J. Agr. Sci. Tech. (2021) Vol. 23(2): 437-446

Efficiency of End-Point PCR Based Detection of Genetically Modified Organisms (GMOs) in Food and Feed

A. Ahatovic[1*], E. Al-Momani[2], K. Bajrovic[1], and A. Durmic-Pasic[1]

ABSTRACT

Since the commercialization of transgenic crops in 1996, the biotech crop planted area has continuously increased. The European consumers have particularly been sceptical about transgenes in food products and EU (European Union) has enacted very complex legislation. The area of food analytics requires continuous development and improvement of detection methods to track the legislative framework and respond to consumers requirements. In the last decade, real-time PCR (polymerase chain reaction) based methods have been the methods of choice for numerous laboratories, but for various reasons, end-point PCR based methods have still been used. In our research, 73 samples of food and feed were analysed for the presence of common elements of transgene construct – Cauliflower Mosaic Virus 35S promoter (P-35S) and *Agrobacterium tumefaciens* Nopaline Synthase Terminator (T-NOS), using end-point PCR based methods. These samples had been previously tested for the presence of the same elements using validated real-time PCR based methods. Comparison of the used methods sensitivity showed that real-time PCR based methods have undeniable advantage. More important factor is specificity, and the fact that the list of approved Genetically Modified Organisms (GMOs) is constantly increasing necessitates updating of validation methods procedures. Considering upward trend of approved GMOs, it is important to pay more attention to the improvement and specialization of GMO detection methods.

Keywords: GMO detection methods, GMO screening, Real-time PCR, Transgenic crops.

INTRODUCTION

In the last two decades, we have witnessed massive development of plant biotechnology based on genetic engineering. International Service for the Acquisition of Agri-biotech Applications (ISAAA) reports more than 191.7 million biotech crop acreage planted in 26 countries in 2018. However, GMOs have been subject of public debate since the very beginning of their commercialization and vast majority of consumers, especially those in the European Union (EU), maintain sceptical attitude about cultivation and consuming GM food. In order to protect consumers' rights as well as protect their health and the environment, EU has enacted complex legislation that prescribes the authorization procedure and labelling requirements for the authorized GM events.

In countries with regulated GMO, implementation of legal framework requires an efficient system of monitoring the market where GMO testing laboratories play the decisive role. The ability of laboratories to reliably detect GMO and, where applicable, identify and quantify GM event in food and feed is essential (Žel *et al.*, 2012). European Union Reference Laboratory for GM Food and Feed (EURL-GMFF) and European Network of GMO Laboratories (ENGL) have headed an international effort to establish a portfolio of validated and

[1] University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina.
[2] University of Sarajevo, Faculty of Science, Zmaja od Bosne 33-35, Sarajevo, Bosnia and Herzegovina.
[*]Corresponding author; e-mail: anesa.ahatovic@ingeb.unsa.ba

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

harmonized methods for detection, identification, and quantification of GMO in food and feed. Those methods are mainly based on real-time PCR and are made publicly available (EURL-GMFF and ENGL 2010; Bonfini *et al.,* 2012). However, given the number of GM events authorized worldwide circulating in the food chain, in many countries the detection methods are the most relevant. According to EU register of authorized GMOs, at this moment, there are 33 authorized single events of soybean and maize only, with ever-increasing number of stacked events. The numbers are even greater when asynchronous authorizations and unauthorized events are taken into account. Laboratories worldwide make organized efforts to navigate this complex field by developing and validating multi-target systems. Most of the approaches are based on PCR Capillary Gel Electrophoresis (PCR-CGE), microarray and Luminex, and prespotted plates based on real-time PCR (Querci *et al.*, 2009; Rosa *et al.*, 2013; Fraiture *et al.*, 2015; Gatto *et al.*, 2015).

Real-time PCR has become the technique of choice for GMO screening/detection methods due to its high specificity and sensitivity. Still, laboratories around the world resort to conventional, end-point PCR based methods to perform GMO screening. The costs of fluorescent probes, training and equipment required for real-time PCR based methods are prohibitively high in certain parts of the world, while conventional end-point PCR is simple to use and has affordable price (Garcia-Cañas *et al.,* 2004). The possibility of using multiplex end-point PCR reactions in GMO detection additionally reduces the price and time required for the analysis (Sarmadi *et al.*, 2016). However, sensitivity of end-point PCR methods is lower and with low concentration of analyte false negatives are expected. Also, the probability of cross-contamination is higher because of electrophoresis detection (Garcia-Cañas *et al.*, 2004).

Here, we aimed to explore reliability of screening approach based on conventional PCR by comparing the results of real-time and end-point PCR based amplification of common elements of transgene construct, and analyzing potential discrepancies among the results obtained by different methods and establishing false negative results rate obtained by end-point PCR based methods. The purpose of our study was to establish whether end-point PCR as low-cost method with lower technical demands was still useful in routine laboratory practice.

MATERIALS AND METHODS

Genomic DNA extracts from 73 samples of food and feed, previously collected within official monitoring of B&H (Bosnia and Herzegovina) market, were recovered from -20°C storage and reanalysed using end-point PCR based methods for the detection of T-NOS and P-35S. The samples were previously screened for these elements using validated real-time PCR based methods with TaqMan probes. Food samples included variety of products with maize or soybean as the main ingredient: flour, polenta, cakes, flips, popcorn, soybean containing lunch meats, and muesli (Table 1).

DNA was extracted according to CTAB precipitation-based protocol, which is most widely used for DNA isolation from plant material (ISO 21571: 2005; Annex A, Part A3). This method is validated by EURL-GMFF for DNA isolation from seeds and flour and is also shown to be efficient for DNA isolation from processed food. The extracted DNA was subjected to spectrophotometric analysis, analysed for taxon specific marker, screened for T-NOS and P-35S using real-time PCR based methods validated by Instituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana (Gatto *et al.,* 2011) and stored at -20°C for six to 12 months. The DNA was not thawed until T-NOS and P-35S reanalysis using end-point PCR based methods.

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

Table 1. Analysed samples of food and feed, including type of product and target plant.

Sample type	Product	Analyte
Food	Grain (1) Flour (6) Cake (4) Flips (5) Popcorn (1)	Maize
	Cake (1) Semi-processed product (5) Drink (4) Pate (1)	Soya
Feed	Grain (5) Flour (10) Mixed feed (5)	Maize
	Grain (2) Flour (1) Grits (17) Mixed feed (5)	Soya

Certified Reference Materials (CRMs) of 10% RoundupReady soybean, GTS 40-3-2 and 1% MON 863 were procured from JRC-IRMM (Joint Research Centre– Institute for Reference Materials and Measurements). CRM DNA was extracted fresh according to the same CTAB based method. Positive control for P-35S contained DNA extract of 10% GTS 40-3-2 CRM as a template, while 1% MON 863 CRM DNA extract was used for T-NOS. Deionised water was used as a negative control template.

End-point PCR methods for T-NOS and P-35S were performed according to Lipp *et al.* (2001). These methods are included in EU Database of Reference Methods for GMO Analysis under codes QL-ELE-00-004 (P-35S) and QL-ELE-00-009 (T-NOS; Bonfini *et al.*, 2012). Specific primer sequences are listed in Table 2. PCR reactions were performed in total volume of 20 µL with 40 ng of DNA template (Table 3). Cycling conditions of PCR reactions are listed in Table 4. The analyses were performed in duplicate accompanied with the compatible CRM and PCR negative controls. Amplicons were visualized using horizontal gel electrophoresis in 2% agarose and SB buffer. All the methods were subject to verification procedure in our laboratory according to the verification of analytical methods for GMO testing when implementing interlaboratory validated methods (ENGL, 2011).

RESULTS AND DISCUSSION

Total of 73 samples were successfully analysed in our survey using end-point PCR based methods.

Both positive and negative controls resulted as anticipated, thus excluding cross-contamination. Positive amplification of P-35S was obtained in 45.2% of samples while 12.3% resulted in positive amplification of low efficiency (low band intensity). For T-NOS, 50.7% of samples tested positive while amplification of low efficiency was obtained for 4.1%. In 27.4% of the samples, both markers were successfully amplified. The results of end-point PCR based analysis were compared with previously obtained results of real-time PCR based amplification (Table 5), which are presented as average Ct values derived from Ct values of duplicates.

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

Table 2. Sequences of primer pairs used in end-point PCR reactions.

Target	Label	Size (bp)	Sequence 5'-3'	Reference
P-35S	P35S-cf3	120 bp	CCACGTCTTCAAAGCAAGTGG	Lipp *et al.* 2001
	P35S-cr4		TCCTCTCCAAATGAAATGAACTTCC	
T-NOS	HA-nos	118 bp	GCATGACGTTATTTATGAGATGGG	
	HA-nos		GACACCGCGCGCGATAATTTATCC	

Table 3. End-point PCR reaction conditions.

MASTERMIX	P-35S	T-NOS
	Volume for one reaction (µl)	Volume for one reaction (µL)
RedTaq mix Sigma[a] (x2)	10	10
$MgCl_2$ (25 mM)	0.8	0.8
Forward primer (10 mM)	1	1
Reverse primer (10 mM)	1	1
ddH_2O	6.2	6.2
DNA (40 ng μL^{-1})	1	1
Total	20	20

[a] Contains 0.06 u μL^{-1} TaqDNA Polymerase, 3 mM $MgCl_2$ and 0.4 mM dNTP.

Table 4. Cycling parameters for end-point PCR reactions.

Step	Temperature (°C)	Time	No of cycles
Initial denaturation	95	3 min	1
Denaturation	95	25 s	
Annealing	62	30 s	50
Extension/Elongation	72	45 s	
Final elongation	72	7 min	1
Standby	4	∞	1

Previous verification study that had been performed in our laboratory had shown that it is more suitable to use Ct≤35 as a positive result for routine analyses because it is closer to LOD_{abs} (absolute Limit Of Detection) value of the method.

Ct values and end-point PCR results of P-35S amplification are harmonized for 69 out of 73 reanalysed samples. As Ct value approaches the LOD_{abs} of the method, efficiency of end-point PCR amplification decreases, which is evident from the low intensity of bands (Table 5). If we take into consideration that LOD_{abs} is the lowest concentration of analyte that can be detected at least 95% of the times, it is understandable that the samples with high Ct value result in positive end-point PCR test. Discordance was observed for samples 2, 16, 17, and 35. Sample 2 tested positive for P-35S end-point PCR based amplification, while amplification was not obtained using real-time PCR. Sample 35 tested negative for end-point PCR based method while Ct=28.5. Although Ct values of samples 16 and 17 indicated target concentration way below LOD_{abs} (Ct= 40 and Ct= 37.5 respectively), both samples yielded strong bend following PCR. Both samples were pure processed soybean. Comparison of the two P-35S detection methods by JRC GMO-Matrix (Angers-Loustau *et al.* 2014) reveals that real-time PCR method has better coverage as it recognizes two maize and one

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

Table 5. Comparative overview of amplification data of end-point and real-time PCR reactions.

Ord	Sample type			P-35S (Ct values)	P-35S (Ct values)	T-NOS (Ct values)	T-NOS (End-point)
1	Food	Maize	Flour	36	-	33.5	+
2	Food	Maize	Flour	0	+	33.5	+
3	Food	Maize	Flour	38	-	34	+
4	Food	Maize	Flour	35.5	+	33.5	+
5	Food	Maize	Flour	35.5	-	33.5	+
6	Food	Maize	Flour	37.5	-	33.5	+
7	Food	Maize	Cake	37	-	34.5	+
8	Food	Maize	Cake	36.5	-	33	+
9	Food	Maize	Flips	36	-	31	+
10	Food	Maize	Flips	37	-	32	+
11	Food	Maize	Flips	36	-	31	+
12	Food	Maize	Flips	35	-	31	+
13	Food	Maize	Flips	37.5	-	32	+
14	Food	Maize	Popcorn	39	-	31.5	+
15	Food	Maize	Cake	40	-	33	+
16	Food	Soya	Cake	40	+	31.5	+
17	Food	Soya	Semi-processed product	37.5	+	34.5	+
18	Food	Soya	Semi-processed product	38	-	34	+
19	Food	Soya	Drink	34.8	-	30	+
20	Food	Soya	Drink	40	-	34.5	+
21	Food	Soya	Drink	42	-	32	-
22	Food	Soya	Pate	39	-	33	-
23	Food	Soya	Semi-processed product	39.5	+/-	34.5	-
24	Food	Soya	Semi-processed product	39	-	34	-
25	Food	Soya	Semi-processed product	0	-	33.5	-
26	Feed	Maize	Flour	28	+	27.5	-
27	Feed	Maize	Flour	33.5	+/-	32.5	-
28	Feed	Maize	Flour	34	+/-	33	-
29	Feed	Maize	Flour	31.5	+/-	30.5	-
30	Feed	Maize	Flour	27	+	26.5	-
31	Feed	Maize	Flour	32.5	+/-	32	-
32	Feed	Maize	Flour	35.2	+/-	31	-
33	Feed	Maize	Mixed Feed	27	+	26	-
34	Feed	Maize	Flour	24	+	23.5	+
35	Feed	Maize	Mixed Feed	28.5	-	27	-
36	Feed	Maize	Mixed Feed	27.5	+	27	+
37	Feed	Maize	Mixed Feed	34.5	-	34	-
38	Feed	Maize	Flour	34.5	-	34.5	-
39	Feed	Maize	Mixed Feed	25.5	+	25	+
40	Feed	Maize	Flour	27	+	26.5	-
41	Feed	Maize	Grain	38	-	34.5	-
42	Feed	Soya	Grits	22	+	21	+
43	Feed	Soya	Grits	21	+	21	-
44	Feed	Soya	Grits	33	+	21	+
45	Feed	Soya	Grits	21.5	+	21	-
46	Feed	Soya	Grits	21.5	+	23	-
47	Feed	Soya	Grits	24	+	23	+
48	Feed	Soya	Mixed Feed	25	+	24	+/-
49	Feed	Soya	Mixed Feed	26.5	+	25	-
50	Feed	Soya	Grits	20.5	+	21	+

[a] +/- low band intensity

Continued…

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

Continued of Table 5. Comparative overview of amplification data of end-point and real-time PCR reactions.[a]

Ord	Sample type			P-35S (Ct values)	P-35S (Ct values)	T-NOS (Ct values)	T-NOS (End-point)
51	Feed	Soya	Grits	21	+	21	+
52	Feed	Soya	Grits	21	+	21	+
53	Feed	Soya	Grits	34	+/-	34	-
54	Feed	Soya	Grits	21	+	21	+
55	Feed	Soya	Grits	21	+	20	+
56	Feed	Soya	Flour	23	+	22.5	+
57	Feed	Soya	Grits	21	+	20	+
58	Feed	Soya	Mixed Feed	33.5	+/-	33	-
59	Feed	Soya	Grits	21	+	21.5	+
60	Feed	Soya	Mixed Feed	26.5	+	25.5	+/-
61	Feed	Soya	Grits	21	+	20.5	-
62	Feed	Soya	Mixed Feed	35.5	+/-	33	+
63	Feed	Soya	Grits	21	+	20	+
64	Feed	Soya	Grits	21	+	20	+
65	Feed	Soya	Grain	26	+	25	-
66	Feed	Soya	Grain	24	+	22.5	-
67	Food	Maize	Cake	0	-	36.5	-
68	Food	Maize	Grain	40	-	35	-
69	Food	Soya	Drink	0	-	0	-
70	Feed	Maize	Grain	0	-	40	-
71	Feed	Maize	Grain	0	-	0	-
72	Feed	Maize	Grain	40	-	36.5	-
73	Feed	Maize	Grain	40	-	35	-

[a] +/- low band intensity

soybean events more than its conventional PCR counterpart.

Interpretation of T-NOS amplification results is more complex because there are discrepancies for 28.77% of samples (Table5). It is hard to perceive some sort of regularity between the results of end-point PCR reactions and previously observed Ct values. Therefore, real-time PCR based method superiority is not only the result of higher sensitivity. It is important to bear in mind that LOD and LOQ (Limit Of Quantification) are method specific values and besides varying between different methods, they also vary between different sample types. If we compare validation procedures of end-point and real-time PCR based methods used in this research, we can notice that end-point PCR methods validation was performed on cookies prepared following very strict recipe, while real-time PCR based methods were validated using CRMs (EURL-GMFF and ENGL 2010; Gatto *et al.*, 2011).

One of the factors that affect end-point PCR sensitivity is sensitivity of agarose gel electrophoresis, which is a commonly used method for visualization of amplicons (Holden *et al.*, 2010). Ethidium bromide has limited sensitivity that, according to various sources, amounts to 1-10 ng per band (Baird *et al.*, 1996; Mahon *et al.*, 1999; Garcia-Cañas *et al.*, 2004). Besides limited sensitivity, major disadvantage of end-point PCR based methods is lack of precise quantitative information related to amplification efficacy. These problems are circumvented with real-time PCR based methods. However, constantly increasing number of commercialized GMOs and emergence of new genetic modifications (ISAAA, 2018) requires over 20 qualitative PCR reactions for identification of specific GMO event, which is time consuming, expensive and burdens laboratory capacities.

Incongruity of our amplification results obtained for different methods could also be caused by specificity differences among

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

these detection methods. Depending on specificity level of the used methods, the results can vary significantly. Considering these facts, EURL-GMFF implemented bioinformatic platform JRC GMO-Matrix, which takes advantage of GMO sequences information stored in CCSIS (Central Core DNA Sequence Information System) as well as primer and probe sequences of available detection methods compiled in GMOMETHODS database aiming to perform *in silico* simulation of PCR amplification (Angers-Loustau *et al.*, 2014; Bonfini *et al.*, 2012). Advantage of JRC GMO Matrix compared to other similar matrices is availability of original sequence data in CCSIS. However, in P-35S detection method instance (QT-ELE-00-004, Pauli *et al.*, 2001), this proved to be a disadvantage because it did not consider actual experience based on laboratory practice. Even though one of the main conditions for GMO commercialization is stability of transgene construct, these regions are not immune to mutation rate characteristic for certain organism. In Morisset *et al.* (2009) research, it was shown that validated and commonly used screening method for detection of TC1507 maize did not provide efficient detection. Comparative study of different detection methods performed on CRMs showed that Pauli *et al.* (2001) method had 16X lower efficiency and sensitivity compared to other screening method – modified Pauli-Alary method (Alary *et al.*, 2002), which targets adjacent region of P-35S promoter. Further research showed that low sensitivity represents result of SNP (Single Nucleotide Polymorphism) that is located in target region of Pauli *et al.* (2001) method. This SNP was not identified in TC1507 maize sequence available in the database or in other available P-35S sequences inserted in different transgene plants (Morisset *et al.*, 2009). Real-time PCR protocols used in our laboratory are IZSLT validated (Gatto *et al.*, 2011) and SNP problem described by Morisset *et al.* (2009) was resolved. Harmonization of screening phase of testing strategy would provide equivalent results of GMO detection in international framework. In spite of developing PCR based methods, it is necessary to use other analytical methods that will enable more precise, validated, and faster results. Using bionformatic tools and applications, it is also possible to contribute the prediction of detection, reduce costs, accelerate and facilitate detection methods.

CONCLUSIONS

Comparison of P-35S and T-NOS end-point and real-time PCR based methods sensitivity showed that, as anticipated, real-time PCR based methods have indisputably higher sensitivity. Sensitivity of end-point PCR based methods, however, is not restrictive for routine analyses. Much more important factor is specificity, because different methods target different regions of the same element of transgene construct, they do not necessarily enable detection of the same GMO events. The list of authorized GMO events is constantly getting longer and, consequently, the process of method validation needs to be revised. It is also important to consider that transgene construct sequences are susceptible to mutations, which can affect analysis efficiency. Therefore, selection of detection method needs to be based on practical specificity testing and not just *in silico*. Considering upward trend of GMO authorizations, the process of analysis is becoming more complex, so, it is important to pay more attention to improvement and specialization of GMO detection methods.

ACKNOWLEDGEMENTS

In part, the research was funded by Federal Ministry of Education and Science of B&H, the project “Harmonization of GMO detection strategies in Federation B&H with the norms of EU Laboratories” in 2012.

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

REFERENCES

1. Alary, R., Serin, A., Maury, D., Jouira, H. B., Sirven, J. P., Gautier, M. F. and Joudrier, P. 2002. Comparison of Simplex and Duplex Real-Time PCR for the Quantification of GMO in Maize and Soybean. *Food Control*, **13(4–5):** 235–244.
2. Angers-Loustau, A., Petrillo, M., Bonfini, L., Gatto, F., Rosa, S., Patak, A. and Kreysa, J. 2014. JRC GMO-Matrix: A Web Application to Support Genetically Modified Organisms' Detection Strategies. *BMC Bioinform.*, **15:** 417.
3. Baird, D. J., Maltby, L., Greig-Smith, P. W. and Douben, P. E. T. 1996. *Ecotoxicology: Ecological Dimensions*. Chapman & Hall, London.
4. Bonfini, L., Van den Bulcke, M. H., Mazzara, M., Ben, E. and Patak, A. 2012. GMOMETHODS: The European Union Database of Reference Methods for GMO Analysis. *J. AOAC Int.*, **95(6):** 1713–1719.
5. EURL-GMFF and ENGL. 2010. *Compendium of Reference Methods for GMO Analysis.* EU-JRCIHCP, QT/GM/005 102–104, QT/ZM/020 54–56. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC64876/gmo-jrc_reference%20report_2011_publ.pdf
6. ENGL. 2011. Verification of Analytical Methods for GMO Testing When Implementing Interlaboratory Validated Methods. Guidance Document from the European Network of GMO laboratories. EUR 24790 EN. Available at: https://publications.jrc.ec.europa.eu/repository/bitstream/JRC64395/lb-na-24790-en-c.pdf
7. Fraiture, M. A., Herman, P., Taverniers, I., De Loose, M., Deforce, D. and Roosens, N. H. 2015. *Current and New Approaches in GMO Detection: Challenges and Solutions*. *BioMed Res. Int.*, Article ID 392872.
8. Gatto, F., Paternò, A., Ciabatti, I., Marchesi, U. and Amaddeo, D. 2011. Report of the collaborative study for the validation of real time PCR methods for GMO screening. Technical Report, Available at: https://www.izslt.it/crogm/wp-content/uploads/sites/5/2019/04/CROGM_report_screening-1.pdf
9. Gatto, F., Rosa, S., Querci, M. and Kreysa, J. 2015. Development and Optimization of the GM Soybean Event-Specific Pre-Spotted Plate (SePSP). Technical Report, Institute for Health and Consumer Protection, Joint Research Centre, European Commission.
10. García-Cañas, V., Gonzales, R. and Cifuentes, A. 2004. Detection of Genetically Modified Organisms in Food by DNA Amplification Techniques. *Crit. Rev. Food Sci. Nutr.*, **44(6):** 425–436.
11. Holden, M. J., Levine, M., Scholdberg, T., Haynes, R. J. and Jenkins, G. R. 2010. The Use of 35S and Tnos Expression Elements in the Measurement of Genetically Engineered Plant Materials. *Anal. Bioanal. Chem.*, **396(6):** 2175–2187.
12. International Service for the Acquisition of Agri-Biotech Applications. 2018. Global Status of Commercialized Biotech/GM Crops in 2018: Biotech Crops Continue to Help Meet the Challenges of Increased Population and Climate Change. Brief 54, Available at: https://www.isaaa.org/resources/publications/briefs/54/executivesummary/pdf/B54-ExecSum-English.pdf
13. International Organization for Standardization. 2005. Foodstuffs. Methods of Analysis for the Detection of Genetically Modified Organisms and Derived Products: Nucleic Acid Extraction. Par. A3, Annex A, EN ISO 21571: 2005.
14. Lipp, M., Bluth, A., Eyquem, F., Kruse, L., Schimmel, H., Van den Eede, G. and Anklam, E. 2001. Validation of a Method Based on Polymerase Chain Reaction for the Detection of Genetically Modified Organisms in Various Processed Foodstuffs. *Eur. Food Res. Technol.*, **212:** 497–504.
15. Mahon, A. R, MacDonald, J. H., Ott, R. J. and Mainwood, A. 1999. A CCD-Based System for the Detection of DNA in Electrophoresis Gels by UV Absorption. *Phys. Med. Biol.*, **44(6):** 1529–1541.
16. Morisset, D., Demšar, T., Gruden, K., Vojvoda, J., Štebih, D. and Žel, J. 2009. Detection of Genetically Modified Organisms-Closing the gaps. *Nat. Biotechnol.*, **27(8):** 700–701.
17. Pauli, U., Schouwey, B., Hubner, P., Brodmann, P. and Eugster, A. 2001. Quantitative Detection of Genetically Modified Soybean and Maize: Method Evaluation in a Swiss Ring Trial. *Mitt. Lebensm. Hyg.*, **92:** 145–158.

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

18. Querci, M., Foti, N., Bogni, A., Kluga, L., Broll, H. and Van den Eede, G. 2009. Real-Time PCR-Based Ready-to-Use Multi-Target Analytical System for GMO Detection. *Food Anal. Method.*, **2:** 325–336.
19. Rosa, S., Gatto, F., Querci, M. and Kreysa, J. 2013. *Development of a Ready-to-Use, Multi-Target Screening Pre-Spotted Plate (sPSP) for GMO Detection*. Technical Report, Joint Research Center, European Commission.
20. Sarmadi, L., Alemzadeh, A. and Ghareyazie, B. 2016. PCR-Based Detection of Genetically Modified Soybean at a Grain Receiving Port in Iran. *J. Agr. Sci. Tech.*, **18(3):** 805–815.
21. Žel, J., Milavac, M., Morisset, D., Plan, D., Van den Ede, G. and Gruden, K. 2012. *How to Reliably Test for GMOs*. Springer Science and Business Media, New York.

کارآیی تشخیص ارگانیسم های تغییر ژنتیکی یافته (GMO) مبتنی بر End-Point PCRدر مواد غذایی و علوفه ای

ا. احدویک، ا. المومنی، ک. باجرویک، و ا. دورمیک پاسیک

چکیده

از زمان تجارتی شدن گیاهان تراریخته در ۱۹۹۶، مساحت زیر کشت گیاهان بیوتک (زیست-تکنیک) به طور پیوسته ای در حال گسترش است. مصرف کنندگان اروپایی به طور مشخصی در مورد ژن های ترازیخت در محصولات غذایی مشکوک بوده اند و اتحادیه اروپا (EU) در این باره مقررات بسیار پیچیده ای به کار بسته است. انجام تجزیه و تحلیل مواد غذایی نیازمند توسعه و بهبود روش های تشخیص برای ردیابی در چارچوب قانونی و پاسخ دادن به نیازهای مصرف کنندگان است. در دهه گذشته، روشهای مبتنی بر real-time PCR (واکنش زنجیره ای پلیمراز) روش انتخابی برای بسیاری از آزمایشگاه ها بوده است، ولی به دلیل های مختلف، روش های مبتنی برEnd-Point PCR همچنان مورد استفاده قرار می گیرند. در این پژوهش، ۷۳ نمونه مواد غذایی و علوفه ای برای تعیین حضور اجزای معمولی ساختار ژن های تراریخته Cauliflower Mosaic Virus 35S promoter (P-35S) و *Agrobacterium tumefaciens* Nopaline Synthase Terminator (T-NOS) با استفاده از روش های مبتنی بر End-Point PCR مورد تجزیه قرار داده شد. این نمونه ها قبلا با روش تایید شده مبتنی بر real-time PCR برای تشخیص همان اجزای ژن های تراریخته مورد آزمون قرار گرفته بودند. مقایسه حساسیت روش های مزبور نشان داد که روش های مبتنی بر real-time PCR ارجحیتی انکار نشدنی دارند. عامل مهمتر در این موارد اختصاصی بودن (specificity) است و نیز این واقعیت که فهرست تایید شده ارگانیسم های تغییر ژنتیکی یافته (GMO) به طور پیوسته در حال افزایش است چنین ایجاب می کند که دستورالعمل روش های

[DOR: 20.1001.1.16807073.2021.23.2.1.6]

تشخیص به روز باشد. با در نظر گرفتن روند افزایشی GMO ، مهم است که به بهبود و تخصصی کردن روشهای تشخیصGMO توجه بیشتری معطوف شود.

[DOR: 20.1001.1.16807073.2021.23.2.1.6]