Rapid detection of genetically modified products based on CRISPR-Cas12a combined with recombinase polymerase amplification

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A B S T R A C T

With the large-scale planting of genetically modified (GM) crops, consumers were more aware of biosafety. Onsite rapid diagnostic methods were advantageous to the regulation of GM products. In this study, a rapid, sensitive and portable detection method based on recombinase polymerase amplification were proposed based on RPA reaction and Cas12a cleavage reaction for GM ingredients, named RPA-Cas12a-GM. The results would be displayed by fluorescence signal (FS) and visual bands of lateral flow strip (LFS). RPA-Cas12a-GM method could be completed within 45 min, and the detection limit was as low as 45 copies/μL of the standard plasmid containing CP4-EPSPS gene and Cry1Ab/Ac gene. Furthermore, the detection coincidence rate of RPA-Cas12a-GM method was 100%. In conclusion, the proposed RPA-Cas12a-GM method based FS and LFS were sensitive, specific, rapid and visible for diagnosis of CP4-EPSPS gene and Cry1Ab/Ac gene without complex equipment, which provides technical support for the regulation of GM products in the field.

1. Introduction

In 2019, 2.7 billion hectares of genetically modified (GM) crops were planted worldwide, which represents an increase of approximately 112 folds the amount planted in 1996 (ISAAA, 2019). The proportion of GM crops insect stacked resistance/herbicide tolerance (IR/HT) traits accounted for 45% of the global GM crop area, making stacked IR/HT traits the most important traits in GM crops (ISAAA, 2019). However, issues related to the public and environmental safety of life and the environment, such as whether GM crops induce toxic effects, induce genetic drift, cause allergies, create antibiotic resistance, and damage ecosystem, have been highly controversial and are not yet conclusive (Muzhinji and Ntuli, 2021). Different countries and regions have adopted different measures to regulate GM crops (Simbo and Maredia, 2016). GM regulatory laws have been implemented in the United States since 2016 (Bovay and Alston, 2018). The minimum labelling threshold set by the EU is 0.9% (Castellari et al., 2018). China has implemented the most stringent, catalog-based, mandatory labeling management (Li and Li, 2017). The development of detection methods is very important to the safety and supervision of GM crops.

The classical detection methods are DNA-based polymerase chain reaction (PCR) (Bogozalec Kostir et al., 2019) and protein-based enzyme-linked immunosorbent assays (ELISA) (Salisu et al., 2017). Nucleic acid-based methods have gradually become the main means of GM crop detection for the high sensitivity, wide range of applications, and simple operation (Mukama et al., 2020). The most commonly used methods for GMO nucleic acid detection was traditional PCR and real-time PCR (qPCR) with a detection limit of 0.05% (Chhaliyil et al., 2020). However, PCR-based methods rely on a variety of instruments and equipment, and have high requirements for skilled operators and laboratory
conditions, making it difficult to be implemented in resource-limited scenarios (Huang et al., 2020). Diagnostics based on recombinase polymerase isothermal amplification (RPA) has emerged as a convincing alternative to conventional PCR protocols (Yoo et al., 2021), because these methods can be conducted at a constant temperature near 37–42 °C using a single-temperature heat source (e.g. water bath) without commercial thermocyclers (Mai et al., 2021). The RPA reaction could be performed at 37 °C and be easily implemented in an outdoor environment.

Recently, CRISPR/Cas-based nucleic acid detection system has been developed (Chen et al., 2018; Ge et al., 2021; Gootenberg et al., 2018). CRISPR-Cas12a can identify and cleavage the target DNA under the guidance of a crRNA to form a 5’ sticky end (Niu et al., 2021), and then non-specific ssDNA can also be trans-cleaved by Cas12a (Nguyen et al., 2020). For that the trans-cleavage activity of Cas12a can only be activated in the presence of crRNA bound to the targeted DNA, ssDNA can be cleverly designed as signal-reporting probes to present results in different ways (Chen et al., 2018). Both RPA and Cas12a trans-cleavage reaction can be conducted at a constant temperature of 37 °C, making them suitable methods for rapid detection in the field. There is an urgent need to establish rapid, sensitive, portable, low-cost, and easy-to-operate methods for the detection of GM crops to guarantee the implementation of legislation (Gao et al., 2019; Wang et al., 2020). The efficient integration of RPA with crRNA-guided CRISPR/Cas12a system has shown great promise in the exploitation of next-generation molecular diagnostics technology due to its high reliability, sensitivity and specificity (Li et al., 2021; Ramachandran et al., 2021).

In this study, RPA and CRISPR/Cas12a-based assays were used in combination to construct a novel lateral flow strip method for transgene detection, named RPA-Cas12a-GM as shown in Fig. 1, which would be evaluated by comparison with the related RPA-AG and RPA-Cas12a-FS fluorescent assay. The CP4-EPSPS and the Cry1Ab/Ac, which has been widely employed in most insect-resistant transgenic crops and glyphosate-resistant crops, were important indicators of the presence of transgenic components and were selected as targets in this strategy. For RPA-Cas12a-GM, non-specific single-stranded reporters labeled with biotin and fluorescein isothiocyanate isomer (FTTC) were designed by Primer Premier 5.0 software (Premier Biosoft, San Francisco, CA, USA) according to the design principles. For CP4-EPSPS and Cry1Ab/Ag gene, 5 pairs of RPA primers were synthesized by Sangon Biotech (Shanghai, China). The synthesis of oligonucleotides and standard plasmid molecules, and the sequencing of amplified products were completed by Sangon Biotech (Shanghai, China).

GM corn, alfalfa, rapeseed, soybeans, and sugar beets were purchased from Institute for Reference Materials and Measurements (IRMm, Geel, Belgium) and American Oil Chemists Society (AOCs, Urbana, IL, USA). Transgenic cotton and non-GM corn and soybeans were provided by the crop Ecology and Environmental Safety Monitoring Center of the Ministry of Agriculture of the people's Republic of China. The materials were listed in Table S1.

2.2. Primer design and RPA assay

Online BLAST was used to compare the homology of candidate sequences with other species for endogenous genes of different lengths of the inserted genes. And sequence alignment analysis of insect-resistant or herbicide-tolerant genes was carried out using DNAMAN software (version 6.0, Lynnon, USA). After that, primers for RPA assay were designed by Primer Premier 5.0 software (Premier Biosoft, San Francisco, CA, USA) according to the design principles. For CP4-EPSPS and Cry1Ab/Ag gene, 5 pairs of RPA primers were synthesized by Sangon Biotech (Shanghai, China), respectively. All primer sequences were listed in Table 1, and optimal primers were further screened according to the amplified products visualized on a 4% agarose gel.

![Fig. 1. The locations of the primers and crRNA on the flanking sequence containing CP4-EPSPS and Cry1Ab/Ag. B: Schematic diagram of the RPA-Cas12a-LFA method.](image-url)
The RPA assay was performed using the TwistAmp basic kit (TwistDX, Cambridge, UK). The 50 μL mixture of RPA reaction consisted of 29.5 μL of RPA buffer, 2.4 μL of forward and reverse primer, 11.2 μL of ddH₂O, 2.5 μL of MgOAc and 2 μL of DNA template. Among them, MgOAc was first added to the lids of the tubes and finally fused with the other components by mixing and centrifugation. The RPA reaction was conducted at 39 °C on a constant temperature amplifier. The RPA products were subsequently purified by phenol-chloroform and then analyzed by agarose gel electrophoresis.

2.3. crRNA design and screening

According to the characteristics of the LbCas12a crRNA recognition target sequence of Cas12a protein, crRNA recognizes PAM sites rich in T at the 5′ end. Three pairs of specific crRNA primers were designed within the RPA amplifiers of CP4-EPSPS and Cry1Ab/Ac genes, as shown in Table S1 and Fig. 1 A. The report probe HEX-12NT-BHQ1 was designed based on the characteristic of Lb Cas12a crRNA recognition and synthesized by Sangon Biotech (Shanghai, China). The sequence is shown in Table 2.

Based on the characteristic of Lb Cpfl (Cas12a) to recognize the T-rich PAM sequence at the 5′ end, three crRNAs targeting the RPA products of CP4-EPSPS and Cry1Ab/Ac genes were designed. Double-labeled ssDNA probes were specially designed as signal reporters, with the HEX-12NT-BHQ1 probe for the fluorescent detection system and the FITC-8NT-Biotin probe for the lateral flow strip assay.

2.4. Cas12a digestion reaction and RPA-Cas12a-GM assays

In general, the detection process of RPA-Cas12a-LFB, as shown in Fig. 1 B, includes rapid DNA extraction, RPA reaction, Cas12a cleavage and test strip assay. The Cas12a cleavage system consisted of 12.5 μL DEPC water, 2 μL buffer, 1 μL Cas12a enzyme, 1 μL crRNA (5 μM), 0.5 μL RNA enzyme inhibitor, 1 μL ssDNA (10 μM), and 2 μL RPA product with a total volume of 20 μL. The Cas12a cleavage reaction was performed at 37 °C for 45 min.

Both fluorescence detection method and lateral flow strip analysis were employed for the detection of GM crops in the RPA-Cas12a-GM strategy. Different reporter probes were employed in the system to observe the results by different means. For fluorescence detection, the cleavage products containing the HEX-12NT-BHQ1 probes were 10-fold diluted and then used for fluorescence signal acquisition on a microplate reader. Identification of transgenes can be performed by comparing the fluorescence signal of negative and positive samples. For test strip analysis, 5 μL of the product was pipetted to the sample pad of the test strip, and the lateral flow assay was performed in 70 μL of running buffer. And the results could be interpreted visually by observing whether there was a distinct T line.

2.5. Specificity validation of RPA-Cas12a-GM

To evaluate the specificity of the RPA-Cas12a-GM system, pima cotton MON89913 containing CP4-EPSPS gene, maize BT-11 containing Cry1Ab/Ac gene, maize A2704-12 and TC1507 carrying PAT gene, oilseed rape MS, oilseed rape RF1, as well as different GM compositions without CP4-EPSPS gene and Cry1Ab/Ac gene were employed. At the same time, different samples of different GM compositions were mixed in a mass ratio of 1:1:1 to investigate the analytical capacity of the system. The specificity of the RPA-Cas12a-GM system was evaluated by

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**Table 1**

RPA primers, crRNA sequences, and reporter ssDNA sequences.

| Primer name   | Sequences (5′-3′) | Amplification length |
|---------------|-------------------|----------------------|
| RPA-Cpfl-F1   | CCAATCCACTACAGGTGTCATGGTTTCCCCGCTTCGACTACA | 372 μL |
| RPA-Cpfl-R1   | CAGCATCGTCTGTTACAGGGTTAGTGCTTGGACACGACTACA | 372 μL |
| RPA-Cpfl-F2   | CCAACCGTTCACAGGGTTAGTGCTTGGACACGACTACA | 372 μL |
| RPA-Cpfl-R2   | CCAACCGTTCACAGGGTTAGTGCTTGGACACGACTACA | 372 μL |
| RPA-Cpfl-F3   | CCAACCGTTCACAGGGTTAGTGCTTGGACACGACTACA | 372 μL |
| RPA-Cpfl-R4   | CCAACCGTTCACAGGGTTAGTGCTTGGACACGACTACA | 372 μL |

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**Table 2**

Comparison of nucleic acid detection methods.

| Analytical method      | Detection target | Sensitivity | Time | Reference                     |
|------------------------|------------------|-------------|------|------------------------------|
| PCR                    | Pst, Bgl, Cry1Ab/Ac, F-3′, T-NOS, CTP2-EPSPS | 0.05% | 1.5 h | Deboe et al. (2018)          |
| Digital PCR            | MON87705, MON8769, DFP36049 | 0.05% | 1.5 h | Koeppel et al. (2015)        |
| LAMP                   | CP4-EPSPS        | 0.5% | 1 h  | Rong et al. (2018)           |
| ELISA                  | Cry1             | 15 ng/mL | 2 h  | Dong et al. (2018)           |
| Electrochemical immunoassay | CP4-EPSPS| 0.72 pg/ml | 2 h  | Zhang et al. (2018)          |
| RPA-Cas12a-GM          | CP4-EPSPS, Cry1Ab/Ac | 45 copies | 45 min | This study                  |

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the analysis of fluorescence values as well as the T-line of the test strips.

2.6. Sensitivity assay of RPA-Cas12a-GM

The RPA products of CP4-EPSPS and Cry1Ab/Ac genes were cloned into pESI-T vectors to construct standard plasmids, respectively. Plasmids were quantified and diluted in 10-fold gradients, and the sensitivity assay of the RPA-Cas12a-GM system was carried out with different plasmid concentrations.

2.7. Sample application of RPA-Cas12a-GM

The practical application performance was one of the important indicators for method evaluation. Genomic DNA from 13 GM crops was rapidly extracted with DNA rapid release agents and subsequently used in RPA reactions. Cas12a cleavage assays were performed by targeting the RPA products of the samples with different probes for fluorescence detection and lateral flow strip analysis, respectively.

2.8. Result determination and data analysis

For this method, the test result is invalid when the C line is not visible on the test strip. In the presence of a C line, a T line that would clearly distinguish from a blank control is a sign of a positive result. To analyze the results, all strips were photographed, and Image J software was used to read the peak value and generate peak areas.

Each experiment was performed in triplicate. Data are expressed as the mean ± standard (SD) after analysis by GraphPad Prism software version 7.0 (GraphPad, CA, USA). Statistically significant differences at P < 0.05 were performed using the one-way analysis of variance (ANOVA), followed by a t-test on SPSS 23.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. RPA primer screening

The highly conserved sequences of herbicide-tolerant gene (CP4-EPSPS) and insect resistant gene (Cry1Ab/Ac) were used to design RPA primers. The five pairs of RPA primers designed were screened by agarose gel electrophoresis of the amplification products. Different specific bands from 140 bp to 206 bp were exhibited in Fig. S1, while there were no non-specific bands in the negative control. The amplified bands of primer 2, 3, and 4 (2F-2R, 3F-3R and 4F-4R) in Fig. S1 A were clear, and the bands of primer 4 were significantly brighter. In Fig. S1 B, the amplified band of primer 4 was brightest. Therefore, for both genes, the fourth primer pair was optimal and was used for subsequent amplification assays.

3.2. Screening of crRNA

Standard plasmids containing CP4-EPSPS and Cry1Ab/Ac genes were used for the screening of the two crRNAs, respectively. Firstly, a large number of amplification products containing the target genes were obtained by RPA reaction. And whether the crRNA works or not was determined by comparing the collected fluorescence signals and visible bands on T-line of positive samples with the blank control. As can be seen in Fig. 2 A, the fluorescence signal of the positive samples was significantly different from the blank control (P < 0.0001) and there was a clear T line of the test strip when CP4-crRNA-1 was employed. For the plasmid containing Cry1Ab/Ac gene, strong fluorescent signals and clear T bands of the test strip could be detected in the system with all three crRNAs, in which the signal of Cry-crRNA-2 was the strongest (Fig. 2 B). Therefore, CP4-crRNA-1 and Cry-crRNA-2 were adopted for the detection of the CP4-EPSPS gene and Cry1Ab/Ac gene, respectively.

![Fig. 2. Results of crRNA screening. A: crRNA screening for CP4-EPSPS gene; A1: Fluorescence results with HEX-12NT-BHQ1 probe; A2: Results of test strips with FITC-8NT-Biotin probe; 1: CP4-crRNA-1; 2: CP4-crRNA-2; 3: CP4-crRNA-3. B: crRNA screening for Cry1Ab/Ac gene; B1: Fluorescence results with HEX-12NT-BHQ1 probe; B2: Results of test strips with FITC-8NT-Biotin probe; 1: Cry-crRNA-1; 2: Cry-crRNA-2; 3: Cry-crRNA-3. NTC: No template control. Note: **** extreme difference (P < 0.0001).](image-url)

3.3. Specificity of the RPA-Cas12a-GM

Ten transgenic and non-transgenic crops were analyzed separately with the RPA-Cas12a-GM system. The results showed that significant fluorescence signals and clear T-lines of the test strips were only produced when the products contained CP4-EPSPS or Cry1Ab/Ac gene, while the negative control and non-GM products couldn’t. And the fluorescence signal could still be detected by mixing the target and non-target GM crops in the same mass ratio of 1:1:1 (Fig. 3 A-2, A-3, B-2, and B-3). All results showed that positive samples of MON88913 containing CP4-EPSPS gene, Bt11 containing Cry1Ab/Ac gene and their corresponding mixed samples displayed positive signals, while other genomes exhibited negative signals, indicating good specificity of the RPA-Cas12a-GM system (Fig. 3).

3.4. Sensitivity of the RPA-Cas12a-GM

Diluted standard plasmid molecules in gradient were used for sensitivity assays of the RPA-Cas12a-GM system. The results showed that the target bands could only be observed in the lanes at concentrations above 450 copies/μL for the CP4-EPSPS plasmid and 45 copies/μL for the Cry1Ab/Ac plasmid, indicating that the detection capability of the RPA-AGE (agarose gel electrophoresis of RPA products) method was 45–450 copies/μL (Figs. 3 A-4 and Fig. 3 B-4). For the RPA-Cas12a-GM system, the fluorescence value and the T-band intensity of the test strip for the plasmid at 45 copies/μL were significantly distinguished from the blank control for both CP4-EPSPS and Cry1Ab/Ac genes, while the plasmids at 4.5 copies/μL could not be detected (Figs. 3 A-5, A-6, B-5, and B-6). Therefore, the detection limit of the RPA-Cas12a-GM system for CP4-EPSPS and Cry1Ab/Ac plasmids was 4.5 copies/μL with strong detection signals.

3.5. Analysis of GM crops

Common GM crops containing the CP4-EPSPS and Cry1Ab/Ac genes were applied to validate the practical applicability of the RPA-Cas12a-GM system. The results in Fig. 4 showed that for all GM crops containing target genes, strong fluorescent signals were collected and the corresponding test strips presented clear C lines. All detection results were consistent with the current issued standard method and the RPA-AGE method, indicating the accuracy and practicality of the RPA-Cas12a-
system in China (Elke and Neumann, 2019). At present, there were many technical support for the implementation of the GM product labeling. The technology was an important tool for GM market regulation and provided urgent to strengthen the safety regulation of GMOs. Detection technology was more time-saving than PCR and Digital PCR methods and more sensitive than RPA and LAMP methods. Over the years, electrochemical biosensors have been rapidly developed and applied to protein and nucleic acid detection. However, the sensors were equipment dependent, and it was more time-consuming to perform the analysis in outdoor environments. By comparison and analysis, the RPA-Cas12a-GM system outperformed most other amplification-based methods (Fu et al., 2011). However, PCR-based methods required a long reaction time, expensive equipment, and trained operators, which couldn’t meet the demand for rapid on-site detection. LAMP, RPA, and other isothermal amplification technologies could be performed under constant temperature conditions with high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. However, PCR-based methods required a long reaction time, expensive equipment, and trained operators, which couldn’t meet the demand for rapid on-site detection. LAMP, RPA, and other isothermal amplification technologies could be performed under constant temperature conditions with high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to high sensitivity of the system.

In this study, the designed crRNA could specifically activate the trans-cleavage activity of Cas12a when target DNA exists (Aman et al., 2020; Zhang et al., 2020). The RPA-Cas12a-GM system exhibited good stability, strong practicability, and efficient amplification of target sequences, and the feasibility and reliability of the method were verified for the detection of GM crops. Although visible bands were present in the T lines of the negative samples, they were significantly weaker compared to those of the positive samples. And as can be seen in Fig. 3 B and Fig. 3 D, the T lines of the plasmids with 45 copies were also very obvious and could be clearly distinguished from the negative ones, indicating the high sensitivity of the system.

Compared with other methods described in Table 2, the proposed RPA-Cas12a-GM system shows significant advantages in terms of detection time and analytical sensitivity. The RPA-Cas12a-GM system was more time-saving than PCR and Digital PCR methods and more sensitive than RPA and LAMP methods. Over the years, electrochemical biosensors have been rapidly developed and applied to protein and nucleic acid detection. However, the sensors were equipment dependent and inconvenient to be performed in outdoor environments. By comparison and analysis, the RPA-Cas12a-GM system outperformed most protein-based assays in terms of detection sensitivity. In addition, the detection methods for GM crops, including protein-based technologies and nucleic acid-based technologies. Although immunochromatographic lateral flow strip methods (Zeng et al., 2021) and nano-enhanced ELISA techniques (Jia et al., 2009) were common assays, protein-based methods were generally less sensitive than nucleic acid amplification-based methods (Fu et al., 2011). However, PCR-based methods required a long reaction time, expensive equipment, and trained operators, which couldn’t meet the demand for rapid on-site detection. LAMP, RPA, and other isothermal amplification technologies could be performed under constant temperature conditions with high specificity. These methods compensated for the deficiencies of PCR method and enabled on-site testing. RPA technology was more suitable for the analysis of real samples than LAMP technology owing to non-specific amplification of LAMP method (Zhang et al., 2014). By combining with the Cas12a cleavage reaction, the RPA-Cas12a system could be applied for rapid and highly sensitive detection of nucleic acid molecules at a constant temperature (Chen et al., 2018).

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proposed strip method has a shorter detection time and better portability than immunosensor-related methods, making it more suitable for on-site testing. In general, compared with the existing methods, the RPA-Cas12a-GM system provides a better tool to realize highly sensitive in-field detection of GM crops.

5. Conclusion

In summary, a new strategy (named RPA-Cas12a-GM) for rapid GM crops detection with high sensitivity, specificity, visualization, and device independence has been successfully constructed. Targeting herbicide-tolerant gene and insect-resistant gene, a low concentration of 45 copies of the standard plasmid could be detected with the novel approach. The system could be conducted in 45 min with the advantages of being rapid, high sensitivity and specificity, as well as easy to operate, which provided technical support for safety regulation of GM crops.

CRediT authorship contribution statement

Jinbin Wang: Conceptualization, Methodology, Writing – review & editing. Xiawan Hu: Data curation, Writing – original draft. Yu Wang: Data curation, Writing – original draft. Haijuan Zeng: Visualization, Investigation. Xiaofeng Liu: Software, Validation. Hua Liu: Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.11.009.

References

Aman, R., Mahas, A., Mansic, T., Hassan, N., Mahfouz, M.M., 2020. Efficient, rapid, and sensitive detection of plant RNA viruses with one-pot RT-RTA-CRISPR/cas12a assay. Front. Microbiol. 11.

Bogotażek Kortis, A., Demtar, T., Stebih, D., Zel, J., Milavec, M., 2019. Digital PCR as an effective tool for GMO quantification in complex matrices. Food Chem. 294, 73–78.

Bovay, J., Alston, J.M., 2018. GMO food labels in the United States: economic interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

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Declaration of competing interest

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Data availability

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.11.009.

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