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PLANT GENETICS

Testing Safety of Genetically Modified Products of Rice: Case Study on Sprague Dawley Rats

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Abstract—Genetic engineering is considered as background for crop protection against pest damage by adding new genes inside the grains. Rice, like other cereals is included in gene engineering experiments. The questions about possible gene transfer related to food safety appear. It is important to find any additional genes or fragments in animal tissues after consumption of genetically modified (GM) food. Therefore, in this study, the remaining of *CryIA(b)* gene and *P35* were assessed in the liver of rats fed with GM rice. This work presents an experimental study with the intervention of GM rice feeding by Sprague Dawley rats. Overall, 20 male and 20 female SD rats were fed by pellets made by GM rice in 50% of needed carbohydrate for 90 days. Then, sampling was done from rats liver. DNA extraction was done based on the protocol. The quality and quantity of the extracted DNA was done by agarose gel electrophoresis and spectrophotometry, respectively. Detection of GM genes residues, including *CryIA(b)*, *P35*, and *T35* was done by Polymerase Chain Reaction using specific primer pairs. The results were analyzed by agarose gel electrophoresis alongside with 50 bp DNA ladder. The results were compared with the ones in control groups with feeding by standard pellet of non-modified rice. All amplification tests were done in triplicates. Analysis of the amplification of *P35*, *CryIA(b)* and *T35* showed no residues inside the liver tissue. The results showed no significant difference in the presence of transgenic gene of *cryIA(b)*, *T35*, and *P35* in the liver tissue between the control and experiment groups. Therefore, this study rejects the possibility of gene settle of GM rice gene residues in liver tissue of the animal model studied.

Keywords: genetically modified rice, transgene, food safety, Sprague Dawley rat, liver, *CryIA(b)*, *P35*, *T35*
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INTRODUCTION

Rice is the staple food of more than half the world's population, with more than 3.5 billions [1]. According to forecasts, by 2025, rice will be the primary food of about 4.3 billion people of the world. This will require an annual production of 880 million tons of rice, which is far higher than the current global production. On the other hand, more than 53% of the world's population exposed to hunger and poverty live in countries

in which rice farming is prevalent, and thus increasing the productivity of rice production in these areas is of greater importance [2–4]. However, rice, like other cereals, is one of the products severely damaged by insects [5].

Approximately 200 species of insects severely damage plants during their growing season. This damage for rice has ranges from 15 to 25% [6]. Various methods are used to reduce crop waste. One of the most recent of these techniques is genetic engineering, which is modifying genomic host by introducing insect-resistant genes by transferring genetic material from any plant, animal, microorganism or artificial material [5, 6]. After such genetic changes, plants show new and desirable properties, such as increased resistance to insects, which can greatly contribute to reducing damage [7]. These kinds of foods are named genetically modified (GM) foods. Safety concerns raised continuing public debated worldwide [3, 7–9]. Here we consider only case of experimental food safety testing on a GM-rice cultivar from point of view of possible gene transfer in animal organism.

Bacillus thuringiensis (Bt) is a microorganism that naturally produces active protein against certain insects [10]. *B. thuringiensis* forms crystals of insecticidal δ-endotoxins (crystal proteins or Cry proteins), which are encoded by *cry* genes. After transferring of these genes to the rice genome, it can be resistant against insects, including types of moths of *Sesamia inferens, Chilo suppressalis, Tryporyza incertulas*, and *Cnaphalocrocis medinalis* [11]. Following GM-rice safety testing on laboratory animals used Sprague-Dawley rats [11, 12] as well as chicken [13].

Generally, when the technology was widely marketed for the first time at the mid-1990s, economic efficiency and product returns were increased [14, 15], such as 3% increasing in crop trade in 2012. There is significant impact on agriculture [16]. Currently, China is the biggest producer of transgenic rice at a limited level. In other countries, such as the United States, Pakistan, Spain, and India, it is cultivated in test scales on farms [1]. In Russia, transgenic plants are used only as a model for biotechnology research [17, 18], especially on tobacco [19, 20].

Cry genes of *Bacillus thuringiensis* were successfully introduced in rice genome in 1993 [21]. The first pest resistant rice from Iran is called Tarom Molaii Cry1Ab that produced from 1998 [22] by Iran Agricultural Biotech Research Institute [23].

Some studies showed the possibility of survival of the transgenic genes in the body of living organisms, especially human [3]. On the other hand, the safety of transgenic food consumption has some controversies for likely risks, including the production of unknown toxins by the transgenic genes resulting in allergy and carcinogenesis [24, 25]. This may happen by insertion of transgenic genes inside the genomic host, by activating silent genes or decreasing the activity of other genes, disrupting the metabolism or producing of new protein toxins in the consumer. The reported data show that meal-derived DNA fragments which are large enough to carry complete genes can avoid degradation and through an unknown mechanism enter the human circulation system [26]. Such concerns demands safety testing of GM-genes presence on laboratory animals [27, 28] including models of *cry* genes on rat [29, 30] and monkey [31, 32].

Based on our knowledge, the prerequisite for the harmfulness of any edible substance, is its absorption through the intestines and the presence of metabolites, whole gene fragments or proteins inside the liver and other tissues. Therefore, in this study, the remaining of *CryIA(b)* gene alongside with *P35*, and *T35* were assessed in liver of rats fed with Iranian GM rice. Based on our knowledge, such study on GM-rice was done for the first time.

MATERIALS AND METHODS

Animal Population Study

This work was an experimental study. The laboratory animal population studied included 20 male and 20 female Sprague Dawley (SD) rats with intervention of feeding by pellets with 50% GM rice in needed carbohydrate. The control group of 20 male and 20 female rats was fed by standard food pellets of the same Tarom Molaii rice without gene modifications. The animals were randomly chosen into the groups. The rats were 4 to 5 weeks old with approximately 200 g in weight. The rats were provided by the Razi Vaccine and Serum Research Institute. To reduce the environmental stress, all rats were kept at animal lab for 5 days before the experiments. Feeding was done for 90 days for all groups *ad libitum*. During the intervention period, the humidity and temperature were maintained by 40–60% and 15–22°C. The light cycle was also set to 12 h on and 12 h off. The rats have been daily clinically evaluated (changes in the skin, fur, eyes, and mucous membranes; respiratory, circulatory, autonomic, and central nervous system function; somatomotor activity and behavior patterns).

Materials and Foods

The foods were prepared by the Razi Vaccine and Serum Research Institute. The food pellets were prepared in two different groups: standard and GM pellets. The last was prepared as 50% of needed carbohydrate was Tarom Molaii GM. The Tarom Molaii GM rice was presented by the Agriculture Biotechnology Research Institute of Iran. The Tarom Molaii GM rice was resistant to stem borer worm by transferring of *CryIA(b)* from *Bacillus thuringiensis*.

Table 1. The primer pairs used in this study [22, 33–36]

Primer name	Primer sequence (5' → 3')	Target gene	PCR product length, bp	References
CryIA(b)FI	CGGCCCCGAGTTCACCTT	*CryIA(b)*	189	[33]
CryIA(b)-RI	CAACAACATCATCCCAGCAG			
CryIA(b)-FII	CCGCACCCTGAGCAGCAC	*CryIA(b)*	420	[34]
CryIA(b)-RII	CCCCTCAGAACAACAACGTGCCACC			
T35-F	CGGGGGATCTGGATTTTAGTA	35S terminator	137	[35]
T35-R	AGGGTTCCTATAGGGTTTCGCTC			
T35S-F	AGGGTTTCTTATATGCTCAACACATG	35S terminator	118	[35]
T35S-R	CACCAGTCTCTCTCTACAAATCTATCAC			
P35-F	GCTCCTACAAATGCCATCA	35S promotor	194	[36]
P35-R	GATAGTGGGATTGTGCGTCA			
CryIA(b)-FIII	ACCGGTTACACTCCCATCGA	*CryIA(b)*	1400	[22]

Table 2. The amplification program for the specific primers used in this study. (Time and temperature of the polymerase chain reaction for specific primers and internal control)

Primer name	Denaturing	Annealing	Extending
CryIA(b)I	30 s at 94°C	30 s at 62/5°C	30 s at 72°C
CryIA(b)II	″	30 s at 60°C	″
T35	″	30 s at 58°C	″
T35S	″	30 s at 59/3°C	″
P35	″	30 s at 55°C	″
CryIA(b)III	″	30 s at 60/5°C	″

The number of cycles for each primer was 32.

Sampling

After 90 days feeding, anesthesia was performed with Ketamine Hydrochloride. Sampling was done from the liver. Samples were stored at −20°C till next steps.

DNA Extraction

DNA extraction was done using Exgene Cell SV kit (GeneAll Biotech Korea, no. 101-106) based on the manufacturer protocol. The quality and quantity of the extracted DNA was estimated using agarose gel electrophoresis and spectrophotometry, respectively.

Detection of Transgenic Targets

In order to ensure the presence or absence of *P35*, *T35*, and *CryIA(b)*, amplification was done using PCR by the specific primer pairs (Table 1).

The amplification programs were done based on the Table 2. Agarose gel electrophoresis (1–2%) was used for amplification analysis alongside with 50 bp DNA ladder. The negative control was the samples with double distilled water instead with DNA. The positive control was the sample with the interested targets. All the amplification tests were repeated in triplicate.

Table 2 shows time and temperature of polymerase chain reaction for the specific primers. Time of first denaturing was 30 s at 94°C. Final extending—5 min at 94°C.

Statistical Analysis

Data were processed using SPSS software (version 16). The statistical tests are not shown since DNA fragment were not found in any group.

RESULTS

Clinical Examination

Clinically, over the 90-days period, no different clinical symptoms were observed in any of the groups.

Quality and Quantity Analysis of Extracted DNA

The quality of extracted DNA showed a large fragment of DNA without any smears. The quantity analysis showed standard values (35 ± 5.6 ng/μL with purification of 1.7 ± 0.16).

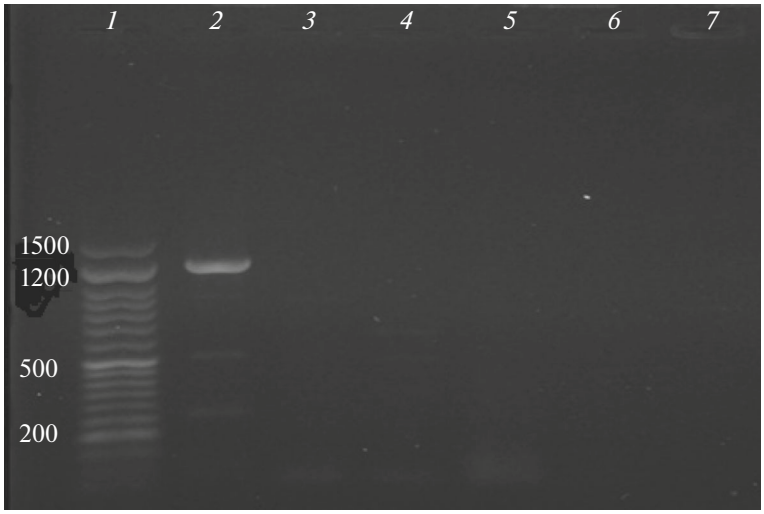

Fig. 1. Agarose gel electrophoresis for assessing the amplification using *CryIA(b)III* primer pair. Column *1*: 50 bp DNA ladder, Column *2*: positive control, Column *3–6*: experimental samples (pooling for *5* animals). Column *7*: negative control.

Detection of Transgenic Targets

The assessing of amplification by all primer pairs used in this study showed no amplicons in comparison with positive control (Fig. 1).

Figure 1 presents results of electrophoresis. We used pooling of the samples (5 animals per one microtube—in total 4 microbubes for group of 20 animals).

DISCUSSION

The results of this study showed no significant difference for survival and presence of transgenic genes *cryIA(b)*, *P35*, and *T35* in liver of rats in both control and experimental groups.

In 2003, research was conducted to investigate the presence of DNA of transgenic plants in rumen, duodenum, milk, feces and blood of lactating beef fed with soybean meal and transgenic maize seeds [37]. The results showed that transgenic genes (*cp4epseps* and *cry1a(b)*) is found only in the rumen and duodenum but has not been found in any of the blood samples found to be consistent with the results of research conducted by Einspanier et al. (2001) [38]. According to researchers, the cause of the absence of DNA components in stool samples may be due to the destruction of DNA in the gastrointestinal tract so that only small pieces are present, which contradicts the results of the study by Einspanier et al. (2001) [38]. In fact, the discovery of any plant DNA in the stool is likely to be affected by a number of factors, including the dietary form (e.g., whole grain or processed feed), or the amount of DNA replication that sometimes results from a small number that replicating parts cannot recognize it [37].

Nemeth et al. (2004) [39] was conducted on broiler chickens, steering cattle, milk cows, and pigs fed with transgenic corn (MON810). They showed that the gene of Rubisco (*rbcl*) present in muscle samples of steering cattle, broiler chickens and pigs, as well as in milk in cattle, but it was not found in the liver. Also, they proved that *P35* was not found in any tissue. We also did not find any *P35* remnants in liver.

There are some documents that show rapid degradation of genes and their relations in the digestive tract of animals which is similar to the results of the study by Phipps et al. (2003) and following papers [37, 39, 40]. A study was done in 2014 [41] on transgenic cabbage-fed rats (caMVP35S). The results show the presence of transgenic gene fragments in the blood, liver, and brain tissues.

In opposite of the above studies, some other ones reported that the genes inside the corn consumed by chicken may be resistant to complete degradation in digestive system. According to researchers from the study, these results indicate cumulative effects of blood, liver, and brain tissues, and contrary to the results of Mazza et al. (2005) [42], which reported a progressive decline in DNA detection in target tissues [40].

CONCLUSIONS

Based on the results of this study, there was no significant difference in the presence and survival of transgenic gene of *cryIA(b)*, *T35*, and *P35* in the liver tissue of the control and intervention groups. Therefore, the present results reject the possibility of transferring the transgenic genes to the consumer organs. The authors aware that Sprague Dawley rats might be not best choice model to estimate food toxicity due to initial development of this rat line for cancer studies [9, 43]. Detailed analysis should be based on modern sequencing technologies. Recent studies rely on next-

gene sequencing and bioinformatics approaches for estimating potential hazards of the transgenes [44, 45] including miRNA profiling in transgene plants [46].

It should also be noted that although the results of this study showed that transgenic genes inside the GM Tarom Molaii rice does not transfer to the liver, but we cannot generalized this results for other events, therefore more experiments is necessary. Recent work on natural genetic heterogeneity [47] shows that some factors that lead to variant overestimation, including issues related to the genetic identity of the background genotype in relation to transgene effects. The variation among conventional cultivars is proposed to be used as a criterion for the safety assessment of GM-rice [48].

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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