Original Article

REAL-TIME PCR FOR TRACEABILITY AND QUANTIFICATION OF GENETICALLY MODIFIED SEEDS IN LOTS OF NON-TRANSGENIC SOYBEAN

PCR EM TEMPO REAL PARA RASTREABILIDADE E QUANTIFICAÇÃO DE SEMENTES GENETICAMENTE MODIFICADOS EM LOTES DE SOJA NÃO TRANSGÊNICA

Joelma LEÃO-BUCHIR[1,2]; Gilberto Vinícius Melo PEREIRA[1]; André Luís Lopes da SILVA[1]; Silvana ALBAN[1]; Maria Carolina ROCHA[3]; Jossimara POLETTINI[4]; Vanete THOMAZ-SOCCOL[1]; Carlos Ricardo SOCCOL[1]

1. Engenharia de Bioprocessos e Biotecnologia, Centro Politécnico, Universidade Federal do Paraná, Curitiba, PR, Brasil. soccol@ufpr.br; 2. Centro de Biotecnologia, Universidade Eduardo Mondlane, Maputo, Moçambique; 3. Departamento de Hidráulica e Saneamento, Centro Politécnico, Universidade Federal do Paraná, Curitiba, Brasil; 4. Universidade do Oeste Paulista, UNOESTE, Presidente Prudente, Brasil

ABSTRACT: The constant presence of genetically modified (GM) soybean in conventional seed lots has become a growing problem for international seed trade. In this context, seed companies have prompted the development of routine tests for accurate genetically modified soybean seeds detection. In this study, a quantitative PCR-based method was standardized in order to detect and quantify mixtures of seeds (i.e. certified seed) or GM grains (i.e. seeds came from field) into samples of non-GM soybean, in a way that soybean lots can be assessed within the standards established by legislation. The method involved the use of p35S-f2/petu-r1 primers targeting CP-4 enolpyruvylshikimate-3-phosphate synthase (cp4-epsps) gene (i.e. that confers herbicide tolerance in Roundup Ready™ (RR)) for real-time PCR detection and quantification through mericon Quant GMO Detection Assay. The results revealed the method efficiency to detect and quantify the presence of even one soybean seed in batch used for routine evaluation of GM seeds. In addition, it was possible to detect of up to 0.1% of transgenic DNA relative to the soybean grains content. Thus, the sensitive GMO quantitative approach described in this study will provide support in supervising activities, and facilitate the process and control of GM soybean.

KEYWORDS: Roundup Ready® soybean. GMP. *Glycine max*. *Cp4-epsps* gene.

INTRODUCTION

Concerns about the intrinsic and extrinsic quality attributes in foods have been intensified due to the entry of genetically modified crops in the global consumer market (SIEW-PING et al., 2011). Many of the current debates on agricultural biotechnology have focused on the potential risks of genetically modified (GM) crops for human health. Some of the health risks pertinent to unapproved genetically modified food and concerned consumers include antibiotic resistance, allergenicity, nutritional changes and the formation of toxins (MAGHARI; ARDEKANI, 2011).

Thus, large soybean importing countries have internally reached an agreement to satisfy the demands of the consumers. Among these demands is the introduction of mandatory labeling of products containing levels of genetically modified organisms above the allowed limits, without thereby restricting imports of soybean complex (VILJOEN et al., 2006). For instance, the labeling thresholds are defined as 0.9% in the European Union (EUROPEAN COMMISSION, 2003), 1% in Brazil (DE MIRANDA et al., 2005), 3% in South Korea (Notification No.2000-31, 2000), and 5% in Japan (MATSUOKA, 1999).

To fulfill labeling requirements, several analytical methods have been used to detect genetically modified crops. Divided in protein-based assays, e.g., ELISA test, provide only quality results about specific proteins contained in the genetically modified material, and DNA-based methodologies, which can be divided into qualitative (conventional PCR) or quantitative (real time PCR) analyses (MAGHARI; ARDEKANI, 2011). The establishment of an adequate, safe and economical method for detection, identification and quantification of GM seeds in conventional batch remains a challenge. Recognizing the importance of new markets and genetically modified products, seed technology must ensure the genetic purity of new biotechnology products, in order to avoid legal proceedings due to incorrect identification of varieties. In this context, the objective of this study was to apply a quantitative PCR-based method for

Received: 10/01/17
Accepted: 05/12/17

detecting and quantifying mixtures of seeds or GM grains into samples of non-GM soybean, in a way that soybean lots can be assessed within the standards established by legislation.

MATERIAL AND METHODS

Sample material and contamination experiment

Samples of GM and conventional soybean seeds (i.e. certified seed) and grains (i.e. seeds came from field) were used in this study. The cultivar used in this research were GM soybean (P98Y30 RR) with the Certified Reference GM-soybean materials were acquired at Pioneer Hi-Bread International, Inc. (USA), while the Certified Reference pure soybean (cv. BRS1010 IPRO) materials (non-GM) were provided by the Department of Agriculture and Supply of Paraná State, Brazil. The sampling procedure was performed in the Laboratory of Products Classification of Paraná (CLASPAR, Curitiba, Brazil) in accordance with the standards established by the International Seed Testing Association (PINTO et al., 2011). The experimental samples were prepared in order to detect contamination of GM seeds within non-GM soybean seed lots. Portions of 500 g of non-GM soybean seed were individually contaminated with 2, 3, 5, 6, 9, 10, 15 and 20 units of GM soybean seeds. The same procedure was applied for soybean grains, except using portions of 100 g of non-GM soybean and contamination levels of 1, 2, 3, 5, 10, 11, 13 and 14 units of GM soybean grains. Each contamination procedure was carried out in triplicate.

DNA extraction

The samples were transported for the Laboratory of Biotechnological Processes, at Federal University of Paraná, Curitiba, Brazil, for further analysis. Genomic DNA was isolated of each contamination experiment using the Cetyl trimethylammonium bromide (CTAB) method (GREINER; KONIETZNY, 2008; Pinto *et al*., 2011). The integrity and concentration of the extracted DNA was measured by electrophoresis and spectrophotometer absorbance at 260 and 280 nm wavelength, respectively.

Qualitative PCR detection of lectin and GM gene sequences

The primers GMO3 (5´-GCCCTCTACTCCACCCCCATCC-3´) and GMO4 (5´-GCCCATCTGCAAGCCTTTTTGTG-3´) were used as control to amplify endogenous *lectin* gene of soybean according with method described by Greiner & Konietzy (2008). The primers p35S-f2 (5´-TGATGTGATATCTCCACTGACG-3´) and petu-r1 (5´-TGTATCCCTTGAGCCATGTTG-3´) targeting CP-4 5-enolpyruvylshikimate-3-phosphate synthase (*cp4-epsps*) gene that confers herbicide tolerance gene in RR soybean (SIEW-PING et al., 2011) were used for the detection of GM soybean.

The PCR was performed using Veriti Thermal Cycler (Applied Biosystems) in a total reaction mixture volume of 10 µL, containing: 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1.5 unit of *Taq* DNA polymerase, 3 µL of DNA extract and 0.5 µM of GMO3/GMO4 (lectin gene reaction) or 0.8 µM of p35S-f2/petu-r1 (RR soybean gene reaction). For GMO3/GMO4 primers amplification, the following cycle program was used: denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 61°C for 30 s, and 72 °C for 1 min; final extension at 72°C for 3 min. The cycle program for primers p35S-f2/petu-r1 was: denaturation at 95˚C for 5 min, followed by 35 cycles of 95˚C for 30 s, 61˚C for 30 s and 72˚C for 30 s; final extension at 72˚C for 3 min. The PCR products were separated by electrophoresis at 80V for 50 min in a 1.5% agarose gel, 1X TBE buffer and stained with ethidium bromide. The PCR products were visualized in a UV-trans illuminator and the images photographed with a digital camera (Loccus biotecnologia L.PIX System).

Real-time PCR

The real-time PCR for quantitative detection of GM content and control lectin gene was carried out in a 7500 Fast Real-Time PCR System (Applied Biosystems) using Mericon Quant GMO Detection Assays kit (Qiagen). A volume of 4.8 µL of prepared samples, positive and negative controls, as well as the provided standards dilutions were added to the reagents according to manufacturer's instructions. PCR amplification were carried out with an initial denaturation for 5 min at 95°C, followed by 45 cycles of 95℃ for 15 s, 60℃ for 30 s and 72℃ for 10 s. The results of the amplification curve and threshold cycle values (Ct) were analyzed using 7500 Software Version 2.05 (Applied Biosystems).

The threshold cycle number (CT) was used for quantification of GMO in lots of soybean seeds and grains, using the ddCT method (i.e. the algorithm for the analysis of quantitative real-time PCR (qRT-PCR)). The mean of CT from prepared samples was compared to the standard curve Ct means. All reactions were performed in duplicate and no template controls were included in each run.

The results are expressed in percentage by comparing the samples with the standard.

RESULTS AND DISCUSSION

DNA extraction and qualitative PCR

In this study, the CTAB method was efficient for extracting DNA from soybean seed and grain varieties, with good quality and appropriate quantity as demonstrated in Table 1. An important point concerning PCR analysis is the presence of PCR inhibitors in the amplification reactions, which could lead to false-negative results (MAFRA et al., 2008). Thus, it becomes crucial to standardize protocols for the extraction of DNA from food samples to avoid PCR inhibitors due to DNA extraction inadequate. The CTAB is a widely used method of DNA extraction and described in the official methods for the detection of GM foods of the German Food Act LMBG §35 (Greiner et al., 2005).

Table 1. Results of the isolated DNA in soybean seeds and grains (cv. P98Y30 RR (GM = genetically modified) and cv. BRS1010 IPRO (non-GM = non-genetically modified)) and qualitative PCR with each primer pair evaluated, being (GMO3/GMO4) for *lectin* gene used as endogenous reference gene (i.e. to confirm the presence of soybean residues in the samples) and (p35s-f2/petu-r1) for *cp4 epsps* gene that confers herbicide tolerance in Roundup Ready™ (RR). SS = Soybean certified seeds and Cc = Soybean grains (i.e. seeds came from field).

Absorbance (nm)					Primers pairs and amplified product (bp)	
260	280	[DNA] (ng/µl))	Sample	Designation	GMO3/GMO4	p35s-f2/petu-r1
0.067	0.039	167.5	C+	GM	+ (118)	+ (172)
0.032	0.027	80	C-	Non-GM	+ (118)	- (172)
0.032	0.016	80	SS2	GM	+ (118)	+ (172)
0.094	0.061	235	SS3	GM	+ (118)	+ (172)
0.064	0.044	160	SS5	GM	+ (118)	+ (172)
0.098	0.062	245	SS6	GM	+ (118)	+ (172)
0.062	0.04	155	SS9	GM	+ (118)	+ (172)
0.024	0.004	60	SS10	GM	+ (118)	+ (172)
0.072	0.04	180	SS15	GM	+ (118)	+ (172)
0.027	0.018	67.5	SS20	GM	+ (118)	+ (172)
0.009	0.005	22.5	Cc1	GM	+ (118)	+ (172)
0.010	0.004	25	Cc2	GM	+ (118)	+ (172)
0.018	0.006	45	Cc3	GM	+ (118)	+ (172)
0.018	0.022	45	Cc5	GM	+ (118)	+ (172)
0.054	0.059	135	Cc10	GM	+ (118)	+ (172)
0.076	0.044	190	Cc11	GM	+ (118)	+ (172)
0.015	0.019	37.5	Cc13	GM	+ (118)	+ (172)
0.012	0.005	30	Cc14	GM	+ (118)	+ (172)

In the qualitative PCR assay, the use of GMO3/GMO4 primers produced a fragment expected of 118 bp from the lectin gene in all analyzed samples (GREINER; KONIETZY 2008). A method for detection of specific species, such as the soybean lectin gene, is necessary to discriminate between negative and positive results due to inhibition in the amplification. In addition to control PCR, specific detection with the primers p35s-f2/petu-r1 yielded a fragment of 172 bp complementary to the CP-4 5-enolpyruvylshikimate-3-phosphate synthase (*cp4-epsps*) gene that confers herbicide tolerance gene in RR soybean (PINTO et al., 2011). The specificity of this reaction was verified in our study as no amplification product was obtained in parallel experiments using wild-type soybean.

A contamination dilution series of GM soybean into lots of wild-type showed that the target fragment was detected from all of the tested levels (Table 1), which indicates the efficiency of the event-specific PCR assay used in this study for detection of transgenic soybean.

Real-time PCR

A quantitative PCR-based method was applied in order to control maximum limits for GM in lots of soybean grains and seeds. The detection of *lectin* gene (control reaction) gave an average Ct value of 23.00 cycles for seeds (R^2 = 0.992) (Table 2) and 24.00 cycles for grains (R^2 = 0.999) (Table 3).

Table 2. Threshold cycle values (Ct) and copy number of lectin and *cp-4 epsps* genes from soybean certified seeds (SS). C+ = positive control and C- = negative control.

	Mericon Assay			
samples	**Lectin**		***cp-4 epsps***	
	Ct	**Copy number**	**Ct**	**Copy number**
STD[1] 1	21.589	81.920	28.721	10.420
STD 2	24.565	10.420	31.008	1280
STD 3	27.22	1280	33.727	160
STD 4	30.64	160	37.949	20
C+	25.242	3,082.271	36.034	25.813
C-	_	_	_	_
SS2[2]	**25.389**	2,090.444	**36.314**	1.50
SS3	25.027	3,073.221	35.5	2.17
SS5	24.052	4,683.718	35.364	464
SS6	22.907	4,806.966	35.221	544
SS9	22.202	5,948.167	34.901	5,444
SS10	22.187	10,161.592	33.793	15,311
SS15	21.33	25,345.625	33.585	119,285
SS20	**21.138**	67,879.562	**32.063**	567,975

[1] Sample with concentration or with copy number known and these samples were used to build a standard curve. [2] The number after the SS means the number of GM seeds intentionally contaminated in a batch of 500 g of non-GM seeds.

Table 3. Threshold cycle values (Ct) and copy number of lectin and *cp-4 epsps* genes from soybean grains (Cc). C+ = positive control and C- = negative control.

	Mericon Assay			
Samples	**Lectin**		***cp-4 epsps***	
	Ct	**Copy number**	**Ct**	**Copy number**
STD[1] 1	22.93	81.920	28.733	10.420
STD 2	25.816	10.420	31.737	1280
STD 3	28.897	1280	34.564	160
STD 4	32.034	160	37.888	20
C+	26.427	3,207.507	37.907	25.813
C-	_	_	_	_
Cc 1[2]	**27.572**	2,090.444	**40**	32.65
Cc 2	27.092	3,073.221	40	51.19
Cc 3	26.702	4,683.718	38.816	65.051
Cc 5	26.461	4,806.966	37.227	75.810
Cc 10	26.136	10,161.592	36.725	233.918
Cc 11	25.294	10,345.625	30.934	468.491
Cc 13	21.087	120,345.625	30.712	993.081
Cc 14	**20.488**	**159,835.344**	**28.389**	**1,430.225**

[1] Sample with concentration or with copy number known and these samples were used to build a standard curve. [2] The number after the Cc means the number of GM grains intentionally contaminated in a batch of 100 g of non-GM grains.

For *cp-4 epsps* gene amplification, that correlated with the amount of GM content present in each lot, the Ct value was 34.00 cycles for seeds (R^2 = 0.952) (Table 2) and 34.00 cycles for grain (R^2 = 0.965) (Table 3). For all reactions, PCR efficiency over 98% was observed which indicates the suitability of this method for detection/quantification of GMO in lots of soybean seeds and grains. These results are similar to those observed by Siew-Ping *et al.* (2011) in quantifying *cp-4 epsps* soybean gene in others foods matrixes.

Table 4 shows the quantification results of the transgenic DNA gene content in each soybean lot intentionally contaminated with GM seeds and grains.

Table 4. Quantitative analysis of the Roundup Ready™ soybean certified seeds (SS) and grains (Cc). % GM = Percentage of detected genetically modified material and C+ = positive control.

Sample	% GM		% GM
Soybean seed		Soybean grain	
C+	0.99	C+	0.99
SS2[1]	0.007	Cc 1	0.1
SS3	0.007	Cc 2	0.1
SS5	0.009	Cc 3	1.3
SS6	0.01	Cc 5	1.5
SS9	0.09	Cc 10	2.3
SS10	0.2	Cc 11	4.5
SS15	0.5	Cc 13	8.2
SS20	0.8	Cc 14	8.9

[1] The number after the SS means the number of GM seeds intentionally contaminated in a batch of 500 g of non-GM seeds.

The good linearity between transgenic DNA quantities and fluorescence values (Ct) confirm that the assay is well suited to quantitative measurements. In relation to seed evaluation, the quantitative PCR assay enabled the detection of even one soybean grain or seed in batch used for routine evaluation of GM soybean, providing a rigorous process in detecting GMO soybean seeds. For soybean grains mensuration, the method was able to detect the presence of up to 0.1% of transgenic DNA. The threshold of 0.1% will be suitable for practical detection of GM soybean grain materials.

This study gave an insight of the occurrence of different levels of transgenic DNA as an alternative way for tracing GMO in lots of seeds and grains. In relation to seeds, the method enables the detection of even one soybean seed in batch used for routine evaluation of GM soybean. Thus, the sensitive GMO quantitative approach described in this study will provide support in supervisory activities, facilitating the process and control of GM soybean.

ACKNOWLEDGEMENTS

The authors are grateful the CB-UEM and Rita Levi-Montalcini Foundation for financial support and CNPq for financial support and master scholarship of the first author.

RESUMO: A constante presença da soja geneticamente modificada (GM) em lotes de sementes convencionais têm se tornado um grande problema para o comércio internacional de sementes. Neste contexto, as empresas de sementes estão em busca de testes de rotina extremamente precisos para a detecção de sementes de soja geneticamente modificadas. Neste estudo, um método baseado em PCR quantitativo foi padronizado para detectar e quantificar misturas de sementes (i.e. sementes certificadas) ou grãos geneticamente modificados (i.e. sementes oriundas do campo) dentro de lotes de soja não transgênica, de um modo que os lotes de soja possam ser avaliados dentro dos parâmetros estabelecidos pela legislação. O método envolveu o uso dos iniciadores p35S-f2/petu-r1 alvejando o gene CP-4 5-enolpiruvil-shikimato-3-fosfato sintase (*cp4-epsps*) (i.e. que confere a tolerância ao herbicida Roundup Ready® (RR)) para detecção e quantificação em PCR de tempo real via Ensaio de detecção *Mericon Quant GMO*. Os resultados revelaram um método eficiente para detectar e quantificar a presença de até mesmo uma única semente de soja no lote usado para a avaliação de rotina de sementes geneticamente modificadas. Adicionalmente, foi possível detectar até 0,1% de DNA transgênico relativo ao conteúdo de grãos de soja. Dessa forma, uma abordagem quantitativa sensível à soja geneticamente modificada foi descrita

nesse estudo e poderá fornecer suporte em atividades de supervisão, além de facilitar o processo de controle da soja geneticamente modificada.

PALAVRAS-CHAVE: Soja Roundup Ready®. Planta geneticamente modificada. PCR em tempo real. *Glycine max*. Gene *cp4-epsps*.

REFERENCES

ABDULLAH, T.; RADU, S.; HASSAN, Z.; HASHIM, J. K. Detection of genetically modified soy in processed foods sold commercially in Malaysia by PCR – based method. **Food Chemistry**, Amsterdam, v. 98, p. 575-579, 2006. https://doi.org/10.1016/j.foodchem.2005.07.035

AERNI, P. Stakeholder attitudes towards the risks and benefits of genetically modified crops in South Africa. **Environmental Science and Policy**, Netherlands, v. 8, p. 464-476, 2005. https://doi.org/10.1016/j.envsci.2005.07.001

ALEXANDER, T. W.; REUTER, T.; AULRICH, K.; SHARMA, R.; OKINE, E. K.; DIXON, W. T.; McALLISTER, T. A. A review of the detection and fate on novel plant molecules derived from biotechnology in livestock production. **Animal Feed Science and Technology**, Amsterdam, v. 133, p. 31-62, 2007. https://doi.org/10.1016/j.anifeedsci.2006.08.003

ASSOCIAÇÃO BRASILEIRA DOS PRODUTORES DE MILHO (ABRAMILHO). Disponível em: <http://www.abramilho.org.br/noticias.php?cod=1405>. Acesso em: 07/2012.

BONFINI, L.; HEINZE, P.; KAY, S.; VAN DEN EEDE, G. Review of GMO detection and quantification techniques**. European Commission**, Italy, 2001.

BRASIL. Decreto no 4689, de 24 de abril de 2003. Regulamenta o direito à informação, assegurado pela Lei no 8078 de 11 de Setembro de 1990, quanto aos alimentos e ingredientes alimentares destinados ao consumo humano ou animal que contenham ou sejam produzidas a partir de organismos geneticamente modificados, sem prejuízo do cumprimento das demais normas aplicáveis. **Diário Oficial da República Federativa do Brasil**, Brasília, DF, 24 abr. 2003. Disponível em: <http://www.anvisa.gov.br/e-legis> Acesso em Junho de 2012.

CENTRO DE INFORMAÇÃO DE BIOTECNOLOGIA (CIB). Disponível em: < http://cibpt.org/inicio/index.php/cib/comunicados/2012/138-culturas-transgenicas-no- mundo-16-7-milhoes-de-agricultores-cultivaram-mais-8-em-2011>. Acesso em: 07/2012.

CHEN, Y.; WANG, Y.; GE, Y.; XU, B. Degradation of endogenous and exogenous genes of roundup-ready soybean during food processing. **Journal of Agricultural and Food Chemistry**, Washington DC, v. 53, p. 10239-10243, 2005. https://doi.org/10.1021/jf0519820

DE MIRANDA, D. M.; TILLMANN, M. A. A.; BALERINI, F.; VILLELA, F. A. Bioensaio na detecção e quantificação de sementes de soja geneticamente modificada resistente ao glifosato. **Revista Brasileira de Sementes**, Londrina, v. 27, n.1, p. 93-103, 2005. https://doi.org/10.1590/S0101-31222005000100012

DINON, A. Z.; TREML, D.; de MELLO, C. S.; ARISI, A. C. M. Monitoring of GMO in Brazilian processed meat and soy-based products from 2007 to 2008. **Journal of Food Composition and Analysis**, Amsterdam, v. 23, p. 226-229, 2010. https://doi.org/10.1016/j.jfca.2009.12.002

EUROPEAN NETWORK OF GMO LABORATORIES (ENGL). Definition of minimum performance requirements for analytical methods of GMO testing, **Community Reference Laboratory**, p.8, 2008.

FERREIRA, R. T. B.; BRANQUINHO, M. R.; CARDARELLI-LEITE, P. Soja geneticamente modificada em alimentos contendo farinha e preparados à base de farinha de trigo. Detecção e adequação à legislação de rotulagem. **Brazilian Journal of Food Technology**, Campinas, v. 12, n. 3, p. 241-248, 2009. https://doi.org/10.4260/BJFT2009800900018

GACHET, E.; MARTIN, G. G.; VIGNEAU, F.; MEYER, G. Detection of genetically modified organism (OGMs) by PCR. A brief review of methodology available. **Trends in Food Science and Technology**, Amsterdam v. 9, p. 380-388, 1999. https://doi.org/10.1016/S0924-2244(99)00002-3

GREINER, R.; KONIETZNY, U.; VILLAVICENCIO, A. L. C. H. Qualitative and quantitative detection of genetically modified maize and soy in processed food sold commercially in Brazil by PCR-based methods. **Food Control**, Amsterdam, v. 16, p. 753-759, 2005. https://doi.org/10.1016/j.foodcont.2004.06.015

HEID, C.A.; STEVES, J.; LIVAK, K. J.; WILLIAMS, P. M. Real time quantitative PCR. **Genome Research**, New York, v. 6, n. 10, p. 986-994, 1996. https://doi.org/10.1101/gr.6.10.986

HOLST-JENSEN, A.; RONNING, S. B.; LOVSETH, A.; BERDAL, K. G. PCR technology for screening and quantification of genetically modified organisms (GMOs). **Analytical and Bioanalytical Chemistry**, Berlin, v. 375, p. 985-993, 2003. https://doi.org/10.1007/s00216-003-1767-7

LEE, S. H.; MIN, D. M., KIM, J. K. Qualitative and quantitative polymerase chain reaction analysis for genetically modified maize MON863. **Journal of Agricultural and Food Chemistry**, New York, v. 54, p. 1124-1129, 2006. https://doi.org/10.1021/jf052199a

LI, X.; PAN, L.; LI, J.; ZHANG, Q.; ZHANG, S.; LV, R.; YANG, L. Establishment and application of event-specific polymerase chain reaction methods for two genetically modified soybean events, A2704-12 and A5547-127. **Journal of Agricultural and Food Chemistry**, New York, v. 59, p. 13188-13194, 2011. https://doi.org/10.1021/jf202806w

MAFRA, I., Silva, S. A., Moreira, E. J. M. O., da Silva, C. S. F., Beatriz, M., Oliveira, P. P. Comparative study of DNA extraction methods for soybean derived food products. **Food Control**, Amsterdam, v. 19, p. 1183–1190, 2008. https://doi.org/10.1016/j.foodcont.2008.01.004

MAGHARI, B. M.; ARDEKANI, A. M. Genetically modified foods and social concerns. **Avicenna Journal of Medical Biotechnology**, Tehran, v. 3, n. 3, p. 109-117, 2011.

MATSUOKA, T.; KAWASHIMA, Y.; AKIYAMA, H.; MIURA, H.; GODA, Y.; SEBATA, T.; ISSHIKI, K.; TOYODA, M.; HINO, A. A detection method for recombinant DNA from genetically modified soybeans and processed foods containing them. **Journal of the Food Hygienic Society of Japan**, Tokyo, v. 40, p. 149-157, 1999. https://doi.org/10.3358/shokueishi.40.149

PINTO, G. B. A.; SILVA, M.; GREINER, R.; KONIETZNY, U.; SOCCOL, C. R.; SPIER, M. R.; FILHO, M. A. S. C.; PANDEY, A.; THOMAZ-SOCCOL, V. Application of Polymerase Chain Reaction for high sensitivity detection of roundup ready^{TM} soybean seeds and grains in varietal mixtures. **Food Technology and Biotechnology**, Kačićeva, v. 49, n. 3, p. 277-285, 2011.

ROTT, M. E.; LAWRENCE, T. S.; WALL, E. M.; GREE, M. J. Detection and quantification of roundup ready soy in foods by conventional and real-time polymerase chain reaction. **Journal of Agricultural and Food Chemistry**, New York, v. 52, p. 5223-5232, 2004. https://doi.org/10.1021/jf030803g

SIEW-PING, K.; YOKE-KQUEEN, C.; SON, R. Quantitative analysis of Roundup Ready soybean content in soy-derived food and animal feed by using Real-time PCR incorporated with cloned DNA fragments. **International Food Research Journal**, Serdang, v. 18. p. 507-514, 2011.

SCHOLTENS, I. M. J.; KOK, E. J.; HOUGS, L.; MOLENAAR, B.; THISSEN, J. T. N. M.; VAN DER VOET, H. Increased efficacy for in-house validation of real-time PCR GMO detection methods. **Analytical and Bioanalytical Chemistry**, Berlin, v. 396, p. 2213- 2227, 2010. https://doi.org/10.1007/s00216-009-3315-6

SHIMIZU, E.; KATO, H.; NAKAGAWA, Y.; KODAMA, T.; FUTO, S.; MINEGISHI, Y.; WATANABE, T.; AKIYAMA, H.; TESHIMA, R.; FURUI, S.; HINO, A.; KITTA, K. Development of a screening method for genetically modified soybean by plasmid- based quantitative competitive polymerase chain reaction. **Journal of Agricultural and Food Chemistry**, New York, v. 56, p. 5521-5527, 2008. https://doi.org/10.1021/jf073348n

TUNG NGUYEN, C. T.; SON, R.; RAHA, A. R.; LAI, O. M.; CLEMENTE MICHAEL WONG, V. L. Detection of genetically modified organisms (GMOs) using molecular techniques in food and feed samples from Malaysia and Vietnam. **International Food Research Journal**, Serdang, v. 15, n 2, p. 155-166, 2008.

VILJOEN, C. D.; DAJEE, B. K.; BOTHA, G. M. Detection of GMO in food products in South Africa: Implications of GMO labelling. **African Journal of Biotechnology**, Nairóbi, v. 5, n 2, p. 73-82, 2006.