Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.69921

Comparison of two methods for the quantitative assessment of genetically modified soybeans

Chen CHEN[1], Yan ZHANG[1], Rui ZHANG[1], Yalun ZHANG[1], Tao ZHANG[1], Zilun ZHANG[1], Guohua SHI[1*], Wei ZHOU[1*]

Abstract

Genetically modified soybean strains are trade monitoring object by China. Currently, the genetically modified soybean MON89788 has been approved in China to be imported as processing raw material. Therefore, there is an urgent need to establish a method to quantitatively assess MON89788. This study used droplet digital PCR technology to quantitatively detect MON89788. The results showed that the genomic DNA concentration and its copy number of genetically modified soybeans showed a certain linear relationship. The formula was y = 2.5967x + 3.1437, where R2 = 0.999. When the same 4.5% genetically modified sample was contained, the results of the droplet digital PCR ratio and linear methods were 5.88% and 3.599%, respectively. Comparing the two results showed that the droplet digital PCR ratio method has a larger error than the linear relationship method. The novel droplet digital PCR linear relationship method established in this study has high sensitivity and specificity, and thus is a good prospect for quantitative research.

Keywords: digital PCR; quantitative detection; MON89788.

Practical Application: This paper provides a new direction for the quantitative detection of transgenic soybean.

1 Introduction

Soybean is the most important oil crop in China, as it is rich in many nutrients. With the introduction of genetically modified foods into the market, their unpredictable risks have become important, causing consumers to pay attention to their safety issues (Cao et al., 2014). To date, genetic modifications have been noted eight times in the No. 1 Central Document by China (Shen et al., 2017). The development of genetically modified reference materials (Zhou, 2014) as well as the establishment of qualitative or quantitative detection technology for genetically modified genes are important to evaluate the safety of genetically modified foods (Yu, 2017). However, some countries have not complied with the corresponding laws and regulations (Tan & Chen, 2014). In foreign international trade, it is necessary for testing agencies to effectively test genetically modified strains and strictly control the related products of genetically modified labels into the Chinese market (Liu et al., 2015). The American Oil Chemists' Society (Cai et al., 2016) developed standard materials covering almost all commercialized genetically modified crops (Wu et al., 2019). China has developed 26 matrix standard materials, 5 plasmid DNA standards, and 5 kinds of genomic DNA standard materials (Wang et al., 2018). The herbicide-resistant genetically modified soybean GTS40-3-2 developed by Monsanto Ltd. and herbicide-resistant strain A2704-12 developed by Bayer (Clive, 2015) are the most widely used in the market. The genetically modified soybean MON89788 is a glyphosate-resistant soybean variety, which has been approved for use in food and feed processing materials in 11 countries (International Service for the Acquisition of Agri-biotech Applications, 2019). At present, 16 lines of genetically modified soybeans in China have been approved as raw materials for processing including MON89788 (Yu et al., 2016; Zhou et al., 2010). Therefore, there is an urgent need to establish quantitative detection methods for genetically modified soybeans.

Transgenic detection technology mainly includes protein, nucleic acid, enzyme activity and real-time fluorescent PCR detection (Fraiture et al., 2015). Nucleic acid detection technology is widely used because of its strong specificity, high sensitivity, high degree of automation, and accurate quantitative analysis (Deng et al., 2002). Common nucleic acid detection technologies include PCR, loop-mediated isothermal amplification, ligase chain reaction, and fingerprinting (Köppel et al., 2012; Feng et al., 2018; Zhou et al., 2017; Li et al., 2012) established a multiplex fluorescence-based real-time quantitative PCR (qPCR) method for four strains, namely MON89788, A5547-127, A2704-12, and GTS40-3-2. Currently, qPCR is widely used for food-borne pathogen detection (Zhang et al., 2016), identification of adulterated food of animal origin (Bharuthram et al., 2014), and many other food safety tests. However, common PCR detection technology has the disadvantage that it cannot be absolutely quantified. Digital PCR uses the Poisson distribution principle to fluorescently label each droplet in the reaction system. It does not require a standard curve or reference gene to directly obtain the DNA copy number, which has the characteristics of absolute quantification. Therefore, this technology is applied to copy

Received 01 Mar., 2021
Accepted 31 Dec., 2021
[1] *Hebei Food Safety Key Laboratory, Hebei Food Inspection and Research Institute, Shijiazhuang, China*
*Corresponding author: shiguohua@nepp.com.cn; zhouwei0311@163.com

number variation (Rodríguez et al., 2005; Wang et al., 2015), transgenic characteristics (Bhat et al., 2012), single nucleotide polymorphisms (Strain et al., 2013), gene expression analysis (Blaya et al., 2016), and microbial detection.

Based on the specific sequence and probe of the genetically modified soybean MON89788, this study established the droplet digital PCR ratio method and linear relationship method detection systems. Through comparison, the results of the linear relationship method is more accurate. The linear relationship between the DNA concentration of genetically modified components and the number of amplified copies was attained, the genetically modified components were precisely quantitated, and a set of rapid and accurate inspection methods were established to overcome the inconsistent gene amplification efficiency caused by different primers. These methods greatly reduce inspection errors and provide a reliable technical means for better monitoring of genetically modified soybeans on the market.

2 Materials and methods

2.1 Experimental materials

The following materials were used in this study: the genetically modified soybean standard product (≥ 99%) and test sample (adulterated amount, 4.5%), primers and probes (Shanghai Bailige Synthetic, Shanghai, China), deep-processed food DNA extraction kit (Tiangen Company, Beijing, China), isopropanol and anhydrous ethanol (Tianjin Komiou Chemical Reagent Co., Ltd., Tianjin City, China), ddPCRTM Supermix for Probes (no dUTP), Droplet Generation Oil for Probes, and ddPCRTM Droplet Reader Oil (Bio-Rad, Hercules, CA, USA). After the test sample was obtained, it was crushed with liquid nitrogen before proceeding with the experiment.

2.2 Laboratory apparatus

The following equipment was used: Proteinase K (Tiangen Company), Sigma 1-15pk refrigerated centrifuge (Sigma, Harz, Germany), QX200 ddPCR droplet generator (Bio-Rad), NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), C1000 Touch Thermal Cycler gene amplification Instrument American (Bio-Rad), and the ME204/02 electronic balance (METTLER TOLEDO Instruments (Shanghai) Co., Ltd., Shanghai, China).

2.3 Experimental method

DNA extraction

GMO1 buffer (500 μL) and proteinase K (40 μL, 20 mg/mL) were added to 100 mg test sample, vortexed for 1 min, and incubated at 56 °C for 1 h with shaking every 15 min. Then 200 μL buffer GMO2 was added to the sample, followed by mixing, vortexing for 1 min, incubation at room temperature for 10 min, and centrifugation at 12000 rpm for 5 min. The supernatant was transferred to another centrifuge tube, and these steps were repeated until the supernatant was clear. Then 0.7 times the volume of isopropanol was added to the supernatant and mixed well, followed by centrifugation at 12000 rpm. The supernatant was removed and the precipitate was saved. Next, 700 μL of 70% ethanol was added and the mixture was vortexed for 5 s, centrifuged at 12000 rpm for 2 min. The supernatant was removed, and the ethanol precipitation steps were repeated. After the sample was thoroughly dried, it was eluted with 200 μL elution buffer and vortexed for 1 min to finally obtain the DNA. The amount and purity of the DNA were measured by an ultraviolet spectrophotometer.

2.4 Primer design

The primers and probes were all diluted to 10 μmol/L. The 5' end of the probe sequence for MON89788-P was labeled with fluorescein amidite, the most commonly used fluorescent dye for labeling, and the probe for the soybean endogenous gene lectin-P 5' was labeled with VIC dye (Table 1).

Fluorescence qPCR and digital PCR reaction procedures

The primers and probes used were first tested by fluorescence qPCR using the following reaction parameters: 40 cycles of 94 °C for 10 min, 94 °C for 3 s, and 60 °C for 1 min. Amplification was done in a total volume of 20 μL and included 10 μL of 2 × ddPCR Super Mix, 10 μmol/L (1.2 μL) each of upstream primer and downstream primer, 10 μmol/L (0.4 μL) probe, 4.0 μL DNA template, and ddH2O to 20 μL. Different concentrations of sample template DNA (20 μL) and 70 μL droplet generation oil were used for the dropletization reaction. The generated droplets were added to a 96-well plate. After the PCR reaction, the QX200 Droplet Reader was used to read the generated thermal-cycled droplets. Quantasoft software was used to calculate the final results according to the probability of the Poisson distribution. The droplet digital PCR reaction parameters were: 40 cycles of 94 °C for 10 min, 94 °C for 3 s, and 60 °C for 1 min; and 98 °C for 10 min.

Ratio method to detect the genetically modified soybean content

The genomic DNA of the transgenic test sample was extracted, and the sample was quantitatively analyzed using a digital PCR instrument. The copy numbers of the exogenous transgene

Table 1. Primer probes used in the experiments.

Primer/probe name	Working concentration	Sequence (5'→3')	Probe labeling
MON89788-F	10 μmol/L	TCCCGCTCTAGCGCTTCAAT	FAM-TAMRA
MON89788-R	10 μmol/L	TCGAGCAGGACCTGCAGAA	
MON89788-P	10 μmol/L	CTGAAGGCGGGAAACGACAATCTG	
Lectin-F	10 μmol/L	CCTCCTCGGGAAAGTTACAA	VIC- TAMRA
Lectin-R	10 μmol/L	GGGCATAGAAGGTGAAGTT	
Lectin-P	10 μmol/L	CCCTCGTCTCTTGGTCGCGCCCTCT	

MON89788 and endogenous gene lectin were measured by micro-drop digital PCR. The quantitative result of the transgenic soybean was the copy number of the exogenous transgene MON89788/endogenous gene lectin × 100%.

Linear relationship method to detect the genetically modified soybean content

The extracted genomic DNA of the genetically modified soybean was diluted to 5, 10, 20, 40, 60, 80, and 100 ng/μL. The DNA concentration and copy number were detected by ddPCR at each concentration in triplicate and linearly analyzed. A linear relationship method for the genetically modified soybean was constructed. Using the relationship between the genetically modified soybean DNA content and its DNA copy number, the copy number of the genetically modified component obtained in the experiment was substituted into the formula to calculate the actual content of the genetically modified component. The content of the genetically modified soybean in the experiment was 100 ng/μL, and the calculated genetically modified soybean content/experimental genetically modified soybean content × 100% was the quantitative result of the genetically modified soybean.

Detection of genetically modified samples

The pre-processing, genomic DNA extraction, dilution, and detection of the test samples were the same as the experimental procedures used for the genetically modified soybean standards. The test sample was independently extracted three times, and each extracted template DNA was subjected to three PCR experiments. When performing detection with a droplet digital PCR instrument, the template DNA of the transgenic sample was diluted to 100 ng/μL, and the obtained copy number was substituted into the established linear relationship to obtain the target gene content, after which the amount of genetically modified ingredients was added.

Data analyses

The results obtained were used for chart editing in Excel.

3 Results and discussion

3.1 Extraction and determination of DNA from soybean samples

To ensure the accuracy and precision of the experiment, the soybean sample was accurately weighed, and the DNA template obtained after extraction was measured with NanoDrop 2000. The optical densitometry values were between 1.8 and 2.0, demonstrating that the DNA extraction method used in this experiment was effective.

3.2 Relationship between sample DNA concentration and copy number

The transgenic soybean MON89788 powder standard was extracted for DNA template purification, and the extracted genomic DNA was diluted to 5, 10, 20, 40, 60, 80, and 100 ng/μL. Set three repeats for each gradient. To obtain accurate and reliable results, each gradient template was accurately diluted, and sterile double-distilled water was used as a negative control for digital PCR detection. The detection results showed that the number of droplets in all samples was above 13000, the experimental data are reliable, and the linear relationship was good, with R2 = 0.999. The copy number results are shown in Table 2, the ddPCR gradient detection map is shown in Figure 1.

3.3 Ratio method

The test samples in this study were first tested with fluorescence qPCR. The experimental results showed that the

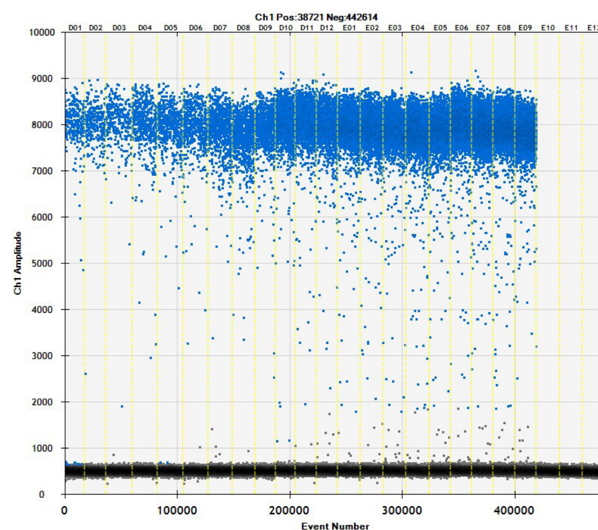

Figure 1. Soybean copy number map under gradient DNA content. D01 to E09 DNA content was 5, 10, 20, 40, 60, 80, and 100 ng/μL; E10–E12 are the ddH2O blank control.

Table 2. Soybean copy numbers under gradient DNA content conditions.

Sample name	ng/μL	Copies/μL #1	#2	#3	Average value (copies/μL)	CV (%)
Genetically modified soybean	5	17.6	18.2	16.6	17.46	4.63
	10	28.5	28.4	29.6	28.83	2.31
	20	49.9	51	54.2	51.7	4.32
	40	108	117	112	112.3	4.01
	60	154	162	151	155.67	3.65
	80	204	204	222	210	4.95
	100	280	256	256	264	5.25

CV: coefficient of variation.

average value of MON89788 soybean exogenous gene was 31.55, and the average value of soybean endogenous lectin gene was 27.78 (Table 3, Figure 2). Digital PCR was used to analyze the copy number of the genetically modified components. Each independently extracted DNA template was subjected to three repeat experiments conducted in triplicate. The average copy number of the MON89788 exogenous gene was 12.49, with a relative standard deviation of 11.19%. The average copy number of the soybean endogenous gene was 212.44, with a relative standard deviation of 8.96%. The droplet digital PCR ratio method uses the exogenous gene/endogenous gene method to measure the droplet number The PCR results showed that the exogenous gene DNA copy number/endogenous gene DNA copy number was 5.88%, with a relative standard deviation of 8.79%. The relative standard deviations of the MON89788 exogenous gene, soybean endogenous gene, and exogenous gene/endogenous gene were all within 15%, indicating the reliability of the experimental data. The specific experimental results are shown in Table 4. Figure 3 is a droplet digital PCR endogenous gene detection map. The endogenous gene map of the test sample was clearly divided between negative and positive droplets, proving that the primers and templates were suitable for this experimental study.

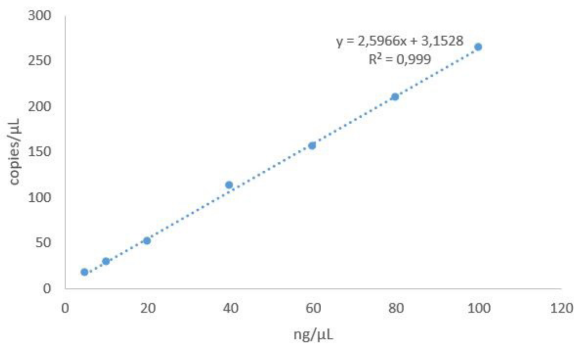

Figure 2. Relationship between DNA content and copy number of transgenic soybeans.

Figure 4 is a droplet digital PCR exogenous gene detection map. G01 to G09 are test samples with obvious negative and positive droplet boundaries. Figure 5 shows the endogenous and exogenous genes. In the droplet-type digital PCR two-dimensional detection map, from the amplification point of view, the reactions in the two channels were well amplified, and the signal value met the analysis requirements.

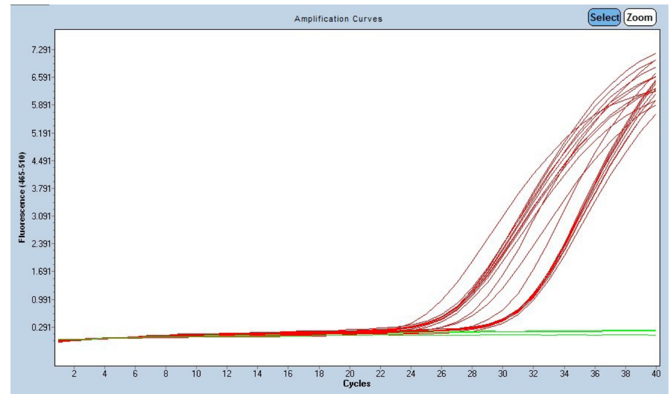

Figure 3. Quantitative fluorescence detection results of test sample.

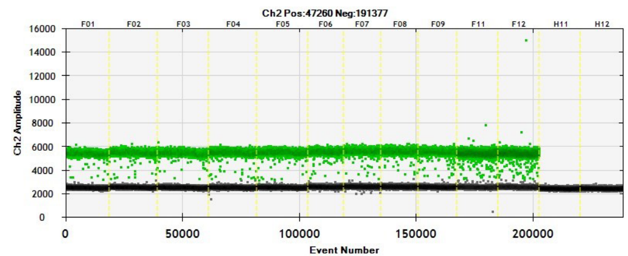

Figure 4. Digital PCR detection map of endogenous gene lectin. F01 to F09 are samples, F11 and F12 are positive controls, and H11 and H12 are ddH2O blank controls.

Table 3. Fluorescence qPCR detection results.

name	Repeat 1			Repeat 2			Repeat 3			Mean	Qualitative results
	1	2	3	1	2	3	1	2	3		
MON89788 foreign gene Ct value	31.23	31.08	31.42	31.55	31.45	31.26	31.77	32.01	32.14	31.55	Transgene positive
Ct value of soybean endogenous gene	27.40	27.39	27.37	27.51	27.52	27.52	28.52	28.39	28.36	27.78	

Table 4. Microbial digital PCR results of transgenic soybeans.

name	Repeat experiment									Mean	RSD
	Repeat 1			Repeat 2			Repeat 3				
	1	2	3	1	2	3	1	2	3		
MON89788 Foreign gene copy number	12.9	12.5	14.6	12.5	14.6	10.7	12.1	11	11.5	12.49	11.19%
Soybean endogenous gene copy number	240	232	238	208	207	194	204	194	195	212.44	8.96%
Exogenous gene/endogenous gene	5.38%	5.39%	6.13%	6.01%	7.05%	5.52%	5.93%	5.67%	5.90%	5.88%	8.79%

RSD: relative standard deviation.

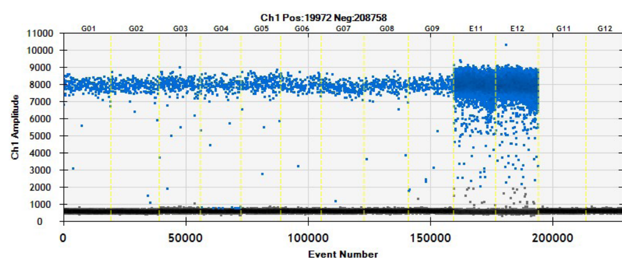

Figure 5. Digital PCR detection map of foreign gene MON89788. G01 to G09 are samples, E11 and E12 are positive controls, and G11 and G12 are ddH2O blank controls.

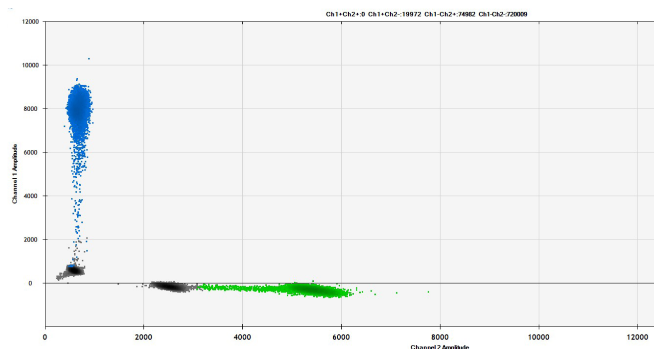

Figure 6. Digital PCR two-dimensional detection map of endogenous and exogenous genes.

3.4 Linear relation method

In this paper, through establishment of the linear relationship between the DNA of different concentration gradients of genetically modified soybean (≥ 99%) and its copy number, the genetically modified content of the test sample was calculated from the copy number. Figure 6 shows the DNA concentration of the genetically modified soybean standard and its copy number. The linear relationship of the copy number was y = 2.5967x + 3.1437, and R2 was 0.999 (x is the DNA concentration of genetically modified soybean, and y is the corresponding DNA copy number). The tested genetically modified soybean samples were quantitatively detected. Within the established linear range, the establishment of the linear relationship method accurately quantified the soybean genetically modified components. The DNA concentration of soybean samples was 100 ng/μL, and the copy number of the genetically modified soybeans detected by the droplet digital PCR method was 12.49 copies/μL. According to the relationship established in this paper, y = 12.49 as calculated to obtain the genetically modified soybean DNA concentration x as 3.599 ng/μL, calculated by detecting the content of genetically modified soybeans (100 ng/μL) and the content of genetically modified soybeans calculated by the formula (3.599 ng/μL); that is, the actual amount of genetically modified soybeans added was 3.599%. The droplet digital relational method gets rid of the influence of the reference gene and amplification efficiency, and it is more accurate.

3.5 Comparison between ratio method and relational method

The establishment of the above two methods show that in samples containing the same 4.5% transgene, the result of the droplet digital PCR ratio method was 5.88%. The result of droplet digital PCR ratio method, although within the detection range, lacked accuracy. The result of the method of constructing the relationship between DNA content and copy number was 3.599%. This method establishes a formula for the copy number and DNA content of genetically modified soybeans to obtain the actual target gene content. Comparing the experimental results of the two methods, the experimental results of the linear relationship method were more accurate. The droplet digital PCR ratio method does not construct a standard curve and uses the ratio method to calculate. The experimental results ignore the influence of different primers' amplification efficiency. However, the constructed droplet digital PCR relational method does not depend on the cycle threshold. The result obtained in the case of amplification efficiency was more accurate than the droplet digital PCR ratio method.

4 Conclusion

The four most widely planted genetically modified crops worldwide are soybeans, cotton, rapeseed, and corn. The soybean imports in China exceeded 71 million tons in 2014. The current status of China for genetically modified soybeans is to allow imports. These genetically modified soybean raw materials are gradually entering people's tables. This requires the establishment and improvement of market access systems and genetically modified food-related labeling systems for genetically modified crops. Therefore, relevant testing technologies need to be established. In this study, the extracted sample DNA was uniformly diluted to 100 ng/μL for testing. The droplet digital PCR detection technology processed the sample to be tested into droplets and distributed it to a large number of independent reaction chambers for PCR amplification. Finally, the number of molecules in each amplification reaction was analyzed to obtain the number of copies required for the experiment. Digital PCR is developed on the basis of the original PCR detection technology. It does not rely on cycle thresholds and can achieve absolute quantification of samples. Establish the relationship curve between the DNA content of soybean components and its copy number, where R2 is 0.999. Compared with the droplet digital PCR ratio method, the detection result of the droplet digital PCR linear relationship method is more accurate, and the results are more reliable. It can better explain the actual genetic modification content. The result of the droplet digital PCR ratio method was 5.88%. The result of droplet digital PCR linear relationship method was 3.599%. In the case of 4.5% of the same amount of transgene added, the results obtained by the droplet digital PCR linear relationship method were more reliable and could accurately simulate the amount of transgene added. Therefore, the droplet digital PCR linear relationship method is more accurate than the droplet digital PCR ratio method, and has great market application value for the actual detection of genetically modified soybean MON89788 ingredients. It provides a technical means for the market supervision of the detection of genetically modified soybeans.

References

Bharuthram, A., Paximadis, M., Picton, A. C., & Tiemessen, C. T. (2014). Comparison of a quantitative Real-Time PCR assay and droplet digital PCR for copy number analysis of the CCL4Lgenes. *Infection, Genetics and Evolution*, 25(7), 28-35. http://dx.doi.org/10.1016/j.meegid.2014.03.028. PMid:24727646.

Bhat, S., Polanowski, A. M., Double, M. C., Jarman, S. N., & Emslie, K. R. (2012). The effect of input DNA copy number on genotype call and characterising SNP markers in the humpback whale genome using a nanofluidic array. *PLoS One*, 7(6), e39181. http://dx.doi.org/10.1371/journal.pone.0039181. PMid:22745712.

Blaya, J., Lloret, E., Santísima-Trinidad, A. B., Ros, M., & Pascual, J. A. (2016). Molecular methods (digital PCR and real-time PCR) for the quantification of low copy DNA of Phytophthora nicotianae in environmental samples. *Pest Management Science*, 72(4), 747-753. http://dx.doi.org/10.1002/ps.4048. PMid:26012497.

Cai, J., Hui, L. I., Long, H. M., Yang, F. U., Wang, L., & Wang, L. (2016). Research progress in techniques for the detection and analysis of genetically modified ingredients. *Journal of Food Safety & Quality*, 7(2), 706-714.

Cao, J. J., Xu, J. Y., Cao, D. M., Zhang, P. Q., Luan, F. X., & Liu, Y. (2014). Detection of genetically modified wheat B73-6-1, B72-8-11b and B102-1-2 strains by real-time fluorescent polymerase chain reaction. *Shipin Kexue*, 8, 156-159.

Clive, J. (2015). Development trend of global biotechnology/GM crop commercialization in 2014. *Chinese Journal of Bioengineering*, 34(1), 1-14.

Deng, P. J., Zhao, J., Liu, J. J., & Fang, S. (2002). Study on the identification of genomic modified foods. *Wei Sheng Yen Chiu*, 31(1), 37-40. PMid:12561571.

Feng, K., Wenzhong, H. U., Jiang, A., Sarengaowa, Yongping, X. U., & Qi, S. I. (2018). Multiplex PCR detection technology for four pathogenic microorganisms in fresh-cut fruits and vegetables. *Shipin Kexue*, 39(6), 276-283.

Fraiture, M. A., Herman, P., Taverniers, I., De Loose, M., Deforce, D., & Roosens, N. H. (2015). Current and new approaches in GMO detection: challenges and solutions. *BioMed Research International*, 2015, 392872. http://dx.doi.org/10.1155/2015/392872. PMid:26550567.

International Service for the Acquisition of Agri-biotech Applications – ISAAA. (2019). *GM approval database*. Retrieved from http://www.isaaa.org/gmapprovaldatabase/

Köppel, R., van Velsen, F., Felderer, N., & Bucher, T. (2012). Multiplex real-time PCR for the detection and quantification of DNA from four transgenic soy Mon89788, A5547-127, Roundup Ready, A2704-12 and lectin. *European Food Research and Technology*, 235(1), 23-28. http://dx.doi.org/10.1007/s00217-012-1730-y.

Li, J., Xiao, Q., Zhou, Z., & Geng, Y. (2012). The status quo of the industrialization of genetically modified crops and my country's development strategies. *Issues in Agricultural Economics*, 6(1), 23-28.

Liu, X., Zhang, G., Zhou, X., Zhu, C., & Huang, M. (2015). Establishment of real-time fluorescent PCR detection method for genetically modified soybean MON89788. *Shipin Kexue*, 36(4), 193-197.

Rodríguez, M. A., Garcia, T., González, L., Hernández, P. E., & Martín, R. (2005). TaqMan real-time PCR for the detection and quantitation of pork in meat mixtures. *Meat Science*, 70(1), 113-120. http://dx.doi.org/10.1016/j.meatsci.2004.12.005. PMid:22063287.

Shen, P., Wu, Y., Liang, J., Lu, X., Zhang, Q., & Wang, H. (2017). Overview of the development and application of genetically modified crops. *Chinese Journal of Bioengineering*, 37(1), 119-128.

Strain, M. C., Lada, S. M., Luong, T., Rought, S. E., Gianella, S., Terry, V. H., Spina, C. A., Woelk, C. H., & Richman, D. D. (2013). Highly precise measurement of HIV DNA by Droplet digital PCR. *PLoS One*, 8(4), e55943. http://dx.doi.org/10.1371/journal.pone.0055943. PMid:23573183.

Tan, T., & Chen, C. (2014). Development path and strategy of the industrialization of genetically modified crops in my country. *Agricultural Technology and Economics*, 1, 22-30. http://dx.doi.org/10.13246/j.cnki.jae.2014.01.003.

Wang, H. Q., Chen, R., Xia-Ying, L. I., Wang, M. Y., & Liu, P. C. (2018). Research progress on the testing technologies for composition in genetically modified products. *Biotechnology Bulletin*, 34(3), 31-38.

Wang, S., Li, Z., & Miao, L. (2015). Comparison of microsphere digital PCR and real-time fluorescence PCR for detection mutton-derived and porcine-derived ingredients in mutton products. *Meat Industry*, 7, 38-41.

Wu, Y., Li, J., Li, X., Zhai, S., Gao, H., Li, Y., Zhang, X., & Wu, G. (2019). Development and strategy of reference materials for the DNA-based detection of genetically modified organisms. *Analytical and Bioanalytical Chemistry*, 411(9), 1729-1744. http://dx.doi.org/10.1007/s00216-019-01576-w. PMid:30707265.

Yu, X. F. (2017). *Fluorescence PCR and digital PCR detection of genetically modified soybeans, genetically modified potatoes and Enterobacter sakazakii* (Master's thesis). Qingdao University, Qingdao.

Yu, X., Gao, H., Xiao, X., & Li, R. (2016). Detection of genetically modified DAS-44406-6 soybean line using fluorescent PCR and digital PCR. *Shipin Kexue*, 37(16), 235-241.

Zhang, Y., Tang, E. T., & Du, Z. (2016). Detection of MET Gene Copy Number in Cancer Samples Using the Droplet Digital PCR Method. *PLoS One*, 11(1), e0146784. http://dx.doi.org/10.1371/journal.pone.0146784. PMid:26765781.

Zhou, T., Li, J., Li, J., Qiao, X., Huang, X., Chen, X., Yang, J., & Guo, Y. (2017). A method for identifying adulteration of meat and meat products based on multiple real-time fluorescent polymerase chain reaction melting curve analysis. *Shipin Kexue*, 38(12), 217-222.

Zhou, X., Zhu, G., Li, X., Wang, Y., & Liu, X. (2010). Principles and applications of real-time fluorescent quantitative PCR technology. *China Animal Husbandry and Veterinary Medicine*, 37(2), 87-89.

Zhou, Y. L., (2014). *Development and application of reference materials for genetically modified organisms*. Beijing: China Quality Inspection Press.