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Review

Genetically modified crops: Detection strategies and biosafety issues

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ABSTRACT

Genetically modified (GM) crops are increasingly gaining acceptance but concurrently consumers' concerns are also increasing. The introduction of *Bacillus thuringiensis* (*Bt*) genes into the plants has raised issues related to its risk assessment and biosafety. The International Regulations and the Codex guidelines regulate the biosafety requirements of the GM crops. In addition, these bodies synergize and harmonize the ethical issues related to the release and use of GM products. The labeling of GM crops and their products are mandatory if the genetically modified organism (GMO) content exceeds the levels of a recommended threshold. The new and upcoming GM crops carrying multiple stacked traits likely to be commercialized soon warrant sensitive detection methods both at the DNA and protein levels. Therefore, traceability of the transgene and its protein expression in GM crops is an important issue that needs to be addressed on a priority basis. The advancement in the area of molecular biology has made available several bioanalytical options for the detection of GM crops based on DNA and protein markers. Since the insertion of a gene into the host genome may even cause copy number variation, this may be uncovered using real time PCR. Besides, assessing the exact number of mRNA transcripts of a gene, correlation between the template activity and expressed protein may be established. Here, we present an overview on the production of GM crops, their acceptabilities, detection strategies, biosafety issues and potential impact on society. Further, overall future prospects are also highlighted.

Contents

Abbreviations: ELISA, Enzyme linked immunosorbant assay; GMV, Genetically modified varieties; PCR, Polymerase chain reaction.

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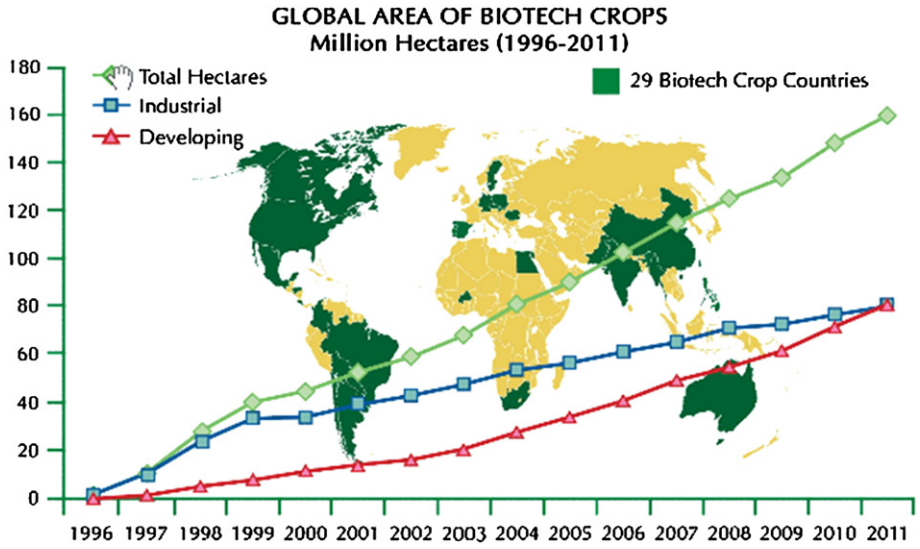

Fig. 1. A graphical representation showing current global status of biotech crops.
Source: James (2011).

1. Introduction

The evolution of GM crops has come a long way (James, 2011) fuelling the processes of their rapid adoption in the context of modern agriculture. Despite this, the global agriculture sector plunged into an enkindled debate over GM crops. Prior to the commercial cultivation of GM crops, consumers' concerns regarding their biosafety have also gained momentum. Arguably, the anti-GM groups (Greenpeace and Gene Campaign) are voicing their reservation fearing the growth of several non-approved varieties and the possibility of cross-contamination of the GM crops (Parlberg, 2002; Smythe et al., 2006). To resolve such issues, International Regulatory (IR) bodies are making efforts to deal with the biosafety measures of the GMOs. This includes corporate council chambers and legislative councils besides research laboratories. These bodies, after due deliberation, regulate the release of GM crops accepted the world over (Codex, 2003; James, 2011; Stewart et al., 2000). Labeling is mandatory to avoid unintended commingling of GM and non-GM crops, thus providing assurance to the consumer (Gruère and Rao, 2007). Creating acceptability of GM crops like that of non-GM ones will continue to remain a challenge.

With an increased acceptability amongst consumers and society, advanced qualitative and quantitative analytical parameters may be developed for the accurate detection of the GM crops carrying multiple traits and events (Que et al., 2010). Currently, bioanalytical

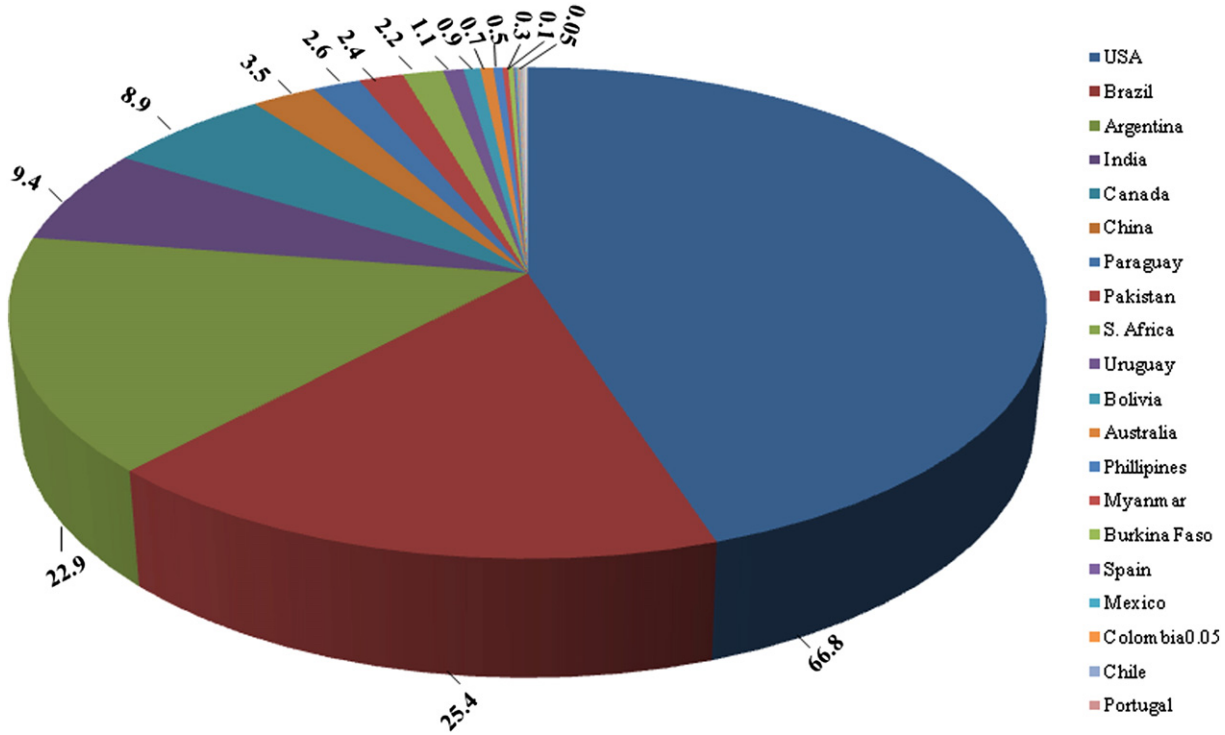

Fig. 2. Diagrammatic illustration representing the area (in million hectares) covered by biotech crops in major countries of the world.

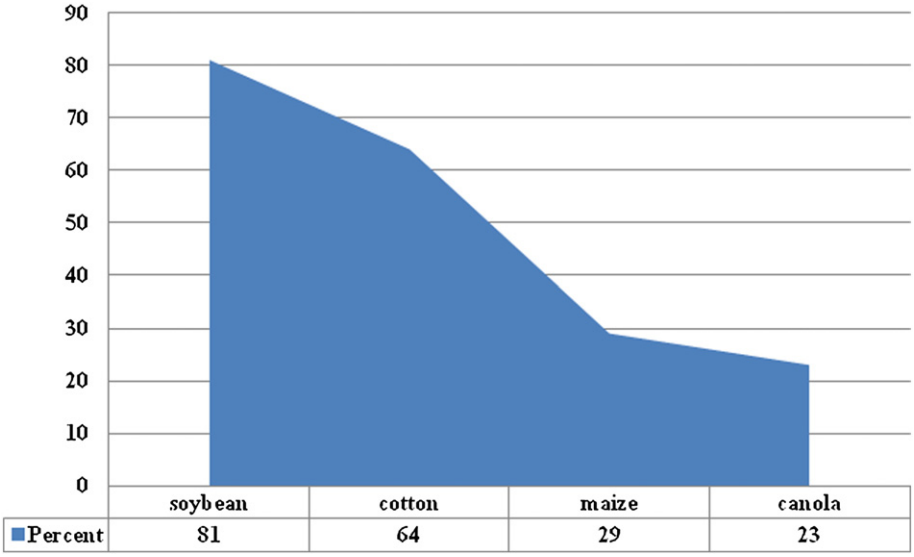

Fig. 3. A graph displaying area (in percent) covered by four major biotech crops worldwide.

tools like PCR, real-time PCR, biosensors, ELISA, immuno-strip and immuno-PCR are routinely used for the detection of DNA/protein. It is envisaged that biosafety and testing procedures both will continue to draw the attention of the policy makers, scientists and consumers alike (Arntzen et al., 2003).

2. Global status of GM crops

During the past sixteen years, the global area of GM crops has markedly increased by 94-fold covering a total of 160 million hectares. About 16.7 million farmers across the world planted GM crops. Of the twenty-nine countries known to have advanced biotechnology, nineteen developing and ten industrial ones (Figs. 1–3) planted GM crops (James, 2011). Of the seven continents, GM crops were grown in the six continents. The United States of America (USA) is the leading producer of GM crops. Brazil is following this trend and had registered the highest absolute growth of 4.9 million hectares. India recorded a phenomenal success of Bt cotton which reflect notable acceptance of GM crops. The European Union (EU) following the approval of the GM crops has reached a record level of 28% of the total production (James, 2011). South Africa is the biggest producer of GM crops in the African continent and economically benefitted from the adoption of GM technology. Mexico had the highest growth rate in the year 2011. GM crop is the fastest adopted crop technology which can contribute to global food security in due course of time (James, 2011).

Table 1
List of the regulatory bodies for GMOs.

Country	Legal regulatory organizations for GMOs
Argentina	National Advisory Commission on Agricultural Biotechnology (CONABIA)
Australia–New Zealand	Australia and New Zealand Food Authority (ANZFA)
Canada	Canadian Food Inspection Agency (CFIA)
European Union	2001/18/EC and 1830/2003
India	Biotechnology Authority of India (BRAI) Act
South Africa	South African GMO Act
USA	Animal and Plant Health Protection Inspection Service (APHIS); The Environmental Protection Agency (EPA); Food and Drug Administration (FDA)

3. International regulations on GM crops

The World Health Organization (WHO) defined GMOs as those organisms in which the genetic material has been altered in a way that does not occur naturally. Together with the sustainability of GM crops in agriculture for food safety, biodiversity and biosafety issues are equally important. Thus, efforts to regulate biosafety measures are vigorously made both at the international and national levels. Accordingly, GMOs are carefully examined and policies are revised regularly by the regulatory bodies to strengthen the system (Stewart et al., 2000).

Worldwide biosafety protocols and amendments on GMOs are strictly implemented. In 1992, the United Nation (UN) conference documented Agenda-21, emphasizing the ecofriendly management of modern biotechnology and the Convention on Biological Diversity (CBD), published the safe guidelines for GMOs (Codex, 2003; Haslberger, 2003; Ladics, 2008). Later, in 1995, the World Trade Organization-Technical Barrier to Trade (WTO-TBT), laid down guidelines for regulations, standards testing, certification process, packaging, marking and mandatory labeling requirements (Codex Alinorm, 06/29/34; Report of the APO Study, 2002). Similarly, the Cartagena Protocol (2000) on biosafety aims at regulating the safe transfer and handling of GMOs protecting the biodiversity (Alexandrova et al., 2005; MacKenzie, 2000). The Codex Alimentarius Commission (CAC) an international governmental body of the FAO and the WHO, established in 1962, promulgated the Codex guidelines (2003), for the food safety assessment and evaluation of the immunogenic potency of GMOs.

Most of the countries have a specific multidisciplinary Inter Institutional advisory group to evaluate scientific and technical issues associated with the GMOs (Table 1). To be effective, these regulatory bodies share overall responsibility of GM crops and their products based on empirical data. In 2005, the Bulgarian Parliament adopted the GMO laws and directed the European Commission (Regulation (EC) 2001/18) to enforce the GM guidelines (Alexandrova et al., 2005). Later on in 2010, the Bulgaria's parliament released a fresh and stringent law and effectively banned GM crops both for commercial reasons and trial purposes (http://www.euractiv.com/cap/bulgaria-approves-law-ban-gmo-cr-news-355729). In 2006, the National Biotechnology Regulatory Authority (NBRA) of India published new legislation known as the Biotechnology Regulatory Authority of India Act regarding GMOs. But under current Indian law, any GM crops before commercialization requires legal approval from the Genetic Engineering Approval Committee (GEAC), the highest body under the Ministry of Environment

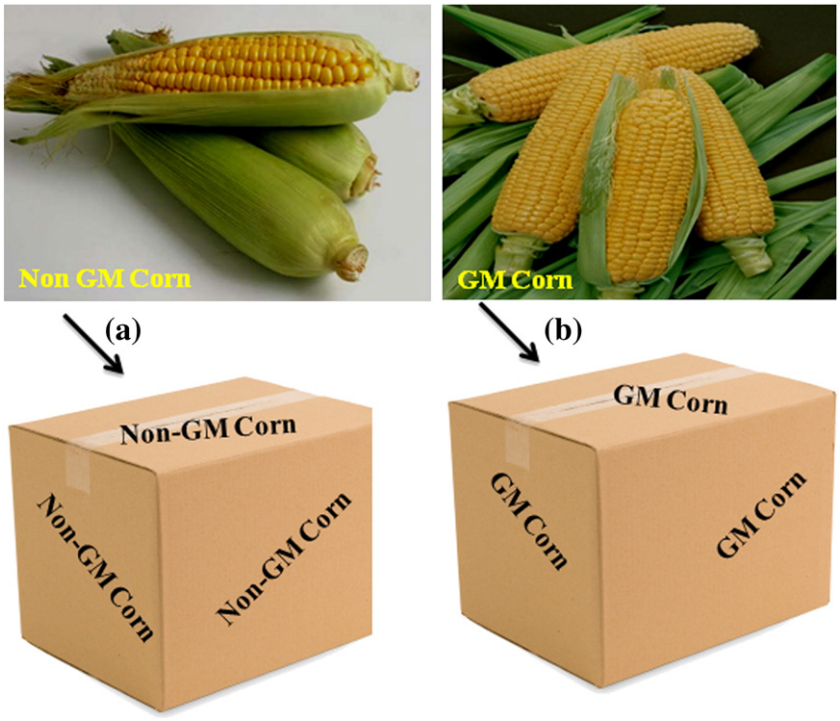

Fig. 4. A pictorial representation of proper labeling of crops: (a) non-GM corn (b) GM corn.

and Forest of India. These regulatory frameworks ensure comprehensive biosafety assessment of GM crops and administer enforcement, compliance, accreditation, and national and international policy coordination through its legal units.

Every year, a number of new GM crops are approved asynchronously (http://ftp.jrc.es/EURdoc/report_GMOpipeline_online_preprint.pdf). Modern biotechnology can benefit mankind employing GM crops to meet the food requirement thus, ensuring the economic prosperity of the teeming millions in the world. Besides this, there is a pressing requirement of unified regulations acceptable to all the countries (Gupta, 2000).

4. International consensus on labeling of GM crops

The labeling of the GM crops is a contentious issue. The international authorities are drafting guidelines for proper labeling of GM crops and their products. Exact labeling requires an extensive identity preservation system from granger to the elevator to grain processor to food processing manufacturer and finally to the consumer through the retailer (Maltsbarger and Kalaitzandonakes, 2000). Labeling of GM crops is compulsory to inform the consumer. Consumers must know that the GM crop has been declared safe by the authority (Fig. 4) (Hansen, 2004; McKay White and Veeman, 2007; Streiffer and Rubel, 2003). Moreover, it helps to enhance surveillance and tracing of GM food. Labeling is required when GM crops are substantially different from its conventional counterpart (e.g. a change in composition, nutritional value or allergenic nature). The FDA stance is that the GM and non-GM crops are substantially equivalent. But it is difficult to label each fruit as it would incur additional prices to the products and at the end be shifted to the consumer (Bansal and Ramaswami, 2007).

GM labeling requirement for food products as a precautionary measure was introduced by the EU (Regulation (EC) 258/97) and approved lawfully to provide safety to society. Thus, biosafety measurement and regulations are made to create a 'safety net' by testing and labeling GM products.

Usually, country specific labeling policies are made. In many countries, the labeling of grains, feed and foodstuffs is mandatory if the GMO content exceeds a certain threshold level as mentioned earlier. The proposed threshold level is 1% but it has been urged to achieve as low as 0.01% (Fig. 4 and Table 2). The threshold value is based on the percentage of GMO material in a non-GM background (Hansen, 2001). Normally, no GM food labeling would be required if the food contains GM material below the threshold level.

Countrywise, the degree of the labeling pattern varies greatly (Bansal and Gruere, 2010; Carter and Gruere, 2003). The Codex Committee on Food Labeling (CCFL) has drafted advanced recommendations on

Table 2
A labeling system and threshold level of GM crops/products in major countries.
Source: (EC), 1829/2003 and 1830/2003 (Regulation (EC) 1829/2003).

Country	Labeling type	Threshold level	Product/process	Country	Labeling type	Threshold level	Product/process
China	Mandatory	0%	Process	Indonesia	Mandatory	5%	Product
EU	Mandatory	0.9%	Process	Taiwan	Mandatory	5%	Product
Russia	Mandatory	0.9%	Product	Thailand	Mandatory	5%	Product
Australia–New Zealand	Mandatory	1%	Product	Canada	Voluntary	5%	Product
Brazil	Mandatory	1%	Process	Hong-Kong	Voluntary	5%	Product
Saudi Arabia	Mandatory	1%	Product	Japan	Mandatory	5%	Product
Israel	Mandatory	1%	Product	Philippine	Mandatory	5%	Product
Korea	Mandatory	3%	Product	South Africa	Voluntary	–	Product
Chile	Mandatory	2%	Product	USA	Voluntary	–	Product
Philippines	Mandatory	5%	Product	Argentina	Voluntary	–	Product

Table 3
Details of stacked traits in GM crops and their products.
Sources: http://cera-gmc.org/index.php?action=gm_crop_database; http://www.bayercropscience.com/bcsweb/cropprotection.nsf/id/BioScience; http://www.syngenta.com/country/us/en/Seeds/Pages/Home.aspx; http://www.dowagro.com/prod/; http://www.monsanto.com/; http://www.pioneer.com/.

GM crops	Trait developer	Product	GM event	Stacked transgenes	Target
Canola	Bayer crop sciences	Invigor, Seed link	$MS_8(DBN_{230\text{-}0028})$ $RF_3(DBN_{212\text{-}05})$	*Bar*, *barnase*, *barstar*	Weeds, male fertility
	Monsanto	Genuity 'RR'	$GT_{73}(RT_{73})$	*cp4-epsps*, *gox*	Weeds
Cotton	Bayer crop sciences	Fiber ax Liberty link Bollgard II	LLCotton25, MON15985	*bar*, *cry1Ac* + *cry2Ab*	Lepidopteron, weeds
	Dow Agro sciences	Widestrike	DAS-21023-5-DAS-24236-5	*pat*, *cry1Ac*, *cry1Fa*	Lepidopteron, weeds
	Monsanto	'RR' Bollgard II	MON531, MON1445-2	*cry1Ac*, *cp4-epsps*	Lepidopteron, weeds
Maize	Dow Agro sciences, Pioneer Hi-Bred	Herculex Xtra	TC1507, DAS-59122-7	*cry 1Fa*, *cry34Ab1*, *cry35Ab1*	Lepidopteron, coleopterans, weeds
	Monsanto	Yieldgard Triple	MON810, MON88017	*cry1Ab*, *cry3Bb1*, *cp4-epsps*	Lepidopteron, coleopterans, weeds
	Syngenta	Agrisure3000GT	GA21, Bt-11, MIR604	*pat*, *cry1Ab*, *cry3Aa*, mutant maize *epsps*	Lepidopteron, coleopterans, weeds

labeling of the biotech products and is directly linked with the WTO through an agreement. The Codex process for standard development is based on developing an international consensus, to protect the consumer and to facilitate trade by developing the best labeling policies for harmonization (Codex, 2003; Haslberger, 2003; Ladics, 2008).

Till date, there is no authentic global approval and legal registration of GM crops and their processed food products. Therefore, GM testing and its legal registration must be made mandatory and operational the world over (Goodman and Tetteh, 2011).

5. *Bt* gene and stacked traits

The modern biotechnological approach allows genes to be introduced into a plant genome. These foreign genes may originate from prokaryotes (bacteria) or eukaryotes either from plants or animals. The first GMO was *Bt*, and due to its wide applications was called *Bt* technology. In its first application, *Bt* genes were transferred into tobacco and tomato (Fischhoff et al., 1987) and following this, many other crops were developed (Jouanin et al., 1988). A GM maize (*Bt*11) has been developed to express the Cry1Ab insecticidal protein. This Cry1Ab was found to be toxic against some lepidopterons *Helicoverpa punctigera*, *Helicoverpa zea* and *Pectinophora gossypiella* insects (Bruderer and Leitner, 2003). Various GM crops harboring *Bt* genes (*cry1Ac*, *cry1Ab*, *cry2Aa*, *cry2Ab*, *cry2Ac*, *cry1F*, *epsps* and *vip-3A*), encoding insecticidal proteins were derived from a ubiquitous soil bacterium *Bt*. These insecticidal proteins generally have molecular weights between 65 kDa and 88 kDa (Hofte and Whiteley, 1989) and are known to be lethal against dipteran, coleopteran and lepidopteron insects.

Since the commercialization of GM crops, herbicide tolerance (HT) has consistently been the dominant trait and is used in soybean, followed by insect resistance used in Bt maize, Bt cotton, and Bt canola (Fig. 3). Such GM crops tolerate more herbicides like glyphosate and ammonium glufosinate and are resistant to different pests. GM crops expressing insecticidal proteins are steadily gaining acceptance and grown throughout the world (James, 2011). GMV that have been commercialized are Bt cotton in five different countries, roundup ready (RR) soybean in Argentina, Bt maize in Canada and Argentina and HT maize in Canada. Argentina gave approval to Syngenta to grow four-stack (GA2 × Bt 11 × MIR60 × MIR162) Viptera maize (Que et al., 2010).

Stacked events are those which in the same plant combine by conventional breeding or re-transformation of one or more existing traits (http://ftp.jrc.es/EURdoc/report_GMOpipeline_online_preprint.pdf). The first generation GM crop has a single *Bt* gene (e.g. Bollgard-I: *cry1Ac*) and now the second and third generations of GM crops were stacked with multiple genes (e.g. Bollgard-II: *cry1Ac* + *cry2Ab*) having one copy of each event to achieve long-lasting resistance. GM maize stacked with thirteen double, three triple and one quadruple event and is currently under EU assessment. The stacked GM crops which are likely to be commercialized are—soybean, maize, cotton, rapeseed, rice and potato (Table 3). A database for GM crops has been established to provide uniform and updated information the world over (http://cera-gmc.org/index.php?action=gm_crop_database).

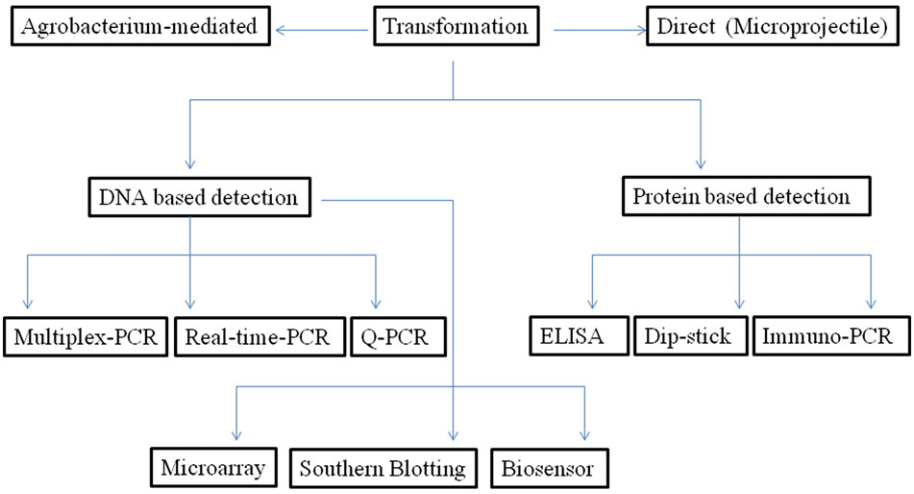

Fig. 5. A schematic view of detection methods for GM crops.

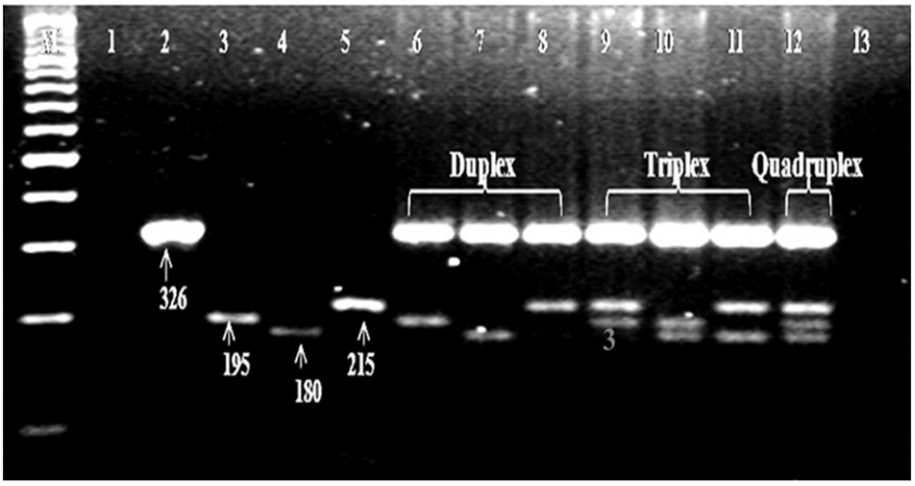

Fig. 6. A multiplex PCR assay showing simultaneous amplification of *cry2Ab* transgene, promoter (*P-35S*), terminator (*T-nos*) and marker gene (*Npt-II*) in GM cotton (MON15985). Lane M, 100 bp DNA ladder; 1, environmental control; standard single PCR, 2–5: 2, *cry2Ab* (326 bp); 3, *P-35S* (195 bp); 4, *T-nos* (180 bp); 5, *npt-II* (215 bp); duplex PCR, 6–8; 6, *cry2Ab* + *P-35S*; 7, *cry2Ab* + *T-nos*; 8, *cry2Ab* + *npt-II*; triplex PCR, 9–11; 9, *cry2Ab* + *npt-II* + *P-35S*; 10, *cry2Ab* + *T-nos* + *P-35S*; 11, *cry2Ab* + *npt-II* + *T-nos*; quadruplex PCR, 12, *cry2Ab* + *npt-II* + *P-35S* + *T-nos*; 13, non-GM cotton.
Source: Kamle et al., (2011a).

6. Necessity of GM crop testing

GM content based verification requires testing of GM products for the presence of foreign DNA or protein. The enforcement of threshold values has created a pressing demand for the development of reliable GM analysis methods of a rapid and inexpensive character. Reliable screening methods are important both for detection of unauthorized GM crops and labeling control (Morisset et al., 2009). Unauthorized GM crops can challenge the present analytical system on the ground of practical application of detection methods such as regulatory sequences common to all GM crops. Different screening methods based on DNA and proteins are employed for the detection of GM crops and their products (Fig. 5).

7. DNA based detection methods

PCR is the preferred method for the identification and quantification of *Bt* gene because of its versatility, sensitivity, specificity, and high throughput applications (Morisset et al., 2009). To detect any *Bt* gene, it is necessary to know the sequence of the genes used in the GM construct. These may include plasmid vector sequences, selectable markers, promoters and terminators.

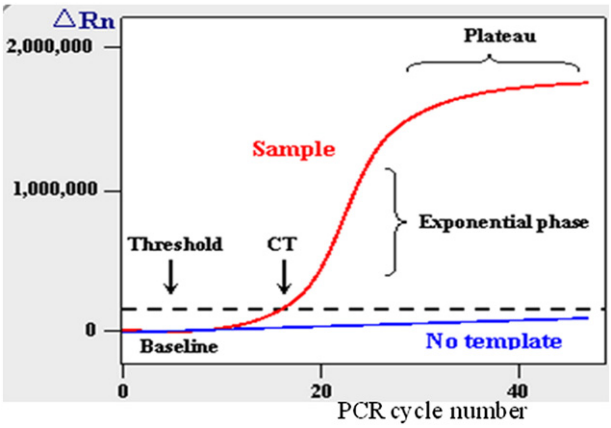

Fig. 7. A schematic plot of real-time quantitative PCR, displaying threshold and CT value.
Source: http://www.ncbi.nlm.nih.gov/projects/genome/probe/IMG/PCR_plot.gif.

7.1. PCR and real time PCR

As mentioned earlier, commonly used detection methods for GM crops is based on PCR (Stull, 2001). To identify GM crops and products, a primer needs to be designed for the amplification of the inserted gene. This basic requirement is ascertained by restriction endonuclease digestion of the gene followed by hybridization with a specific DNA probe. Alternatively, the PCR product itself may be used for direct sequencing.

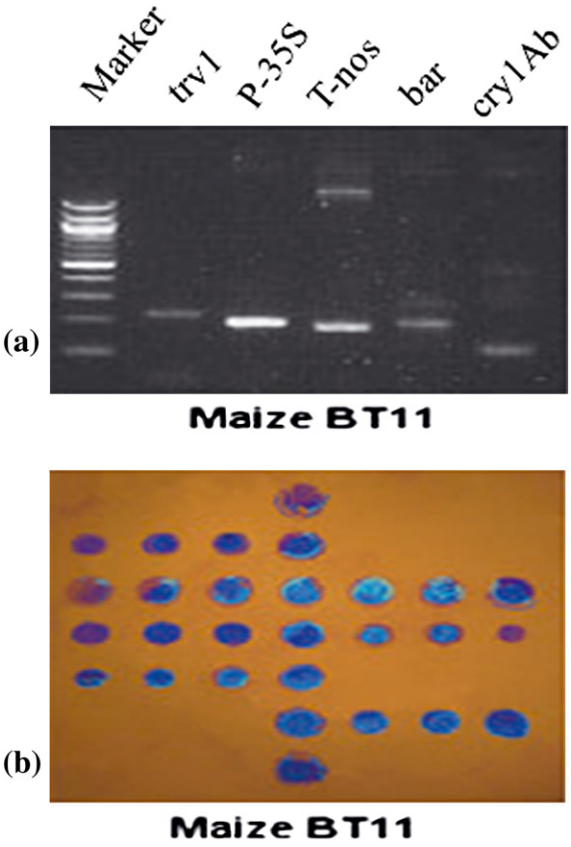

Fig. 8. A comparative view of detection of Bt maize (*cry1Ab*): (a) PCR based detection; (b) biosensor based detection.
Source: Bai et al. (2007).

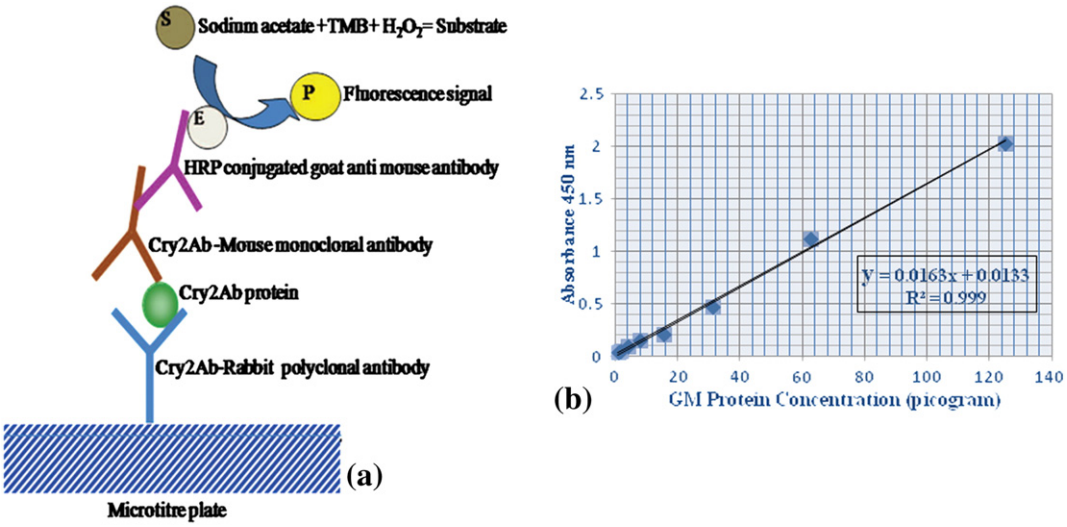

Fig. 9. (a) A schematic view of Cry2Ab sandwich ELISA; (b) a linear graph representing absorbance vs GM protein concentration. Source of panel a: Kamle et al. (2011b).

In addition, a nested PCR in which two sets of standard primers are used that bind specifically to the target sequences may also be employed. A multiplex and transgene construct specific PCR assays for *cry1Ab*, *cry1Ac*, *cry2Ab* and *vip-3A* transgenes (Fig. 6) have been reported (Kamle et al., 2011a; Randhawa et al., 2010).

Real time PCR is used to quantify a targeted DNA molecule. For detection of the products, sequence specific oligonucleotides labeled with a fluorescent reporter are used which allow the detection of the amplified product as the reaction advances (Fig. 7). Real-time PCR has great value in validating and estimating the number of copies of inserted genes into the host genome (Bonfini et al., 2002; Zhang et al., 2003). This has been reported for several GM crops such as maize, cassava, rapeseed, wheat, cotton and brinjal (Aguilera et al., 2008; Ballari et al., 2013; Beltrán et al., 2009; Lee et al., 2006; Li et al., 2004; Wu et al., 2007). Furthermore, a sensitive loop mediated isothermal amplication method employed for the detection of three GM rice events has been reported (Chen et al., 2012b; Kiddle et al., 2012).

Besides these techniques, microarray based detection systems are under development. *Bt*-176 transgenic maize (*cry1Ab*) was quantified by ligation detection reaction (LDR) combined with a universal array approach (Bordoni et al., 2004).

7.2. Biosensors

A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component. GM detection has encouraged the development of sensitive sensor technology that promises to generate quick results. Biosensors' prominent attribute is the immobilization of the

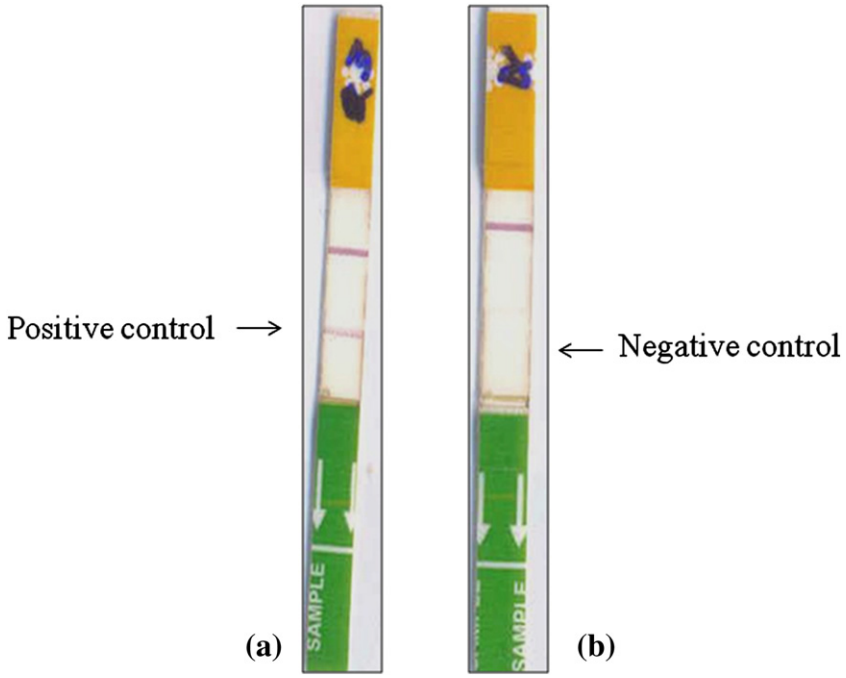

Fig. 10. A display of immuno-strip: (a) GM sample having protein of interest (two bands—positive control); (b) non-GM sample having no protein (single band—negative control) of interest.

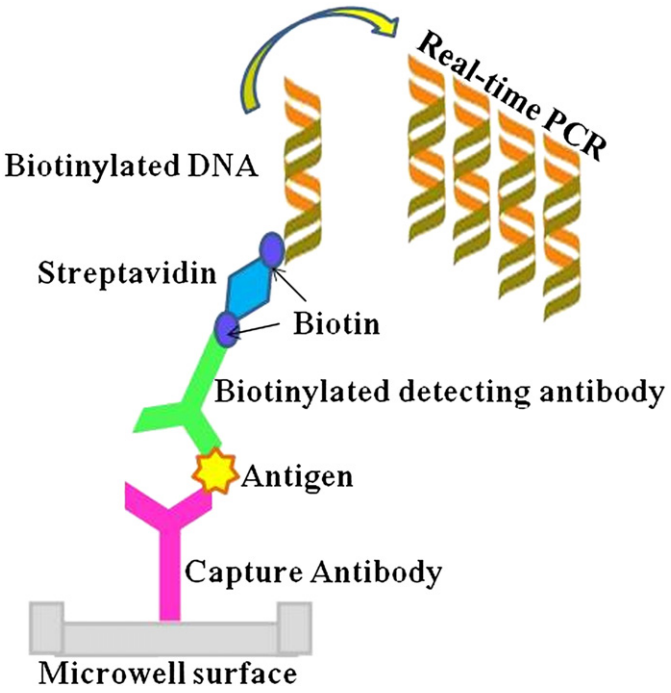

Fig. 11. A schematic representation of immuno-PCR. Source: http://sites.mc.ntu.edu.tw/sysdata/81/81/doc/c55122547d93a327/attach/1003.png.

probe on an electrode surface like altered cysteamine gold. Currently, different types of biosensors (electrochemical sensors, pizeoelectric biosensors, surface plasmon resonance/optical biosensors) are used to detect transgenes (Fig. 8) in GM crops like soybean, maize, cotton, rice, tomato and canola (Bai et al., 2007; Feriotto et al., 2003; Mariotti et al., 2002; Stobiecka et al., 2007; Tichoniuk et al., 2008). Recently, in Surface Enhanced Raman Scattering Spectroscopy (SERS), a barcoded nano-sensor has been developed to detect *cry1Ab* and *cry1Ac* transgenes in GM rice (K. Chen et al., 2012; X. Chen et al., 2012).

8. Protein based detection

An immunoassay technique based on antibodies is a standard approach for qualitative and quantitative detection of protein of a known target analyte (Brett et al., 1999). Both monoclonal (highly specific) and polyclonal (often more sensitive) antibodies can be used depending on the specificity of the detection system. On the basis of typical concentrations of a transgenic material in plant tissues (>10 μg per tissue), the limit of detection (LOD) of a protein immunoassay can predict the presence of recombinant protein in up to 1% of GMOs (Stave, 2002).

8.1. ELISA

ELISA has a significant advantage of protein analysis in GM crops and their products. A sandwich ELISA is the preferable immunoassay used for the detection of Bt protein, where an analyte is sandwiched in between the two antibodies; a capture antibody and the detector antibody. In sandwich ELISA protein concentration is directly proportional to the color intensity (the higher the protein concentration, the greater will be the color intensity). ELISA was successfully used for the detection of protein encoded by *cp4-epsps* gene in a RR soybean (Rogan, 1999). Also, monoclonal antibodies are being used for the development of sensitive and single epitope specific immunoassays for the detection of Bt proteins like Cry1Ac and Cry1Ab (Vázquez-Padrón et al., 2000). For the detection of Cry1Ab, a capillary electrophoresis competitive immunoassay and a highly sensitive quanti-dot based fluorescence linked immunosorbant assay have been developed (Giovannoli et al., 2008; Zhu et al., 2011). Similarly, a monoclonal antibody based sandwich immunoassay (Fig. 9) having a 100 ng/g LOD for Cry1Ac and a 1 pg/g LOD for Cry2Ab in cotton seed/leaf samples have been reported (Kamle et al., 2011b, 2013; Shan et al., 2007).

8.2. Immuno-strip

Use of a different format like ELISA, using a nitrocellulose-strip rather than microtiter wells, led to the development of lateral flow strip/dipstick/immuno-strip technology. Immobilized double antibodies, specific to recognize expressed protein are conjugated to a color reactant (gold nano-particles) and incorporated into a nitrocellulose strip. This nitrocellulose strip when dipped in the protein extract of plant tissue (e.g. GM cotton leaf) harboring a GM protein, leads to an antibody reaction releasing color. This red colored gold conjugated complex flows to the other end of the strip through capillary movement to a porous membrane that has two captured antibody zones. One zone is specific for the GM protein and the other one is specific for untreated antibodies coupled to the reagent (Fig. 10). The immuno-strips can give results as either 'Yes' or 'No' within 5 to 10 min. The immuno-strip is an economical, easy and field tractable detection method. These immuno-strips are commercially available to detect Cry1Ab, Cry1Ac, Cry2Ab and CP4-EPSPS (Lipton et al., 2000; Fagan et al., 2001).

8.3. Immuno-PCR

Immuno-PCR potentially offers a sensitive and specific method for detecting the antigen, in which a specific DNA molecule is used as a marker (Fig. 11). It combines the specificity of an ELISA with the sensitivity of the assay using PCR (Sano et al., 1992). An immuno-PCR assay has been reported for the detection of Cry proteins expressing GM crops such as Cry1Ac (Allen et al., 2006; Zhang and Guo, 2011).

9. Future prospects

The first generation of *Bt* crops (MON810) have been extraordinarily successful with a few examples of pest populations evolving resistance. These crops are already being replaced with a second or third generation of GM crop varieties having two or more traits/events. Even, this is not a matter of complacency and still needs more efficacious and potent *Bt* strains to meet the future requirement (Christou et al., 2006; Crickmore, 2006).

An engineered Cry1AMod toxin lacking helix α-1 has been reported, which does not bind with the receptor-cadherin and therefore kills even insects that are resistant to the parent toxin Cry1Ab (Muñóz-Garay et al., 2009). New *Bt* strains using a proteomics method can be screened for the presence of the novel toxin Cry60Ba from *Bt* serovar *malayensi*. Incidentally, this is also a mosquitocidal toxin (Sun and Park, 2010). A recent report showed that the isolated strain LLP29 from the phylloplane of *Magnolia denudate*, produces a novel toxin (Cyt1Aa6) which is lethal to mosquito larvae (Zhang et al., 2010). This has far reaching implications to control mosquitoes.

10. Conclusions

With the development of newer transgene crops, detection methods are also likely to be improved. The International Regulations and the Codex guidelines acting together with the biosafety issues and labeling of the GMOs seems to be a promising proposition towards the acceptance of GM crops.

Conflict of interest

The authors declare they have no conflict of interest.

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