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Investigation of genetically modified (GM) ingredients in packaged maize containing food products in Indian market: A regulatory study

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In compliance with the regulatory status of genetically modified (GM) maize and derived food products in India, checking unauthorized presence of GM maize in packaged food products from marketplace is required. This article details a pilot research to track the GM status of fifty samples of food products with maize as an ingredient where 12 were manufactured in the countries where GM maize products are commercially available. Amplifiability of DNA was first verified using endogenous gene-specific polymerase chain reaction (PCR) or real-time PCR, or by inhibition test. Four transgenic elements were selected for the GMO tests, which could screen for 90% of the globally approved GM events of maize. PCR assays targeting screening elements, *Cauliflower Mosaic Virus* (*CaMV)* 35S promoter (*P-35S*) and nopaline synthase terminator (*T-nos*), followed by real-time PCR assays targeting *ctp2-cp4epsps* and *cry1Ab/Ac,* were used to monitor their GM status. None of these samples were found GM positive based on the tests conducted indicating unauthorized presence of GM maize ingredients was not detected. Such regulatory studies will provide quality assurance that there are no unapproved GM events in the food products in the marketplace and the regulatory system is in place in the country.

Keywords: GM detection, Inhibition test, Maize products, Polymerase chain reaction (PCR), Real-time PCR

Worldwide genetically modified (GM) crops have been commercially grown in an area of about 206 million hectares (mha)[1]. GM maize or corn with diversified traits has been approved in several countries. GM maize covers an area of approximately 60 mha globally, contributing to around 32% of global maize production[2]. Maize has the highest number of approved GM events, comprising more than 45% of total approved GM events of different crops[3]. In India, ICAR-National Bureau of Plant Genetic Resources, New Delhi facilitates the import of GM seeds for the research purposes after technical approval from the Review Committee on Genetic Manipulation (RCGM), and the highest number of imports comprises of GM maize[4]. All the food products with 1.0% or more of any GM ingredient need to be labelled as *"Contains genetically modified organisms"* as per the *Draft Regulations known as the Food Safety and Standards (Genetically Modified Foods) Regulations* by the Food Safety and Standards Authority of India (FSSAI)[5].

Since some of the packaged food items with plant ingredients are imported from the countries where the GM events of the respective food crop are approved, it is therefore necessary to check the unauthorized entry of GM food products for regulatory purpose in India. DNA-based techniques are preferred for testing of genetically modified organisms (GMO) in processed food products as there may be a possibility of protein degradation due to intense processing procedures. However, while subjected to high temperature and pressure during processing or packaging steps or due to complex composition, the quality of DNA may also get impeded occasionally. Thus, choosing an appropriate DNA extraction procedure for a particular food product is critical for GM detection[6,7].

A few reports on GMO testing of maize products from the marketplace are available, which have been conducted in different countries including Algeria[8], Portugal[9], Singapore[10] and Turkey[11]. Keeping in view of the regulatory status of GM maize in India, this study has focused on checking the GM ingredients in fifty samples of maize containing packaged food products employing PCR and real-time PCR based assays targeting four transgenic elements (*P-35S* promoter, *T-nos* terminator, *ctp2-cp4epsps*, *cry1Ab/Ac*) covering GMO screening for over 90% of globally approved GM maize events.

Materials and Methods

Collection of samples of food products

Fifty samples of maize containing food products including chips, flakes, flour, instant mixes, popcorn,

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and starch, were purchased from the local markets/ retail outlets or through online shopping in New Delhi or National Capital Region (NCR), Delhi, India. Thirty-eight of these products were of Indian origin and twelve were manufactured in different countries, namely, the United States of America (USA), Malaysia, and Thailand, where GM maize has been approved for commercial use. The details of purchased samples with respect to the country of origin, GMO labeling if any, manufacturer company and ingredients are summarized in Table 1.

Table 1 — Description of food products used in this study along with the summary of quality check and GMO tests results

Sample Name	Origin	Composition *w.r.t.* maize as ingredient	Test for *Adh1* PCR (135 bp band)	Test for *Adh1* Real-time PCR Ct value	Test for *Adh1* Real-time PCR Tm (°C)	GMO Tests PCR (*P-35S*, *T-nos*)	GMO Tests Real-time PCR (*cry1Ab/Ac*, *cp4-epsps*)
*Crusties	India	Corn grifts, Corn oil	Present	15.807	79.460	Absent	Absent
* Crisps (Cheese and Herbs)	India	Corn (70%), Corn oil, Corn starch	Present	20.797	79.018	Absent	Absent
* Crisps (Sizzlin Jalapeno)	India	Corn (70%), Corn oil, Corn starch	Present	22.241	79.018	Absent	Absent
*French Butter & Pink Salt Popcorn	India	Corn (77%)	Present	17.552	79.018	Absent	Absent
*Sour Cream &Wasabi Cheese Popcorn	India	Corn (62%)	Present	15.005	79.018	Absent	Absent
*Orange Chilli Caramel Popcorn	India	Corn (33%), Corn syrup	Present	16.779	80.049	Absent	Absent
Popcorn Butter Flavour	India	Popping Corn (60.62%)	Present	17.090	78.870	Absent	Absent
Cheese Balls	Malaysia	Corn Grifts	Present	16.852	79.312	Absent	Absent
Super Ring	Malaysia	Corn	Present	20.190	79.165	Absent	Absent
Corn Puffs	India	Corn Grifts	Present	15.656	79.460	Absent	Absent
Mexilla (Tortilla Chips	India	Corn (70%)	Present	37.169	78.873	Absent	Absent
Sweet Corn Vegetable Soup	India	Corn (7%), Maize Starch	Present	37.715	78.873	Absent	Absent
*Cornado	India	Corn Flour, Edible Corn Starch	Present	29.153	79.779	Absent	Absent
American Corn Snack	Thailand	Corn Flour (61%)	Present	30.181	78.420	Absent	Absent
Corn Dots	India	Corn	Present	31.241	78.571	Absent	Absent
Corn Nut	India	Spanish Corn (90%)	Present	28.655	78.420	Absent	Absent
Corn Nut	India	Spanish Corn (80%)	Present	29.020	78.722	Absent	Absent
Roasted Spanish Corn Nut	India	Spanish Corn	Present	29.703	79.024	Absent	Absent
Popcorn Maize	India	Corn	Present	29.243	79.628	Absent	Absent
Sweet Corn Chat Masala	India	Sweet Corn	Present	37.515	79.024	Absent	Absent
Instant Popcorn	India	Popping Corn	Present	17.473	79.460	Absent	Absent
Golden Sizzle	India	Popping Corn	Present	10.698	79.312	Absent	Absent
Popcorn	USA	Corn	Present	17.987	79.312	Absent	Absent
Cornflour	India	Cornflour	Present	18.777	79.165	Absent	Absent
Cornflour	India	Cornflour	Present	18.398	79.312	Absent	Absent
Cornflour	India	Cornflour	Present	13.661	79.312	Absent	Absent
Corn Starch	USA	Corn Starch	Present	22.673	79.298	Absent	Absent
Corn Starch	USA	Corn Starch	Present	17.946	79.445	Absent	Absent
*Corn Flour	India	Edible corn starch	Present	22.877	79.151	Absent	Absent
Corn Flour	India	Maize starch	Present	17.401	79.298	Absent	Absent
Diet Popcorn	India	Popping corn (73.35%)	Present	38.734	78.720	Absent	Absent
Popcorn Hot & Spicy	USA	Corn	Present	39.018	78.720	Absent	Absent
Coated Green Peas Hot & Spicy	India	Corn Starch	Present	35.190	78.869	Absent	Absent
Coated Green Peas Wasabi	India	Corn Starch	Present	39.379	78.720	Absent	Absent
Corn Flakes	India	Corn Grits (62%)	Present	34.332	78.571	Absent	Absent
Organic Corn Atta	India	Organic Corn	Present	37.113	78.423	Absent	Absent
American Sweet Corn	India	Corn	Present	37.252	78.571	Absent	Absent
Chips	Malaysia	Corn Flour	Present	26.731	78.274	Absent	Absent
Chips	Malaysia	Corn Flour	Present	29.392	77.977	Absent	Absent
Chips	Malaysia	Corn Flour	Present	25.945	77.829	Absent	Absent

(*Contd*.)

Table 1 — Description of food products used in this study along with the summary of quality check and GMO tests results (*Contd.*)

			Test for *Adh1*			GMO Tests	
			PCR	Real-time PCR		PCR	Real-time PCR
			(135 bp band)	Ct value	Tm (°C)	(*P-35S*, *T-nos*)	(*cry1Ab/Ac*, *cp4-epsps*)
Chips (Fusion Chutney Flavour)	Malaysia	Corn Flour	Present	25.060	77.977	Absent	Absent
Chips(Sour Cream & Onion Flavour)	Malaysia	Corn Flour	Present	26.845	77.977	Absent	Absent
Mixed Vegetable Soup	India	Corn (4.7%), Maize starch	Present	42.408	75.897	Absent	Absent
Mexican Tomato Corn Soup	India	Corn (5.7%), Maize starch	Present	39.278	79.611	Absent	Absent
Sweet Corn Chicken Soup	India	Corn (5.8%), Maize starch	Present	40.061	79.463	Absent	Absent
*Corn Chips Cheese and Herbs	India	Corn (78%)	Present	28.970	78.274	Absent	Absent
*Corn Chips Hawaiian Barbeque	India	Corn (78%)	Present	33.036	77.976	Absent	Absent
Corn Flakes	India	Corn Grits (90%)	Present	33.963	78.125	Absent	Absent
*Corn chips salt and truffle	India	Corn (81%)	Present	29.826	78.125	Absent	Absent
Corn chips	India	Corn (79%)	Present	25.281	78.273	Absent	Absent
Positive Control			Present	19.752		Present	Present
Non-template Control (Water + Master Mix)			Absent	nd		Absent	Absent
Negative Control (Non-GM cotton DNA + Master Mix)			Absent	nd		Absent	Absent

[*Non GMO label available on the packet]

Isolation of DNA

The whole content of the packet of food products was ground to fine powder using an electric grinder (Phillips, India). Some of the samples such as instant popcorn that were hard to grind, were additionally crushed using liquid nitrogen for the final preparation of sample. The samples such as flour, starch, already available in powdered form, were used directly for DNA isolation.

Total DNA from the powdered food products was isolated using modified DNeasy® Mericon Food Kit (Qiagen, Hilden, Germany) protocol[7]. Certified reference material (CRM) of MON810 (MON-ØØ81Ø-6) and NK603 (MON-ØØ6Ø3-6) (procured from the European Reference Material, Joint Research Centre-European Commission, through Sigma Aldrich), reference material of MON531 (MON-ØØ531-6) from M/s Mahyco Private Limited, Jalna, India and non-GM maize were used as respective positive and negative controls, in order to eliminate the effect of false positive and/ or negative on the certainty of the results. DNA from the CRM, reference material and non-GM maize was isolated using DNeasy® Plant Mini Kit (Qiagen, Hilden, Germany) protocol as per the manufacturer's instructions.

Quantification and quality assessment of DNA

Quantity and quality of DNA samples was evaluated by using a UV-VIS spectrophotometer (Eppendorf, Hamburg, Germany). A_{260}/A_{280} ratios between 1.8 and 2.0 were considered to indicate that samples were devoid of protein and other contaminating elements[12]. DNA extracts were diluted to a concentration of 20 ng/µL. The quality of DNA was ensured using PCR and real-time PCR assays targeting the endogenous gene of maize or through inhibition test. Amplifiability of DNA extracts of food products was confirmed by PCR assay targeting *alcohol dehydrogenase 1* (*Adh1*), an endogenous gene of maize[13]. Details of primers used are provided in Table 2. PCR was done in Veriti® Thermal Cycler (Applied Biosystems, Foster City, CA, USA) in 20 µL reaction mix comprising of 100 ng of template DNA, 1× polymerase buffer, 1.5 mM of $MgCl_2$, 200 µM of dNTP mix, 0.25 µM each of forward and reverse primers, and 0.5 units of Taq DNA polymerase (ThermoFisher Scientific, MA, USA). The thermal cycling profile used was 94 °C for 10 min (initial denaturation), and 40 cycles of 94 °C for 30 s (denaturation), 56 °C for 1 min (primer annealing), 72 °C for 1 min (primer extension), and 72 °C for 7 min (final extension). The PCR amplified products were resolved on 2.0% (w/v) agarose gel (G-Biosciences) in 1× Tris-acetate-EDTA (TAE) running buffer by horizontal electrophoresis. The amplification pattern of products was then visualized using an UV Gel Documentation Imaging System (Alpha Innotech, SL, USA).

Real-time PCR was carried out in 20 µL reaction mix containing 100 ng of DNA template, 1× SYBR™ Green PCR master mix (Qiagen, Hildon, Germany), 0.4 µL of ROX dye and 0.25 µM of forward and reverse primers for *Adh1* gene. Real-time PCR was performed on Quant Studio3 Real-time PCR System (Thermo Fisher Scientific, MA, USA). The thermal profile used was: 2 min at 50 °C, single cycle of DNA pre-activation for 10 min at 95 °C followed by 40 amplification cycles of 15 s at 95 °C (denaturation step), and 1 min at 60 °C (annealing-extension step). Specificity of amplified products was confirmed using melt curve analysis based on their melting temperature (Tm) values. The PCR products were heated to 95 °C for 15 s, cooled at 60 °C for 1 min, and then slowly heated back to 95 °C at 1% ramp rate for melting curve analysis.

The presence or absence of PCR inhibitors in selected food products (chips with cornflour, nacho crisps, corn nuts and orange chilli caramel popcorn) was further verified by testing a range of serial dilutions (100-0.01 ng) of individual food samples. The test was conducted using real-time PCR targeting *Adh1* endogenous gene by making tenfold serial dilutions of DNA sample of each food product in molecular grade water to form working DNA concentration of 100 ng, 10 ng, 1.0 ng, 0.1 ng, and 0.01 ng, respectively. Each serial dilution was evaluated in 3-4 replications. Reaction mixtures and cycling conditions were same as those used for real-time PCR analysis for *Adh1* gene to assess DNA quality. Efficiency (E) and correlation coefficient (R^2) were estimated based on the slope and intercept of the standard curve obtained. The slope of the inhibition curve must be in the range $-3.1 \leq \text{slope} \leq -3.6$, corresponding to the efficiency of 110–90%. However, for food samples, which are highly processed, a slope of the inhibition curve within range between -4.1 and -3.1 is acceptable[14]. The value of R^2 should be greater than or equal to 0.98.

Selection of transgenic elements for GM detection

Screening targets for the detection of GM maize ingredients in food products were selected based on the GMO matrix for globally approved GM maize events previously developed by our laboratory as decision support system[15]. Two regulatory elements, namely, *P-35S* promoter and *T-nos* terminator, along with *cry1Ab/Ac* and *ctp2-cp4epsps* transgenic sequences were selected to check the GM ingredients in maize containing food products as they covered screening for presence or absence of more than 90% of globally approved GM maize events. Details of primers for *P-35S* promoter[16], *T-nos* terminator[16], *cry1Ab/Ac*[17] and *ctp2-cp4epsps*[18] sequences used for GMO screening are provided in Table 2. Primers were synthesized by M/s Pivotal Marketing, India.

Qualitative PCR-based GMO testing of food products

The GM status of the food products was first determined using PCR assays targeting *P-35S* promoter and *T-nos* terminator. PCR reaction was set up in 20 µL reaction mix comprising of 100 ng of template DNA, 1× polymerase buffer, 1.5 mM of $MgCl_2$, 200 µM of dNTP mix, 0.25 µM each of forward and reverse primers of *P-35S* promoter and 0.5 U of Taq DNA polymerase (ThermoFisher Scientifc, MA, USA). For *T-nos*, 0.5 µM each of forward and reverse primers were used. The PCR condition used was: 94 °C for 5 min, and 35 cycles of 94 °C for 30 s, 60 °C for 50s, 72 °C for 1 min, and 72 °C for 7 min. The amplified products were resolved on 2.0% (w/v) agarose gel (G-Biosciences) in 1×TAE running buffer by horizontal electrophoresis. The amplification pattern of products was then visualized using UV Gel Documentation Imaging System. DNA sample of NK603 event of maize was used as positive control for *P-35S* and *T-nos*, whereas non-GM maize and non-template (water) control were used as negative controls.

Real-time PCR-based GMO testing of food products

Real-time PCR was used for the detection of transgenic sequences, namely, *ctp2-cp4epsps* and *cry1Ab/Ac*. DNA extract of NK603 event was used as positive control for *ctp2-cp4epsps*, MON810 event of GM maize with *cry1Ab* gene and *Bt* cotton event MON531 with *cry1Ac* gene were used as positive controls for *cry1Ab/Ac*, whereas non-GM maize and

Table 2 — Primers used in the study

Target	Forward Primer (5'-3')	Reverse Primer (5'-3')	Product size
P-35S	GCTCCTACAAATGCCATCA	GATAGTGGGATTGTGCGTCA	195 bp
T-nos	GAATCCTGTTGCCGGTCTTG	TTATCCTAGTTTGCGCGCTA	180 bp
Adh1	CGTCGTTTCCCATCTCTTCCTCCT	CCACTCCGAGACCCTCAGTC	135 bp
cry1Ab/Ac	GAGGAAATGCGTATTCAATTCAAC	TTCTGGACTGCGAACAATGG	74 bp
ctp2-cp4epsps	GGGATGACGTTAATTGGCTCTG	GGCTGCTTGCACCGTGAAG	88 bp

non-template control were used as negative controls. Real-time PCR was carried out in 20 µL reaction mix containing 100 ng of DNA template, 1× SYBR™ Green PCR master mix (Qiagen), 0.4 µL of ROX dye and 0.25 µM of forward and reverse primers. Real-time PCR was performed on Quant Studio3 Real-time PCR System (Thermo Fisher Scientific, MA, USA). The thermal profile used was: 2 min at 50 °C, single cycle of DNA pre-activation for 10 min at 95 °C followed by 40 amplification cycles of 15 s at 95 °C, and 1 min at 60 °C. Specificity of amplified products was confirmed using melt curve analysis based on their melting temperature (Tm) values. The PCR products were heated to 95 °C for 15 s, cooled at 60 °C for 1 min, and then slowly heated back to 95 °C at 1% ramp rate for melting curve analysis.

Statistical analysis

The GMO tests were performed in two replications along with suitable positive and negative controls to avoid the chances of Type I (false positives) and Type II (false negatives) errors. This Type I amd II error analysis was done in order to ensure that the test results comply scientifically and not by chance factor.

Results and Discussion

In compliance with the regulatory status of GM maize and maize derived food products in the country, a pilot study for GMO testing in fifty maize products was undertaken using PCR assays targeting *P-35S* promoter and *T-nos* terminator, and real-time PCR assays targeting *cry1Ab/Ac* and *ctp2-cp4epsps* to track unauthorized entry of GM maize in the marketplace.

Quality checking of DNA extracts

Detection of transgenes might be difficult in case of highly processed foods, as the efficiency of DNA-based tests, relies on the quality of DNA. Inhibitors may also interfere with the PCR reaction components, affecting the ability of DNA polymerase to amplify target DNA, interaction with the template DNA, or reducing the effectiveness of enzyme cofactors such as Mg^{2+} ions[19]. In this study, as the food products procured from the marketplace were constituted of complex ingredients so the column based kit protocol using DNeasy® Mericon Food Kit was used to isolate the DNA of acceptable concentration and purity (Supplementary Table S1).

The amplifiability of isolated DNA is required to be ensured before performing a GMO test, to minimize the chances of false negatives for the respective test. Amplifiability of DNA extracts of maize containing products was confirmed using PCR assay targeting *Adh1*, an endogenous gene of maize. PCR product of 135 bp of *Adh1* gene was detected in all the maize products (Table 1, Suppl. Fig. S1). The results were further confirmed by real-time PCR assay targeting *Adh1* gene and the amplification curves with appropriate cycle threshold (Ct) values were detected in all the samples (Suppl. Fig. S2). Ct values of the samples ranged from 10.698 to 38.408 and the melting curve was found relatively similar for all the samples, thereby confirming the specificity of products. The use of endogenous gene-specific assay as an internal experimental control in GMO testing ensures the certainty of the results for better interpretation.

Checking inhibition in selected food matrices

Inhibition tests were conducted for the DNA extracts of four of the maize products, namely, chips with cornflour, nacho crisps, corn nuts and orange chilli caramel popcorn. These products were selected as representative of highly processed samples with maize as a major ingredient. In chips, nachos crisps and caramel popcorn samples, the amplification was detected in the test samples with working concentration of 100, 10, 1 and 0.1 ng of DNA with R^2 value of 0.986, 0.989 and 0.993 and, slopes of -3.177, -3.348 and -3.309 respectively, indicating that samples can be efficiently detected from a range of 0.1 to 100 ng of DNA concentration without any inhibition (Table 3, Fig. 1). The amplifiability of these samples was in line with the acceptance requirements by ENGL[16] [$-3.1 \leq slope \leq -3.6$ (in general), and $-3.1 \leq slope \leq -4.1$ (for highly processed samples), amplification efficiencies of 110 to 90% and $R^2 \geq 0.98$].

In corn nuts, amplification was detected in 100-0.1 ng of serial dilution series with R^2 value of 0.981. However, slight deviation observed in the value of slope (-2.848) and corresponding value of efficiency (124.446 %), may be attributed to highly processing steps such as thermal treatments or the complex composition. Similar observation was also reported for potato chips where slight deviation was found in value of slope (-2.534) because of complex constituents and thermal treatments[7]. Such deviations might be considered in case of complex matrices for the qualitative tests.

PCR-based GMO screening of food products

An initial screening for two regulatory transgenic elements, *i.e.*, *P-35S* promoter and *nos* terminator was

Table 3 — Summary of real-time PCR based inhibition test results of selected food products

Sample	Dilution	Mean Ct ± SD	Mean Tm ± SD	R^2	Slope	% Efficiency
Chips with corn flour (n=4)	100 ng	25.391±0.114	78.989±0.077	0.986	−3.177	106.411
	10 ng	28.602±0.194	79.107±0.004			
	1.0 ng	31.989±0.461	78.989±0.081			
	0.1 ng	34.853±0.772	79.186±0.400			
Nacho Crisps (n=4)	100 ng	23.976±0.034	78.939±0.400	0.989	−3.348	98.927
	10 ng	26.960±0.052	79.215±0.339			
	1.0 ng	30.524±0.236	79.254±0.401			
	0.1 ng	33.948±0.803	79.175±0.212			
Corn Nuts (n=3)	100 ng	26.492±0.214	78.652±0.358	0.981	−2.848	124.446
	10 ng	30.054±0.072	79.042±0.356			
	1.0 ng	32.907±0.151	78.952±0.226			
	0.1 ng	34.856±0.268	78.420±0.423			
Orange Chilli Caramel Popcorn (n=4)	100 ng	23.352±0.050	79.235±0.151	0.993	−3.309	100.534
	10 ng	26.338±0.136	79.511±0.091			
	1.0 ng	29.584±0.212	79.652±0.204			
	0.1 ng	33.301±0.521	79.652±0.158			

[n: number of replications of each dilution]

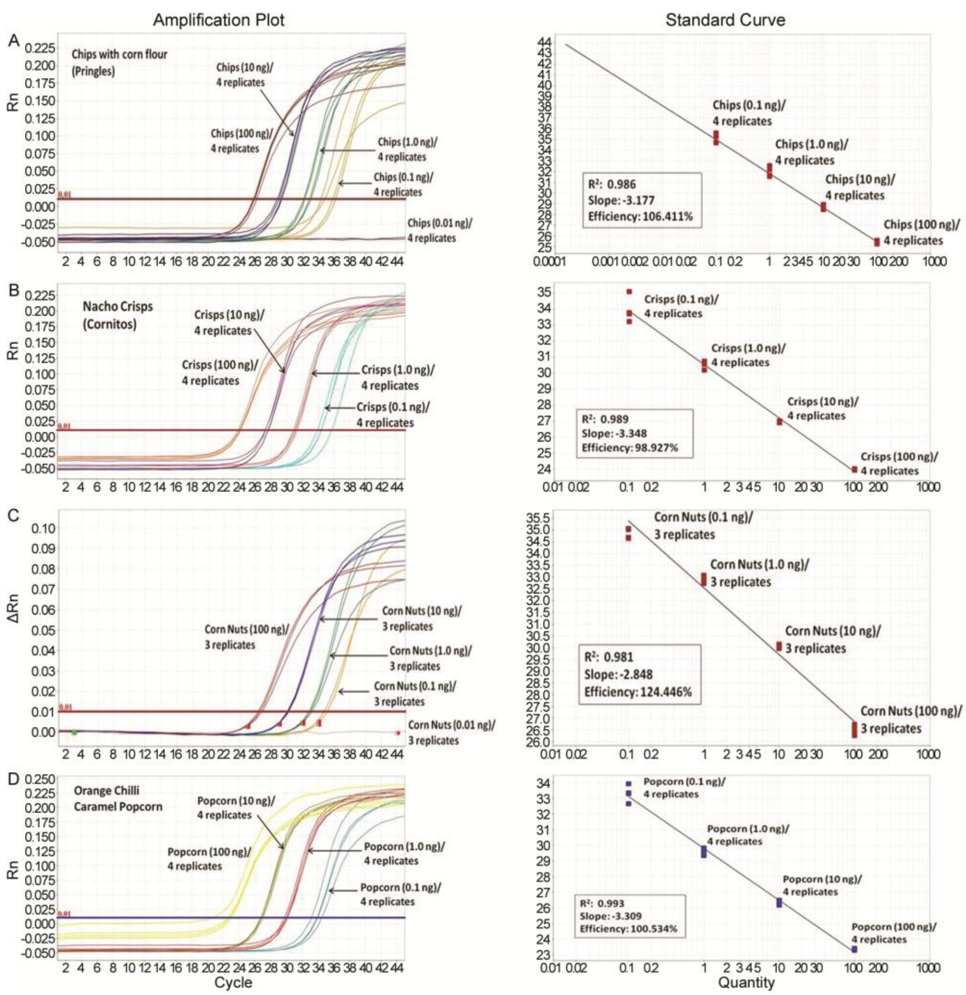

Fig. 1 — Real-time PCR amplification profiles and respective standard curves generated on inhibition test targeting *Adh1* endogenous gene to check the inhibition in selected samples. (A) Chips with corn flour, (B) Nacho Crisps, (C) Corn Nuts, (D) Orange Chilli Caramel Popcorn

performed using conventional PCR. The primers used for PCR analysis were also in compliance with the ISO 21569:2005 standard protocol[20], with limit of detection up to 0.1%. This initial screening using conventional PCR could cut down the cost of GMO testing. Each sample of maize food product was analyzed in two replications, and appropriate positive and negative controls were run during each test as internal controls. On PCR-based screening tests targeting 195 bp region of *P-35S* promoter sequence and 180 bp region of *T-nos* terminator, amplification products of expected size were detected in respective positive controls only, whereas no amplification was detected in any of the test samples and the negative controls (Suppl. Fig. S3 & S4).

Real-time PCR-based GMO testing of food products

Additionally, gene-specific real-time PCR tests targeting *ctp2-cp4epsps* and *cry1Ab/Ac* were conducted where none of the samples were found positive for these specific targets (Fig. 2, Suppl. Fig. S5). For *ctp2-cp4epsps*, no amplification was detected in any of the test samples of food products and negative controls including non-GM maize and non-template control. However, the positive fluorescent signal as amplification curve was detected in the positive control, *i,e*, replicates of GM maize event NK603 carrying *ctp2-cp4epsps* trangenic sequence with mean Ct value of 29.76 in the run for 1-25 samples and Ct of 30.42 in the run for 26-50 samples (Fig. 2). Further, specificity of the products was verified with respective meting curve. Similary, for *cry1Ab/Ac*, the florescent signal as amplification and melting curves was detected in positive control only whereas no amplification was observed in any of the test samples and negative controls (Suppl. Fig. S5).

GM detection is a stepwise approach that includes DNA extraction followed by required quality checks and GMO testing. GMO testing involves both the qualitative as well we quantitative analysis as per the requirement. Qualitative GMO tests can be perfomed using end-point PCR and real-time PCR, which may include the screening tests targeting regulatory sequences or marker genes/ transgenes or the event-specific tests. GMO screening tests are preferred for initial screening in order to reduce the time and cost of GMO testing by simultaneously covering the screening for presence or absence of a range of GM events, The present study covers the qualitative analysis for the GM status of 50 samples of packaged food products with maize as an ingredient, procured from the marketplace in India. The end-point PCR tests targeting two common regulatory elements, *i.e*., *P-35S* promoter and *T-nos* terminator, followed by real-time PCR tests targeting specific transgenic sequences, *i.e*., *cry1Ab/Ac* and *ctp2-cp4epsps*m were employed herein.

For the isolation of DNA from food products, we have selected column-based method using DNeasy® Mericon Food Kit protocol with slight modifications[7]. The DNA samples were firstly amplified using PCR and real-time PCR assays for maize-specific *Adh1* endogenous gene to ensure that these samples were suitable to be used as template DNA for further GMO

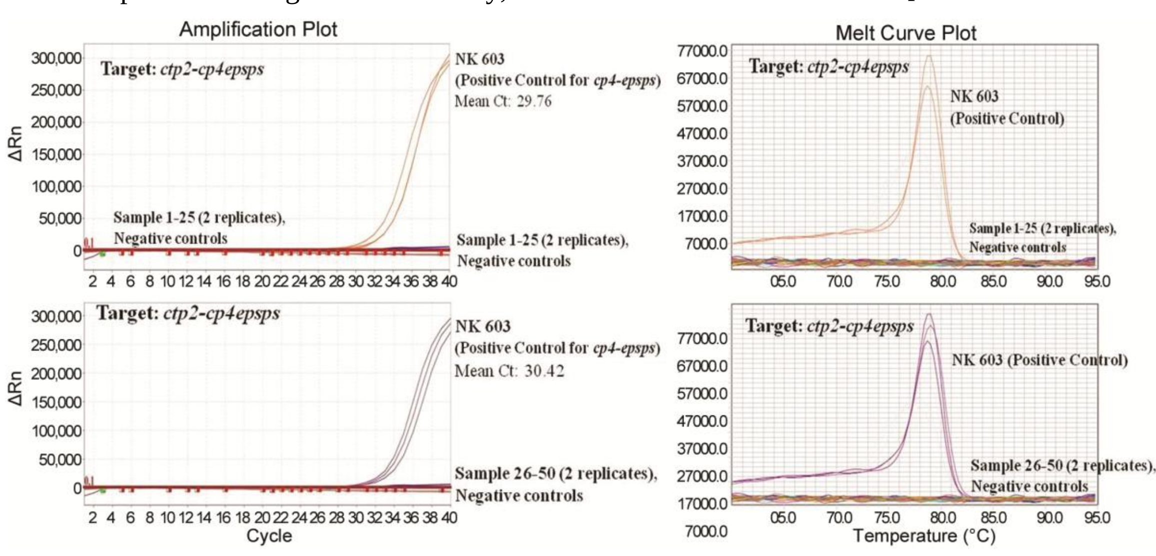

Fig. 2 — Real-time PCR amplification and melting curve profiles for checking presence or absence of *ctp2-cp4epsps* gene in fifty samples of food products in two replicates of each

analysis. The results confirmed the amplification of desired product of *Adh1* gene in all the samples. These assays were included to reduce the chances of false negatives as well as to ensure the quality of DNA. Similar results for confirming the amplifiability have been reported for food and feed products of maize, soybean, and rice using assays for respective endogenous genes, namely, zein, lectin and sucrose phospahe synthase[11].

Once quality check was completed, the DNA samples were subjected to GMO analysis using PCR targeting regulatory transgenic elements, *P35S* and *T-nos*. The results showed that none of the samples were detected positive for these two screening targets. The findings of GM detection in maize were in accordance with the study from Singapore where none of the tested samples of food products of maize were found positive for uapproved GM events based on the GM targets screened[10]. Such reports could be useful to the regulatory bodies and consumers by giving quality assurance to the food products in the market in terms of non-GM in the countries where these products are not commercialized. However, in few of the reports, unauthorized presence of GM maize in food or feed products was detected. A set of 119 maize products were analyzed where 4% of these food products contained more than the GMO threshold of 0.9% GM maize[9].

Further more sensitive real-time PCR tests were performed targeting specific transgenic sequences, namely, *cry1Ab/Ac* and *cp4-epsps*. More than 70% of the globally approved GM events of maize can be efficiently screened by checking these two transgenic sequences[3]. These tests could screen the GMOs with more specificity. Based on the real-time PCR tests conducted, none of the samples were found GM positive for these targets. The results were confirmed by the presence of amplification only in the positive control. In this case, as the samples were not detected positive for the GM targets screened so further GMO quantification was not performed. In another report, among the 11 GM events tested, six GM events of maize (Bt11, DAS59122, GA21, MON810, NK603, TC1507) were detected in the samples, which were then quantified where high levels of GMOs were detected[8]. The unauthorized presence of GMOs in the marketplace needs to be monitored, particularly for the imported samples and stringent regulatory policies and GMO labeling system need to be in place with its effective implementation.

A systematic study was reported herein comprising of screening and gene-specific tests for qualitative checking of GM ingredients in 50 samples of maize containing food products from the marketplace. The unauthorized occurrence of GMOs was not detected in these samples, which ensured that the regulatory system is in place. In India, there is stringent regulatory mechanism for the GMOs. Furthermore, the FSSAI Draft regulations, if implemented in the country, would provide a way of informed choice to the consumers.

Conclusion

In conclusion, fifty samples of maize containing food products purchased from marketplace in Delhi or nearby areas were tested for the presence or absence of transgenic elements commonly present in >90% of globally approved GM maize events. Based on the tests conducted for *P-35S* promoter, *T-nos* terminator, *cry1Ab/Ac and ctp2-cp4epsps* transgenic regions, none of the samples were detected positive for these GM targets, which could give an indication that unauthorized GMOs are not present in the marketplace. Such studies, including a recent report on rice[21], need to be undertaken routinely for the suspected packages or consignments to track the unauthorized entry of GMOs as GM food products are regulated in the country.

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Conflict of interest

The authors declare no competing interests.

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