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Validation of a Rice Specific Gene, *Sucrose Phosphate Synthase*, Used as the Endogenous Reference Gene for Qualitative and Real-Time Quantitative PCR Detection of Transgenes

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With the development of transgenic crops, many countries have issued regulations to label the genetically modified organisms (GMOs) and their derived products. Polymerase Chain Reaction (PCR) methods are thought to be reliable and useful techniques for qualitative and quantitative detection of GMOs. These methods generally need to amplify the transgene and compare the amplified result with that of the corresponding reference gene to obtain reliable results. In this article, we reported the development of specific primers and probe for the rice (*Oryza sativa*) *sucrose phosphate synthase* (*SPS*) gene and PCR cycling conditions suitable for the use of this sequence as an endogenous reference gene in both qualitative and quantitative PCR assays. Both methods were assayed with 13 different rice varieties, and identical amplification products were obtained with all of them. No amplification products were observed when DNA samples from other species, such as wheat, maize, barley, tobacco, soybean, rapeseed, tomato, sunflower, carrot, pepper, eggplant, lupine, mung bean, plum, and *Arabidopsis thaliana*, were used as templates, which demonstrated that this system was specific for rice. In addition, the results of the Southern blot analysis confirmed that the *SPS* gene was a single copy in the tested rice varieties. In qualitative and quantitative PCR analyses, the detection sensitivities were 0.05 and 0.005 ng of rice genomic DNA, respectively. To test the practical use of this *SPS* gene as an endogenous reference gene, we have also quantified the *β-glucuronidase* (*GUS*) gene in transgenic rice using this reference gene. These results indicated that the *SPS* gene was species specific, had one copy number, and had a low heterogeneity among the tested cultivars. Therefore, this gene could be used as an endogenous reference gene of rice and the optimized PCR systems could be used for practical qualitative and quantitative detection of transgenic rice.

KEYWORDS: ***Oryza sativa*; rice; *sucrose phosphate synthase*; qualitative PCR; quantitative PCR; endogenous reference gene; GMOs detection**

INTRODUCTION

Attempts are being made to solve more and more agricultural production problems with genetically modified (GM) plants. The number of licensed GM plants worldwide is growing rapidly (*1*). Twenty-six GM crops were approved worldwide at the end of 2002 (*2*). Up to now, six GM crops, such as two kinds of insect resistant cottons, virus resistant sweet pepper, cauliflower mosaic virus resistant tomato, shelf life-altered tomato, and color-altered petunia, have been approved for commercialization in China (*3*−*5*). Moreover, GM soybean, maize, and rapeseed products are being imported from other countries to China. Pressures from consumer groups and public demands have led several countries, including China, to require labeling for the presence of GM organisms (GMOs) in foods.

In conjunction with these labeling policies, polymerase chain reaction (PCR) methods are considered to be the most common DNA detection method for identifying the presence of GMOs. They are very sensitive, and very small aliquots of vegetal material are required for the analysis. PCR methods are not only

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Table 1. Primer Pairs and Fluorogenic Probes for Qualitative and Quantitative PCR

name	orientation	sequence (5′–3′)	length (bp)	position (bp)
SPSF	forward primer	TTGCGCCTGAACGGATAT	18	1055–1072
SPSR	reverse primer	CGGTTGATCTTTTCGGGATG	20	1116–1135
SPSP	forward probe	GACGCACGGACGGCTCGGA	19	1096–1114
GUSF	forward primer	TTTCTTTAACTATGCCGGAATCCATC	26	697–722
GUSR	reverse primer	CACCACGGTGATATCGTCCAC	21	758–778
GUSP	forward probe	AATGCTCTACACCACGCCGAACACCTG	27	730–756

used for identification of GM products but also for quantification purposes (*6*). To make the screen procedure more normative, one should detect the target sequences and plant species specific endogenous reference gene. Among the PCR detection methods, real-time quantitative PCR is considered to be an easy-to-use, accurate, specific, quantitative method (*6*, *7*). A unique feature of this PCR technique is that the amplification of the target DNA sequence can be followed during the whole reaction by indirect monitoring of the products accumulation. Using this method, a target gene can be quantified by preparing a standard curve from known quantities of additional endogenous gene and extrapolation from the linear regression line. This system requires both the primers specific for the transgene and the species specific primers for an endogenous reference gene. In practice, accurate relative quantification can be achieved by a combination of two absolute quantification reactions: one for the target specific gene and a second for the crop reference gene (*6*, *8*).

For this purpose, much effort has been made to obtain reference genes from crops. Now some genes have been used as the reference gene of some crops, for example, the *invertase I* (*9*, *10*, *18*), *10 kDa zein*, and *hmg-A* genes (*11*−*14*, *18*) for maize; *lectin* (*9*, *11*, *15*, *18*) and *β-actin* (*16*) genes for soybean; and *cruciferin* (*16*) and *BnACCg8* (*18*) genes for rapeseed. However, there has been no report about the endogenous reference gene for the detection of GM rice. As an important foodstuff, rice varieties have been widely planted in the world, especially in Asia. Up to now, there are two GM rice varieties that have been authorized for commercialization. One is from BASF Inc., which is tolerant to the imidazolinone herbicide, imazethapyr, induced by chemical mutagenesis of the *acetolactate synthase* enzyme using ethyl methanesulfonate. This variety has three lines, i.e., Event CL121, CL141, and CFX51. The other is from Aventis Crop Science, is glufosinate ammonium herbicide tolerant, and is produced by inserting a modified *phosphinothricin acetyltransferase* encoding gene from the soil bacterium *Streptomyces hygroscopicus* (*17*). This variety has two transgenic lines, i.e., Event LLRICE06 and LLRICE62.

In this article, we reported the specific primers and the probe for the rice (*Oryza sativa*) *sucrose phosphate synthase* (*SPS*) gene and PCR cycling conditions suitable for the use of this sequence as an endogenous reference gene in both qualitative and quantitative PCR assays. *SPS* (EC 2.4.1.14), a key enzyme in the sucrose biosynthetic pathway, catalyzes the formation of sucrose phosphate from UDP-glucose and fructose 6-phosphate. A growing body of evidence indicates that *SPS* together with fructose 1,6-bisphosphatase regulates the synthesis of sucrose in plants (*19*). In addition, we showed that this optimal quantitative PCR condition could be used for practical detection of the *GUS* gene in transgenic rice.

MATERIALS AND METHODS

Materials. The 13 nontransgenic rice varieties were provided by The Crop Research Institute of Agriculture Science (SAAS). Seven of them were cultivars of *O. sativa* L. ssp. *Japonica*, such as Hanfeng, Nonghu No. 6, 9520, Xiushui 04, Taihunuo, 20566, and P2C1 Huayu No. 1. Six of them were cultivars of *O. sativa* L. ssp. *indica*, for example, D5 Heiheaihui, C43*β*g94-1, C26 PR106, D20 BaSmati, C21 Zhongyuzao 18, and D57 kholt marshi. Other DNA samples of different species such as wheat (*Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), tobacco (*Nicotiana tabacum*), soybean (*Glycine max*), rapeseed (*Brassica napus*), tomato (*Lycopersicon esculentum*), sunflower (*Helianthus annuus*), carrot (*Daucus carota*), pepper (*Capsicum annum*), eggplant (*Solanum melongena*), lupine (*Lupinus albus*), mung bean (*Phaseolus aureus*), plum (*Prunus domestica*), and *Arabidopsis thaliana* were collected by our laboratory. Transgenic rice plants used in this work containing *GUS* genes were conserved by our laboratory.

DNA Extraction. The plant genomic DNA samples used for qualitative and quantitative PCR detection were extracted and purified using a DNA extraction kit, which was developed by Shanghai Ruifeng Agro-tech Co. Ltd. (Shanghai, China). The plant genomic DNA samples used for the Southern blot analysis were extracted and purified from fresh leaves according to the CTAB method (*22*). Genomic DNA was quantified spectrophotometrically using a DUR 640 DNA/Protein calculator (Beckman Company) and analyzed by 1% agarose gel electrophoresis in 0.5 × TBE with ethidium bromide staining.

Southern Blot. Ten micrograms of each DNA from seven different nontransgenic rice varieties (Hanfeng, Nonghu No. 6, 9520, Xiushui04, D5 Heiheaihui, C43*β*g94-1, and C26 PR106) were completely digested with *Hin*dIII and *Eco*RI, respectively. The digested DNA was resolved in a 0.8% agarose gel electrophoresis and then transferred onto a nitrocellulose membrane, which was purchased from Gene Company Co., Ltd. (Shanghai, China). A 1026 bp DNA fragment (from 1058 to 2084) of the *SPS* gene was used as the hybridized probe. This DNA fragment was labeled by α[^{32}P]dCTP using the Random primer DNA labeling kit ver. 2 from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Hybridization was performed at 62 °C for 24 h, and the filter was washed at room temperature with 2 × SSC/0.1% SDS and 1 × SSC/0.1% SDS for 10 min each and at 60 °C with 0.2 × SSC/0.1% SDS for 30 min. DNA markers (*λ* DNA digested with *Hin*dIII and *Eco*RI) were run on the same gel.

Oligonucleotide Primers and Probes. The oligonucleotide primers and TaqMan fluorescent dye-labeled probes between the primers were designed by using Primer Premier 5.0 software and Beacon Designer 2.0 software (PREMIER Biosoft International). The corresponding nucleotide sequences of the *SPS* gene and the *GUS* gene are listed in **Table 1**. All PCR primers and fluorogenic probes were synthesized from Shanghai Shenyou Co., Ltd. (Shanghai, China). The *SPS* and *GUS* probes were labeled with the fluorescent reporter dyes 5-hexachloro-fluorescein and 6-carboxy-fluoroscein, respectively, on the 5′ end and both of the fluorescent quencher dyes 6-carboxytetramethylrhodamine were located on the 3′ end of the probes.

We used primers SPSR and SPSF for *SPS* qualitative PCR and primer pair SPSR/SPSF in combination with the SPSP probe for quantitative PCR. Both qualitative and quantitative PCR gave rise to an 81 bp amplification product. In addition, the *GUS* quantitative PCR used primer pair GUSF/GUSR and the GUSP probe gave rise to a 149 bp product.

PCR Conditions. Qualitative PCR was run in a PTC-100 (MJ Research, Waltham, MA) thermocycler. Each reaction mixture had 1 × PCR buffer, 0.2 mM dNTP, 1 *μ*M each primer, 5 ng of each DNA sample, and 1.5 units of Taq DNA Polymerase; the final volume was 30 *μ*L. All reagents were from TaKaRa Biotechnology Co., Ltd. except for the primers. This reaction ran using the following cycle condi-

tions: denaturing of DNA at 94 °C for 10 min, 35 cycles of 30 s at 94 °C, 30 s at 56 °C, 30 s at 72 °C, and a final extension at 72 °C for 5 min. Amplification products were analyzed by 2% agarose gel electrophoresis (in 0.5 × TBE) and stained with ethidium bromide.

Quantitative PCR was carried out in a fluorometric thermal cycler (Rotor Gene 2000; Corbett Research, Australia) using the TaqMan system. Each reaction mixture contained 1 × PCR buffer, 5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, and dGTP, 0.4 mM dUTP, 5 ng of each DNA sample,1.5 units of Taq DNA Polymerase, 0.2 unit of Amperase uracil *N*-glycosylase (UNG), 750 nM each SPSF and SPSR primers, and 300 nM fluorogenic SPSP probe. The final volume of the PCR reaction was 25 μL. The amplification conditions were 5 min at 95 °C, 50 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C. Each sample was quantified in triplicates. The PCR reactants were purchased from Gene Company Co., Ltd. The results were analyzed using a sequence detection system provided by the Software Rotorgene 4.6 (Corbett Research). In addition, to test the sensitivity of the two *SPS* PCR assays and to generate a standard curve, the extracted DNA was serially diluted to a final concentration of 500, 50, 5, 0.5, 0.05, 0.005, and 0.0005 ng/μL. The amount of DNA per reaction is 500, 50, 5, 0.5, 0.05, 0.005, and 0.0005 ng. Using the quantitative PCR assay, these plots were analyzed repeatedly and reproducibly.

Multiplex quantitative PCR with two primer pairs, SPSF/SPSR and GUSF/GUSR, and probes, SPSP and GUSP, labeled with different fluorescent dyes were also performed to detect the amount of transgenic rice. We performed the multiplex PCR with serial dilution of transgenic rice ranging from 0.005 to 50 ng, with each dilution containing 50 ng of total rice DNA. Besides, each reaction mixture also contained 1 × PCR buffer, 5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, and dGTP, 0.4 mM dUTP, 1.5 units of Taq DNA Polymerase, 0.2 units of Amperase UNG, 750 nM each SPSF and SPSR primer, 300 nM fluorogenic SPSP probe, 500 nM each GUSF and GUSR primer, and 150 nM GUSP probe. The final volume of the PCR reaction was 25 μL. The amplification conditions were 5 min at 95 °C, 50 cycles of 30 s at 94 °C, 30 s at 56 °C, and 30 s at 72 °C. Each sample was quantified in triplicate.

RESULTS

Selection of a Suitable DNA Fragment as a Specific Gene for Qualitative and Quantitative PCR Detection of Rice. To make the GMO detection more reliable and precise, a reference gene is indispensable. A reference gene has three requirements: species specific, low copy number (to obtain high sensitivity, a single copy reference gene is the best), and low heterogeneity among cultivars. To select a suitable endogenous reference gene of rice for quantitative and qualitative PCR amplification, we searched the public gene databanks (EMBL) for DNA sequences with a single copy gene in the rice genome. After selection of several candidate genes, we chose a DNA fragment encoding *SPS* (GeneBank No. U33175), which can catalyze the formation of sucrose phosphate from UDP-glucose and fructose 6-phosphate. Then, we designed the specific primers and probe on this DNA sequence and tested them for species specificity in both qualitative and quantitative PCR assays.

Qualitative and Real-Time Quantitative PCR Assay Detection. We used the primer pair SPSF/SPSR in qualitative PCR and the combination of SPSF/SPSR primers and the SPSP probe in quantitative PCR. The primer pair gave rise to an 81 bp amplification product. To test the specificity of the *SPS* gene, we ran the qualitative and quantitative PCR reaction by using 5 ng of DNA from 15 different plant species, which included three gramineous varieties such as wheat, maize, and barley and other dicotyledon species such as tobacco, soybean, rapeseed, tomato, sunflower, carrot, pepper, eggplant, lupine, mung bean, plum, and *A. thaliana*, respectively, as the templates. The electrophoresis results of qualitative PCR showed that there were no amplification products observed with any of the species tested other than rice and also there were no fluorescent signals detected with any of the species tested other than rice in quantitative PCR (**Figure 1**). These indicated that the *SPS* DNA fragment was highly specific for rice in both qualitative and quantitative PCR in the tested species.

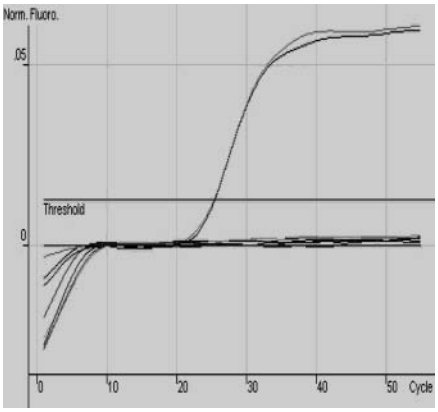


Figure 1. Specificity analysis of the rice *SPS* gene using quantitative PCR: amplification plot generated from 15 kinds of different plants (such as wheat, maize, barley, tobacco, soybean, rapeseed, tomato, sunflower, carrot, pepper, eggplant, lupine, mung bean, plum, and *A. thaliana*) and rice with the SPSF/SPSR primer pair and the SPSP probe.

Additionally, as an ideal reference gene, the *SPS* gene should not exhibit allelic variation and should have a consistent copy number among different rice varieties. To test the nonspecificity of the *SPS* gene in different rice varieties, we used 10 ng of DNA from 13 different rice varieties mentioned above as templates to run qualitative and quantitative PCR. As shown in **Figure 2**, PCR products of identical size and relative intensity were obtained for all of these tested varieties in quantitative PCR. This indicated that there were no major sequence differences among the different varieties in this amplified region. Likewise, quantitative PCR analysis performed with DNA extracted from these rice varieties exhibited similar Ct values, which varied from 28.62 to 29.29 (**Figure 3**). These results indicated that the copy number of the *SPS* gene was similar and without allelic variation among these different rice varieties.

Sensitivities of both Qualitative and Quantitative PCR Assays. To test the sensitivities of the two *SPS* PCR assays, we carried out a PCR reaction using the known DNA amounts ranging from 500 ng to 0.5 pg. Qualitative PCR allowed detection of the *SPS* DNA fragment in 50 pg of rice genomic DNA (**Figure 4**). This meant that 50 pg of rice genomic DNA could be detected using the SPSF/SPSR primer pair in qualitative PCR. On the basis of the rice genome size of 0.52 pg per haploid genome (*21*), the sensitivity of qualitative PCR corresponds to an average of 100 copies in the templates. As to quantitative PCR assay, the fluorescent signal could be detected when the amount of the DNA template was lowered to 5 pg, i.e., 10 copies of rice genomic DNA (**Figure 5A**). Also, to test the accuracy of the quantification system for relative quantitative analysis, we constructed a standard curve of the *SPS* gene by using the quantitative PCR system. A linear relationship ($R^2 = 0.9990$) with a slope of -0.287 was obtained between the Ct value and the starting DNA quantity (**Figure 5B**). This demonstrated that there was a high correlation between the tested rice DNA amounts and their corresponding Ct values.

Reproducibility of Quantitative PCR. To further validate the quantification accuracy, we generated the standard curve three times, and each time, we had three repetitions. The

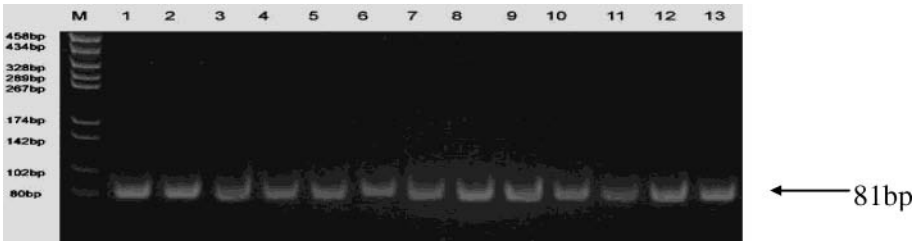


Figure 2. Nonspecific detection of the *SPS* DNA sequence of the 13 tested different rice cultivars using qualitative PCR: lanes 1–13 corresponded to Hanfeng, Nonghu No. 6, 9520, Xiushui04, Taihunuo, 20566, P2C1 Huayuyihao, D5 Heiheaihui, C43βg94-1, C26 PR106, D20 BaSmati, C21 Zhongyuzao 18, D57 kholt marshi, and M:pGEM-7Z(f).

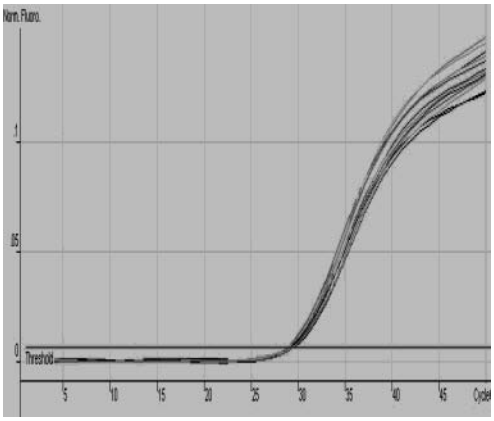


Figure 3. Allelic variation of the *SPS* gene among rice cultivars: amplification plot generated from 13 kinds of different rice cultivars described in **Figure 2** with the SPSR/SPSF primer pair and the SPSP probe.

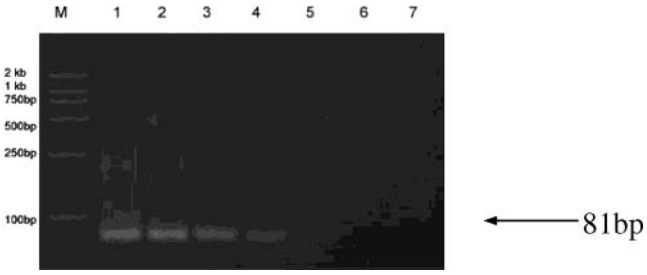
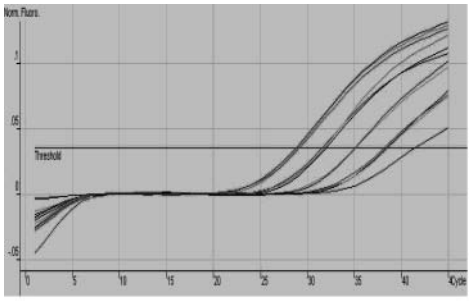


Figure 4. Sensitivity detection of the rice *SPS* DNA fragment using qualitative PCR: lanes 1–6 corresponded to 500, 50, 5, 0.5, 0.05, and 0.005 ng, and line 7 was a negative control; M, DL2000 marker.

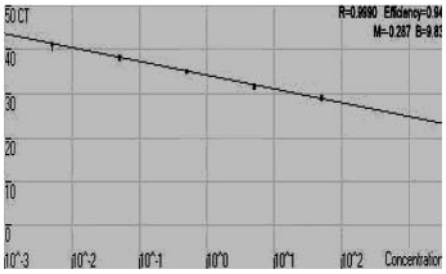
reproducibility of this quantitative PCR system was estimated with five rice DNA dilutions ranging from 50 to 0.005 ng. As the result, the Ct values ranged from 28.20 to 42.29, the coefficient of variation (CV%) values varied from 0.76 to 5.46, and the standard deviation (SD) values ranged from 0.00 to 0.16, respectively (**Table 2**). These data showed that the CV values and the SD values derived from these tests were relatively small and that this quantitative PCR detection system worked stably and reliably.

Copy Number of the Rice *SPS* Gene Confirmed by Southern Blot. To analyze the copy number of the *SPS* gene in different rice varieties, we performed a Southern blot analysis in which the rice genomic DNA was digested with *Eco*RI or *Hin*dIII and hybridized with the 1026 bp (from 1058 to 2084 bp) *SPS* DNA probe. Only one hybridized band was detected in the *Eco*RI- or *Hin*dIII-digested rice genomic DNA (data not shown), which confirmed that the *SPS* gene was a single copy gene per rice haploid genome.

Quantitative Detection of the *GUS* Gene in Transgenic Rice. To ensure the practical use of the *SPS* gene, we performed



(A)



(B)

Figure 5. Sensitivity detection of the rice *SPS* DNA fragment using quantitative PCR. (**A**) Amplification plot generated by serial dilution of rice DNA ranging from 50 to 0.005 ng with the SPSF/SPSR primer pairs and the SPSP probe. (**B**) Standard curve generated from the amplification data given in **A**.

Table 2. Reproducibility of the Ct Measurements of Replicate Standards from 50 to 0.005 ng of the Nontransgenic Rice DNA Content Using the TaqMan Assay System

	Ct value for reaction					
DNA amount (ng)	1	2	3	mean	SD[a]	CV%[b]
50	28.25	28.20	28.37	28.27	0.07	4.12
5	31.6	31.99	31.8	31.80	0.16	1.03
0.5	35.21	35.21	35.23	35.22	0.01	5.40
0.05	38.75	38.90	38.93	38.86	0.08	5.46
0.005	42.28	42.28	42.29	42.28	0.00	0.76

[a] Standard deviation. [b] Coefficient of variation.

multiplex quantitative PCR to detect the amount of *GUS* transgenic rice (**Figure 6**). We performed the multiplex PCR with serial dilution of transgenic rice ranging from 50 to 0.005 ng with each dilution containing 50 ng of total rice DNA, and the results of relative quantification were achieved with the ΔCt method (*23*). Therefore, the transgenic amount of the *GUS* gene was calculated based on the difference between the Ct values of the target *GUS* and the endogenous reference *SPS* gene (**Figure 6D**). Additionally, in this multiplex PCR using primer

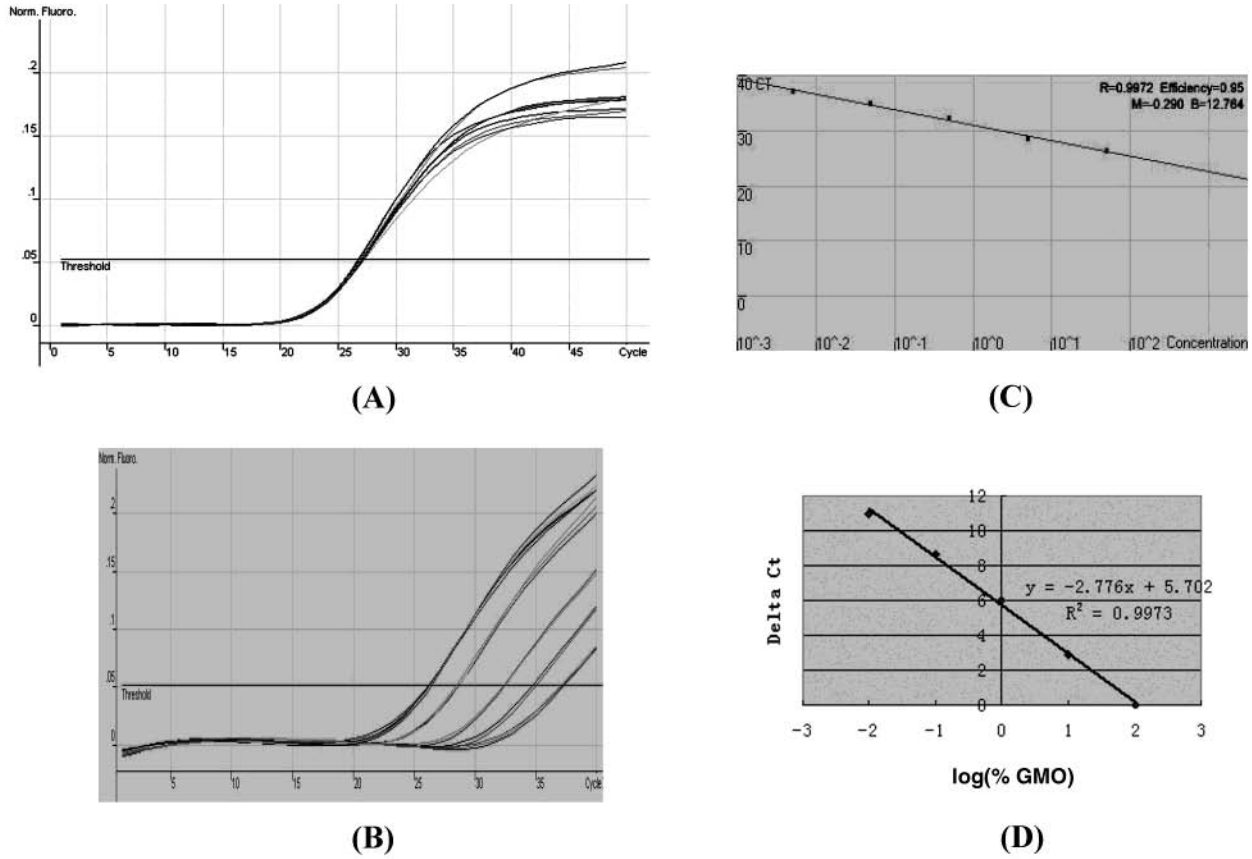


Figure 6. Multiplex quantitative PCR to detect the *SPS* and *GUS* genes using serial dilutions (10-fold) of transgenic rice. (**A**) Amplification plot of the endogenous *SPS* gene. Each dilution contains 50 ng of total rice DNA. (**B**) Amplification plot of the transgenic *GUS* gene and serial dilutions of transgenic rice ranging from 50 to 0.005 ng. (**C**) Initial transgenic DNA concentration vs Ct standard curve. (**D**) Standard curve, plotting log(GMO amount) vs ΔCt ($R^2 = 0.9972$, reaction efficiency = 0.95, three replicates per dilution).

pairs of the *SPS* and *GUS* genes, we were able to detect the *GUS* gene using 5 pg of initial transgenic rice genomic DNA, i.e., about 10 copies of the target *GUS* gene (**Figure 6C**). These results proved that the *SPS* gene could be used as the rice endogenous reference gene for GM rice detection.

DISCUSSION

In this article, we successfully validated a *SPS* gene as an endogenous reference gene of rice. All of the results in this paper demonstrated that this *SPS* DNA fragment met the requirements of the reference gene, i.e., rice species specificity, one copy number in the rice genome, and low heterogeneity among the different tested rice cultivars.

To set up the qualitative and quantitative PCR detection system using this endogenous reference gene, we designed a primer pair (SPSF/SPSR) to be used in the two PCR assays. The amplicons for the PCR products with an 81 bp length and its related qualitative and quantitative PCR cycling conditions were suitable for GMOs detection, which were similar to those of other widely used endogenous genes, such as maize *Zein* and soybean *Lectin* in GMOs detection (*11*). In addition, the sensitivities of 50 pg of rice genomic DNA for qualitative PCR and 5 pg of DNA for quantitative PCR were acceptable in GMOs detection (*6*). Similar results were also reported for GM soybean, maize, and rapeseed detection using *Lectin* (*20*), *Zein* (*14*), and *BnACCg8* as endogenous reference genes (*18*).

To assay the accuracy of the quantification system, we generated a standard curve by Ct value against initial rice DNA quality. The R^2 of the standard curve was 0.9990. When the amount of DNA was lowered to 5 pg or less, the Ct value would slightly separate from the calculated standard curve, which indicated that in such a quantitative PCR system using a combination of the SPSF/SPSR primer pair with the SPSP probe at such levels of DNA, quantification was not accurate. So to obtain the precise quantification results, the starting DNA template amount should not be less than 5 pg.

Additionally, to test the stability of the quantitative PCR system, we tripled all of the quantitative experiments. Comparing the Ct values of the three reactions, we got similar results. The CV values just varied from 0.76 to 5.46%, and the SD values were between 0 and 0.16. So the quantitative PCR system could be considered to work reliably and stably.

For practical use of the *SPS* gene as an endogenous reference gene of rice, we detected the amount of *GUS* transgenic rice by using multiplex quantitative PCR. The multiplex system is useful because one primer set can be used to detect the amount of a transgene that is variable and another can be used to detect an endogenous gene that is relatively constant, used as a reference, and has the advantage of not requiring the construction of standard curves for both genes of the endogenous reference gene and transgene. Instead, it requires a validation experiment to demonstrate that reaction efficiencies for the transgene and the endogenous reference gene are identical or very close (*24*). A sensitive method for assessing if two amplicons have the same efficiency is to examine how ΔCt varies (Ct target $-$ Ct reference) with template dilutions (*23*). Because the reaction efficiencies of the target *GUS* gene (0.95) and reference *SPS* gene (0.94) in our tests were similar, the ΔCt calculation for the relative quantification of the *GUS* gene

could be used. Moreover, we also used the *SPS* gene as the internal control gene to quantify the hygromycin phosphotransferase gene (*HPT*) in transgenic rice, and similar quantification results as that of the *GUS* gene detection were obtained (data not shown). All of these results demonstrated that the *SPS* gene could be used as the endogenous reference gene for GM rice detection.

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