Chapter

Genetic Modified Food for Ensuring Food Security Issues

Sujay Kumar Bhajan, Mohammad Mahmood Hasan, Md. Anwarul Haque and Mohammad Nurul Islam

Abstract

Genetic modification is a technology that allows incorporating beneficial genes from other organisms. One of the major challenges of current era is feeding the consisting growing population of the world. Reduced cultivatable land and climate change have made it even more challenging. Through genetic engineering technology it is possible to develop high yielding, disease resistance and climate resilient crop varieties within shorter period of time than the conventional ways. Current global status of GM crop cultivation and production has already proved that GM crops have the potential to achieve food security for the future world. However, in most of the countries, public has misperception of its risks on human health and environment. Due to such misconception most of the countries have not adopted GM crops yet which could make it difficult to utilize GM crops to achieve food security. Therefore, in this book chapter we discussed on how genetically modified crops are developed, what beneficial traits are usually incorporated, how they are assessed for human health risks and regulations for selling genetically modified foods in the market. Such discussion would help common people to understand how this technology can help us ensuring food security of the world.

Keywords: genetically modified organisms (GMOs), genetically modified foods (GMF), food security, safety assessment, biosafety

1. Introduction

The growth of the human population will increase day by day and create significant challenges for agricultural production. Now a days food security is one of the major concerns for the growing global population. Nearly 870 million people suffer from malnutrition; most of them are living in the developing countries of Africa, Asia, and South America [1]. Additionally, climate change and environmental deterioration are currently reducing the available agricultural land, creating additional challenges to fill the increasing food demand [2]. The use of modern biotechnology, including genetic modification techniques, has been proposed as a way to reduce the environmental footprint, by improving food quality and increasing productivity [3]. Genetically Modified Food (GMF) means any food containing or derived from a genetically engineered organism [4]. The majority of the biotech crops available on the global market have been genetically manipulated to express one of these basic traits: resistance to insects or viruses, tolerance to certain herbicides and nutritionally enhanced quality. In 2019, the 24th year of commercialization of biotech crops, 190.4 million hectares of biotech crops were planted by up to 17 million farmers in 29 countries. From the initial planting of 1.7 million hectares in 1996 when the first biotech crop was commercialized, the 2019 planting indicates ~112-fold increase Thus, biotech crops are considered as the fastest adopted crop technology in the history of modern agriculture [5]. Major producers of GM crops include USA, Argentina, Canada, and China. In the US, about 80% of maize, cotton and soya are biotech varieties [6]. Several safety issues are considered for the release or commercialization of genetically modified crops such as safety issues for human health, toxicity analysis, allergenicity analysis and biosafety assessment of genetically modified foods. This book chapter discusses the overall process for developing of genetically modified crops, brief history of global adoption of genetically modified crops, main agricultural traits and current status of the approved genetically modified crops and its role in food Security.

2. The overall process for developing of genetically modified crops

The crop plants developed through modification of genetic composition using genetic engineering techniques are referred to as genetically modified (GM) crops, transgenic crops or genetically engineered (GE) crops. The overall process for developing of genetically modified crops illustrated in (**Figure 1**) [7]. Different steps are involved in the genetic transformation work like Identification and isolation of gene of interest (transgene), cloning into suitable plasmid vector and transformation of desired gene into the plant cells, confirmation of transformation by molecular techniques.

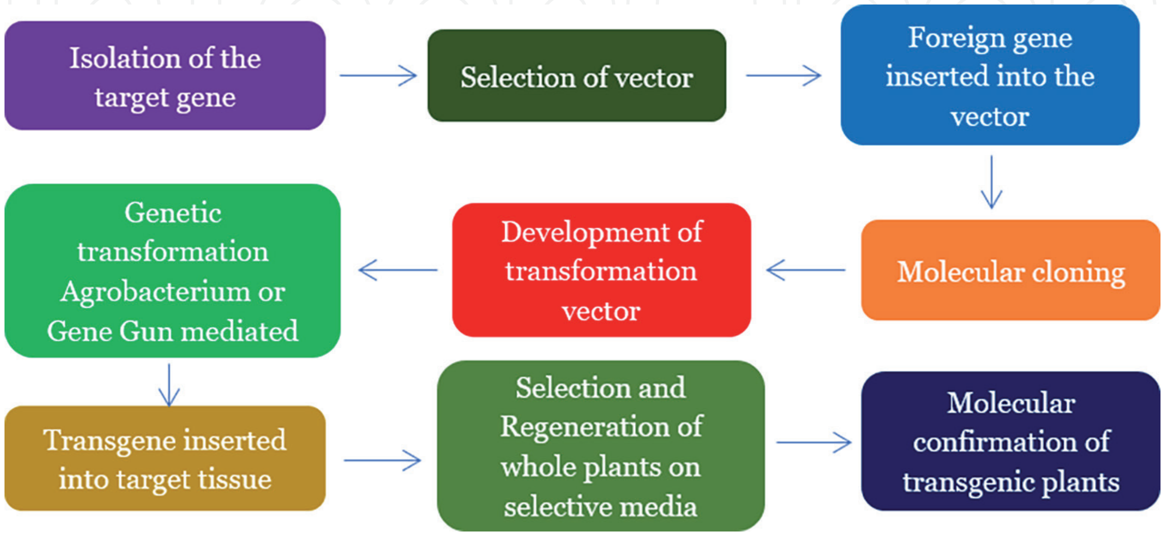

Figure 1.
The overall process for developing of genetically modified crops.

2.1 Identification and isolation of the target genes

To produce a GM plant, scientists first identify the desired trait missing in the cultivar, such as resistance to biotic and abiotic factors (salt, cold, drought, herbicides, pesticides, insects, nutritional quality etc.). Then, by using existing knowledge they find an organism (plant, animal, or microorganism) processing the desired trait. Finally, the desired genes are isolated using suitable molecular tools.

2.2 Molecular cloning of target gene and development of plant transformation vector

A proper gene construct is crucial for the success of producing ideal transgenic line. A vector is a DNA molecule that has the ability to replicate inside the host to which the desired gene has integrated for cloning. A vector acts as a vehicle that transports the gene of interest into a target cell for replication and expression [8]. Common vector consists of three components: an origin of replication, multiple cloning site or recombination site and selectable marker gene. The common vectors include-plasmids, cosmids, bacteriophages, bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC), etc. which is used for the production of genetically modified crops [9]. The most commonly used plant transformation vectors are Ti plasmid binary vectors of *Agrobacterium tumefaciens.*

2.3 Transformation of desired gene into the plant cells

Transformation of transgenes into the plant cells can be carried out through indirect or direct gene transfer methods. An indirect gene transfer (vector-mediated) method involves the introduction of exogenous DNA into the plant genome via biological vectors, whereas direct gene transfer methods involve the introduction of exogenous DNA directly into plant genome through physical or chemical reactions. The important physical and chemical gene transfer methods are electroporation, microinjection, particle bombardment, Polyethylene glycol and Liposome mediated gene transfer. *Agrobacterium*-mediated transformation is the most common technique used in plant transformation as it is efficient and effective in a wide range of plants [10]. This method had successfully transformed a broad variety of plants such as rice, maize, barley, and tobacco. Among the physical methods particle bombardment biolistic is the most common one used in plant transformation [11]. Currently, transient and stable transformation can be achieved through the *Agrobacterium*-mediated and biolistic methods. In the *Agrobacterium*-mediated method, the T-DNA region is inserted into the plant genome forming a stable transformant, whereas the non-integrated T-DNA plasmid expresses the transgene transiently. In the biolistic method or other direct gene transfer methods such as electroporation, transient and stable expression of the transgenes are usually dependent on the plasmid or transgene constructs.

2.4 Confirmation of transgene integration by molecular techniques

Confirmation of transgene integration has to be done through an appropriate method based on the transgene constructs, selectable marker, and reporter gene used. The antibiotic resistance genes are screened by the addition of herbicides or antibiotics to the growing media to distinguish transformed plant cells from the non-transformed plant cells. However, this method requires a large quantity of antibiotics and herbicides that are expensive and worsen by the risk of horizontal gene transfer to other bacteria.

An alternative screening method such as polymerase chain reaction (PCR) and reporter gene expression are used for the identification of transgenic plants. Some reporter genes such as the GFP, GUS, CAT, LacZ and Luc are used for the expression of these genes could be observed visually or directly under microscopy [12]. The GUS expression can also be detected through histochemical assay in which the localization of the transgene can be observed. Southern blotting is a molecular method which is generally used to identify the number of transgenes (copy number) inserted into the host genome as well as for the detection of transgene integrity and transgene rearrangement [13]. The polymerase chain reaction (PCR) method is one of the most sensitive and easiest methods among all the molecular techniques employed for the verification of the transgene. The PCR is generally done with primers specific to the site of plasmid constructs and gene of interest used for development of the transgenic plants. Successful amplification of the DNA fragment with expected band indicates the possible presence of transgene, and this DNA fragment is further confirmed through DNA sequencing. A real-time PCR provides fast, sensitive, and high-throughput molecular PCR–based analysis compared to the traditional Southern blot analysis especially in the area of transgene copy number and zygosity detection in transgenic plants [14]. In recent years, the availability of NGS tools and bioinformatics resources facilitates the study of genome and molecular characterization of complex traits. Hence, NGS approach provides an alternative high-resolution analysis tool for transgenes insertion in GM crops [15].

2.5 Regeneration of the whole plant from the transformed cells

Expression of transgene and production of functional protein is important for development of genetically modified foods. Successful genetic engineering of plants often requires the regeneration of whole plants from transformed plant cells. Plant regeneration usually involves culturing plant tissue in the presence of specific plant hormones under sterile conditions.

3. Brief history of global adoption of genetically modified crops

The history of GM crop in agriculture started with Flavr Savr™ Tomato, the first ever GM crop approved for human consumption in 1994 by the Food and Drug administration, USA [16]. This GM tomato was developed by former Monsanto Company (currently Bayer Crop Science) with a longer shelf life. Later on, in 1995 Mexico and Canada adopted this GM tomato for consumption. Till then many countries started to adopt GM crops and according to ISAAA, in 2019, a total of 71 countries have adopted GM crops among which 29 countries directly cultivated different types of GM crops including cereal, vegetables, oilseed, fruits, and flowers, whereas rest of the countries imported GM food [17]. Most crops have been genetically modified for herbicide tolerance and insect resistance [18], however, several crops have been also targeted for modified quality, for example, modified flower color, modified nutrient quality, longer shelf life and so on [19].

4. Commercially approved GM crops and their commercial traits

The commercially approved GM crops around the world and their desired traits according to the reports of ISAAA are described below [17].

4.1 Cereal crops

Some important cereal crops such as maize, rice and wheat are approved for their commercial traits. Maize is the second most cultivated GM crop in the world and USA is the major cultivator and producer of GM corns. Maize has been genetically modified mainly for herbicide tolerance and insect resistance. Several commercial GM corns are stacked with the aforementioned traits, for instance, Agrisure® Duracade™ 5222 by Syngenta. Few are modified for better quality, such as, Enogen™, that contains a synthetic gene derived from *Thermococcales* spp.

Like maize, rice has also been genetically modified for herbicide tolerance and insect resistance. Liberty Link™ rice developed by Bayer Crop Science is a herbicide tolerance variety containing a gene (*bar*) from *Streptomyces hygroscopicus.* It was first approved in the USA in 2000 and later on other countries have also approved. Till date, 9 more countries including Canada, Argentina, Australia, Colombia, Honduras, Mexico, New Zealand, Philippines, Russia and South Africa have approved this GM rice. The commercially available insect tolerant GM rice is BT Shanyou 63 that was only approved by China in 2009. Another GM rice of similar trait is Huahui-1 that was approved in the USA in 2018. The most famous GM rice would be the Golden Rice. It is adopted for cultivation in Philippines in 2018, however, in 2017-2018, USA, Canada, Australia and New Zealand have also approved this as food and feed.

Roundup Ready™ wheat, a glyphosate herbicide resistant variety is the only GM wheat in the world developed by Monsanto Company. Initially, in 2004, 4 countries including Australia, Colombia, New Zealand, and the United States approved this for direct use and processing as food, however, Monsanto reportedly stopped pursuing the regulatory process and other countries also have withdrawn.

4.2 Oilseeds crops

Among the oilseed crops soybean, canola and sunflower are more important. Soybean is the most adopted and most produced GM crop in the whole world. The herbicide tolerant varieties Liberty Link® soybean and Contrivance were developed by BASF group. It is approved to be used as food and feed in more than 20 countries. Some other herbicide tolerant varieties are Liberty Link® GT27™, Roundup Ready™ soybean, Genuity® Roundup Ready™ 2 Xtend™, Genuity® Roundup Ready 2 Yield™, and Optimum GAT™. Apart from these, Conkesta Enlist E3™ Soybean and Intacta™ Roundup Ready™ 2 Pro, developed by Dow AgroSciences LLC and Monsanto Company, respectively, are stacked with herbicide tolerance and insect resistance capacity. The Vistive Gold™ developed by Monsanto Company and the Plenish™, developed by DuPont (Pioneer Hi-Bred International Inc.) are stacked with herbicide tolerance and enhanced oleic acid content. These are approved in more than 15 countries. Lastly, the Verdeca HB4 Soybean is an abiotic stress tolerant GM soybean developed by Verdeca. It is currently approved in 6 countries for food and feed purposes.

The genetic modification in Argentine Canola (*Brassica napus*) is more focused on herbicide tolerance. To name a few herbicides tolerant GM canola varieties would be Optimum® Gly canola, Roundup Ready™ Canola, Liberty Link™ Independence™, InVigor™ Canola, Navigator™ Canola, etc. Though there is no insect resistant GM canola available few have been genetically modified for improved quality. For example, Laurical™ Canola developed by Monsanto Company has increased levels of a triglyceride, lauric acid. However, it is only adopted for cultivation in the USA and Canada. There is only one GM Polish Canola (*Brassica rapa*) is available named Hysyn 101 RR Roundup-Ready™. It was developed by the University of Florida and only adopted for cultivation and approved for use in Canada (1997). GM safflower is adopted for cultivation only in Australia and it was approved in 2018 for food and feed purposes. This has a down regulated expression of *fatB* and *fad2.2* genes for lowering the fat content.

4.3 Vegetables and fruits

Commercially important vegetables and fruits are potato, tomato, eggplant, common bean, squash, apple, papaya, plum and pineapple. Primary potato trait that was focused for GM potatoes is modified product quality, such as, Starch potato, Amflora™, for reduced amylose and increased amylopectin content; Innate®, Cultivate, Innate® Generate, Innate® Accelerate, and Innate® Invigorate for lower reducing sugar. Several modified product quality GM potatoes are stacked with disease resistance capacity against blight (Innate® Acclimate, Simplot Innate) and potato virus Y (Hi-Lite NewLeaf™ Y potato). Commercially available coleopteran insect resistant GM potatoes are Superior NewLeaf™ potato, Elizaveta plus, Lugovskoi plus, Atlantic NewLeaf™ potato, etc.

According to ISAAA, 11 GM tomatoes have been developed so far for commercial purposes and 9 of them are for a single trait, that is, delayed fruit ripening or delayed fruit softening for longer shelf life. FLAVR SAVR™ by Monsanto Company and three others developed by Zeneca Plant Science and Petoseed Company followed the same strategy for delayed fruit softening. These four are only cultivated in the USA, however, Canada and Mexico have approved these for food purposes around 1995–1996. Some other GM varieties were developed by targeting the genes that are responsible for ethylene production, the fruit ripening hormone. This one was adopted and approved as food and feed in the USA in 1996. China approved 2 GM tomato varieties for cultivation and domestic uses with delayed ripening in 1997 and 1999 that was developed by the Huazhong Agricultural University (China) and Institute of Microbiology, CAS (China), respectively. The DNA Plant Technology Corporation (USA) developed another GM tomato by reducing the expression of *ACC synthase* gene resulting in reduced ethylene production, thus, delayed fruit ripening. Monsanto Company developed another one with resistance capacity against lepidopteran insects using *cry1Ac* gene and was approved in 1998 and in 2000 for Canada.

The only available GM eggplant is BARI bt Begun developed by Maharashtra Hybrid Seed Company (MAHYCO). It mainly contains *cry1Ac* gene from *Bacillus thuringiensis* subsp. Kurstaki strain HD73 that confers resistance against lepidopteran insects by selectively damaging their midgut lining. Bangladesh is the only country that approved the cultivation of this GM eggplant in 2013. Recently, Philippines approved it for food and feed uses but did not adopt for cultivation. In Bangladesh it is only approved for direct use or processing as food only.

BRS FC401 RMD is the only commercially available GM common bean (*Phaseolus vulgaris*). It was developed by EMBRAPA (Brazil) and is resistant against Bean Golden Mosaic Virus (BGMV). It inhibits the replication of viral protein. Brazil is the only country to cultivate and use this GM crop as food and feed. Seminis Vegetable Seeds (Canada) and Monsanto Company (Asgrow) developed the GM squash that is stacked with resistance capacity against three plant viruses, e.g., *Cucumber mosaic cucumovirus* (CMV), *Zucchini yellow mosaic* Potyvirus (ZYMV) and *Watermelon mosaic potyvirus 2* (WMV2). Cultivation of this variety was stared in 1996 in the USA and approved for food and feed usage in 1997. No other country has approved this one yet. Canada approved it food use in 1998.

Fruits.

Apple: Arctic™, Arctic™ "Golden Delicious" Apple, and Arctic™ Fuji Apple, all these 3 GM apple varieties has been developed for non-browning phenotype by Okanagan Specialty Fruits Incorporated. Arctic™ Fuji Apple was first adopted in Canada in 2018 and then in the USA in 2019. The other 2 are also approved only in these 2 countries and their cultivation started in 2015.

Papaya ringspot virus is the greatest concern of papaya cultivation. Therefore, all the GM papayas are aimed to disease resistance trait. Pathogen derived resistance mechanism is used in these GM papayas to check the viral infection. The USA and Japan started cultivation in 1996 and 2011, respectively. Canada only approved its use for food purposes in 2003. Another commercially available one is Huanong No. 1 developed by South China Agricultural University for the same disease resistance purpose. China is the only country that started its cultivation in 2006.

The only GM pineapple is Rosé, developed by Del Monte Fresh Produce Company. It has traits of delayed ripening, modified fruit color, and increased level of lycopene and beta-carotene. It is approved only in the USA (2016) and Canada (2021).

4.4 Sugar crops

Brazil and Indonesia are the only 2 countries to cultivate GM sugarcane. Brazil started the cultivation insect resistant GM sugarcane and approved its usage for food and feed purposes from 2017. It was developed by Centro de Tecnologia Canavieira (CTC) that contains *cry1Ab* gene for conferring resistance against lepidopteran insects. Canada and United States have approved its usage as food. On the other hand, Indonesia adopted the cultivation of drought resistant GM sugarcane and approved to be used as food and processing food products from 2011. It contains *EcBetA* gene catalyzes the production of the osmoprotectant compound glycine betaine conferring tolerance to water stress. Only 3 countries adopted the cultivation of GM sugar beet, Roundup Ready™ sugar beet. Australia and the USA approved in 2005 and Japan in 2007. However, Australia, Canada, China, Colombia, European Union, Japan, Mexico, New Zealand, Philippines, Russia, Singapore, South Korea, Taiwan and USA have approved its use as direct food or processed food. This GM sugar beet was developed by Monsanto Company. There are two more commercially available GM sugar beet plants are present, they are InVigor™ sugar beet developed by Novartis Seeds and Monsanto Company and Liberty Link™ sugar beet developed by Bayer CropSciene.

4.5 Others

Monsanto Company and Forage Genetics International have jointly developed GM alfalfa plants for herbicide tolerance (Roundup Ready™ Alfalfa) and reduced content of guaiacyl (G) lignin (HarvXtra™). These are approved in Australia, Canada, Japan, Mexico, New Zealand, Philippines, Singapore, South Korea and the United States of America in the years ranging from 2004 to 2008. Sweet peppers Approved for cultivation in China in 1998, developed by Beijing University. The GM sweet pepper confers resistance against the Cucumber Mosaic Virus (CMV) through pathogen derived resistance mechanism. Tobacco has been genetically modified for cultivation purposes targeting 2 commercial traits. First one by SEITA S.A. (France) for oxynil herbicide tolerance and the second one for reduce nicotine content developed by Vector Tobacco

Inc. (USA). The first one was approved in 1994 for the European Union and the latter one in 2002 for the United States.

5. Worldwide production and consumption of genetically modified foods

In 2019, According to ISAAA, 29 countries cultivated GM crops among which the USA is the largest producer of the GM crops covering 37.9% of the total GM crop area in the world [5]. USA has adopted the highest number of biotech crops (**Table 1**). Canada is another major GM crop grower that has reached 95% adoption rate of GM canola. In Latin America, 10 countries cultivate biotech crops, they are, Mexico, Costa Rica, Honduras, Columbia, Chile, Bolivia, Argentina, Uruguay, Paraguay, and Brazil. Brazil is the top biotech crop cultivator developing country. In Europe, only two countries adopted the cultivation of GM crop, Spain, and Portugal. These 2 countries only cultivate GM Maize. Whereas, in Africa, most of the countries cultivate GM cotton, like, Nigeria, Sudan, Ethiopia, Malawi, and Eswatini. South Africa cultivates three different GM crops, they are soybean, maize, and cotton. In Asia, Pakistan, India, Myanmar cultivates GM cotton. Bangladesh is the only country to cultivate BT Brinjal in the whole world. Indonesia only cultivates GM sugarcane, whereas Vietnam and Philippines are only focused on the cultivation of GM maize. China cultivates GM papaya and GM cotton. In Oceania, Australia is the only country to cultivate GM crops, like GM cotton, GM alfalfa and GM safflower. Australia is the exclusive grower

Country	Total area cultivated (Million hectares)	GM crops
USA	71.5	Maize, soybeans, cotton, alfalfa, canola, sugar beets, potatoes, papaya, squash, apples
Brazil	52.8	Soybeans, maize, cotton, sugarcane
Argentina	24.0	Soybeans, maize, cotton, alfalfa
Canada	12.5	Canola, soybeans, maize, sugar beets, alfalfa, potatoes
India	11.9	Cotton
Paraguay	4.1	Soybeans, maize, cotton
China	3.2	Cotton, papaya
South Africa	2.7	Maize, soybeans, cotton
Pakistan	2.5	Cotton
Bolivia	1.4	Soybeans
Uruguay	1.2	Soybeans, maize
Philippines	0.9	Maize
Australia	0.6	Cotton, canola, safflower
Myanmar	0.3	Cotton
Sudan	0.2	Cotton
Mexico	0.2	Cotton
Spain	0.1	Maize
Colombia	0.1	Cotton, maize

Country	Total area cultivated (Million hectares)	GM crops
Vietnam	0.1	Maize
Honduras	<0.0.5	Maize
Chile	<0.05	Maize, canola
Malawi	<0.05	Cotton
Portugal	<0.05	Maize
Indonesia	<0.05	Sugarcane
Bangladesh	<0.05	Brinjal
Nigeria	<0.05	Cotton
Eswatini	<0.05	Cotton
Ethiopia	<0.05	Cotton
Costa Rica	<0.05	Cotton, pineapple
Total	**190.4**	

Table 1.
List of GM crops grown in different countries with total biotech crop area [5].

of the GM safflower. Since 1996, a total of 2.7 billion hectares of biotech crops are planted. In India, 6 million farmers planted Bt cotton in 11.9 million hectares in 2019. Malawi, Ethiopia, and Nigeria are comparatively new GM crop growers, started from 2019. Interestingly, Japan has approved the cultivation of GM crops, but they have not planted any yet [5]. The top 5 countries to plant biotech crops are USA- 71.5 million hectares (95% adoption), Brazil-52.8 million hectares (94% adoption), Argentina-24 million hectares (~100% adoption), Canada- 12.5 million hectares (90% adoption) and India –11.9 million hectares (94% adoption). According to the report of ISAAA in 2019, biotech soybean is the most cultivated GM crop with 48.2% coverage of the whole biotech crop land, followed by biotech maize (32%), biotech cotton (13.5%), Biotech canola (5.3%) and all other biotech crops cover around 1% of the total biotech crop land [20].

6. Human health risk and safety assessment of genetically modified crops

General people consider foods obtained from conventionally developed crops are safe and GM crops possess risks to human health and the environment. Previously risk assessment used to be carried out based on some common principles that were introduced in 1993 [20]. Later on, an international body called 'Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology of the Codex Alimentarius Commission', jointly established by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) and developed a guideline for help the national authorities to assess the human health risk of GM foods. The three main concerns for human health from GM foods are (i) allergenicity—the transgene should not be allergenic (currently no allergic effect was recorded), (ii) gene transfer—from GM food to the body cells or to the bacteria of gut microbiome; particularly concerning if antibiotic resistant genes are used. Therefore, any technique used in GM crop development that involves antibiotic resistant genes are discouraged, and (iii) outcrossing—several cases have been reported where transgene from GM crops approved for animal feed or industrial purposes [21]. According to the Codex Alimentarius, the human health risk assessment of GM crops is based on the principle that a newly developed GM crop should be compared with the conventional counterpart with a history of safe use [22]. The whole idea is that the newly developed GM crop is not compositionally different than its conventional counterpart except the newly introduce gene product/s [23].

According to the studies of National Cancer Institute, from 1975 to 2011, some specific types of cancer incidence has increased and some other has decreased in the USA. No absolute pattern was observed in the incensement or reduction of cancer incidence. If GM foods were a factor to increase cancer incidence in human, then there supposed to be a substantial increase in cancer incidence from 1995 when USA introduced GM crops in the market [24]. Another study published data on cancer incidence worldwide from 1980 to 2010 on breast and cervical cancer. This report showed that the incidence of these 2 cancers has increased in a similar pattern in the USA, Canada, and Europe. Interestingly, GM crops are not generally consumed in the European Union [25]. Epidemiological data was examined in the USA to find out the correlation between GM foods and chronic kidney disease (CKD). The prevalence of all stages CKD in the USA has increased 2% between 1994 and 2004. After that the prevalence did not increase significantly. Therefore, this increase is not correlated with the consumption of GM foods [26]. Similarly, no relationship was found between obesity and type-II diabetes with the consumption of GM foods. Similar conclusion was also made for gastrointestinal tract diseases, celiac disease, and food allergy and autism. Spectrum disorder [27].

The consumption of genetically modified foods direct human health safety assessment is considered as it is safe or unsafe. Many organizations such as the U.S. Environmental Protection Agency (EPA), the U.S. Department of Agriculture (USDA), and the U.S. Food and Drug Administration (FDA) other agencies in other countries or by companies, nongovernmental organizations (NGOs), and academic institutions conducted several research for human health safety assessment. American Association for the Advancement of Science says crop improvement by the modern molecular techniques of biotechnology is safe [28]. Genetically modified foods currently available on the international market have passed safety assessments and are not likely to present risks for human health World Health Organization [21]. All the available GM crops in the market did not show any kind of major human health risks till now. Also, newly developed GM crops must go through proper risk and safety assessments for better acceptability. It suggests the necessity of labeling GM food so that consumers can make their own choice [29].

7. Safety assessment process to register or import GM products

Delivering GM products to market requires time and investment to ensure safety. Use of genetically modified crops as human food and animal feed several studies are required for registration or import into the country. Many countries have very strict rules and regulations for release of GM crops and also a trade barrier in some situations. Hence introduction of GM crops and their products as human food and animal feed some reliable methods such as molecular analysis, safety assessment, toxicological test, nutritional quality analysis, genotoxicity analysis, allergenicity etc. are essential for import or release of new genetically modified crops.

A detailed molecular characterization is a common study in the safety assessment of GM crops.

7.1 Molecular analysis

GM crops can be tested by several molecular levels such as at DNA, mRNA and protein level. Polymerase Chain Reaction (PCR) is the primary method for screening of GM crops at DNA level. This method has found very broad and wide applications in GMO detection and in this method target gene multiplied to millions or billions by using gene specific primers [30]. Southern blotting is another important method for the identification of specific DNA fragments transformed into the genome of transgenic plants or its products which was described by Southern in 1975 [31]. This is very reliable method that provides the molecular evidence of the transgene integration and also estimates the copy number of introduced gene into the host genome. DNA microarray is another method used to identify the expression of more than one gene in a single test. This test method has been used in GMO screening as a method for simultaneous detection of more than 250,000 targets in single assay/chip [32]. In case of RNA based methods real-time PCR, northern blotting techniques etc. are used to monitor and study the gene expression in GM crops. RT-PCR method is based on reverse transcription of mRNA and synthesis of complementary DNA (cDNA) which is then used as template in PCR amplification of target gene. In real-time assay of transgene in GMOs, the amplification and detection occur simultaneously [33]. In northern blotting also requires mRNA as tested material from GMOs. This is a standard method for the analysis of size and level of target RNA in a complex GMO sample. It gives comparative amount of gene expression at the RNA level. Protein based test methods enzyme linked immune sorbent assay (ELISA) and western blot methods have been used for the protein analysis in GMOs. In this assay protein specific antibody coated multi-well plate is used to identify and quantify the specific protein [34].

7.2 Toxicological studies

All toxicity assessment for GM material should be performed based on a case-by-case approach, considering the toxicological profile of new introduced substances [35]. The purpose of toxicological studies is to characterize intended changes and detect active substances or compounds that could have unexpected toxic effects for non-targeted organisms [36]. The assessment of toxicity analysis in animal new strategies have been identify for GM feed and foods. Research on the in-planta metabolism pathway, such as "-omics" techniques that may generate a better understanding of the complex pleotropic effects of new plant cultivars [37]. Additionally, *in vitro* assays with gastric enzymes, cultured cell lines, receptor proteins, and *in vivo* animal studies can be performed [36]. When performing *in vivo* studies, toxicology acute (14 days studies), subacute (28 days studies), chronic (90 days studies), or specific toxicity (reproductive, mutagenicity, etc.) assessment can be considered [38]. In long term studies (>100 days), no toxic effects were found in cattle and chickens fed with Bt maize. The toxicological evaluation of proteins introduced into GM crops must be carried out for introduced or release of GMO crops and carefully assess the modification of amino acid sequences that make a non-toxic protein to toxic protein [39].

7.3 Nutritional studies of GMO crops

It is important to assess the nutritional quality of genetically modified foods that consumed in human and animal feed. Assessment of nutritional quality composition of transgenic wheat (*Triticum durum* L.), corn (*Zea mays* L.), and tomato (*Lycopersicum esculentum* Mill.) were done compare with the non-transgenic control with a similar genetic background. No significant differences were observed for qualitative traits analyzed in wheat and corn samples [40]. Many studies have also been carried out with feed derived from GM plants with agronomic input traits in target animal species to assess the nutritive value of the feed and their performance potential. Studies in sheep, pigs, broilers, lactating dairy cows, and fish, comparing the *in vivo* bioavailability of nutrients from a range of GM plants with their near isogenic counterpart and commercial varieties, showed that they were comparable with those for near isogenic non-GM lines and commercial varieties [41]. In case of proteomic analysis creation of respective databases and algorithms of plant proteomic analysis is among the most important avenues of fundamental science aimed to predict the functions of genes and the properties of the products encoded by them [42].

7.4 Assessment of potential genotoxicity

Assessment of potential genotoxicity of the foods obtained from GM crops for human health safety assessment is important. Genotoxic studies include the assessment of genetic material at various stages of DNA, chromosomes etc. [43]. Accordance with the "Food Additive Risk Assessment Guidelines" of the Japan Food Safety Commission were assessed the genotoxicity of 30 food-flavoring chemicals in GM crops. Of the 30 food-flavoring chemicals, three yielded a positive result in both Ames and CA tests [44]. Another 11 chemicals yielded positive results in the CA test. However, none of the chemicals yielding positive *in vitro* test results yielded positive results in the *in vivo* tests. These findings indicate no genotoxicity concerns of the food-flavoring chemicals belonging to the above mentioned 18 chemical classes used in Japan unless there are other structural modifications.

7.5 Assessment of potential allergenicity

The assessment of allergenicity reaction is important to release a GMO crop. A variety of factors considered for an overall assessment of allergenic potential is conducted. This assessment includes a safety of the genes source, protein structure (e.g. amino acid sequence identity to human allergens) and stability of the protein to pepsin digestion *in vitro*, heat stability of the protein, glycosylation status, and specific IgE binding studies with sera from relevant clinically allergic subjects [45]. Since GM crops were first commercialized over 20 years ago, there is no proof that the introduced novel protein(s) in any commercialized GM food crop has caused food allergy. The process by which allergy assessment has been conducted since the 1990s has involved guidance from several expert scientific bodies, including the FAO/WHO [46], and Codex [22]. However, several animal models are important for investigating the etiology of food allergy as well as evaluating advances in immunotherapy techniques to induce desensitization and ultimately, tolerance to food allergic reactions [47]. Now days, Bioinformatics tools has led to advances in predicting the allergenicity of novel proteins.

7.6 Analysis of sensitive biomarkers

Some marker genes are introduced into the gene construct for identification of transgenic plants. So, it is necessary to assess the effect of biomarker in human or other plants. A machine learning methodology would enable the identification of potential biomarkers associated with the potential adverse health effects related to GMO exposure. This broad approach will allow for the collection and analysis of vast quantities of data for biomarker identification. Researchers were not successful in their search for biomarkers cause any health effects [48].

8. Methods of identification and detection of GMO

The number and diversity of genetically modified organisms (GMOs) for the food and feed market is increasing day by day. So, it is important to efficient identification and detection of GMO in food and feed products. Several strategies have been developed to detect GMO in food/feed samples by using different technologies. In case of the protein-based approaches, ELISA technique and mass spectrometry-based technology are used to characterize GM crops [49, 50]. Protein based detection method is not applicable if the genetic modification has no impact at the protein level. Currently, Many DNA based GMO detection methods have been developed such as quantitative PCR (qPCR), loopmediated isothermal amplification (LAMP), PCR capillary gel electrophoresis (CGE), microarray, Luminex, digital PCR (dPCR), DNA walking and Next Generation Sequencing (NGS).

8.1 qPCR technology

Event-specific PCR detection technology is commonly employed for GMO testing due to its ability to specifically detect each transgenic event simply by targeting their unique junction between the host genome and the transgenic cassette [51]. Currently, different event-specific qPCR (quantitative) technology has been designed for transgene detection from GM Corn, Cotton, Canola, Rapeseed, and rest of the crops [52]. The qPCR system, which is the most common strategy, allows detecting, identifying, and quantifying GMO via the SYBR Green or TaqMan chemistries in agricultural and food products [53]. Though, the (qPCR) methods have more reliable, accuracy and greater sensitivity but its success largely relies on various factors, e.g., its throughput strategy is often restricted to one marker per reaction. Due to continuous growth in GMO production, new/additional detection markers (for specific detection of new transgene) are required to be designed continuously and used to completely cover their identification. In case of multiplex PCR-based methods, several DNA targets can be detected in a single reaction.

8.2 Capillary gel electrophoresis (CGE)

CGE technique was developed by Heide et al. to identify various transgenic events in one reaction [54]. The basic principle of this technique is to carry out multiple PCR reaction using forward primers which are fluorescently labeled and discrimination of amplimer of similar magnitude by executing CGE. As compared to the electrophoresis gel, CGE system has higher resolution power to clearly detect PCR products from a multiplex assay. Using the CGE system, eight GM maize were identified via a nonaplex PCR including event-specific, construct-specific, and taxon-specific methods

[55]. Similarly, one pentaplex PCR and two hexaplex PCR were also developed to detect specifically four GM maize and five GM cotton [56]. However, CGE has some disadvantages as it requires extensive labor for designing of primer as well as the optimization when performing the analyses for detection of a new event. Its implementation also requires specialized apparatus which may not always be available.

8.3 Loop mediated isothermal amplification (LAMP)

Loop mediated isothermal amplification (LAMP) is an emerging technology which was developed an easy detection of the transgenic event in a given sample [57]. The main idea is the amplification and identification of the desired nucleic acid sequences at a steady temperature and at some specific stage of the experiment. This novel approach of GMO detection involves the utilization of four distinct primers which identify at least six different segments of the desired DNA. Varieties of LAMP markers were designed for quantitative detection of transgenic GM events [58]. The advantage of this study is simplicity, time-efficiency, and ability to withstand different PCR inhibitors, for instance, acidic polysaccharides. However, this technique has some limitations, e.g., designing four primers per sequence and detection of different GM events employing multiplex approach is also a problem.

8.4 Digital PCR (dPCR)

Digital PCR technology (dPCR) is one of the most reliable techniques among the currently used technology for GMO quantification. The process is accomplished through dividing the mixture of PCR into a sizeable number of distinct reactions which include null, single or least target DNA copies. After completion of PCR, the positive (i.e., observed replicated desired segments) and negative (i.e., observed non-replicated segments) samples are analyzed and then the total copy number of the desired gene in an original sample is determined by the application of binomial Poisson statistics [59]. Most recently, duplex assays, including one GMO specific marker with one soybean, maize, or rice taxon specific marker, were performed by using the dPCR system to quantify 12 GM soybean, 16 GM maize, and two GM rice events [60].

8.5 Microarray technology

A microarrays technique (DNA chips or biochips) is an advanced technology for high-throughput detection of GMO. It can evolve together with the growing number of newly developed GMO in the food and feed markets. With the microarray technology applied to GMO detection, GM targets are amplified by PCR, using target-specific and/or universal primers, prior to being hybridized on the array, allowing the simultaneous detection of more than 250,000 targets in one assay [61]. DNA chip technology coupled with multiplex PCR can be used in the identification of different transgenic events from GMOs by employing multiplex PCR approaches. Nucleic acid array in combination with multiplex PCR has been used successfully for identification of different types of events from GM crops like corn and cotton [32].

8.6 Next generation sequencing (NGS)

Next generation sequencing (NGS) is a promising technology with the detection of transgenic events that allows for massively parallel DNA segment sequencing resulting in millions of sequencing reads [62]. NGS is an efficient tool for transgenic events detection even in the absence of sequence information of such events. NGS is being efficiently employed for characterization of site addition, flanking regions, accidental addition as well as the determination of transgene copy number [63]. Two main approaches are (targeted sequencing strategy) or (whole genome sequencing (WGS) strategy), for samples sequencing which has been enriched previously with desire sequence regions have been identified [64]. Molecular analyses of transgenic varieties from GM soy and GM rice have successfully been achieved using this strategy [65]. An approach based on initial enrichment of the targets using DNA walking starting from transgenic elements covering a large spectrum of GMO has been proposed to characterize unknown sequences including the transgenic inserts and junctions [66]. DNA walking technology will become more sensitive and more suitable and that could provide a more promising solution for the recent challenges of GMO analysis in the near future.

9. GM crops for food security

With the current population growth, it is expected that the world population will reach 9 billion by 2050 and current food production rate is not sufficient to feed the future population [67]. On top of that, 40% of the total arable land of the world is lost over the last 40 years because of erosion and pollution. Heavy utilization of arable lands reduces organic matter and nutrients from the soil; therefore, farmers need to rely on heavy fertilizer input to reintroduce nutrients in the soil for better crop yield [68]. In addition to heavy input of synthetic fertilizers, farmers also need to rely on insecticides and pesticides as every year farmers face 20–30% due to insects, animals, and weeds [69, 70]. Therefore, the most sustainable way to ensure food security is to develop high yielding, enhanced product quality, disease resistance and climate resilient crop varieties. Plant breeders try to develop new superior plant varieties; however, it is limited by the huge number of crosses needed to be done, prolonged and labor intense selection process, transfer of undesired genes with the desired ones, random combination of genes from two parents and so on [71]. In contrast, GM crops are developed by incorporating only the desired gene/s in the target crop, even from a distantly related organism, in a shorter period of time and in more precise manner. GM crops have a lot more to offer than the conventionally developed varieties. Biotech crops are already proven to be more economically beneficial as it requires less chemical input such as herbicide tolerant and insect tolerant GM crops. GM crops are the fastest adopting technology in the world. If farmers have not decided to cultivate GM crops, in order to obtain present day crop yield, we would have needed an additional 13 million hectares of land [72]. This amount of land would come at the cost of destroying rainforests which would eventually increase carbon emission. Most of the biotech crops are cost saving and some are with higher yield capacity. As a result, the farmers are getting more return from the GM crops [73]. GM crops have all the potential to achieve food security, however, lack of societal acceptance of GM crops is the major obstacle as many farmers fear that if they grow GM crops then consumers may not buy it. Also, most of the people mistrusts the benefits of GM crops because they think GM crops are actually harmful, but developers of new GM technologies and GM varieties hides for profit [68]. In real life, this is not the case. Thousands of studies are present that showed GM crops are not harmful at all. It is the duty of the scientific community to make general people understand the science and safety of the GM crops in an easier and clearer manner.

10. Bio-safety assessments of genetically modified food

Biosafety refers to the safe management of living organisms and genetic material, including pathogens and genetically modified organisms. Under international environmental law and policy, biosafety refers to the need to protect the environment and human health from the possible adverse effects of genetically modified organisms (GMOs) and products resulting from modern biotechnology. Currently, at the core of the international regime on biosafety is the 2000 Cartagena Protocol on Biosafety, adopted under the Convention on Biological Diversity (CBD). Now a days dozens of genetically modified (GM) food crops and animals have been developed and commercialized in different countries. That's why several biosafety concerns like- risk to human health, risk to environment, ecological concern has been raised after the rapid commercialization of GM crops every year across the world. So strict biosafety guideline must be followed before introducing a GM crop into the market. According to Cartagena Protocol on Biosafety (CPB) take appropriate measures to regulate, manage or control the risks that may arise due to use and handling of living modified organisms (LMOs) that may pose some threats to biological and to ensure the safe handling, transport, and use of LMOs. Different countries and organizations developed their own biosafety guidelines for safety assessment of genetically modified foods. The safety assessment is undertaken in accordance with internationally established scientific principles and guidelines developed through the work of the Organization for Economic Cooperation and Development (OECD), Food and Agriculture Organization (FAO) of the United Nations, World Health Organization (WHO) and the Codex Alimentarius Commission. Risk assessment must be completed on the basis of scientific evidence to identify and evaluate the possible impacts of GMO on the conservation and sustainable use of biodiversity as well as risks to human health [74]. Risk assessment identifies potential hazards and/or adverse impacts of GM crops or derived product on non-target organisms and/or environment. This involves a number of coordinated steps like risk identification, risk characterization and risk categorization. Risk management involves strategic techniques to reduce the adverse effect of GM crops and associated products on non-target species or environment and also to reduce the chances of development of resistance in target pest population [75]. Risk management is a follow-up to the implementation of a risk assessment that includes the establishment of appropriate mechanisms, steps, and strategies for managing and controlling the risks identified in the risk assessment. The obligation arising from the application of risk management to these parties is to establish and implement a regulatory system with sufficient capacity to manage and control these risks. Regulation of GMO handling, transport, packaging, and utilization is part of efforts to ensure the safety of GMO development in accordance with the requirements of relevant international standards. In case of USA, the U.S. Food and Drug Administration (FDA), U.S. Environmental Protection Agency (EPA), and U.S. Department of Agriculture (USDA) ensure that GMOs are safe for human, plant, and animal health [76]. Safety assessment of GM foods should be carried out on a case-by-case basis comparing the properties of new food with those of conventional counterpart.

11. Conclusion

Genetic modified crops have all the potential for ensuring food security. There is a misconception present surrounding the GM crops that it is harmful for the nature and

GM food brings harms to human health including cancer. This is due to total ignorance towards the scientific proofs that GM crops do not possess any of these threats under proper guidelines. The organic foods do not require synthetic fertilizer or insecticides or herbicides, but it is limited by the low productivity. It is easily understandable that organic farming cannot meet the required crops yield. High yielding varieties developed through conventional breeding methods, most of the time require heavy synthetic chemical inputs, including herbicides, pesticides, fertilizers. The harms that are brought to the environment through the heavy application of such synthetic chemicals is far more harmful. In recent years, GM crops with stacked traits has become more popular. In conventional ways, it requires a lot of time to develop a variety with multiple desired characteristics. That means GM crops can prepare us for future food security challenges. The GM crops need to be gone through proper regulations before being introduced in the market including health and environmental risk assessments. By far no GM crops available in the market for commercial use showed any signs of harm to human health. GM foods coming from GM crops are also properly regulated and labeled. The scientific community must work hard to make general people understand about this technology and its benefits to eradicate the misconceptions about GM crops and foods, whereas regulatory bodies should work through proper guidelines to gain people's trust so they can be attracted to GM crops or GM foods. Therefore, it can be concluded that with proper social acceptance, genetic modified crops can ensure food security for the future world.

Author details

Sujay Kumar Bhajan[1*†], Mohammad Mahmood Hasan[2†],
Md. Anwarul Haque[3] and Mohammad Nurul Islam[4]

1 Department of Microbiology, Rabindra Maitree University, Kushtia, Bangladesh

2 Department of Microbiology and Cell Science, University of Florida,
Gainesville, FL, USA

3 Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia,
Bangladesh

4 Plant Breeding and Biotechnology Laboratory, Department of Botany, University of
Dhaka, Dhaka, Bangladesh

*Address all correspondence to: skbhajan.tech@gmail.com

† These authors contributed equally to this work.

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