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Screening of Exist Genetically Modified Elements in Local and Commercial Rice Available in Baghdad Markets Using PCR and Real-time PCR

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Abstract: Rice is one of the staple foods of the Iraqi population; therefore large amounts of rice cultivated and imported in Iraq. Because of increasing the production of GM crops especially rice crop, it was necessary to investigate if there is any genetically modified rice (GM rice) in Baghdad markets. Conventional PCR and Real-Time PCR used to create this investigation. Genomic DNA extracted from 7 samples of rice seeds that cultivated in Iraq; 31 samples of commercial rice seeds and 4 samples of kids' food, where rice is one of their ingredients. The primers RM 171 selected to amplify the Rice Microsatellite Region (RM) for the DNA integrity inspection and rice species detection especially samples of kids' food, as well as, the primers P35S selected to amplify the CaMV35S promoter region (P35S) for Genetically Modified elements (GM elements) detection. There was no existence of GM elements in all samples except one, which was one of kids' food samples. These results indicate that the ability to produce GM crops and monitoring of the GM food entry are limitable in Iraq. Moreover, no commitment in the labeling regulations of genetically modified food.

Keywords: Conventional PCR, GM rice, GM elements, labeling regulations, P35S

INTRODUCTION

Rice has been considered as staple foods consumed by Human beings, where more than half of the world population used rice as a daily essential food, especially in Asia and the third world countries such as Iraq (Kameswara and Jackson, 1997; Londo *et al*., 2006). Rice has been chosen as a model organism for the molecular biology research because of the economic importance and the small size of its genome (just 430Mb), where it was the first crop that its genome sequence was completed (Bennetzen, 2002; Chao *et al*., 2003).

The rice cultivation faced many problems such as shortage of water resources or drought; lower yield; diseases and insects' infections and other problems (Zhang, 2007). All these0020problems can be solved by improving agricultural practices; using traditional plant breeding techniques or modifying the genetic code using modern scientific technologies, these technologies those known as gene technology or genetic modification technology capable of overcoming the problems more quickly than traditional methods (Verma *et al*., 2011).

The scientists developed a rice crop using genetic modification technology, where they include many traits to rice such as quality improvement; yield enhancement, drought tolerance and insects and diseases resistance as described in the following studies: Paine *et al*., (2005), Bandyopadhyay *et al*. (2007), Kanneganti and Gupta (2008), Tang *et al*. (2006) and Datta *et al*. (2002) respectively. The products of these studies known as genetically modified foods (GM Foods) or Genetically Modified Rice (GM Rice).

Genetically modified food is a term referring to genetically modified plants or/and the food contains components of genetically modified plants, as well as products of genetically modified organisms and this food uses for human and animal consumption (Whitman, 2000; Verma *et al*., 2011). GM Food is one of the best methods that have solved many problems such as nutritional values, world hunger, environmental contamination and other global issues. Since 1996, the cultivation of genetically modified crops has been growing; which, as a result, led to a need of global legislation to regulate the presence of genetic modification in food, rate of genetic modification, labeling for GM foods and methods of detection (James, 2003; Rizzi *et al*., 2004).

The most common methods of GMOs detection are Enzyme Linked Immunosorbant Assay (ELISA) and Polymerase Chain Reaction (PCR), but the best method is PCR because it is more specific and sensitive, despite being more expensive, time consuming and cannot be done on site (Brandner, 2002; Jasbeer *et al*., 2008; Vidhya *et al*., 2012). There are many types of PCR such as conventional PCR, which is used for GMOs detection (qualitative) and Real-Time PCR, which is used for GMOs detection and rate of genetic modification determination (qualitative and quantitative) (Rizzi *et al*., 2004; Abdel-Mawgood *et al*., 2010). Moreover, Real-Time PCR is preferred in routine work because it shorter time if compared to conventional PCR that needs gel electrophoresis to analyze the results (Vidhya *et al*., 2012).

The aim of this study is determined if there is any GM rice in Baghdad markets using conventional PCR and Real-Time PCR.

MATERIALS AND METHODS

Sampling and genomic DNA extraction: The samples of rice seeds that are cultivated in Iraq collected from the State Board for Agricultural Research (SBAR) and AL-Mashkhab Rice Research Station (MRRS) while the samples of commercial rice seeds collected randomly from local market in Baghdad during the period between January-May, 2013. Table 1 shows the details of 42 rice samples including 7 samples of rice seeds that cultivated in Iraq; 31 samples of commercial rice seeds and 4 samples of kids' food, where rice is one of their ingredients. Standard protocol (The isolation of genomic DNA from plant tissue protocol) of wizard genomic kit (DNA purification kit, Promega, USA) was used for DNA extraction from 60 mg of ground seeds. The quality and quantity of extracted DNA was determined by using ActGene micro-volume UV/Vis spectrophotometer (Avans, Taiwan).

Polymerase Chain Reaction (PCR):

Primers: The primers were selected according to previously published studies Hashemi *et al*., (2009) and Tung *et al*. (2008). These primers amplified the Rice Microsatellite Region (RM) for rice species detection and the CaMV35S promoter region (P35S) for GMOs detection. The sequences of PCR primers were as following: (RM171 F: 5'-ACG AGA TAC GTA CGC CTT TG-3'); (RM171 R: 5'-AAC GCG AGG ACA CGT ACT TAC-3'); (P35S 1-5: 5'-ATT GAT GTG ATA TCT CCA CTG ACG T-3') and (P35S 2-3: 5'-CCT CTC CAA ATG AAA TGA ACT TCC T-3'). All primers were supplied by Alpha DNA Company, Canada.

Conventional PCR (reactions and programs): In 25 µL of PCR reaction, 1.5 µL DNA template (100 ng/µL) was amplified using 12.5 µL of Go *Taq*® green master

Table 1: The details of rice samples and the PCR results

					PCR results	
No.	Name	Origen	Sours	Type of food	RM171	P35S
1	Amber	IRAQ	SBAR and MRRS	Rice grains	+	-
2	Yasmin	IRAQ	SBAR and MRRS	Rice grains	+	-
3	Furat	IRAQ	SBAR and MRRS	Rice grains	+	-
4	Mashkhab -1-	IRAQ	SBAR and MRRS	Rice grains	+	-
5	Mashkhab -2-	IRAQ	SBAR and MRRS	Rice grains	+	-
6	Brnamge -4-	IRAQ	SBAR and MRRS	Rice grains	+	-
7	AL-abasia	IRAQ	SBAR and MRRS	Rice grains	+	-
8	NAFEES	IRAQ	Iraqi market-Baghdad	Rice grains	+	-
9	AHMAD	INDIA	Iraqi market-Baghdad	Rice grains	+	-
10	AL-MURAD	INDIA	Iraqi market-Baghdad	Rice grains	+	-
11	KOHINOOR	INDIA	Iraqi market-Baghdad	Rice grains	+	-
12	Punjabi Al-Muhaidib	INDIA	Iraqi market-Baghdad	Rice grains	+	-
13	ABU ARABA	INDIA	Iraqi market-Baghdad	Rice grains	+	-
14	KARMAN	INDIA	Iraqi market-Baghdad	Rice grains	+	-
15	AL.AILA	INDIA	Iraqi market-Baghdad	Rice grains	+	-
16	DAAWAT	INDIA	Iraqi market-Baghdad	Rice grains	+	-
17	MAHMOOD	INDIA	Iraqi market-Baghdad	Rice grains	+	-
18	FAYAH	INDIA	Iraqi market-Baghdad	Rice grains	+	-
19	TILDA	INDIA	Iraqi market-Baghdad	Rice grains	+	-
20	SULTANI	INDIA	Iraqi market-Baghdad	Rice grains	+	-
21	ALWALIMAH	INDIA	Iraqi market-Baghdad	Rice grains	+	-
22	272 (Blue bag)	INDIA	Iraqi market-Baghdad	Rice grains	+	-
23	272 (Red bag)	INDIA	Iraqi market-Baghdad	Rice grains	+	-
24	Abu al-dahab	INDIA	Iraqi market-Baghdad	Rice grains	+	-
25	DURRA	INDIA	Iraqi market-Baghdad	Rice grains	+	-
26	Basmaki	INDIA	Iraqi market-Baghdad	Rice grains	+	-
27	AL-TAIYEB	INDIA	Iraqi market-Baghdad	Rice grains	+	-
28	AL-REEF	INDIA	Iraqi market-Baghdad	Rice grains	+	-
29	INDIA GATE	INDIA	Baghdad International Fair	Rice grains	+	-
30	1121	INDIA	Baghdad International Fair	Rice grains	+	-
31	Palakkad red mutta	INDIA	India market	Rice grains	+	-
32	R2	URUGUAY	Iraqi market-Baghdad	Rice grains	+	-
33	R9	URUGUAY	Iraqi market-Baghdad	Rice grains	+	-
34	R10	URUGUAY	Iraqi market-Baghdad	Rice grains	+	-
35	STAR	PAKISTAN	Iraqi market-Baghdad	Rice grains	+	-
36	RONI Bakliyat	ITALY	Iraqi market- Anah	Rice grains	+	-
37	MAXIM' S	USA	Iraqi market-Baghdad	Rice grains	+	-
38	Kids Against Hunger (other ingredients)	USA	Food aid-Baghdad	Dehydrated components	-	+
39	Kids Against Hunger (Rice ingredient)	USA	Food aid-Baghdad	Rice grains	+	-
40	COCO POPS	SPAIN	Iraqi market-Baghdad	Chocolate toasted rice	+	-
41	Nesquik	TURKEY	Iraqi market-Baghdad	Cereals chocolate flavored	+	-
42	SAHHA	JORDAN	Iraqi market-Baghdad	Infant cereal rice	+	-

Table 2: The PCR programs conditions

	Time and temperature			
Steps	RM171 F/ RM171 R		P35S 1-5/P35S 2-3	
Initial denaturation	5 min at 94°C		5 min at 95°C	
Denaturation	1 min at 94°C		1 min at 95°C	
Annealing	1 min at 60°C	33 cycles	1 min at 55°C	35 cycles
Extension	1 min at 72°C		1 min at 72°C	
Final extension	7 min at 72°C		7 min at 72°C	

mix 2X (Promega, USA) and 0.33 µL of each primer of RM 171 primers (10 pmol/µL) or 0.5 µL of each primer of P35S primers (10pmol/µL),up to the final volume 25 µL with nucleases free water. PCR programs were set on Lab net International thermal-cycler (Multigene TM Gradient Thermal Cycler, Korea); Table 2 described the conditions of these programs (Younan *et al*., 2012; Aarif *et al*., 2013).

Real-time PCR (reactions and program): The Real-Time PCR reaction was performed using *AccuPower* GreenStar qPCR PreMix Kit (Bioneer-Korea), this pre-mix pellet containing all required components for qPCR reaction with the exception of the DNA template and primers. Furthermore, this pre-mix containing SYBR Green dyes for monitoring the amplification process. For each reaction, 0.5 µL of each P35S primers (10 pmol/µL) and 1.5 µL of DNA (100 ng/µL) were added. After that, the final volume was adjusted to 20 µL with DEPC-distilled water. The Real-Time PCR program conditions for GMOs detection were carried out, initial denaturation, at 95°C for 5 min (1 cycle), followed by 45 cycles of denaturation: at 95°C for 30 Sec, annealing: at 60°C for 30 Sec, extension: at 70°C for 30 Sec and scan the fluorescent of SYBR Green dye. Subsequently, the PCR products exposed to melt by increasing 1°C every 2 Sec starting from 60 up to 95°C to make sure the products specificity.

All PCR runs, conventional PCR and Real-Time PCR, included negative and positive control samples. To detect any contamination, negative control reaction was set in each PCR experiment, a negative control reaction containing all components of the reaction without DNA template. On the other hand, a positive control was prepared to determine the effectiveness of the conditions of PCR reaction and program. A positive control reaction containing all components of the reaction with DNA template of standard sample, which containing the target regions (RM 171 and/or CaMV35S promoter).

Agarose gel electrophoresis analysis: The PCR products and 100 bp DNA ladder bands (Promega, USA) were separated using 2% agarose gel electrophoresis, then stained the gel with ethidium bromide and visualized under the ultraviolet light (302nm) (Sambrook and Russell, 2001). The expected molecular size of PCR products were about 305-331 bp for primer RM171 and about 101-110 bp for primer P35S.

RESULTS AND DISCUSSION

To detect if there is any GM rice grain in Baghdad markets, 42 samples were collected from different regions of Baghdad; all collected samples were known origin and certified. DNA extracted using DNA purification kit (Promega, USA); this commercial kit was suitable method for DNA extraction from dry seeds comparing with the traditional methods. Moreover, this method is not time-consuming as traditional methods. DNA yield and purity ranged between (435.5-1141 ng/µL) and (1.32-1.98), respectively.

The PCR results of RM171 detection used to confirm that the studied samples were rice and/or the rice was one of food ingredients, as well as to confirm the DNA integrity for being used in PCR experiments. All tested samples show positive results (band with size 330 bp), for RM171marker except sample no. (38) (Fig. 1) because this sample was the food ingredients of Kids against Hunger food that prepared from the following components: Textured Vegetable; Soy; Modified Corn Starch and other materials. That means all samples contain rice genome and the extracted DNA was apt for using in PCR experiments. This result is similar to those of previously published studies those used different species specific genes such as *lectin* and *zein* genes to detect species of soybean and maize, respectively (Al-Rousan *et al*., 2010; Yoke-Kqueen *et al*., 2011), also to confirm the DNA integrity to be used in PCR experiments.

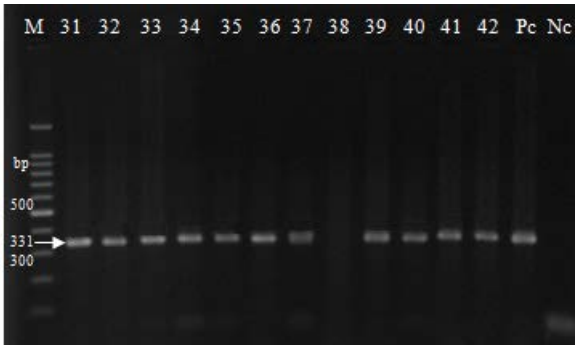

Fig. 1: PCR amplification of RM171. PCR products run in (2%) agarose gel using 0.5X TBE (5V/cm for 2hr.). Lanes M: 100 bp DNA ladder; Nc: PCR negative control; Pc: PCR positive control; Lanes 31 to 42: samples (Table 1)

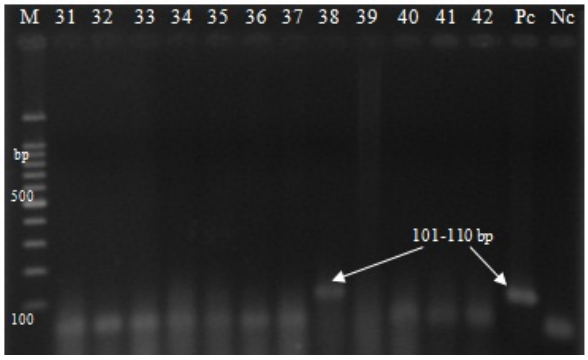

Fig. 2: PCR amplification of 35S promoter. PCR products run in (2%) agarose gel using 0.5X TBE (5V/cm for 2hr.). Lanes M: 100 bp DNA ladder; Nc: PCR negative control; Pc: PCR positive control; Lanes 31 to 42: samples (Table 1)

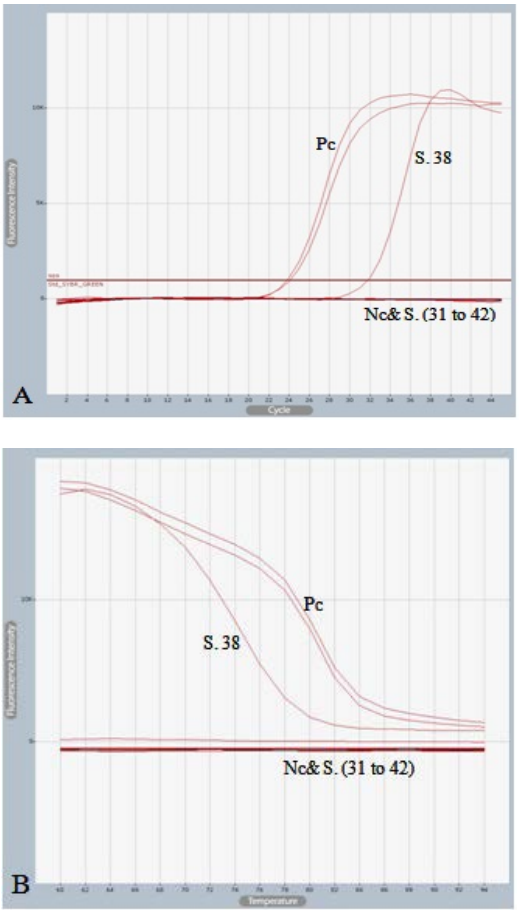

Fig. 3: Graphs of (A) real-time PCR amplification-curve and (B) melt-curve of 35S promoter for samples (31 to 42); Positive control (Pc) and Negative control (Nc)

The 35S promoter and the NOS terminator exist in most of the transgenic crops and these elements known as the GM elements (Abdel-Mawgood *et al*., 2010; Alaraidh *et al*., 2011; Vidhya *et al*., 2012). In this study, the PCR experiments were performed using primers (P35S) to detect the presence of GM element (P35S)

Table 3: The cycle threshold value (Ct) of 35S promoter for samples (31 to 42); positive control (Pc) and negative control (Nc)

Samples	Replicates	Ct	Average
31-37	1	0.000	0.000
	2	0.000	
38	1	31.76	30.63
	2	29.50	
39-42	1	0.000	0.000
	2	0.000	
Pc	1	23.80	23.96
	2	24.12	
Nc	1	0.000	0.000
	2	0.000	

and only one out of 42 tested samples gave a positive result (Fig. 2). GMO element detected with sample no. (38); this sample contains modified corn starch as one of the food ingredients; therefore, this result was expected.

Real-Time PCR experiments have shown the same results of conventional PCR. Sample no. (38) gave the highest peak in the amplification-curve as an indicator of exist the GM element (P35S) comparing with the other samples as shown in Fig. 3A, this peak almost similar to the transgenic standards (Positive control samples (Pc)) peak.

Furthermore, the cycle threshold value (Ct) was determined to detect the amplification efficiency. The average Ct value of sample no. (38) was 30.63 compare with the average Ct value of transgenic standards, or positive control (Pc) was 23.96 while average Ct value was undetermined for the other studied samples and negative control sample (Nc) that indicated the absence of GM elements (P35S) in their DNA. Two replicates were performed for each sample with each run as evident from Table 3.

Figure 3B shows the melt-curve to the products specificity because SYBR Green Dye may bind to double strand DNA such as specific/non-specific PCR products or primer-dimmers (Vidhya *et al*., 2012). If the PCR products were specified, they would have the same length; thus the melt peaks of these products were at the same temperature while the non-specific PCR products have different temperature depending on the product's length and other factors. These results came to confirm the results of conventional PCR and to detect the SYBR green kit effectiveness for using in the future in the routine work for detecting GM Food. This method is a rapid process for food testing, where it gives a reliable result within a few hours without the need for agarose gel electrophoresis analysis (Rizzi *et al*., 2004).

As expected, all Iraqi studied samples were not GM Crops because these samples are local and ancient Iraqi varieties and there was no ability to produce the GM Crops in Iraq. The common indicator of GMOs (P35S) was not detected in the rest of the samples studied, some of these samples certified and labeled from their sources as products that are not genetically

modified. Sample no. (38) is food from the organization of Kids Against Hunger as a food aids for kids in developing countries. This kind of food has been introduced to Iraq without any GM food detection, although there is decision no. 128 that issued from the Advisory Board of the Food in July 2002 ban the import; introduce; use and transfer of GM foods to Iraq (Aarif *et al*., 2013).

When making contact with the Kids against Hunger organization clarify if there is any permission for using GM crops by the authorized agencies such as United States Department of Agriculture (USDA) and if there was permission, why their products did not label from these responsible agencies? The answer was as following: "We are working on that as we speak. It is very involved process to get them to approve anything. We are hoping to have this done by the end of the summer!" (Private connection by e-mail). That means there is no allowance till now to marketing this product.

In these days, there are no rules for regulating the GM food introduction and their products into Iraq; thus, it is easy to enter such foods. This study is not the first in GM food detection in Iraq, but it is the first in GM rice detection in Iraq. There are a number of GM food detection researches with other types of food, where Aarif *et al*. (2013) study indicated that there are GM Corn (*Zea mays*) in the Iraqi markets while Hassan and Ali (2012) study observed presence of the transgenic sequences of maize in three samples of milk that collected from the Sulaimani market in Iraq.

CONCLUSION

In this study, genetically modified elements were detected in rice samples using PCR and Real-Time PCR. The PCR techniques used in the GM food detection has been sensitive and effective. All the Iraqi rice varieties were not GM crops that mean there was no ability to produce any GM crops in Iraq. Also, we can conclude that the monitoring of the GM food entry to Iraq is limitable and there is no commitment neither in labeling some foods that genetically modified, nor in taking approvals from responsible organizations or agencies.

REFERENCES

Aarif, G.L., B.K. Sulaiman and Z.M. Alkhafaji, 2013. Screening of genetically modified corn (*Zea mays*) *in* Iraqi market. J. Biotechnol. Res. Center, 7(1): 21-29.

Abdel-Mawgood, A.L., M.A. Gassem, A.A. Alsadon, S.S. Alghamdi and A.A. Al-Doss, 2010. Monitoring of genetically modified food in Saudi Arabia. Afr. J. Food Sci., 4(8): 536-540.

Alaraidh, I., A. Amid, A. Farouk, M.M. Ibrahim, S. Bazaid, R. Greiner and A. Alghunaim, 2011. Detection of genetically modified rice: Collaborative validation study of a PCR based detection of genetically modified rice *Oryza sativa* commercially available in Saudi Arabia. IUFS J. Bio., 70(1):11-17.

Al-Rousan, H., N. Al-hmoud, B. Hayek and M. Ibrahim, 2010. A study on the occurrence of genetically modified soybean and maize feed products in the Jordanian market. J. Cell Mol. Bio., 8(2): 87-94.

Bandyopadhyay, A., K. Datta, J. Zhang, W. Yang, S. Raychauhuri, M. Miyao and S.K. Datta, 2007. Enhanced photosynthesis rate in genetically engineered *indica* rice expressing *pepc* gene cloned from maize. Plant Sci., 172: 1204-1209.

Bennetzen, J., 2002. The rice genome-opening the door to comparative plant biology. Science, 296: 60-63.

Brandner, D.L., 2002. PCR-Based detection of genetically modified foods. Proceedings of the 23rd Workshop/Conference of the Association for Biology Laboratory Education (ABLE), pp: 69-84.

Chao, Y., C. Chen, H. Chen, T. Chow, M. Chung, J.S. Hsieh and Y.I. Hsing, 2003. Rice structural and functional genome research in ASPGC, academia sinica. J. Genet. Molecul. Bio., 14(4): 201-206.

Datta, K., N. Baisakh, K.M. Thet, J.Tu and S.K. Datta, 2002. Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. Theor. Appl. Genet., 106(1): 1-8.

Hashemi, S.H., S.A.M. Mirmohammadi-Maibody, G.A. Nematzadeh and A. Arzani, 2009. Identification of rice hybrids using microsatellite and RAPD markers. Afr. J. Biotechnol., 8(10): 2094-2101.

Hassan, K.I. and A.A. Ali, 2012. Detection of genetically modified DNA in milk using species specific PCR. J. Am. Sci., 8(9): 248-252.

James, C., 2003. Global review of commercialised transgenic crops. Proceeding of International Service for the Acquisition of Agri-Biotech Applications (ISAAA). Ithaca, New York.

Jasbeer, K., F.M. Ghazali, Y.K. Cheah and R. Son, 2008. Application of DNA and immunoassay analytical methods for GMO testing in agricultural crops and plant-derived products. ASEAN Food J., 15(1): 1-25.

Kameswara, R.N. and M.T. Jackson, 1997. Variation in seed longevity of rice cultivars belonging to different isozyme groups. Genet. Resour. Crop Ev., 44: 159-164.

Kanneganti, V. and A.K. Gupta, 2008. Over expression of OsiSAP8, amember of stress as sociated protein (SAP) gene family of rice confers tolerance to salt, drought and cold stress in transgenic tobacco and rice. Plant Mol. Biol., 66(5): 445-462.

Londo, J.P., Y. Chiang, K. Hung, T. Chiang and B.A. Schaal, 2006. Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. PNAS, 103(25): 9578-9583.

Paine, J.A., C.A. Shipton, S. Chaggar, R.M. Howells and M.J. Kennedy, 2005. A new version of golden rice with increased pro-vitamin a content. Nat. Biotechnol., 23: 482-487.

Rizzi, A., C. Sorlini and D. Daffonchio, 2004. Practicality of detection of Genetically Modified Organisms (GMOs) in food. Agric. Biotech. Net., 6: 1-9.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Tang, W., H. Chen, C. Xu, X. Li, Y. Lin and Q. Zhang, 2006. Development of insect resistant transgenic *indica* rice with a synthetic *cry1*C* gene. Mol. Breed, 18:1-10.

Tung, N.C., R. Son, A. Raha, O. Lai and V. Clemente, 2008. Detection of Genetically Modified Organisms (GMOs) using molecular techniques in food and feed samples from Malaysia and Vietnam. Int. Food Res. J., 15: 155-166.

Verma, C., S. Nanda, R.K. Singh, R.B. Singh and S. Mishra, 2011. A review on impacts of genetically modified food on human health. Open Nutraceuticals J., 4: 3-11.

Vidhya, S.S., P.H. Ramanjini Gowda, K.N. Yogendra, T.M. Ningaraju and T. Salome, 2012. Detection of genetically modified cotton seeds using PCR and real-time PCR. Indian J. Biotechnol., 11: 176-181.

Whitman, D.B., 2000. Genetically Modified Foods: Harmful or Helpful? Retrieved form: http:// www.csa.com/hottopics/gmfood/overview.html.

Yoke-Kqueen, C., C. Yee-Tyan, K. Siew-Ping and R. Son, 2011. Development of multiplex-PCR for Genetically Modified Organism (GMO) detection targeting EPSPS and Cry1Ab genes in soy and maize samples. Int. Food Res. J., 18: 515-522.

Younan, H.Q., B.K. Sulaiman and A.A. Al-kazaz, 2012. Identification of several rice genotypes (*Oryza sativa*) using DNA markers based methods. Proceeding of the 4th Kurdistan Conference on Biological Sciences. University of Duhok, Duhok, Iraq, pp: 307-316.

Zhang, Q., 2007. Strategies for developing green super rice. PNAS, 104(42): 16402-16409.