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DETECTION OF RICE *SPS* GENE BY SIMPLEX PCR METHOD

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ABSTRACT

The study include 14 samples were chosen for *sucrose phosphate synthase* - *sps* gene. Molecular characterization which were characterized by PCR using *SPS* primer to identify up to generic level. Genomic DNA purification of 14 parallel Rice samples was carried out by DNeasy Plant Kit. The Yields and purities of the purified genomic DNAs were measured by spectrophotometer. The purities of all samples were higher than 1.7, which clearly demonstrates that the purified genomic DNA is of high quality. The integrity of the purified genomic DNA was also analyzed by agarose gel electrophoresis. The genomic DNA was used as template for PCR utilizing primers specific for the *sps* gene, and the amplified PCR products size were about 251base pair and the results demonstrate that the purity of the purified genomic DNA is sufficiently high for a sensitive PCR analysis. Detection primer with the optimized quantity of 10 pmole, were used in the PCR. PCR products treated with that primer pairs were observed only in rice samples. The present study confirmed that detection primer pair could efficiently amplify the corresponding gene. Nonspecific bands were not present at the annealing temperature 60 °C and the *sps* internal gene was amplified in all the rice samples with a size of 251 bp. For rice specific genes. PCR products treated with SPS primer pairs were observed. We showed that the PCR detection system used in the present study under optimized condition could be an appropriate tool, without primer interference or dimerization, for the detection of GM rice events. Here, we present results that will be useful for GM crop assessment in detecting multiple targets simultaneously with simplex PCR-based methods for various purposes, such as a part of the approval process or post-market monitoring.

INTRODUCTION

Polymerase chain reaction (PCR) methods are considered to be one of the most common DNA detection methods 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 used for identification of GM products but also for quantification purposes (1). 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 (2). 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 (3).

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Some genes have been used as the reference gene of some crops, for example, the *in* V*ertase I* (4, 5), 10 kDa *zein*, and *hmg-A* genes (6, 5) for maize; *lectin* (4) and *â-actin* genes for soybean; and *cruciferin* (7) and *BnACCg8* genes for rapeseed (5). 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. 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 for qualitative PCR assays. *SPS* (EC 2.4.1.14), a key enzyme in the sucrose biosynthetic pathway. A growing body of evidence indicates that *SPS* together with fructose 1, 6-bisphosphatase regulates the synthesis of sucrose in plants (8).

MATERIALS AND METHODS

Samples collection

Rice samples from different Iraqi local and non-Iraqi market was analyzed to detect the genetic modification. Rice samples were collected randomly (9), as shown in table (1) which illustrate some observations about the models that have been collected.

Table 1: Sampling areas and some of the specifications.

N	Sample	Product	Date of collection	Place the sample collection
1	ROYAL	INDIA	20-1-2013	Iraqi local markets
2	BASMATIC RICE	INDIA	20-1-2013	Iraqi local markets
3	SEVEN STAR	BACSTIN	20-1-2013	Iraqi local markets
4	AHMAD	INDIA	20-1-2013	Iraqi local markets
5	CHOPSTICK	U.S.A	20-1-2013	Iraqi local markets
6	UNCLE BENS BROUN RICE	U.S.A	20-1-2013	Iraqi local markets
7	DAAWAT BROUN RICE	INDIA.	20-1-2013	Iraqi local markets
8	ELDOHA golden rice.	Egypt.	5-2-2013	Egyptian local markets
9	LAASTURIANA	SPAIN	3-3-2013	Jordanian local markets
10	Long grain white rice Jerash good	Thailand	3-3-2013	General Company for Trade grain- Iraqi Ministry of Trade
11	U.S. rice	Argoae	20-3-2013	General Company for Trade grain- Iraqi Ministry of Trade
12	Thai rice mark(R)	Thailand	20-3-2013	General Company for Trade grain- Iraqi Ministry of Trade
13	NAFEES	Iraqi	20-3-2013	Iraqi local markets
14	BAGHDADY	Iraqi	3-4-2013	Iraqi local markets

Genomic DNA extraction from rice event

About 160 µg rice samples were ground using electric blender, and then treated with the DNA easy Plant Kit (promega, Germany). Isolated genomic DNA from various different rice samples were resuspended in double distilled water and quantified by a UV spectrophotometer. Appropriate concentration of each DNA was determined and applied to PCR.

Primer design for simplex or multiplex PCR

Primers for multiplex PCR as well as simplex PCR were designed according to the nucleotide sequence database NCBI (National center for Biotechnology information). The nucleotide sequences of the primer set are shown in Table (2). For detecting a rice endogenous gene as an internal positive

control, sucrose phosphate synthase (SPS) gene was chosen and its nucleotide sequences were used for primer design.

Table 2: Primer pairs for PCR detection of rice sample.

Name	Sequence (5'to3')	Target	Amplicon (bp)	Specificity
SPS-F SPS-R	AGCAACAGTCCAGTAAAAAGAGAGCCCCGAAC GAGAGGAAAGGGAAAAAAGCGTCACGTACCA	SPS	251	SBS formed

PCR amplification

Conventional PCR was carried out to verify the proper amplification with the respective primer. Genomic DNA from rice samples was used as the template for the PCR. The reaction mixture for PCR contained 25 µL of 2XMax Taq Hot start master mix (Bio Quest, Korea), 10 Pmole of each primer pairs, 20 ng of template, and distilled water at a final volume of 50 µL. PCR was performed in a thermal cycler (BioRad, Hercules, CA). Pre-treatment was executed at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min 30 s. Further elongation was performed at 72°C for 10 min. PCR amplicons were separated in 1% r agarose gel (CAMBREX, East Rutherford, NJ) at 100 V for 30 min and then visualized by ethidium bromide staining. As a reference, a 100-bp DNA ladder (Fermentas, Burlington, Ontario) was used for size comparison.

RESULTS AND DISCUSSION

Table (3) refers to results for yield and purity DNA of all rice samples. Most of the DNA extracted by CTAB methods in this study showed a high molecular weight and high purity with A260/ A280 rang varies between 1.7-1.9.

This result is agreed with those of Romano and Brasileiro (10) and Couto *et al.* (11) A ratio of higher than 2.0 generally indicated RNA contamination, while the ratio lower than 1.7 normally indicated protein contamination during extraction process, so the good quality DNA should be ranged between 1.70- 2.00 (Table 3).

Table 3: Yields and purities of the genomic DNA from Rice samples

Sample no.	Rice type	Purity (260 nm/280 nm)	Concentration (µg/ml)	Yield (µg)
1	ROYAL	1.9	19	1.9
2	BASMATIC RICE	1.9	19	1.9
3	SEVEN STAR	1.9	19	1.9
4	AHMAD	1.7	23	2.3
5	CHOPSTICK	1.7	23	2.3
6	UNCLE BENS BROUN RICE	1.8	23	2.3
7	DAAWAT BROUN RICE	1.8	17	1.7
8	ELDOHA golden rice.	1.8	24	2.4
9	LAASTURIANA	1.8	24	2.4
10	Long grain white rice Jerash good	1.8	24	2.4
11	U.S. rice	1.9	19	1.9
12	Thai rice mark(R)	1.9	19	1.9
13	NAFEES	1.8	1.91	1.62
14	BAGHDADY	1.8	18.1	1.81

Of the 35 samples, 14 samples were chosen for *sps* gene molecular characterization and were further characterized by PCR using *SPS* primer to identify up to generic level (Table 3). Genomic DNA purification of 14 parallel samples from Rice samples was carried out by DNeasy Plant Kit (promega,

Germany). Before genomic DNA purification samples were treated with lysozyme enzyme to break the cell walls. Yields and purities of the purified genomic DNAs were measured by spectrophotometer. The integrity of the purified genomic DNA was also analyzed by agarose gel electrophoresis (Fig.1).

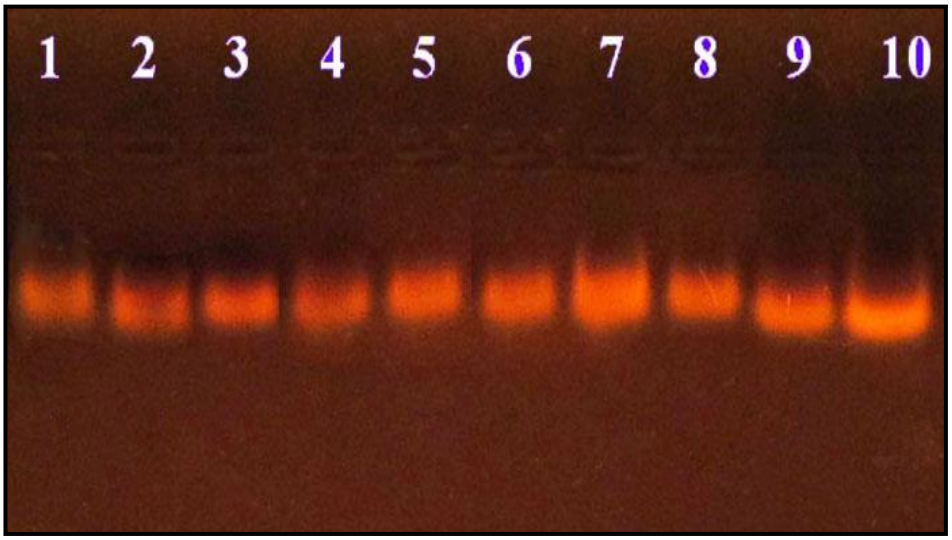

Figure 1: Agarose gel electrophoresis (1% agarose gel) analysis of the genomic DNA purified from Rice samples with DNeasy Plant Kit (promega, Germany) 1-10 = ten Rice samples of genomic DNA eluates, stained with ethidium bromide at 80 volts/cm for 1 hour Photographed under UV light.

All samples within this range demonstrating that DNA of sufficient integrity for PCR analysis. To find out the efficiency of the extraction process and to get positive results for rice specific gene before screening for genetic modification, specific primer for Endogenous rice gene should be used.

The fourth samples were chosen for *sps* gene molecular characterization by conventional PCR using *SPS* primer. The results revealed that the *sps* internal gene was amplified with expected size of 251 bp (Figure 2). Detection primers with the optimized quantity of 10 pmole, were used in the PCR for rice specific genes.

The results of this study confirmed that detection primer pair could efficiently amplify the corresponding gene in all tested samples. The present data came in agreement with the findings by Ding *et al.* (12) 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.

Other study for efficient simplex PCR detection of rice crops improvements in the number of target genes, the specificity of the primers, the prevention of primer dimerization, as well as optimization of the PCR conditions need to be considered (13, 14).

The specificity detection of the different GM elements relies on the fact that the method exploits two independent specific steps: PCR-specific assay conducted with specific primers as mentioned by Hamels *et al.* (15).

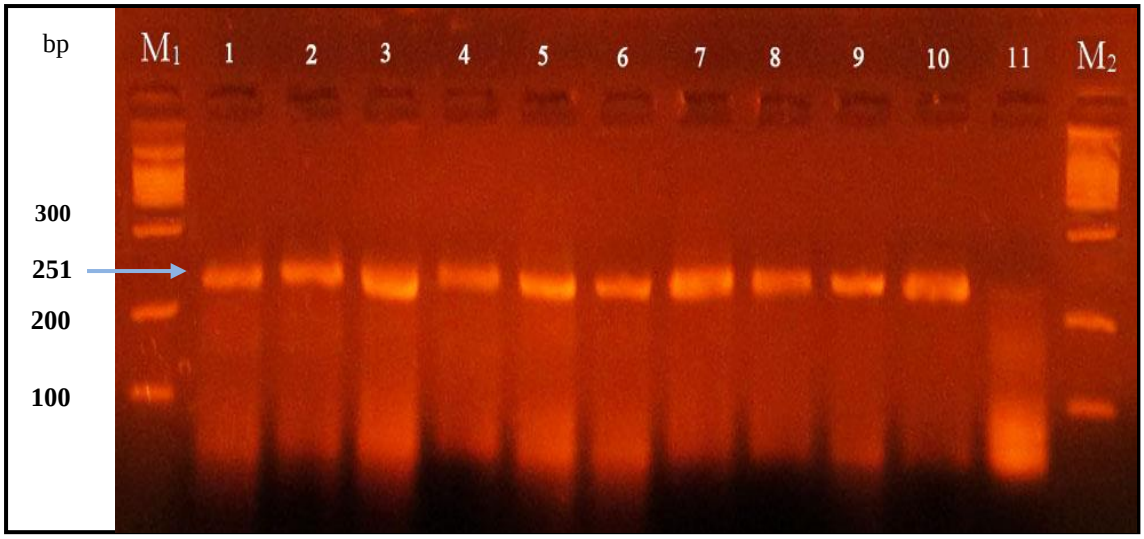

Fig.2: Detection of the PCR product DNA bands of Nonspecific detection of the *SPS* DNA different samples rice cultivars using conventional PCR, The amplified fragments were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide at 90. volts/cm for 1 hour. Photographed under UV light.

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الكشف عن جين*sps* في الرز باستخدام طريقة التفاعل الإنزيمي المتضاعف التسلسلي المبسط

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الملخص

تضمنت الدراسة انتخاب أربعة عشر أنموذجاً لتحديد وتشخيص جزيئي لجين إنزيم بناء السكر الفوسفاتي (*sps* gen) واستخدام تقانات التفاعل الإنزيمي المتضاعف التسلسلي (PCR) وبادئ جين *sps* للتشخيص والتعرف على ألجين بصورة أدق على المستوى الجيني. أجريت عملية استخلاص وتنقية الحامض النووي الرايبوزي منقوص الأوكسجين (DNA) لأربعة عشر أنموذجاً للرز وفي وقت واحد (بصورة متوازية) باستخدام استخلاص وتنقية DNA النباتي. النماذج التي تعطي كثافة ضوئية أعلى من 1.7 تعني الحصول على DNA بنقاوة عالية. تم تحليل DNA المنقى باستخدام تقانة الترحيل الكهربائي لهلام الاكاروز. استخدم DNA المنقى كقالب في جهاز PCR والاستفادة من الباديء الخاص للجين *sps* لمضاعفة نسخ الجين و الحصول على منتج حجمه بمقدار 251 زوجاً قاعدياً. تشير نقاوة DNA العالية إلى حساسية التحليل جهاز PCR ونوعية وكمية باديء الكشف المثلى وهي 10 بيكومولات المستخدم والخاص بـ DNA الرز فقط. تؤكد دراستنا إن زوج الباديء المتخصص يمكن أن يؤدي إلى تضاعف كفوء للجين الهدف، وعدم وجود حزم غير متخصصة (تداخلات) عند درجة حرارة الارتباط 60 م، فضلاً عن مشاهدة تضاعف جين *sps* في كل نماذج الرز بحجم 251 زوجاً قاعدياً والخاصة بجينات الرز المعاملة بزوج باديء SPS المنتجة باستخدام جهاز PCR المبسط. نشاهد أن نظام الكشف باستخدام PCR المبسط وبظروفه المثلى المستخدم في دراستنا يمكن أن تكون أداة مناسبة للكشف عن الرز المحور جينياً دون ظهور تداخلات للباديء أو ظهور ازدواجات. نتقدم بنتائجنا التي يمكن أن تساعد في الكشف عن أهداف متعددة أحياناً مع طرائق معتمدة على PCR المبسط لأغراض متعددة جزءاً من عمليات المراقبة للصلاحية أو السوق ما بعد الإنتاج.

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