked to these alterations and show natural variations in protein configurations due to certain environmental conditions.

The partial acquaintance of the usual variations in the plant proteins configurations demands the formation of up-to-date databases and further optimization of the techniques. In order to study interesting aspects of proteomics, it would be better to concentrate on the proteins that took part in significant metabolic pathways, linked proteins and on the immuno-blotting micro-arrays.

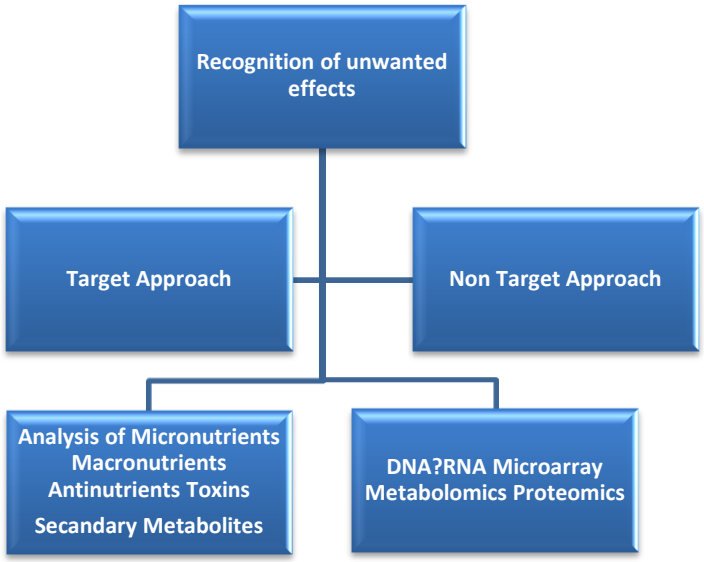

Fig.1: Safety Analysis of Genetically Modified Foods(Kuiper et al., 2003)

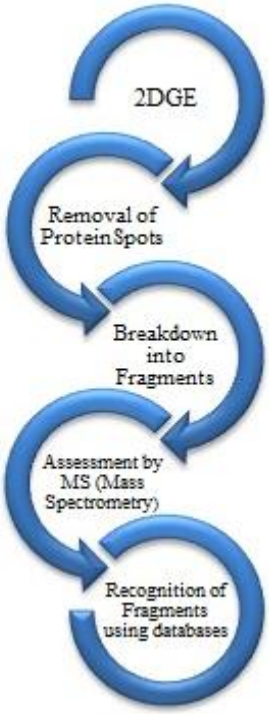

Fig.2: Safety Analysis by Proteomics
(Kuiper et al., 2003)

Recognition of Changed Gene Expression

In the history of analysis of gene expression, the microarray technology is considered as the most recent advancement. This technology can analyze expression of a large number of different genes at the same time and can detect the alterations to the core underlying the genes directly (Maurer *et al.,* 2005; Kruse & Stewart, 2007). This technology is being tested and applied (Lawrie *et al.,* 2007) in many fields like botany (Arabi *et al.,* 2015, Tokimatsu *et al.,* 2005), humans nutrition (Van-Beek *et al.,* 2008) and medical sciences (Pepperkok & Ellenberg, 2006). Construction of array is one of the most important steps in recognizing the altered gene expression through this technology. Moreover, various array systems are accessible commercially that contain a huge number of genes which can be expressed in a particular organism. However, this number is considered extremely less in food crops.

In order to make informative arrays, various research projects have been initiated on potato and tomato as model experiments on food crops. cDNA libraries which are used for the construction of array in case of tomatoes have been attained and these have particular cDNAs for red and green levels of ripening. The library that is red-specific microarray may have cDNAs linked with metabolism of flavonoids and vitamins in tomatoes, while the library that is with green-specific microarray may have cDNAs associated with the production of natural toxins, tomatin in tomatoes (Arabi*et al.,* 2015).

The cDNA libraries have been constructed following an idea that genetic alterations may cause changes in vital metabolic pathways associated in the formation of toxins or some health favorable compounds. Moreover, a potato-library has been made in order to illuminate metabolic pathways of naturally produced toxins (glycoalkaloids i.e. solanine and chaconine) of potato. Furthermore, additional potato-libraries are now being constructed for potato plant and tubers. Through this process, the resultant array will not only be able to recognize changed gene expression in tuber pathways but can also be capable of detecting activation of metabolic pathways that are related to other plant parts.

There are some other methods of recognition of gene expression, in which hybridization of correctly selected mRNAs is of great importance. In this technique, it is pertinent to compare the gene expression of plant tissues growing at the same developmental stages, under natural and in laboratory conditions. In order to estimate microarray fluorescent configurations it would be essential to possess adequate knowledge of the variations produced naturally in the gene expression throughout different steps of formation of tissues under diverse environmental circumstances. Hence, alterations which are identified between two different plant generations i.e. in genetically modified (GM) and under naturally produced parent organisms, can be analyzed against generally predicted background configurations. Efforts are being carried out to identify the naturally occurring variations in particular tissues of plants and the results of these evaluations will highlight the significance of microarray technology to recognize the various outcomes in genetically modified (GM) food varieties. This technique can efficiently observe projected outcomes in genetically modified (GM) varieties of tomato and can be used to monitor or identify the unwanted effects of the processes related to plant breeding in specific plants (Lawrie *et al.,* 2007).

Metabolomics

It is a branch of biochemistry which deals with the study of specific sets of metabolites that exist in a cell, tissue or an organism. To detect changes in a genetically modified (GM) food, the composition of the cellular compounds such as acids, fats, sugars and metabolites would be better to evaluate. In order to perform analysis, metabolic profiling techniques have been developed on the basis of mass spectrometry (MS), high performance liquid chromatography (HPLC), gas chromatography (GC) and nuclear magnetic resonance (NMR)(Raamsdonk *et al.,* 2001; Smits *etal*., 2006;Wenig & Odermatt, 2010). These techniques may serve many purposes such as detection, quantification, identification and resolving of compounds in a specified sample(Lenucci *et al.,* 2012). A new methodology has also been introduced named as “metabolite profiling methodology” in which rice is used as a standard crop(Rohloff, 2015). Therefore, through this methodology, fractionalization of entire rice samples is performed which enable the assessment of a wide range of the major and minor components present in rice. The profiles of the methylated or silylated compounds of plants can be obtained through gas chromatography and mass spectrometry associated with flame-ionization detection techniques. The significance of association of gas chromatography and mass spectrometry to the instantaneous evaluation of a wide range of polar and non-polar metabolites in

potato tubers and *Arabdidopsis thaliana* leaves has already been described by Fiehn *et al.* (2000) and Lisec *et al.* (2006). In order to characterize the genotypes of modified potatoes in sucrose metabolism, metabolomics was used by Hall *et al.*2005). This technique also showed the presence of novel unpredicted metabolites in chromatograms belonging to transgenic tubers.

There are some limitations of this technique such as it is necessary to fractionate compounds for making them volatile and it is necessary to reabsorb them from their liquid solution from column in order to perform further evaluation. These limitations can be overcome through the use of metabolite profiling techniques(Intoh *et al.,* 2009). The combination of nuclear magnetic resonance (NMR) and liquid chromatography was previously in use for the evaluation of GM varieties of tomato (with moderate ripening factors attained by the modification of anti-sense RNA exogalactanase) and their unaltered counterpart (Anonymus, 2008; Ren *et al.,* 2009). α-lycopene existed in anti-sense fruit two to four times more in concentration than present in the parental line and it was revealed by ^{1}H-NMR spectra of pre-fractionated extracts. This variation is not a proposed target of alteration but it seems to be a result of moderate ripening procedure. The above mentioned techniques used for metabolite profiling are the great tools for neutral evaluation of a wide-range of metabolites present in plants.

Data Evaluation

The use of molecular profiling methods to the fewer samples produces a hugedata. The significant evaluation of profiles of genetically and non-genetically modified foods should be established by keeping all the responsibilities in mind. Therefore, these multi-variate techniques i.e. hierarchical cluster analysis (HCA) and principal component analysis (PCA) are normally used for data evaluation (Fiehn *et al.,* 2000; Hall *et al.,* 2005). It is very useful to apply multi-variate techniques but it may not always be able to differentiate between wanted and unwanted effects of genetically modified genes in plants. The large amount of data which is being generated through the use of profiling techniques should be analyzed as early as possible regarding their biological significance. Therefore, a system of linked databases containing protein profiles, metabolite profiles and gene expression, indicating distinct developmental steps and environmental circumstances, where necessary(Shulaev, 2006; Medvedeva *et al.,* 2005).

CONCLUSION

The use of profiling methods for safety analysis of GM foods may produce related information about changes in gene expression and linked metabolic outcomes due to the genetic alterations. A neutral comparison between GM and non-GM organisms may provide us with changes observed at different integration stages of cells and the tissues. But there is a restriction in applying these profiling methods that is the production of large data used to analyze specific genetically modified generations and natural complications in producing a significant explanation. The absence of up-to-date associated databases contains information of profiles variations linked with related developmental steps and environmental circumstances are another problem. The ability of profiling techniques to identify unwanted effects linked with genetic alterations is apparent but in order to ensure sensitivity and specificity, more investigation is required to discuss their importance for the safety analysis of GM foods.

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