UDC 575.630
https://doi.org/10.2298/GENSR1903895S
Original scientific paper

DEVELOPMENT OF AN EFFICIENT ELEMENT-SPECIFIC PCR METHOD FOR THE DETECTION OF GENETICALLY MODIFIED RICE, SOY & CORN

Neda SEPAHIAN[1], Zahra NOORMOHAMMADI[1]*, Masoud SHEIDAI[2], Hamid-Reza ZAMANIZADEH[3]

[1] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Faculty of Biological Sciences and Technology, Shahid Beheshti University, Tehran, Iran
[3]Department of Plant Pathology, Agriculture and Natural Resources Faculty, Science and Research Branch, Islamic Azad University, Tehran, Iran

Sepahian N., Z. Noormohammadi, M. Sheidai, H.-R. Zamanizadeh (2019): *Development of an efficient element-specific PCR method for the detection of genetically modified rice, soy & corn*.- Genetika, Vol 51, No.3, 895-905.

With the ever-increasing number of GM crops introduced to the market, it is required to respect customers' freedom of choice and their perception toward GMOs. According to obligatory demand by food and drug administration for GM testing on raw and processed materials, efficient and robust analytical approaches are needed for screening and identification of GM crops. This article describes the development and applicability of a PCR-based GMO testing of the most frequent constructs used in transgenic soy, corn and rice varieties which include CaMV35S promoter and the NOS terminator and also plant endogenous sequences Lectin, Zein, SPS as internal controls. A total of 2866 different sample inputs collected during the year 2015 to 2017 including GM and non-GM materials have been tested. Results obtained from qualitative PCR test on rice, maize and soybean-containing food samples showed that in total, 1.59% of maize samples and

Corresponding author: Zahra Noormohammadi, Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran, E-Mail: marjannm@yahoo.com, z-nouri@srbiau.ac.ir

7.53% of soya samples were GM positive and no GM positive rice sample was detected. The presented method showed high specificity and sensitivity offering an absolute limit of detection of 0.25% GM construct in the samples and sufficient reproducibility of the method was proved. This method fits the purpose of GMO testing laboratories due to its convenience and robustness.

Keywords: CaMV, Genetically modified organisms, 35S Promoter, T-NOS

INTRODUCTION

With the advent of various genetic engineering techniques and adoption of GM technology since the first GMO approval in 1996, the total acres of the land area dedicated to cultivation of the genetically modified organisms (GMOs) and the number of GMOs introduced to the market have been growing exponentially (ROSA *et al.*, 2016). In parallel, GMO detection methods have been upgraded enormously in order to meet the consumers' expectations and to respect their freedom of choice and their perception toward GMOs and to overcome the need for traceability, screening, and labeling. This in turn has led to the establishment of regulatory frames (ŽEL *et al.*, 2012). Another side, these newly engineered crops, and organisms may have novel and sometimes unique characteristics which either cannot be found in nature or may take a very long time to be evolved during natural selection (STEIN and RODRIGUEZ-CEREZO, 2009). On the other hand, new challenges are being imposed to develop and optimize more efficient detection methodologies for the analysis of the diverse and growing number of GM events (ŽEL *et al.*, 2012).

Several methodologies have been introduced for detection of GM crops which can be divided into two main categories: 1- DNA-based methods, and 2- protein-based detection methods.

In protein-based methods, the immunoassays are employed for detection of the recombinant protein that is expressed in GMOs. In DNA-based methods which are very sensitive, the detection target is the recombinant DNA itself (ŽEL *et al.*, 2012; AHMED, 2004).

Protein-based methods have a lower price compared to DNA-based methods, and can be used as rapid tests in the field for examining the raw material. However, these methods may suffer from some limitations, like, the expression of recombinant proteins may vary greatly in various tissues, or transgenic proteins may not be produced due to gene silencing, etc. (ŽEL *et al.*,2012; AHMED, 2004) .

The regulatory frameworks implemented in many countries around the world and also in Iran, are generally based on very sensitive methods and precise determination of the GMOs' content, which in turn guarantees the efficient traceability of GMOs in the raw and processed materials used as food and feed.

In DNA-based methods, molecular laboratory protocols are the proper choice for the screening and quantification of the GM constructs (FRAITURE *et al.*, 2016).

Since the introduction of the labelling rules of GM products, different analytical methodologies have been used to identify the GMOs. The most accurate and commonly used detection methods implemented in different laboratories are based on the amplification of the specific DNA targets by using the PCR technique (MIRAGLIA, 2004; RODRÍGUEZ-LÁZARO, 2007). Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and Nopaline Synthase Terminator (T-NOS) are the most widely used transgenic elements in the usual GM events (RUTTINK *et al.*, 2010).

Various approaches have been used to detect transgeneic elements, for example, Eagster and colleagues (EUGSTER, 2014) used a tetraplex real-time PCR screening assay targeting P-35S and T-NOS regulatory genes in maize, soy, rapeseed and tomato samples. They also tested Figwort Mosaic Virus promoter (P-FMV) and T-35S elements. Similarly, Fu *et al.* 2015, employed digital PCR technology in nine different food and feed input samples to check the presence of the CaMV35S promoter and the NOS terminator in different events. WU *et al.* 2014, used Taqman probes to detect the P-35S promoter in a qualitative PCR assay in 23 unique GMO events with high specificity and sensitivity, while BARBAU-PIEDNOIR *et al.* (2010), employed SYBR Green q PCR screening methods with CaMV35S promoter and NOS terminator as GM targets in their assays. Moreover, validated screening methods based on PCR have been published by Joint Research Center, European Union reference laboratory for GM food and feed database (http://gmo-crl.jrc.ec.europa.eu/gmomethods).

In this paper, we present an efficient PCR-based technique for screening of P-35S and T-NOS in different raw and processed food and feed samples. The limit of detection of the employed technique has been evaluated and is in accordance with GM test analytical requirements.

The reason for selection of rice samples to be checked for GMOs is that this crop constitutes the dominant part of Iranian people's food. Besides, soy and corn samples were selected because these crops are among the four main GM crops in the world. The larger number of rice samples for GM evaluation compared to soya and maize samples was due to this crop's strategic place in Iranian people food chain and its economic value for the country.

MATERIALS AND METHODS

Samples

Certified reference materials (CRM) of GM [ERM-BF410a (Roundup Ready Soybean), ERM-BF417d MON863 x MON 810 Maize, ERM-BF420c Maize, ERM-BF419b Sugar beet and 0306-I8 LLRice62 were obtained from the American Oil Chemists' Society (AOCS, Urbana, IL, USA) and the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). Sixty-three soybean-containing food samples consist of soy protein isolate, chocolate, soybean, Lecithin, soy milk, or concentrate, oil, fiber were used. Also two-thousand-seven-hundred-and-ten rice samples as well as ninety-three maize containing food and feed samples (corn seeds, powder, corn oil, frozen and dried samples) collected. All samples were received from Food and drug administration, different companies, or taken from domestic markets during three years (2015 – 2017).

DNA isolation

The studied samples' genetic material was extracted using the QIAamp DNeasy Mini Kit (QIAGEN, Germany) that works based on silica gel membrane technology which allows an efficient recovery of complete DNA from plant tissues and processed food samples.

Target selection

We used two different classes of targets to accurately detect and identify the GM material in different samples: 1) Plant endogenous sequences were used specifically to detect soya, maize, and rice. This not only provides information on the host species of the transgenic material but also implies possible plant cross-contamination in the case of existence in the raw

material. Besides, it is useful to check the probable amplification inhibition during PCR reaction. 2) Cauliflower Mosaic Virus 35S promoter (CaMV P-35S), and Nopaline Synthase Terminator of *Agrobacterium tumefaciens* (T-NOS), were used as key indicators of GM material existence, as these elements are frequently used in GM production.

All the primers used in this study are listed in Table 1. The primer pair SPS-F and SPS-R, as well as the primer pair 35S-1 and 35S-2 were designed by Oligo7 Primer Analysis Software (Molecular Biology Insights, Inc. Colorado Springs, CO, USA). These were then used as rice species internal control and for CaMV P-35S amplification, respectively.

The primer pairs Lectin-1 and Lectin-2, NOS-F and NOS-R, Zein-1 and Zein-2 were selected from ISO 21569, Amendment 1, 2013-04-01. All oligonucleotides were ordered to Metabions, Germany as customized assays.

Table 1. Primers for 35S Promoter, T-NOS and species internal control amplification used in this study

Primer	Sequence	Product size	Reference	Targeted sequence
SPS-F	5'-ATCATTCGTGCTCTCAAGG-3'	204 bp	This study	Sucrose phosphate synthase gene-Endogenous control gene in rice
SPS-R	5'-GGTAAGTTCGTGGTAGTGG-3'		This study	Sucrose phosphate synthase gene-Endogenous control gene in rice
35S-1	5'-TCATTGCGATAAAGGAAAGG-3'	189 bp	This study	pCaMV35s element
35S-2	5'-TCTTGCGAAGGATAGTGG-3'		This study	pCaMV35s element
Lectin-1	5'-CCTATAATGCCGCCACGAG-3	Size:216 bp	ISO 21569, Amendment 1, 2013-04-01	Lectin (Le1) gene-Endogenous control gene in soybean
Lectin-2	5'-TGCTGCTACCATCTGACAAC-3'		ISO 21569, Amendment 1, 2013-04-01	Lectin (Le1) gene-Endogenous control gene in soybean
NOS-F	5'-GAATCCTGTTGCCGGTCTTG-3'	177 bp	ISO 21569, Amendment 1, 2013-04-01	T-NOS element
NOS-R	5'-TAATCCTAGTTTGCGCGCTA-3'		ISO 21569, Amendment 1, 2013-04-01	T-NOS element
Zein-1	5'-TGCTTGCATTGTTCGCTCTCCTAG-3'	329 bp	ISO 21569, Amendment 1, 2013-04-01	Delta Zein structural10 gene-Endogenous control gene in corn
Zein-2	5'-GTCGCAGTGACATTGTGGCAT-3'		ISO 21569, Amendment 1, 2013-04-01	Delta Zein structural10 gene-Endogenous control gene in corn

PCR amplification

All PCR amplifications were performed using an ABI Simpliamp System (Life Technologies, USA). Each amplification reaction contained 1X reaction buffer, 0.2-0.4 mM of each primer; 0.5-1 U *Taq* DNA polymerase (Sinaclon), 1.5 mM MgCl2, 0.8 mM of each dATP, dCTP, dGTP, and dTTP (Sinaclon, Iran) for soybean-specific amplifications. The same reaction was carried out for maize and rice-specific amplifications except dNTPs (0.4 mM and 0.2 mM respectfully). PCR thermal programms were as follows: Denaturation step for 3 min at 95ºC; 35 cycles (30 cycles for rice) of 30 sec denaturation step at 95ºC, 30 sec an annealing step at 55ºC, and 60-120 sec (Rice 30 s) at 72ºC; and a final extension of 3-5 min at 72ºC.

Amplified DNA fragments were electrophoresed on 2% agarose gels containing safe dyes and 1X TAE buffer was used for this purpose, and bands then were visualized by UV trans-illumination system.

To assess the limit of detection of the method, Since the LOD of an assay is the amount of the analyte which can be detected by the analytical method at least 95% of the time, at least 20 replicates of transgenic samples were tested for each target in at least three different runs done in 3 different days by different operators.

RESULTS

The endogenous sequences were successfully amplified in GM plant samples studied (Table 2. This indicates the specificity of the assay, as the GM samples contained 1, 2, and 5% of GM content (ERM-BF410a (Roundup Ready Soybean), along with some animal DNA (caw, pig, horse, mouse, sheep, goat and chicken and turkey). All samples did not produce any amplicon based on animal DNA.

Table 2. Tested samples for GMOs through 2015-2017.

Year /Sample	Rice	Soya	Maize
Year 2015	1183	39	30
Year 2016	1072	12	9
Year 2017	455	12	54
Total	2710	63	93
GM No./Percentage	0 (0%)	1 (1.59%)	7 (7.53%)

All the samples which contained 5% to 0.0625% of GM content produced visible bands after amplification and horizontal gel electrophoresis (Figure 1). These results indicated the sensitivity of the assay used.

It was estimated that the assay has the capability of detection of GM material (LOD of 0.25 %), which is known to meet the purpose of the detection of very low levels of residual transgenic plant DNA even in highly processed food products, such as protein isolate, chocolate, Lecithin, soy concentrate, oil and starch (LAUBE *et al.*, 2010).

We used a negative control, a non-template control (distilled water) and a positive control in each run and in all the assays. The P-35S, T-NOS, and the endogenous sequences were successfully amplified in the positive control, with no amplification in GM targets in negative

control and no signal was obtained with NTC. These results show that the presented method is quite reliable.

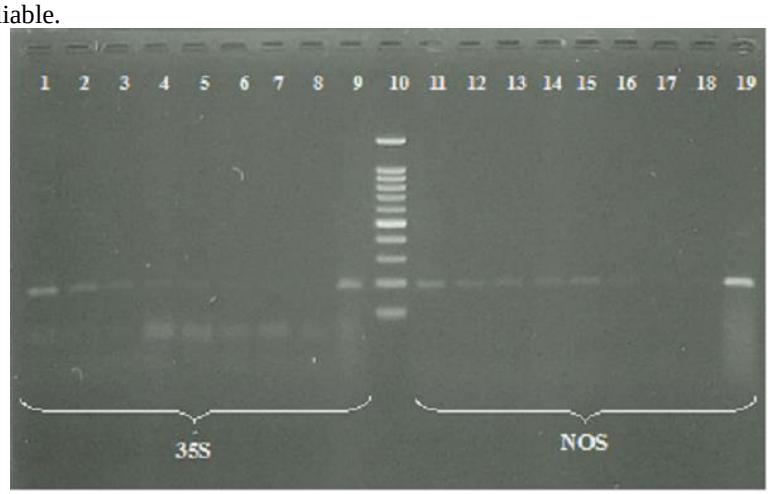

Fig. 1. Sample serial dilution for limit of detection determination. Lanes 1 to 9: 35S Amplicons; L1: 5%, L2: 2%, L3: 1%, L4: 0.5%, L5: 0.25%, L6: 0.125%, L7: 0.0625%, L8: NTC, L9: Positive control (10%) ERM-BF410a (Roundup Ready Soybean), L10: 100 bp DNA Size marker, Lanes 11 to 20: NOS Amplicons; L11: 5%, L12: 2%, L13: 1%, L14: 0.5%, L15: 0.25%, L16: 0.125%, L17: 0.0625%, L18: NTC, L19: Positive control (10%).

GM target and indigenous gene detection

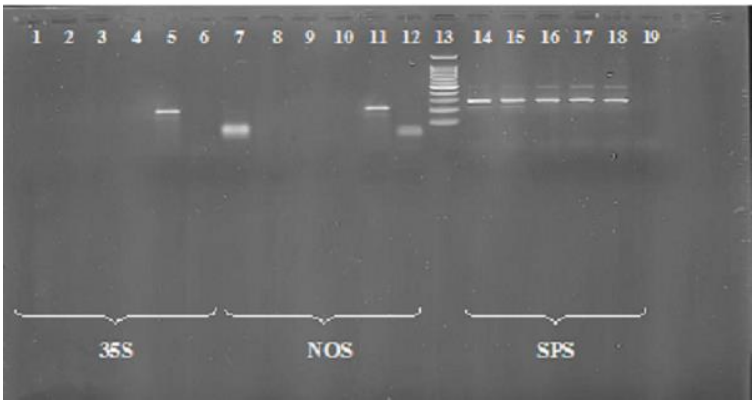

Fig.2. Rice samples checked with Primer pairs targeting 35S, NOS, SPS. Lanes 1 to 6; 35S Amplicons; L1 through L4: Rice samples, L5: Positive control 0306-I8 LLRice62 (10%), L6: NTC, Lanes 7 to 12: NOS Amplicons; L7 through L10: Rice samples, L11: Positive control (10%), L12: NTC, L13: 100 bp DNA Size marker, Lanes 14 to 19; SPS Amplicons; Lanes 14 to 17: Rice samples, L18: Positive control (10%), L19: NTC.

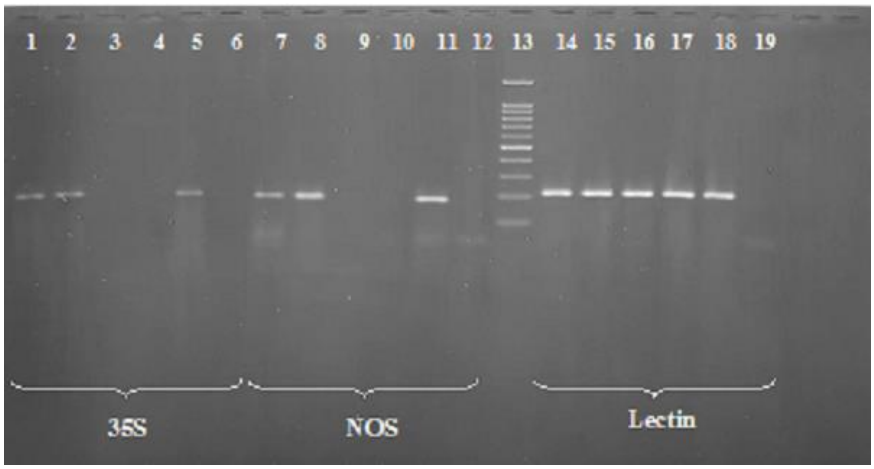

Fig.3. Soya samples checked with Primer pairs targeting 35S, NOS, SPS. Lanes 1 to 6; 35S Amplicons; L1 through L4: Soya samples, L5: Positive control ERM-BF410a (Roundup Ready Soybean) (10%), L6: NTC, Lanes 7 to 12 NOS Amplicons; L7 through L10: Soya samples, L11: Positive control (10%), L12: NTC, L13: 100 bp DNA Size marker, Lanes 14 to 19; Lectin Amplicons; Lanes 14 to 17: Soya samples, L18: Positive control (10%), L19: NTC.

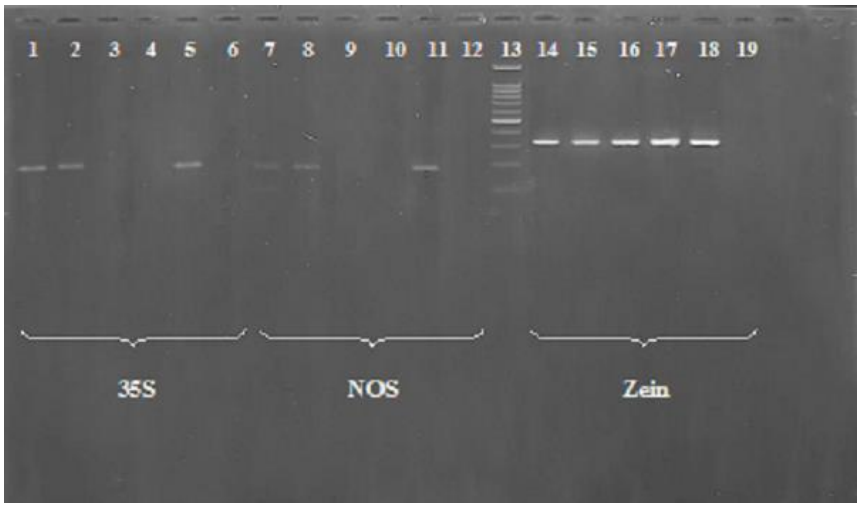

Fig.4. Maize samples checked with Primer pairs targeting 35S, NOS, SPS. Lanes 1 to 6; 35S Amplicons; L1 through L4: Maize samples, L5: Positive control ERM-BF420c Maize (10%), L6: NTC, Lanes 7 to 12 NOS Amplicons; L7 through L10: Maize samples, L11: Positive control (10%), L12: NTC, L13: 100 bp DNA Size marker, Lanes 14 to 19; Zein Amplicons; Lanes 14 to 17: Maize samples, L18: Positive control (10%), L19: NTC.

The extracted DNA samples with good quality and purity were subjected to PCR amplification to identify Plant endogenous sequences as well as cauliflower mosaic virus 35S promoter (pCaMV 35S) and *Agrobacterium tumefaciens* Nopaline Synthase Terminator (T-NOS) specific targets. The agarose gel electrophoresis of the PCR products showed the anticipated bands of 189 bp and 199 bp for the introduced genes of the 35S promoter and NOS terminator, respectively. Besides, fragments of SPS, Lectin, and Zein genes were also amplified to prove the presence of rice, soya and maize in the samples. Product sizes of each of these indigenous genes are 277 bp, 216 bp, 329 bp respectively (Figures 2-4). The results of gel electrophoresis for DNA templates isolated from the samples tested with CaMV35s and NOS primer pairs are shown in figure 1. The results obtained from qualitative PCR test on two-thousand-seven-hundred-and-ten rice samples, Sixty-three soybean-containing food samples and Ninety-three Maize containing food and feed samples containing SPS, Lectin, Zein genes, respectively showed that in total, 1.59% of Maize samples and 7.53% of Soya samples were GM positive and no GM positive rice sample was detected.

DISCUSSION

Although recombinant DNA can be detected in all of the cells of a GM crop grown from a GM seed, the recombinant proteins expression can vary largely in various tissues and even transgenic proteins may not be produced due to some mechanisms such as gene silencing. In second method, some immunoassay based GM tests is applicability restricted to raw materials and beyond that, only those plant tissues that sufficient GM proteins are expressed within them (VAN DEN EEDE *et al.* 2007).

Furthermore, protein-based approaches for GM detection are limited by some chemical and thermodynamic properties of the proteins such as thermostability and expressivity, epistatic gene interactions, and gene silencing, etc. (VAN DEN EEDE *et al.* 2007). Besides, the sensitivity of protein-based methods is generally lower than DNA-based methods (AHMED 2004).

The presented protocol used in this study is a fast, inexpensive and easy to perform and can be employed with minimal facilities like a thermal cycler. It can be adopted with automated DNA extraction and presence of various GM events can be screened simultaneously within one working day.

Since 2016, thirty-three percent of all maize and 78 percent of soy cultivated worldwide is GM (ISAAA Briefs, 2016), so there is a significant probability that the tests' results come positive especially in the case of soybean and the low percentage of GM soybean and maize samples can be related to the restricted number of samples sent for our laboratory or precision of choice of the government and the importers for choosing which country or provider to choose to import non-GM crops since the labeling was not obligatory for food products consist of GMOs at the time of wring this paper (http://bch.cbd.int/database).

The tested targets for GMO detection used in the developed PCR method were chosen due to their frequency of presence in the production of transgenic crops. The obtained detection limits of our developed PCR assay is 0.25% of GM element in the sample which is fulfilling to meet the legal requirements of labeling threshold (2%) in Iran (http://bch.cbd.int/database).

It should be mentioned that even when using a very complex matrix like processed food, Lecithin, and oil as starting materials, the developed DNA extraction system revealed reliable results. The sensitivity of the developed method is high enough for traceability of GM elements in food and feed samples (GHAZIZADEH *et al*., 2014).

It should be borne in mind that although the presented PCR-based method can be used for the screening of widely used targets in GM events in different samples, in those cases which quantification of the GM content is required, other applicable approaches such as Real-Time PCR, Digital PCR, etc can be employed.

As a conclusion, the optimized PCR-based method described in this article offers an efficient, easy to use and cost-efficient procedure in the screening of common GMOs produced worldwide. The described method allows the detection of three routine types of samples to obtain their content. This method fits the purpose of being used as a routine testing procedure in GMO laboratories due to its convenience and robustness, high sensitivity and specificity and it complies with the legal and analytical requirements described by different authorities.

ACKNOWLEDGMENTS

We would like to thank Parsian Biotech Lab's general manager Mr. Hessam Shabani and Parsian Biotech laboratory personnel Ms. Ziba Bakhtiar for their collaboration. We also appreciate Mrs. Mahdizadeh, Mr. Mousavi, GMO Reference laboratory of Food and Drug Administration of The Islamic Republic of Iran, for their valuable contribution.

Received, September 12th, 2018
Accepted August 18th, 2019

REFERENCES

AHMED, F.E. (2004): Testing of Genetically Modified Organisms in Foods, © 2004 by Food Products Press®, an imprint of The Haworth Press, Inc., 10 Alice Street, Binghamton, NY 13904-1580.

BARBAU-PIEDNOIR, E., A., LIEVENS, G., MBONGOLO-MBELLA, N., ROOSENS, M., SNEYERS, A., LEUNDA-CASI, M., VAN DEN BULCKE (2010): SYBR_Green qPCR screening methods for the presence of ''35S promoter'' and ''NOS terminator'' elements in food and feed products. Eur. Food Res Technol., *230*: 383–393.

EUGSTER, A., P., MURMANN, A., KAENZIG, A., BREITENMOSER (2014): Development and Validation of a P-35S, T-nos, T-35S and P-FMV Tetraplex Realtime PCR Screening Method to Detect Regulatory Genes of Genetically Modified Organisms in Food. Chimia., *68*: 701–704.

FU, W., P., ZHU, C.H., WANG, K., HUANG, Z.H., DU, W., TIAN, Q., WANG, H., WANG, W., XU, S.H., ZHU (2015): A highly sensitive and specific method for the screening detection of genetically modified organisms based on digital PCR without pretreatment. Sci Rep., *5*: 12715.

FRAITURE, M.A., N.H.C., ROOSENS, I., TAVERNIERS, M.D., E LOOSE, D., DEFORCE, P.H., HERMAN (2016): Biotech rice: Current developments and future detection challenges in food and feed chain. Trends Food Sci Technol., *52*:66-79.

GHAZIZADEH E., A., MOUSAVI, F., HADI (2014): A quantitative competitor PCR assay to detect genetically modified roundup-ready soybeans in commercially sold foods in Iran. P Bio Sci., 4(2): 2014/235-243.

ISAAA Briefs, Global Status of Commercialized Biotech/GM Crops: (2016) brief 52.

EU Database of Reference Methods for GMO Analysis, Joint Research Center, European Union Reference Laboratory for GM Food and Feed database, http://gmo-crl.jrc.ec.europa.eu/gmomethods.

LAUBE, I., H., HIRD, P., BRODMANN, S., ULLMANN, M., SCHÖNE-MICHLING J., CHISHOLM, H., BROLL (2010): Development of primer and probe sets for the detection of plant species in honey. Food Chem., *118*: 979–986.

MIRAGLIA, M., K.G., BERDAL, C., BRERA, P., CORBISIER, A., HOLST-JENSEN, E.J., KOK, J., ZAGON (2004): Detection and traceability of genetically modified organisms in the food production chain. Food Chem Toxicol., *42*: 1157–1180.

RODRÍGUEZ-LÁZARO, D., N., COOK, M., HERNÁNDEZ (2007): Real-time PCR in Food Science: PCR diagnostics. Curr Issues Intest Microbiol., *15*: 39-44.

RUTTINK, T., R., DEMEYER, E., VAN GULCK, B., VAN DROOGENBROECK, M., QUERCI, I., TAVERNIERS, M., DE LOOSE (2010): Molecular toolbox for the identification of unknown genetically modified organisms. Analytical and Bioanalytical Chemistry., *396*: 2073–2089.

ROSA, S.F., F., GATTO, A., ANGERS-LOUSTAU, M., PETRILLO, J., KREYSA, M., QUERCI (2016): Development and applicability of a ready-to-use PCR system for GMO Screening. Food Chemistry, *201*: 110–119.

STEIN, A.J., E., RODRIGUEZ-CEREZO (2009): The global pipeline of new GM crops: Implications of asynchronous approval for international trade. Luxembourg: Office for Official Publications of the European Communities; Report No.: EUR 23486 EN.

VAN DEN EEDE, G., A., DE SCHRIJVER, D., DE BERNARDI, M., DEVOS, G., YANN, L., MBONGOMBELLA, W., AMAYA, M., MOENS (2007): Sneyers. Detection of genetically modified plant products by protein strip testing: An evaluation of real-life samples. Eur Food Res Technol., *225*:49-57.

WU, Y., Y., WANG, J., LI, W., LI, L., ZHANG, Y., LI, X., LI, J., LI, L., ZHU, G., WU (2014): Development of a general method for detection and quantification of the P35S promoter based on assessment of existing methods. Sci Rep., *4*: 7358.

ŽEL, J., M., MILAVEC, D., MORISSET, D., PLAN, G., K., VAN DEN EEDE (2012): Gruden. How to Reliably Test for GMOs. Springer Briefs in Food, Health, and Nutrition, *1*:100-109.

RAZVIJANJE EFIKASNE ELEMENT-SPECIFIČNE PCR METODE ZA DETEKCIJU GENETIČKI MODIFIKOVANOG PIRINČA, SOJE I KUKURUZA

Neda SEPAHIAN[1], Zahra NOORMOHAMMADI[1]*, Masoud SHEIDAI[2], Hamid-Reza ZAMANIZADEH[3]

[1] Departman za biologiju, Ogranak za nauku i istraživanje, Islamski Azad Univerzitet, Teheran, Iran

[2]Fakultet za biološke nauke i tehnologiju, Shahid Beheshti Univerzitet, Teheran, Iran

[3]Departman za biljnu patologiju, Fakultet za poljoprivredu i prirodne resurse, Ogranak za nauku i istraživanje, Islamski Azad Univerzitet, Teheran, Iran

Izvod

Bez obzira na sve veći broj GM useva koji su predstavljeni na tržištu, zahteva se da se poštuje sloboda izbora kupaca i njihova percepcija prema GMO-u. Prema obaveznim zahtevima uprave za hranu i lekove za GM ispitivanje sirovih i prerađenih materijala, potrebni su efikasni i robusni analitički pristupi za skrining i identifikaciju GM useva. Ovaj rad opisuje razvoj i primenjivanje PCR-a zasnovanog na GMO ispitivanju najčešćih konstrakata korišćenih u transgenim sortama soje, kukuruza i pirinča koje uključuju CaMV35S promotor i NOS terminator i takođe biljne endogene sekvence Lektin, Zein, SPS kao unutrašnje kontrole. Ukupno je testirano 2866 različitih uzoraka prikupljenih tokom 2015. do 2017. godine, uključujući GM i ne-GM materijale. Rezultati dobijeni kvalitativnim PCR testom na uzorcima hrane od pirinča, kukuruza i soje pokazali su da je ukupno, 1,59% uzoraka kukuruza i 7,53% uzoraka soje bilo GM pozitivno i da nije utvrđen GM pozitivan uzorak pirinča. Predstavljeni metod je pokazao visokuspecifičnost i osetljivost nudeći apsolutnu granicu detekcije 0,25% GM konstrukta u uzorcima i dokazana jje dovoljna ponovljivost.

Primljeno 12.IX 2018.

Odobreno 18. VIII 2019.