Absolute quantitative detection of genetically modified soybean MON87708×MON89788 with stacked traits by digital polymerase chain reaction

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A B S T R A C T

The main advantage of digital PCR (dPCR) is that it facilitates absolute quantification of the target without reference to the standard/calibration curve. Crystal droplet dPCR has a three-color staining detection function, which enables multiplex PCR reaction. In this study, this technique was used to establish triple dPCR detection for the genetically modified soybean MON87708 × MON89788 with stacked traits. Specific absolute quantitative detection was accomplished for the genomic DNA extracted from the homogenized seeds of GM stack MON87708 × MON89788 soybean. Our results can serve as a reference for the absolute quantitative detection of stacked events of genetically modified crops.

1. Introduction

It has been 24 years since the commercialization of genetically modified organisms (GMOs) (Hinchee et al., 1988). At the core of modern biotechnology, transgenic technology plays an important role in ensuring food security, protecting ecological security, and expanding agricultural functions. However, at the same time, it also raises potential security issues. GMOs may have potential toxic, allergic, or other harmful effects on humans and animals, and they may also produce “gene drift” and “gene pollution” phenomenon, which in turn can affect the ecological environment (Gao et al., 2020; 2011a and 2011b). Therefore, several countries have established corresponding laws and regulations to regulate GMOs and their products.

However, with the increasing demand for agricultural development, genetically modified crops with a single trait cannot meet the needs of modern agriculture, and hence, genetically modified crops with stacked traits have been developed. According to the official statistical report of the International Service for the Acquisition of Agri-biotech Applications (ISAAA) (ISAAA 2019), the area planted with genetically modified crops with stacked traits was 80.514 million ha in 2018, which accounts for 42% of the global area planted with genetically modified crops. Genetically modified crops with stacked traits show promising applications by combining traditional breeding methods with modern genetic breeding to develop multi-trait crops. Nevertheless, as an emerging technology, it poses potential risks to the environment and human health, and the safety aspects of genetically modified crops have always been a major concern for the public. Unlike genetically modified crops with a single trait, those with stacked traits involve multiple genes and thus require higher safety evaluation standards. Indeed, objective evaluation and testing of genetically modified crops have become the basis for the safety management of such crops (Liu et al., 2016).

Over the past two decades, several methods of GMO detection and quantification have been developed, of which, polymerase chain reaction (PCR) is the most widely used one (Demeke and Dobnik 2018). Digital PCR (dPCR) is regarded as the third-generation PCR (Miraglia et al., 2004; Holst-Jensen et al., 2012). The most significant advantage of dPCR is that it can perform absolute quantification of the target without reference to the standard/calibration curve. Therefore, the effect of matrix differences between standards and test samples can be effectively minimized (Bhat et al., 2009; Corbisier et al., 2010; Morisset et al., 2013). Based on the principle of a high level of sample assignment, dPCR can yield highly accurate results even with a low copy number of targets (Bhat et al., 2009; Corbisier et al., 2010), thereby ensuring reliable detection of the target genes in the background of a high concentration of non-target DNA (Whale et al., 2013).

dPCR systems for GMO detection can be categorized into droplet dPCR and chip-based dPCR (Baker 2012). Stilla's Naica system provides a combination of the droplet and chip-based technology platforms and is

Table 1
Samples and sources.

Serial number	Event	Code	Species	Source
1	MON87708 × MON89788	–	soybean	Positive samples kept in this laboratory
2	MON87708	0311-A	soybean	AOCS
3	MON89788	0906-B2	soybean	AOCS
4	DAS 68416-4	BF432d	soybean	IRMM
5	FG72	0610-A4	soybean	AOCS
6	GTS-40-3-2	BF410ep	soybean	IRMM
7	MON87751	0215-A	soybean	AOCS
8	DAS-44406-6	BF436e	soybean	IRMM
9	DAS-81419-2	BF437e	soybean	IRMM
10	MON87769	0809-B	soybean	AOCS
11	MON87705	0210-A	soybean	AOCS
12	CV127	0911-D	soybean	AOCS
13	MON87701	0809-A	soybean	AOCS
14	DP305423	BF426d	soybean	IRMM
15	DP356043	BF425d	soybean	IRMM
16	A5547-127	0707-C3	soybean	AOCS
17	A2704-12	0707-B11	soybean	AOCS
18	Non-genetically modified soybean	0906-A	soybean	AOCS
19	GA21	0407-B	corn	AOCS
20	MIR604	0607-A2	corn	AOCS
21	Non-genetically modified corn	0406-A	corn	AOCS
22	GT73	0304-B2	cole	AOCS
23	Ms8	0306-F7	cole	AOCS
24	Rf1	0711-B2	cole	AOCS
25	Rf2	0711-C3	cole	AOCS

Table 2
Primers and probes.

Name	Sequence (5′ to 3′)	Fluorescence labelling
87708-f12	GATCTCCATGAGCATCCA	/
87708-r12	TTTCCCGCCTTCAGTTTA	/
87708-p1	TATCCACGAGCATCCACGAGC	5′CY5, 3′BHQ2
89788-f1	AACGCTCAGACTCTAGTG	/
89788-r1	CAGCTATCACATATGGTTGAA	/
89788-p1	CCACCTTCACTCTCCTCAAGCA	5′FAM, 3′BHQ1
lectin-F	CCAGCTTCGCCGCTTCCTTC	/
lectin-R	GAAGGCAAGCCCATCTGCAAGCC	/
lectin-P	CCTCACCTTCTATGCCCCTGACAC	5′VIC, 3′BHQ2

called crystal dPCR (Madic et al., 2016). The platform has a tricolor detection function that enables multiplex PCR reactions. The tricolor fluorescence channel allows the simultaneous detection of three different target DNAs. The application of Naica's technology has enabled us to perform dPCR quantitative detection of genetically modified stacked events. Since the flanking sequences of GMOs with stacked traits are similar to those of GMOs with the corresponding single trait, no suitable method exists to accurately perform their quantitative detection.

In this study, crystal dPCR was used to establish a triple dPCR for the detection of the genetically modified soybean MON87708 × MON89788 with stacked traits. This system can perform specific absolute quantitative detection of genomic DNA extracted from the homogenized seeds of the GM stack MON87708 × MON89788 soybean. It can serve as a novel technical approach for the accurate detection of the stacked event of genetically modified soybean MON87708 × MON89788, and can provide technical support for the supervision of genetically modified crops.

2. Materials and methods

2.1. Samples

Genetically modified MON87708 × MON89788 soybean stacked event was sourced from soybeans shipments for testing purposes. Other genetically modified plants were obtained from the American Oil Chemists Society (AOCS) or the European Reference Materials (ERM), as indicated in Table 1.

2.2. Reagents

TaKaRa MiniBEST Plant Genomic DNA Extraction Kit (9768, TaKaRa (Dalian) Co., Ltd.), Tris-EDTA (TE) solution (10 × TE, pH 7.6) (RYX267, Qingdao JISSKANG Technology Co., Ltd.) and PerfeCTa Multiplex qPCR ToughMix (5 ×) premix (Quantabio, USA) were used.

2.3. Instruments

ND-1000 ultra-micro spectrophotometer (Nanodrop, USA), 5910R high-speed refrigerated centrifuge (Eppendorf, Germany), Naica Crystal Digital PCR System (Stilla Technologies, France) were used to do the experiments.

2.4. Primers and probes

The lectin was selected as the soybean endogenous control gene. According to the specificity of the genetically modified MON87708 × MON89788 soybean hybrid genomic sequence, the single-copy DNA integration boundary region and the inserted sequence element region were selected to design the primers and TaqMan hydrolysis probes, which were then used for the specific detection and quantification of the MON87708 and MON98788, respectively. The primers and probes were designed using the Beacon Designer 7 software (Version 7.9). The TaqMan hydrolysis probe was labeled with FAM, CY5, and VIC fluorescence dyes at the 5′-end and the quenched groups BHQ1 and BHQ2 at the 3′-end, respectively (Table 2). The abovementioned primers and probes were synthesized by Biotech Bioengineering (Shanghai) Co. Ltd.

2.5. dPCR detection and data analysis

The triple dPCR reaction mixture was prepared as follows: The total reaction volume was 25 μL, which included 5 μL of PerfeCTa Multiplex qPCR ToughMix (5 ×) premix (Quantabio, USA) and 1 μL of each primer (final concentration of 400 nM) and probe (final concentration of 200 nM). Twenty-five microliters of the premix were loaded into the microarray (Stilla Technologies, France), the droplets in a Naica Geode thermal cycler were separated, and the PCR reaction was performed. The conditions were as follows: 95 °C for 5 min; 95 °C for 15 s, 58 °C for 30 s, 45 cycles. After amplification, information was collected from the microarray using a Naica Prism3 reader. Statistical analyses were carried out using the Poisson distribution principle with the Crystal Miner Software, Version 2.3.0 (Stilla Technologies, France).

2.6. Optimization of triple dPCR system

DNA from the genetically modified soybean MON87708 × MON89788 stacked event samples was used as a template for optimizing the triple dPCR system. Using the same template, the annealing temperature, and the primer and probe concentrations (The dMIQE Group, 2020) were adjusted for dPCR amplification to obtain the optimal reaction conditions for genetically modified MON87708 × MON89788.

2.7. Specificity of triple dPCR

Triple dPCR (MON87708/MON89788/lectin) was used to test the DNA extracted from the genetically modified soybean MON87708, MON89788, MON87708 × MON89788 stacked event, non-genetically modified soybean, other genetically modified soybean events, genetically modified corn, and cole, among others (Table 1).

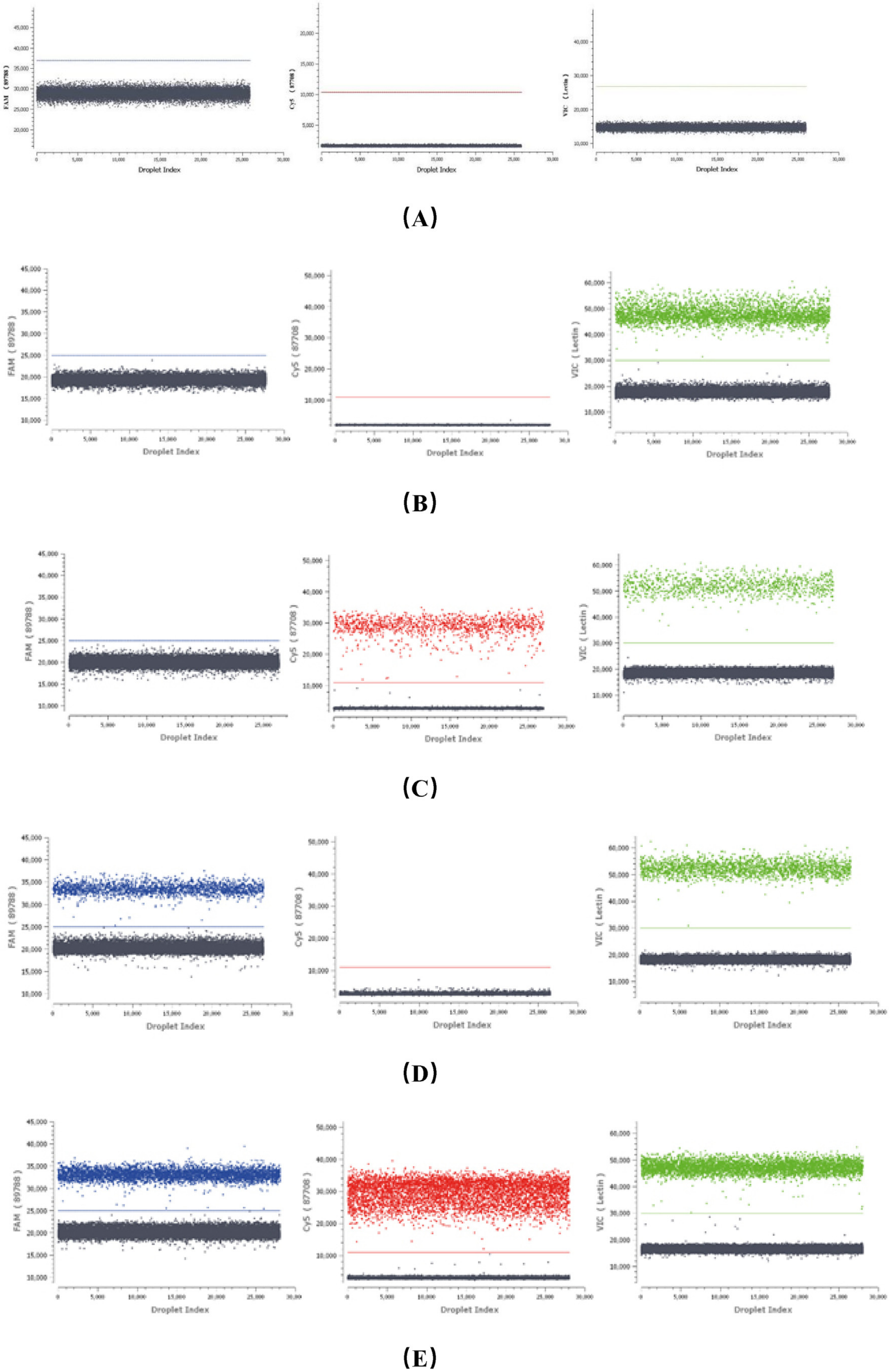

Fig. 1. One-dimensional diagram of triple dPCR detection. (A) blank control (B) non-genetically modified soybean (C) MON87708 (D) MON89788 (E) MON87708 × MON89788.

Table 3
The copy number results of triple dPCR detection.

Sample	Total droplets	MON89788 (copies/μL)	Positive partitions of MON89788	MON87708 (copies/μL)	Positive partitions of MON87708	Lectin (copies/μL)	Positive partitions of Lectin	Lectin/MON89788 ratio	Lectin/MON87708 ratio
Blank control	25372	0	0	0	0	0	0	/	/
non-genetically modified soybean	27252	0	0	0	0	193.9	2927	/	/
MON87708	27048	0	0	80.2	1242	76.5	1185	/	0.95
MON89788	27612	166.4	2565	0	0	162.9	2514	0.98	/
MON87708 × MON89788	25599	334.6	4557	383.7	5154	369.4	4982	1.10	0.96

Table 4
Five times repeatability verification results of the MON87708 × MON89788 stacked event.

Sample	Target gene	R1	R2	R3	R4	R5	Mean	RSD%	Lectin/MON89788	Lectin/MON87708
		(copies/μL)								
MON87708 × MON89788	MON89788	327.5	332.6	321	324.6	327.5	326.64 ± 3.82	1.17	1.07	0.94
	MON87708	377.1	372.5	375.5	364.6	377.1	373.36 ± 4.69	1.26		
	Lectin	352.3	347.4	344.4	356.7	352.3	350.62 ± 4.28	1.22		

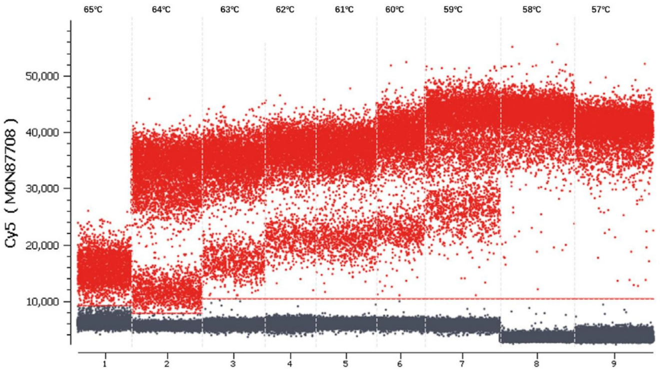

Fig. 2. Optimization of the annealing temperature used in the experiments.

Table 5
dPCR chip-layout plan.

Reaction pore	Chipset 1 (58 °C)	Chipset 2 (58 °C)	Chipset 3 (58 °C)
A	Primer 300 nM; Probe 100 nM	Primer 300 nM; Probe 200 nM	Primer 300 nM; Probe 300 nM
B	Primer 400 nM; Probe 100 nM	Primer 400 nM; Probe 200 nM	Primer 400 nM; Probe 300 nM
C	Primer 500 nM; Probe 100 nM	Primer 500 nM; Probe 200 nM	Primer 500 nM; Probe 300 nM
D	Primer 600 nM; Probe 100 nM	Primer 600 nM; Probe 200 nM	Primer 600 nM; Probe 300 nM

2.8. Sensitivity of triple dPCR

The genetically modified soybean MON87708 × MON89788 stacked event genomic DNA (300 ng/μL) was sequentially diluted with ultra-pure water at 10-fold (30 ng/μL), 10-fold (3 ng/μL), 5-fold (0.6 ng/μL), 5-fold (0.12 ng/μL), 2-fold (0.06 ng/μL), 2-fold (0.03 ng/μL), and 2-fold (0.015 ng/μL) dilution. The dPCR reaction was performed using 5 μL of DNA for each of the above dilutions as the template, and three replicates were set up for each concentration.

The mean value and relative standard deviation (RSD) of the copy number of genes tested for each concentration of DNA were calculated. RSD = SD / $\bar{x}$*100%, SD is standard deviation, $\bar{x}$ is the mean value of three repetitions. The linear correlation curves for the quantitative range

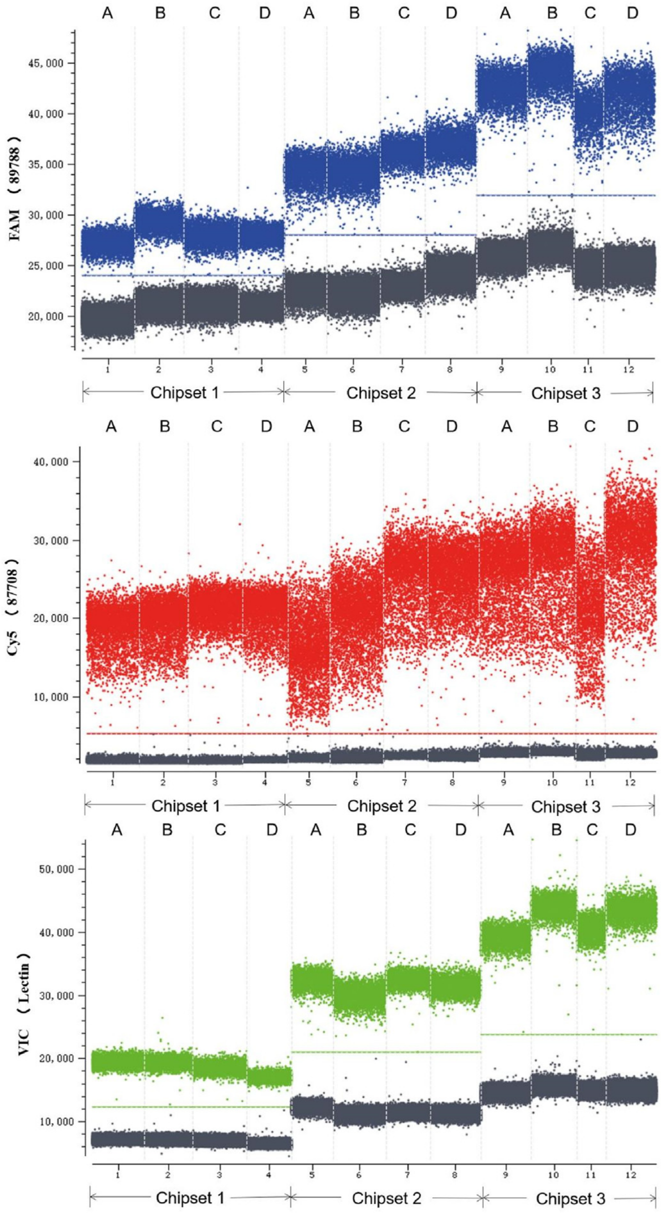

Fig. 3. Optimization of the primer and probe concentrations.

Table 6
Triple dPCR detection results of 25 samples.

No.	Sample	Total droplets	MON89788 (copies/μL)	Positive partitions of MON89788	MON87708 (copies/μL)	Positive partitions of MON87708	Lectin (copies/μL)	Positive partitions of Lectin	Lectin/MON89788 ratio	Lectin/MON87708 ratio
1	MON87708 × MON89788	25270	12.7	175	17.6	242	15.4	212	1.21	0.88
2	MON87708	25813	0	0	79	1090	83.1	1145	/	1.05
3	MON89788	25736	155.2	2090	0	0	169.1	2270	1.09	/
4	DAS 68416-4	20821	0	0	0	0	216.2	2318	/	/
5	FG72	26105	0	0	0	0	893.1	10073	/	/
6	GTS-40-3-2	26976	0	0	0	0	546.5	6958	/	/
7	MON87751	26087	0	0	0	0	86.8	1207	/	/
8	DAS-44406-6	26496	0	0	0	0	506.9	6405	/	/
9	DAS-81419-2	26342	0	0	0	0	134.1	1860	/	/
10	MON87769	27557	0	0	0	0	217.1	3080	/	/
11	MON87705	26460	0	0	0	0	317	4205	/	/
12	CV127	26169	0	0	0	0	63.1	886	/	/
13	MON87701	22549	0	0	0	0	222.9	2583	/	/
14	DP305423	27255	0	0	0	0	121.5	1749	/	/
15	DP356043	25865	0	0	0	0	139.1	1891	/	/
16	A5547-127	23563	0	0	0	0	57.2	724	/	/
17	A2704-12	26112	0	0	0	0	262.9	3491	/	/
18	Non-genetically modified soybean	26770	0	0	0	0	314.4	4222	/	/
19	GA21	25703	0	0	0	0	0	0	/	/
20	MIR604	25837	0	0	0	0	0	0	/	/
21	Non-genetically modified corn	25604	0	0	0	0	0	0	/	/
22	GT73	25084	0	0	0	0	0	0	/	/
23	Ms8	26831	0	0	0	0	0	0	/	/
24	Rf1	25395	0	0	0	0	0	0	/	/
25	Rf2	24471	0	0	0	0	0	0	/	/

of the genetically modified soybean were plotted using DNA concentration as the horizontal axis, and the detected copy number concentration of the target gene, with RSD not exceeding 25% (European Network of GMO Laboratories 2015), as the vertical axis.

3. Results

3.1. Establishment of triple dPCR system

The DNA samples of genetically modified soybean MON87708 × MON89788 stacked event, genetically modified MON87708 event, genetically modified MON89788 event, non-genetically modified soybean and ultra-pure water for blank control were used as templates for triple dPCR amplification. The results are shown in Fig. 1. The endogenous gene lectin was detected in all four soybean samples, but not found in the blank control. Furthermore, 87708 gene and 89788 gene were positive in MON87708 × MON89788 stacked event, only 87708 gene was positive in MON87708 single event, only 89788 gene was positive in MON89788 single event, and only lectin gene was positive in the non-genetically modified soybean negative control; all genes were negative in the blank control (89788 in FAM channel, 87708 in Cy5 channel, and lectin in VIC channel). In the stacked event, the ratio of the endogenous gene lectin to the exogenous gene copy number was almost 1:1, and the copy number results are provided in Table 3. The repeatability verification of MON87708 × MON89788 stacked event was performed 5 times, and the ratios of an endogenous gene to the exogenous gene were 1.07 and 0.94 (CV = 1.82% and 2.27%), respectively, indicating that the genetically modified MON87708 × MON89788 stacked event was homozygous, as shown in Table 4.

3.2. Data analysis

When the total number of droplets is > 20,000, the test is deemed effective. The well-optimized assays have a clear difference in fluorescence intensity between the positive and negative partitions, with no or a small amount of rain. The threshold was set manually in the middle of the positive and negative partitions to separate different clusters.

3.3. Optimization of triple dPCR system

The DNA of the genetically modified soybean stacked event MON87708 × MON89788 was used as the template to verify the amplification performance of triple dPCR, and it was discerned that as the template concentration increased, two positive bands appeared in the detection channel of MON87708 (Cy5). The 2-D plot analysis revealed that this region was a double positive region for MON87708 and MON89788, which could be attributed to the fact that the presence of both MON87708 and MON89788 in the same microdrop (microreactor) may affect the amplification of MON87708. Hence, we optimized the assay conditions by adjusting the annealing temperature and the concentrations of the primer and probe. The annealing temperature was gradually decreased from 65°C to 57 °C. With the decreasing temperature, the rain level also decreased gradually. When the annealing temperature was 58 °C, the positive partition could be distinguished most clearly, such that 58°Cwas selected as the annealing temperature of the detection system (as shown in Fig. 2). The final concentration of the primer was set at 300, 400, 500, and 600 nM, and the final concentration of the probe at 100, 200, and 300 nM. The primer and probe were combined for dPCR assays (as shown in Table 5). The optimization

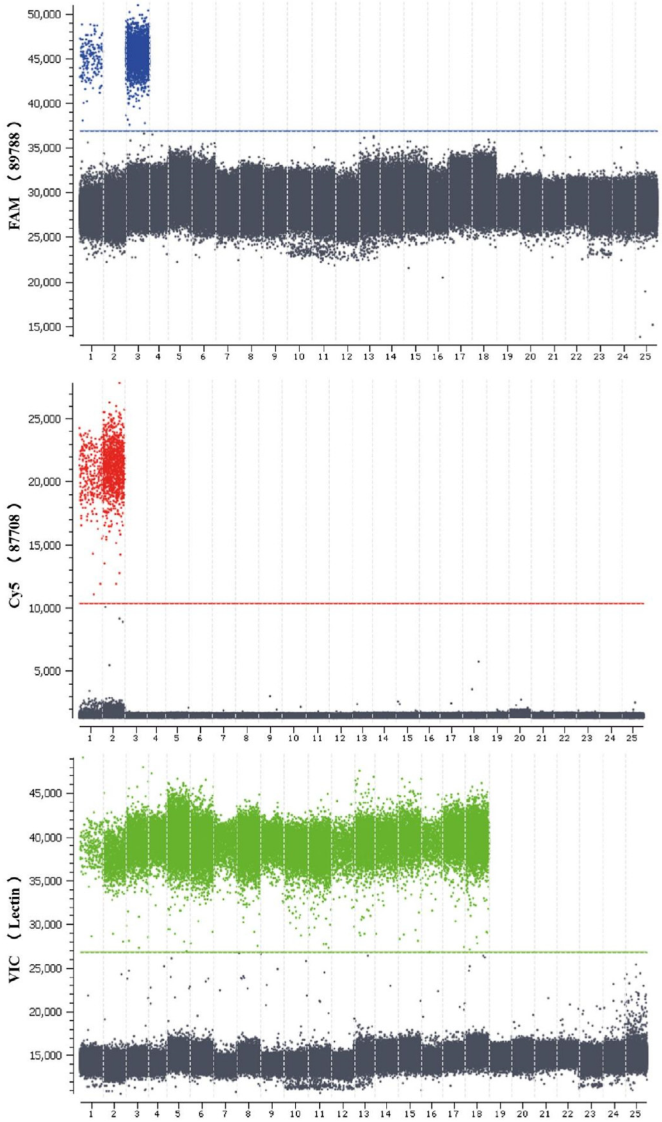

Fig. 4. Triple dPCR specificity test.

results were shown in Fig. 3. According to the distribution of the positive and negative partitions and the rainy partition, two primer-probe combinations were selected for the second optimization. Combination 1: Cy5 channel for MON87708 detection, primer 500 nM, probe 100 nM; FAM channel for MON89788 detection, primer 500 nM, probe 200 nM; Vic channel for lectin detection, primer 600 nM, probe 200 nM. Combination 2: Cy5 channel for MON87708 detection, primer 500 nM, probe 100 nM; FAM channel for MON89788 detection, primer 300 nM, probe 300 nM; Vic channel for lectin detection, primer 300 nM, probe 300 nM. These results revealed that with the Cy5 channel for MON87708 detection, primer 500 nM, probe 100 nM, FAM channel for MON89788 detection, primer 300 nM, probe 300 nM,Vic channel for lectin detection, primer 300 nM, probe 300 nM, the positive and negative partitions could be distinguished most clearly, and the distribution of raindrops was the least. Therefore, we selected this as the best primer-probe concentration for subsequent assays.

3.4. *Specificity of triple dPCR*

The genomic DNA of different genetically modified events of soybean, corn, and cole and the non-genetically modified samples were detected by the proposed triple dPCR system. When the target gene was detected in the sample, if the copy number of the result was lower than the limit of detection, the sample was determined to be negative. If the detected copy number was higher than the limit of detection, the sample was deemed positive. All reactions were performed twice. The copy number concentration of triple dPCR for 25 samples were listed in Table 6. As observed (Fig. 4), the lectin gene was positive in all soybean samples. Moreover, 87708 gene and 89788 gene were positive in MON87708 × MON89788 stacked event, only 87708 gene was positive in MON87708 single event, only 89788 gene was positive in MON89788 single event, and only lectin gene was positive in the non-genetically modified soybean negative control; all genes were negative in other types of test samples. Accordingly, the proposed triple dPCR detection system displays high specificity toward the genetically modified soybean stacked event MON87708 × MON89788.

3.5. *Sensitivity of triple dPCR*

The experimental results of triple dPCR detection sensitivity are furnished in Fig. 5. RSD $\leq$25% was set as the evaluation standard for the precision of the quantitative results (European Network of GMO Laboratories 2015). The relative standard deviation of test results was obtained under 3 repetitions. The limit of quantification (LOQ) was the lowest concentration or quantity of the nucleic acid target sequence per difined volume that can be measured with reasonable statistical certainty consistently under the experimental conditions specified in the method. As can be seen in Table 7, the LOQ of dPCR for MON89788 was 5.42 copies per reaction, and the RSD was 18.97%; the LOQ of dPCR for MON87708 was 6.08 copies per reaction, and the RSD was 12.70% (Table 8); the LOQ of dPCR for lectin was 4.83 copies per reaction, and the RSD was 8.79% (Table 9). The DNA concentration was considered as the abscissa and the detected copy number concentration of the target gene as the ordinate to draw the linear range fitting curve. As shown in Fig. 6, linearity was noted in the copy numbers of MON89788, MON87708, and lectin over the DNA template concentration, and the correlation coefficients reached >0.99. Moreover, the digital PCR method established in this experiment revealed a good quantitative linear correlation that was suitable for the quantitative detection of copy number concentrations of MON89788, MON87708, and lectin.

4. Discussion

As products of genetic modification and hybrid breeding integration, genetically modified crops with stacked traits have been rapidly developed in recent years. According to ISAAA (Soybean (Glycine max L.) GM Events (41 Events)), 16 genetically modified soybeans with stacked traits have been commercialized so far. However, factors such as the level of technological development, economic benefits, and public acceptance have led to different evaluation models for genetically modified plants with stacked traits in different countries. To date, there is no unified standard for the safety evaluation of breeding complex genetically modified plants. Since the flanking sequences of GMOs with stacked traits are similar to those of GMOs with the corresponding single trait, no suitable method is available to accurately detect them.

Akiyama et al., (2005) proposed a detection technique for genetically modified crops with stacked traits based on single and multiplex PCR (regarded as single multiplex PCR). In this method, the genome of a

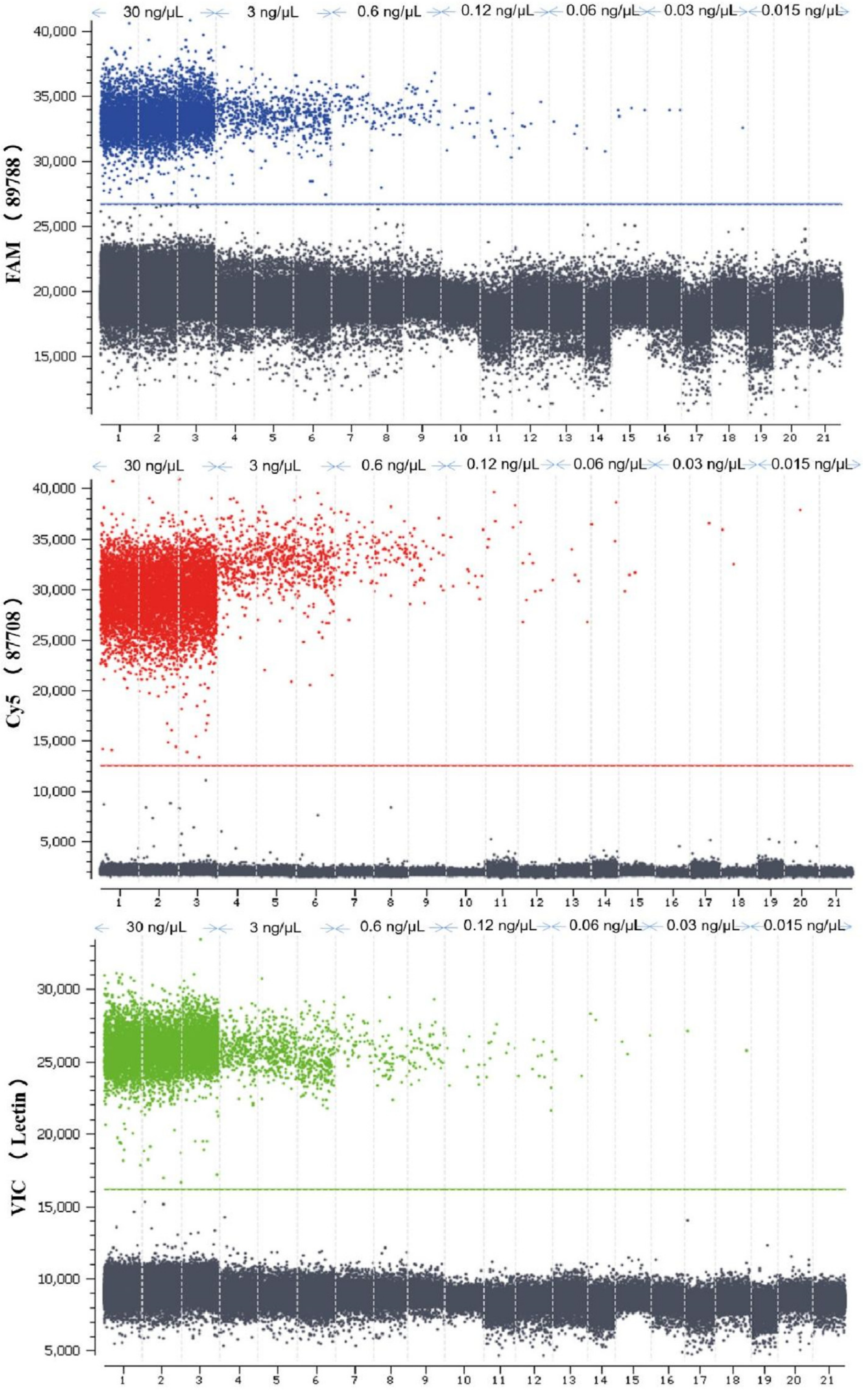

Fig. 5. One-dimensional diagram of triple dPCR sensitivity.

single crop sample is extracted and its DNA is amplified by multiplex PCR. The number and position of the target bands are employed to determine the presence of genetically modified crops with stacked traits. Xu et al., (2009) investigated the use of multivariate statistical quantitative PCR. In this method, the samples are initially subjected to high-throughput screening by multiplex PCR with universal adapters, followed by quantitative PCR for specific primers of transformation events. Finally, the absolute content of each component in the mixed

Table 7
Linear range of dPCR for MON89788.

DNA dilution times	Mass concentration of DNA template (ng/μL)	MON89788					
		Repeat 1 (copies/μL)	Repeat 2 (copies/μL)	Repeat 3 (copies/μL)	AVG	SD	RSD%
10	30	3647.50	3505.00	3405.00	3519.17	99.51	2.83
100	3	350.00	327.50	317.50	331.67	13.59	4.10
500	0.6	95.75	71.50	63.25	76.83	13.79	17.95
2500	0.12	15.50	14.75	11.50	13.92	1.74	12.47
5000	0.06	5.25	4.25	6.75	5.42	1.03	18.97
10000	0.03	3.50	0.00	1.75	1.75	1.43	81.65
20000	0.015	0.00	0.00	0.00	0.00	0.00	/

Table 8
Linear range of dPCR for MON87708.

DNA dilution times	Mass concentration of DNA template (ng/μL)	MON87708					
		Repeat 1 (copies/μL)	Repeat 2 (copies/μL)	Repeat 3 (copies/μL)	AVG	SD	RSD%
10	30	4110.00	4292.50	4240.00	4214.17	76.71	1.82
100	3	392.50	410.00	350.00	384.17	25.19	6.56
500	0.6	86.25	89.00	55.25	76.83	15.30	19.92
2500	0.12	15.50	11.00	14.75	13.75	1.97	14.32
5000	0.06	6.75	6.50	5.00	6.08	0.77	12.70
10000	0.03	0.00	2.00	3.25	1.75	1.34	76.49
20000	0.015	0.00	1.75	0.00	0.58	0.82	141.42

Table 9
Linear range of dPCR for Lectin.

DNA dilution times	Mass concentration of DNA template (ng/μL)	Lectin					
		Repeat 1 (copies/μL)	Repeat 2 (copies/μL)	Repeat 3 (copies/μL)	AVG	SD	RSD%
10	30	4097.50	3987.50	3962.50	4015.83	58.64	1.46
100	3	410.00	407.50	400.00	405.83	4.25	1.05
500	0.6	70.25	87.25	78.00	78.50	6.95	8.85
2500	0.12	14.00	18.50	16.50	16.33	1.84	11.27
5000	0.06	5.25	4.25	5.00	4.83	0.42	8.79
10000	0.03	0.00	2.00	1.75	1.25	0.89	71.18
20000	0.015	0.00	0.00	0.00	0.00	0.00	/

samples is obtained by multivariate data analysis. Using this method, absolute quantification of components with stacked traits in mixed samples can be performed.

Crystal dPCR with tricolor detection enables the recognition of three different target DNAs simultaneously without the negative impacts of other dPCR platforms while performing multiplex detection methods. This technique has already exhibited a unique advantage in the field of medical testing. For instance, Gao et al., (2020) evaluated the differences in the expression of chimeric miniSOG-PrP in brain tissues of different Tg mice through Stilla NaicadPCR analysis. Pomari et al., (2019) indicated that dPCR could be expected to be an effective new molecular technique for parasite detection and quantitative analysis of GMO in the future. For GMO detection, Dobnik et al., (2016) comprehensively evaluated several parameters in multiplex dPCR for the first time and developed two four-plex assays to quantify eight different DNA targets (seven genetically modified corn events and corn endogenous genes).

In this study, specific absolute quantitative detection of genomic DNA extracted from the homogenized seeds of GM stack MON87708 × MON89788 soybean was performed by crystal dPCR. During the developmental process, various factors affecting the triple dPCR reaction and its quantitative results were optimized, including the specificity, repeatability, and concentration of MON89788 and MON87708 primer and probe and the annealing temperature of PCR. The established triple dPCR can perform specific absolute quantitative detection of genomic DNA extracted from the homogenized seeds or leaves of GM stack MON87708 × MON89788 soybean. However, triple dPCR detection showed some limitations. For instance, only the DNA extracted from the homogenized matrix can determine whether it was a stacked event. In the case of a mixed sample, it cannot be completely and accurately determine whether it was a stacked event or a mixture of two single events. Nevertheless, the proposed method is a useful attempt and can serve as a reference for the absolute quantitative detection of a stacked event of genetically modified crops.

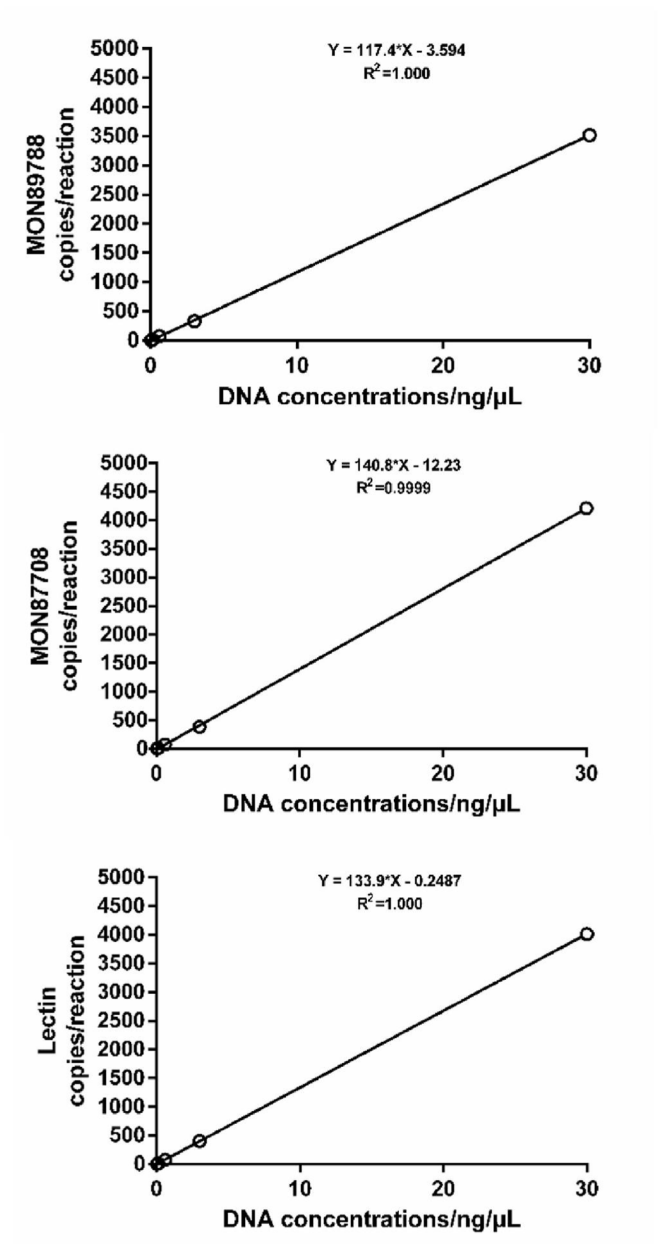

Fig. 6. Linear range of genetically modified soybean MON87708 × MON89788 detected by triple dPCR.

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