

## Food & Health

### An efficient method for identification and quantification of genetic modification of local, imported and food products of maize in Saudi Arabia using PCR-based markers and real-time PCR

Abdulaziz M. Al-Swailem\*, Maher M. Shehata, Omar H. Shair, Saeed A. Sabaan, Ibrahim O. Al-Anazi and Turki A. Al-Shammari

Biotechnology and Genetic Engineering Program, Natural Resources and Environment Research Institute, King Abdulaziz City for Science and Technology, Riyadh 11442, P.O. 6086, Saudi Arabia. \*e-mail: alswailem@kacst.edu.sa

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#### Abstract

In recent years food crops have been engineered to express novel genes which impart new characteristics. The genetically modified corn "Maximizer" is resistant to the European corn borer due to the insertion and expression of the *cryIA(b)* gene from *Bacillus thuringiensis* ssp. Kurstaki. It is already widely cultivated in the U.S.A. and may find its way to Europe and Middle East as a component of a variety of food and feedstuffs. According to EU regulations, novel foods and ingredients must be labeled to allow consumers to make informed decisions regarding the foodstuffs they purchase. Analytical methods are thus necessary to show the presence or absence of GMO in raw materials and food, and need to be continuously up-dated. Consequently, the aim of this work has been to develop a qualitative and quantitative PCR method to detect genetically modified maize. We carried out a preliminary laboratory trial using conventional and real-time PCR assays to detect and identify purified extracts from pure samples of four cultivars (obtained from Ministry of Agriculture) and two processed food products (collected from the markets) of maize.

The screening of 25 RAPD primers allowed selection of 18 primers, which revealed that the loci tested were polymorphic and the results are reproducible. The different profiles obtained among all samples allowed the grouping into 2 main clusters. Cluster A includes American maize 1 and froot loops with 0.47-0.51 similarity matrix. Cluster B includes American maize 2, local maize 1 and 2. Cluster B consisted of two subgroups. Subgroup 1 includes American maize 2 and subgroup 2 includes local maize 1 and 2. Only corn flakes were assigned outside of groups, suggesting that, it may be obtained from another cultivar or subjected to highly processing. Quantification methods were optimized through different real-time PCR chemistries. The correlation coefficients between *ivr1* and *cryIA(b)* genes copy numbers ranged from -0.99 to 1.00, respectively, thus enabling calculation of the number of *ivr1* copies by performing RTQ-PCR. The No. of copies of the *ivr1* and *cryIA(b)* genes are  $1.44 \times 10^6$  and  $1.93 \times 10^5$ , respectively. The percentage of *cryIA(b)* is 3.84% and the rest genes of maize constitutes 96.16%. The detection limit of the method was 0.01%, which is far below the established threshold for accidental presence of genetically modified organisms (GMO). This method is specific, highly sensitive and reliable for both identification and quantification of DNA. Therefore, it is suitable for use in routine GMO analysis. Comparison of the LightCycler system and the well-established conventional PCR revealed no statistically significant differences with respect to sensitivity and reproducibility.

From our preliminary results, we can conclude that, conventional PCR method is rapid and suitable for characterization, establishment of taxonomic position and detecting maize materials and products. Real-time PCR method is highly rapid, sensitive and can detect trace amounts of GM-DNA (as low as 0.01~1%). The assays proved to be suitable for analytical purposes, with excellent limits of detection and quantification. They are also effective in indicating that commercially available maize materials are usually not mixed with GM-maize, but food products may be mixed with GM maize in Saudi Arabia. Our laboratories are continuously working on the development of other new quantitative detection methods of GM-maize. Thus, the method may be applied for control purposes and specific labeling.

**Key words:** Genetic modification, maize, PCR-based markers, real-time PCR, RAPD primers, quantification and detection limit.

#### Introduction

Maize is a gigantic domesticated grass (*Zea mays* L.) of tropical Mexican origin. The plant is used to produce grain and fodder that are the basis of a number of food, feed, pharmaceutical and industrial manufactures. Cultivation of maize and the elaboration of its food products are inextricably bound with the rise of pre-Colombian Mesoamerican civilizations. Due to its adaptability and productivity the culture of maize spread rapidly around the globe after Spaniards and other Europeans exported the plant from the Americas in the 15<sup>th</sup> and 16<sup>th</sup> centuries. Maize is currently produced in most countries of the world and is the third most

planted field crop (after wheat and rice). The bulk of maize production occurs in the United States, Peoples Republic of China and Brazil, which together account for 73% of the annual global production of 456.2 million tons. Mexico, the world's fourth largest producer of maize, currently produces approximately 14 million tons of grain annually on 6.5 million hectares (3% of world production on 5% of the world's land devoted to maize production) <sup>1</sup>.

In order to improve product quality, agronomic traits, as well as develop resistance to pests, genetic modification of agricultural

crops has become a predominant activity of research departments in the agricultural industry. The development of transgenic corn lines with new traits has become one of the main activities of research departments of the agro-industry. A number of transgenic corn lines, developed by different companies, have been approved in various countries<sup>2</sup>. GM soybean and maize are the most extensively cultivated GMO, with the traits introduced in these lines being, basically, resistance to herbicides and increased tolerance to insects and pests. The genetically modified maize "Event 176" or "Maximizer", better known as Bt-176 maize was the first GMO maize approved in the EU<sup>3</sup>. Bt-176 maize carries a gene that codes for the *cryIA(b)*  $\delta$ -endotoxin, derived from the bacterium *Bacillus thuringiensis* ssp. *Kurstaki*, which confers resistance to the European corn borer. This plant pest leads to yield losses and high costs for crop protection. Additionally, Bt-176 maize is herbicide-tolerant to ammonium glufosinate, and carries a marker gene expressing resistance to the antibiotic ampicillin. This gene is under the control of a 35S-promoter from a cauliflower mosaic virus and flanked by intron 1 of the corn-specific heat shock protein 70 hsp70 at its 59-site<sup>4</sup>. The termination signal T-NOS was taken from the nopaline synthase gene of *Agrobacterium tumefaciens*. The MaisGard line MON810 containing only the *cryIA(b)* gene cassette has been approved for food use by the Advisory Committee on Novel Foods and Processes (ACNFP) in the United Kingdom and is the subject of a petition recently submitted in Switzerland.

In Europe, the marketing and labelling of genetically modified organisms (GMO's) is regulated by the Novel Food Directive 258r97<sup>5</sup> or by national Food Ordinances. The Swiss Food Ordinance, for example, requires the labeling of genetically modified products, based on the detectability of the modified gene sequences. According to the Novel Food Directive 258r97, labeling will also be mandatory for certain foodstuffs within European Community EC countries. According to the Swiss and EU law<sup>6</sup>, foods and additives with more than 1% relative amount of genetically modified organisms must be labeled as GMO products. This situation clearly emphasizes the need for specific detection methods to control adherence to national or international regulations.

Worldwide commercialization of GMO has led to the approval of labeling regulations in several countries intended to protect the Consumer's right to information. EEC<sup>7</sup> and Japanese Ministries of Health and Welfare (MHW) and Agricultural Food and Fisheries (MAFF)<sup>8</sup> regulations have established the compulsory labeling of foods containing more than 1 or 5% GMO, respectively, which is considered to be the upper threshold for an accidental GMO contamination during field culture or seed transport.

Due to the ongoing debate surrounding food containing genetically modified foods, various countries (e.g., Europe) established, or are currently in the process of establishing, regulatory frameworks dedicated to GMOs. In order to take such frameworks into account, reliable qualitative methods for GMO screening in food products are required. The more robust GMO detection methods currently in use are those based on DNA amplification, because of the high stability of the DNA molecule under the harsh conditions used in food processing<sup>9,10</sup>. The detection of the introduced genetic elements at the level of the deoxyribonucleic acids DNA using the polymerase chain reaction

(PCR) has been shown to be the most useful method of identifying genetically modified foodstuffs<sup>11</sup>. Quantitative real-time PCR methods have been developed for several GMO events including the maize 176<sup>12</sup>, MON810<sup>13,14</sup>, Bt-11<sup>15</sup>, CBH-351<sup>16</sup> events, the soybean GTS 40-3-2<sup>12,17-19</sup> event, and also a durum transgenic wheat line, transformed to express a tobacco gene (*rab1*)<sup>20</sup>. All these methods are based on the parallel amplification of transgene specific sequences and an endogenous reference gene, which serves as an internal control for the quality of the extracted DNA, and as an estimation of the total amount of target plant DNA present in the sample. Nucleic acid quantification in real-time PCR assays is performed in a closed tube through fluorometric analyses, which reduces the risk of cross contamination and allows easy automation of the method. Different fluorometric detection systems have been developed to the present. The TaqMan chemistry (Applied Biosystems, Foster City, CA, USA) is one of the most commonly used for GMO detection and is based on the simultaneous addition of two primers and a specific probe that yields fluorescence emission upon DNA synthesis<sup>21</sup>. The Amplifluore Universal Amplification and Detection System (Intergen Co., Purchase, NY, USA) is based on a universal hairpin primer (Uniprimere). It anneals to the Z sequence appended to one of the gene specific oligonucleotides and produces fluorescence upon incorporation into the amplification product<sup>22</sup>. The intercalating dye SYBR Green I (Molecular Probes, USA), which exhibits fluorescence enhancement upon binding to the double-stranded amplification product<sup>23,24</sup>, offers an inexpensive and sequence unspecific alternative. In all three types of reaction, the fluorescence signal produced directly correlates with the accumulation of PCR product at each cycle, thus providing a quantification of the amount of template DNA in the reaction.

Many countries have developed standard methods for the detection of GM foods in order to comply with labeling regulations. Germany, for instance, has published methods for the detection of the Event176, MON810, Bt11 and T25 GM-maize. Japan has also developed PCR-based methods for GM-maize of the Event176, MON810, Bt11, CBH-351, GA21 and T25 varieties. Further, to meet the needs of the crop market, the US GIPSA (Grain Inspection, Packers and Stockyards Administration) set up a reference laboratory in 2000 to evaluate GMO detection methods and to certify laboratories with detection capabilities. Saudi Arabia is a large country without permanent rivers, and less than two percent of its land surface is under cultivation. Saudi agricultural development is now considered one of the major accomplishments of modern agriculture in the Middle East. To date, no literature is published in Saudi Arabia regarding the detection of GM-maize and the maize-containing raw materials of the Event176. The main objective of this study is a preliminary laboratory trial to detect and identify four cultivars (obtained from Ministry of Agriculture) and two processed food products (collected from the markets) of maize using conventional PCR (to identify the source of food products and detect genetic modification) and real-time PCR (detection and quantification of genetic modification) assays. The other objective is to demonstrate the picture of GM distribution in such samples in Saudi Arabia.

## Materials and Methods

**Materials:** Four cultivars of maize (two local designated as local 1 and 2, two imported from U.S.A. designated as American 1 and 2) were obtained from Ministry of Agriculture. Two food products (corn flakes and froot loops) were obtained from the market. DNA Isolation, LightCycler GMO Screening and LightCycler-GMO Maize Quantification Kits were obtained from Roche Applied Science. PCR primers for *cryIA(b)* and *ivr1* genes were obtained from the same company.

**DNA isolation and quantitation:** Samples of approximately 200 mg were used. DNA was extracted from the raw and food products of maize using CTAB method<sup>25</sup> and DNA isolation kit (Roche) according to the manufacturer's instructions. The quantitation of DNA by UV light at 260 nm was conducted using a GeneQuant II spectrophotometer (Pharmacia Biotech Europe GmbH, Germany) with 0.5 mm disposable quartz capillaries. Each measurement was repeated three times and the average was used to calculate the concentration of DNA. DNA purity was assessed by OD<sub>260/280</sub> measurements. The integrity of the isolated DNA was checked on a 0.5% agarose gel.

**Conventional PCR:** Polymerase chain reactions were carried out on a thermal cycler (Techne, Flexigene) with hot top assembly. For confirmation of the optimized protocol, the PCR reactions were conducted on another cycler (Clemens). All PCR reactions were performed in final volumes of 25  $\mu$ l in 0.5 ml tubes containing 200 ng (0.5  $\mu$ l) of DNA, 1x reaction buffer (50 mM KCl; 10 mmol Tris-HCl, pH 9; 0.2 mg BSA), 1.5 mM MgCl<sub>2</sub>, 0.2 mM each of dATP, dGTP, dTTP and dCTP; 0.5 mM of each primer (1.6  $\mu$ l) and 1 U of *Taq* polymerase (Pharmacia Biotech Germany). The amplification products were separated using a 2% agarose gel (MS agarose, Boehringer Mannheim Switzerland) in 1x TBE buffer (0.045 mM Tris-borate; 1 mM EDTA, pH 8.3), and visualized by UV after staining for 15 min. in a 1 mg ethidium bromide solution<sup>26</sup>. Amplification reactions were run using the following conditions: first denaturation 5 min. at 94°C, 40 cycles of 1 min. at 94°C, 1 min. at 36°C, 2 min. at 72°C and terminal elongation 10 min. at 72°C.

The PCR products of *cryIA(b)* and *ivr1* (reference gene) have a size of 187 and 211 bp, respectively. The results of PCR fingerprinting were collected into two different matrices indicating the presence or absence (scored as 1 or 0, respectively) of specific bands in each PCR analysis. Simple matrices were obtained by comparing pairs using the simple matching coefficient and dendrograms were constructed using the unweighted pair group method with arithmetic mean (UPGMA).

**RTQ-PCR (Real-time quantitative PCR):** This is the first time in the Arab World that an Amplifluore-based real-time PCR assay is optimized for GMO quantification. Real-time screening and quantitative PCR on the LightCycler instrument was performed as recently described with slight modifications in a total volume of 25  $\mu$ l in the presence of 5  $\mu$ l of DNA (or water). The threshold cycle ( $C_T$ ) values were determined. The  $C_T$  value is defined as the actual PCR cycle when the fluorescence signal increases above the background threshold. It indicates the number of target gene copies. The copy number of *ivr1* and *cryIA(b)* genes was determined assuming that, based on the molecular weight of corn

genome, 1ng of DNA equals  $6 \times 10^5$  times the entire genome and that both *ivr1* and *cryIA(b)* genes are single-copy genes. To quantify DNA by RTQ-PCR, an increase in fluorescence emission during PCR proportional to the initial copy number of the target gene is generated and detected. Fluorescent dsDNA-binding dyes and fluorogenic probes were applied. Fluorescence emission is detected for each PCR cycle. Samples were quantified by interpolation in a standard regression curve of  $C_T$  values generated from DNA samples of known concentrations. Quantification was performed using ten-fold dilution series from 10  $\mu$ mol/ $\mu$ l to 0.0001  $\mu$ mol/ $\mu$ l (representing  $6 \times 10^6$  to 60 copies/ $\mu$ l).

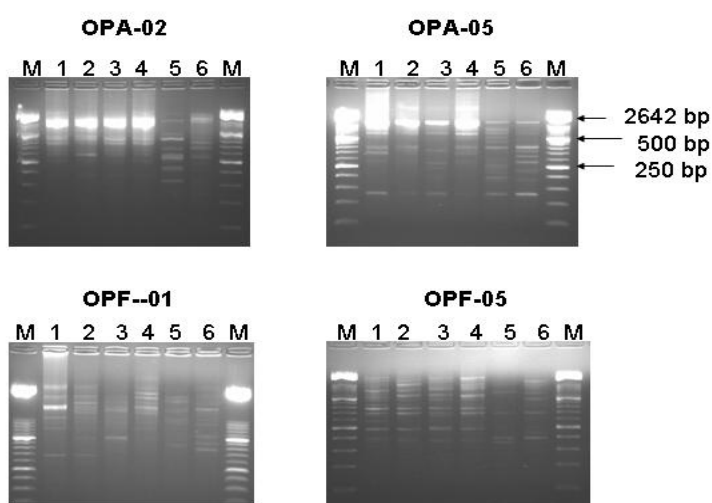
## Results and Discussion

Because there has been no researcher or related authorities proceeding in methodology development or conducting detection of GM-maize on market available processed maize products, the present study employed the methods established herein to detect four varieties of maize and two food products. The reason was speculated to be due to the fact that samples were collected from processed products, not from materials made of a single cultivar of GM-maize. Some modifications were practiced to serve as a double-checking means, and to get an insight on the percentage of the GM-maize content in the products. Experiences from our laboratories found that for maize raw materials, there is no significant difference between the DNA extracted with CTAB procedure or commercial kits procedures. However, for the processed products, especially for highly processed products, the DNA Isolation Kit (Roche) is recommended for DNA extraction.

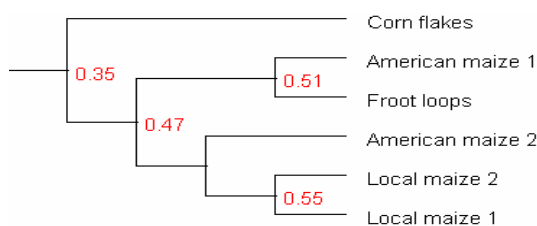
The conventional PCR and LightCycler methods each have their own specialty in corn modification detection. Specificity, precision, speed and cost are the factors that need to be considered when selecting the detection method. The PCR method has become a basic technique and equipment in life science laboratories. This method is capable of detecting target regions in different test samples using different conditions and primers<sup>27</sup>. As the LightCycler system is rather new equipment, its performance was compared to that of the well-established conventional PCR. Experiments to measure the quantification sensitivity and repeatability of results were conducted on both systems. Regarding repeatability, no significant distinction was found between the two machines. The sensitivity of the RTQ-PCR assay was higher than conventional PCR, suggesting a slightly lower efficiency for the conventional PCR. The security of the PCR result is assured through a positive and negative control reaction. The detection of the GMO and the reference gene PCR products are done using labeled hybridization probes. This reaction is then further controlled by another modified DNA. The test is positive when both the GMO {*cryIA(b)*} and the reference gene (*ivr1*) are detected. Generally, for a control to be negative the extinction must be  $\leq 0.2$  and for a control to be positive the extinction must be at least double as high as the negative control. Additionally the extinction value of the positive control must be  $> 0.2$ . Detection of *Ivr* gene in all maize samples (GM and non-GM) is a validation system for protocols, chemicals and instruments.

The screening of 25 RAPD primers allowed selection of 18 primers, which revealed that the loci of tested samples were polymorphic and the results are reproducible. To ensure

reproducibility and reliability of the RAPD markers, PCR reactions were repeated twice with each primer using two different thermal cyclers (Flexigene and Clemens). Different primers produced a different level of polymorphism. The number of polymorphic bands per primer varied between 6 and 19 with a mean of 3 major bands per primer (Fig. 1). The different profiles obtained among all samples using UPGMA cluster analysis allowed the grouping into 2 main clusters. Cluster A includes American maize 1 and froot loops with 0.47-0.51 similarity matrix. Cluster B includes American maize 2 and local maize 1 and 2. Cluster B consisted of two subgroups. Subgroup 1 includes American maize 2 and subgroup 2 includes local maize 1 and 2. Only corn flakes were assigned outside of groups, suggesting that it may be obtained from another cultivar or subjected to highly processing (Fig. 2). The result suggests that RAPD analysis could be used for an efficient identification and fingerprinting of cultivars and food products of maize.



**Figure 1.** RAPD fingerprints detected in four varieties and two food-products of maize (lanes: 1-6) using four decamer primers (OPA-01 & -05, OPF-01 & -05). Lane M: DNA molecular size marker. Lane 1: Local maize 1, lane 2: local maize 2, lane 3: American maize 1, lane 4: American maize 2, lane 5: Corn flakes, lane 6: Froot loops.



**Figure 2.** Dendrogram of four varieties and two food products of maize using eighteen decamer primers.

A sample is *Bt 176* corn-positive when the extinction values for the *ivr*-reference gene, the inhibition control and the *cryIA* (b)-detection gene are  $>0.2$  and at minimum twice as big as the equivalent negative control. When the result of the sample DNA is negative, the absorption value of the inhibition control (sample DNA + positive control DNA) must be at least 50% of the positive control DNA value. If this is not the case, the PCR inhibition factors must have been present in the sample. A true result is not possible under these conditions while false negatives are possible.

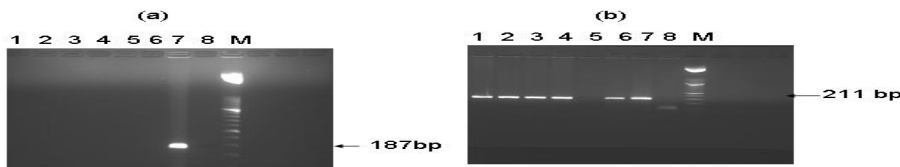
An optimization of the DNA isolation and purification is required. The cycle number at which the amplification plot crosses the threshold is defined as  $C_t^{28}$ . The amount of DNA in an unknown sample is measured by interpolation from a standard curve of  $C_t$  values generated from known concentrations of starting DNA. The corresponding real time PCR efficiency (E) of one cycle in the exponential phase, calculated according to the equation  $E = 10^{[-1/\text{slope}]}$ , was high, with value of 187. Analysis of replicated samples also showed good reproducibility, with standard deviations ranging from 0 to 0.96. Furthermore, the system was totally specific for the target DNA sequences, with none of the replicates of control samples of non-modified DNA giving an amplification signal.

According to the results of LighCycler technique, there is no need for labeling of samples with less than 1% GM gene, while those with more than 1% GM gene must be labeled. The No. of copies of the *Ivr1* and *cryIA*(b) genes detected are  $1.44 \times 10^6$  and  $1.93 \times 10^5$ , respectively. The percentage of *CryIA*(b) is 3.84% and the rest genes of maize constitutes 96.16% (Table 1). The linear regression curve derived from the *cryIA*(b) and *ivr1* reactions showed both very efficient amplification rates (slopes of 3.57 and 3.023, respectively) and a high correlation coefficient ( $R^2$  of 1.00 and 0.99, respectively). These results, besides being consistent with the data provided by Monsanto <sup>30</sup>, demonstrate a low percentage of GA21 maize in the sample analyzed. Our results are in accordance with that of Yanglin et al. <sup>31</sup>, who indicated that methods for the detection of DNA of RR soya and *Bt*-176 using real-time PCR are much more sensitive and robust than the conventional PCR methods. This allowed to conclude that real-time PCR is suitable to be used for quantification assay.

The results of conventional PCR showed that none of the samples were detected to contain the *Bt*-176 except the positive control (Fig. 3a). All real-time reactions yielded negative fluorescence signal corresponding to *cryIA*(b) of cultivars and food products of maize except froot loops which yielded positive signal with 0.014% *cryIA*(b) gene (Table 1, Fig. 4). All real-time

**Table 1.** Quantification of *Ivr1* and *CryIA*(b) genes in modified and non-modified DNA of raw and food products of maize.

Sample	Known conc.	Calculated conc.	Crossing point
Standard			
<i>Ivr1</i> gene	1 5.000E+05	1.627E+06	23.29
serial	2 2.500E+05	6.160E+05	24.48
dilution	3 1.250E+05	4.047E+05	25.08
	4 6.250E+04	2.041E+05	26.06
	5 -	1.176E+05	3.125E04
+ve control <i>Ivr1r</i> gene	-	1.438E+06	-
-ve control <i>Ivr1</i> gene	-	-	-
Local maize 1 <i>Ivr1</i> gene	-	3.049E+06	-
Local maize 2 <i>Ivr1</i> gene	-	1.280E+06	-
American maize 1 <i>Ivr1</i> gene	-	1.012E+06	-
American maize 2 <i>Ivr1</i> gene	-	2.429E+06	-
Food product 1 (Corn flakes) <i>Ivr1</i> gene	-	9.601E+06	-
Food product 2 (Froot loops) <i>Ivr1</i> gene	-	3.644E+03	-
Standard	1 5.000E+05	1.716E+05	26.31
<i>CryIA</i> (b) gene	2 2.500E+05	7.767E+04	27.44
serial	3 1.250E+05	4.742E+04	28.15
dilution	4 6.250E+04	1.818E+04	29.52
	5 3.125E04	8.321E+03	30.6426.14
+ve control <i>CryIA</i> (b) gene	-	1.930E05	26.14
-ve control <i>CryIA</i> (b) gene	-	-	-
Local maize 1 <i>CryIA</i> (b) gene	-	-	-
Local maize 2 <i>CryIA</i> (b) gene	-	-	-
American maize 1 <i>CryIA</i> (b) gene	-	-	-
American maize 2 <i>CryIA</i> (b) gene	-	-	-
Food product 1 (Corn flakes) <i>CryIA</i> (b) gene	-	-	-
Food product 2 (Froot loops) <i>CryIA</i> (b) gene	-	5.729E+02	34.47



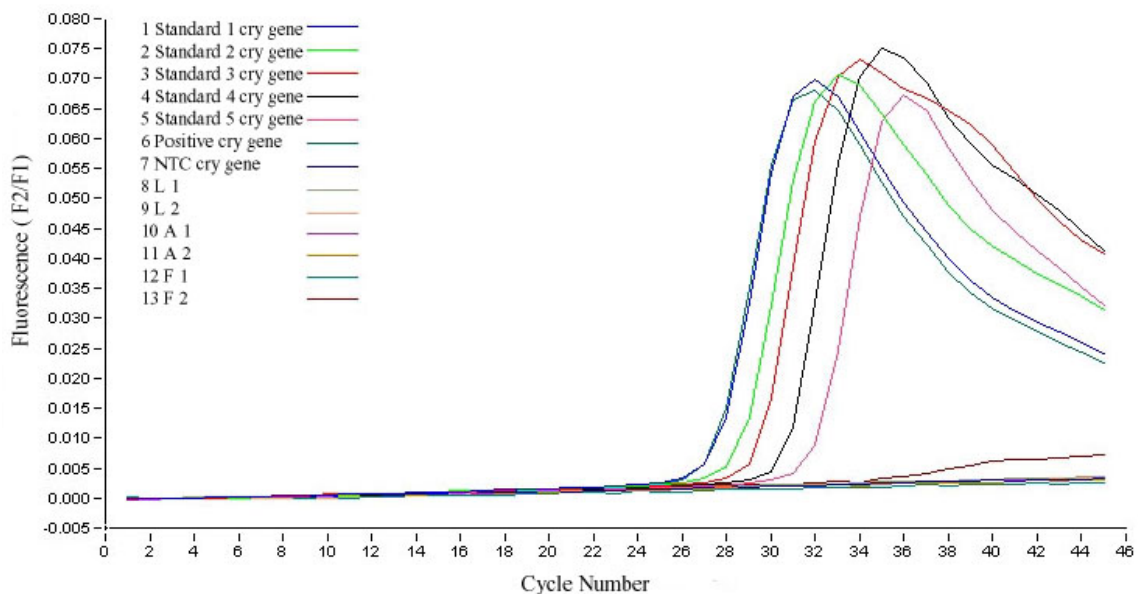
**Figure 3.** PCR products of *cryIA(b)*-modified (a) and *Ivr1*-reference (b) genes of four varieties and two food-products of maize (lanes: 1-6). Lane M; Molecular size marker. Lane 1: Local maize 1, lane 2: local maize 2, lane 3: American maize 1, lane 4: American maize 2, lane 5: Corn flakes, lane 6: Froot loops, positive control of Lane 7: *cryIA(b)*-modified gene, lane 8 (NTC): non-template control sample.

reactions of cultivars and food products of maize yielded positive fluorescence signal corresponding to *ivr1*. Conventional PCR yielded positive results of this gene only in maize cultivars but no 211 bp product was found in the negative control and food products (Fig. 3b), suggesting that food products may be subjected to highly processing which affects the integrity of genes. Ahmed<sup>32</sup> postulated that, in conventional PCR, products of the reaction are measured at a single point in the reaction profile. Plotting the concentration of reactions shows that proportionality between DNA concentration (dynamic range) and PCR products occurring over a limited range of DNA concentrations leads to loss of precision in quantitation. However, it has been shown empirically that the concentration of DNA in real-time PCR reaction is proportional to PCR cycle number during the exponential phase of PCR<sup>33</sup>. This result indicates that the real-time system is more sensitive, specific and has a detection limit of 0.01% GMO with 1 ng of starting maize template DNA. The specificity was ensured by using primer pairs which bind to a particular combination of genetic elements that does not occur in conventional products or in other approved genetically modified corn lines. Hubner et al.<sup>9</sup> reported that the sensitivity of quantitative PCR detection methods can be expressed in terms of LOQ (Limits of Quantification) and LOD (Limits of Detection). These two parameters are dependent on the amount of genomic DNA template used in the analysis, on the plant genome size and on the number of transgenes per genome. These authors indicate 35 genome copies as the theoretical limit of quantification. Based on the assumption that 100 ng plant DNA template is used for PCR and referring to genome size of maize, the theoretical LOQ

for our analysis, calculated according to Hubner et al.<sup>9</sup> is 0.38%. Analysis of samples containing varying amounts of DNA from GM-corn ranging from 10 pg to 100 ng showed that positive results were obtained over the whole range. These data suggest that LOD of our PCR methods is at the single genome copy level. However, no quantitation should be attempted below the LOQ. This is the same result as reported by other researchers<sup>34</sup>. Thus, the method may

be applied for specific labeling and control purposes. It may also be used as a basis for quantitative, competitive PCR detection methods, similar to those developed for other genetically modified crops. The real-time PCR method developed in this study allows as low as 0.01~1% GM-maize to be detected indicating this method is sensitive enough to fit in with the GMO labeling regulation set by Europe (1%)<sup>35</sup> and Japan (5%)<sup>36</sup>.

From our preliminary results, we can conclude that, conventional PCR method is rapid and suitable for characterization, establishment of taxonomic position and detecting maize materials and products. Real-time PCR method is highly rapid, sensitive and can detect trace amounts of GM-DNA (as low as 0.01~1%). The assays proved to be suitable for analytical purposes, with excellent limits of detection and quantification. They are also effective in indicating that in Saudi Arabia, commercially available maize materials are usually not mixed with GM-maize but food products may be mixed with GM maize. Our laboratories are continuously working on the development of other new quantitative detection methods of GM-maize. Thus, the method may be applied for control purposes and specific labeling. It may also be used as a basis for quantitative, competitive PCR detection methods, similar to those developed for other genetically modified crops in different labs<sup>17, 37</sup>. The future work in our laboratory will include evaluation of a DNA extraction method and test of different primers, GMOs or processed foods and other detection methods in addition to PCR and real-time methods.



**Figure 4.** Quantification of *cryIA(b)* modified gene. Samples 15: serial dilution series of *cryIA(b)* gene. Sample 6: positive control of *cryIA(b)*. Sample 7: negative control. Samples 8-13: local maize 1, local maize 2, American maize 1, American maize 2, corn flakes and froot loops, respectively.

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