Food and Chemical Toxicology 62 (2013) 390–396

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effects of 90-day feeding of transgenic Bt rice TT51 on the reproductive system in male rats

Er Hui Wang [a,b], Zhou Yu [b,c], Jing Hu [a,b], Hai Bin Xu [b,c,*]

[a] National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, No. 7 Pan Jia Yuan Nan Li, Beijing 100021, China
[b] National Center for Food Safety Risk Assessment, Beijing, China
[c] Key Laboratory of Food Safety Risk Assessment, Ministry of Health, Beijing, China

ARTICLE INFO

Article history:
Received 18 January 2013
Accepted 16 August 2013
Available online 4 September 2013

Keywords:
Transgenic rice
Bacillus thuringiensis
Reproductive toxicity
Sperm parameter

ABSTRACT

Rice is a staple food crop; however, the threat of pests leads to a serious decline in its output and quality. The CryAb/CryAc gene, encodes a synthetic fusion *Bacillus thuringiensis* (Bt) crystal protein, was introduced into rice MingHui63 to produce insect-resistant rice TT51. This study was undertaken to investigate potential unintended effects of TT51 on the reproductive system in male rats. Male rats were treated with diets containing 60% of either TT51 or MingHui63 by weight, nutritionally balanced to an AIN93G diet, for 90 days. An additional negative control group of rats were fed with a rice-based AIN93G diet. Body weights, food intake, hematology, serum chemistry, serum hormone levels, sperm parameters and relative organ/body weights were measured, and gross as well as microscopic pathology were examined. No diet-related significant differences in the values of response variables were observed between rats that were fed with diet containing transgenic TT51, MingHui63 and the control in this 90-day feeding study. In addition, necropsy and histopathology examination indicated no treatment-related changes. The results from the present study indicated that TT51 does not appear to exert any effect on the reproductive system in male rats compared with MingHui63 or the control.

1. Introduction

Rice is an important source of food for over 3 billion inhabitants on the earth, providing 21% of calories for the global population (Zhou et al., 2011). However, rice is also one of the crops most severely damaged by insects (Cao et al., 2011). Insect resistant crops usually engineered to produce one or more insecticidal proteins that are toxic to target insects. The latter proteins are usually Bt proteins, so named because they are structurally similar to naturally-occurring Cry proteins from a soil bacterium, *Bacillus thuringiensis*. Hence these crops are also called Bt crops (Carman et al., 2013).

TT51 is a new type of genetically modified Bt rice currently under development in China, created by inserting a synthetic fusion gene of CryAb/CryAc into parental rice MingHui63, in order to provide farmers an alternative means to control Lepidopteran rice pests. Fields tests indicated that TT51 is resistant to major Lepidopteran rice pests and thus has the potential to decrease yield losses and reduce the use of chemical insecticides (Lu, 2010; Li et al., 2012). However, potential unintended effects may have occurred with the insertion of the foreign gene in this transgenic rice. As with all transgenic crops, potential unintended effects due to the insertion of foreign gene are to be evaluated to ensure safety of the crop. With the Ministry of Agriculture released a biosecurity certificate for this transgenic Bt rice in late 2009. China is on the threshold of becoming the first country to allow commercialization of transgenic rice (Lu, 2010; Manimaran et al., 2011). However, since then acute debate on safety of this transgenic rice has not stopped in China (Lu, 2010). People are still uncertain about the potential risks of transgenic crops. Considering rice is the most widely consumed staple food, if the product was toxic or allergen the outcome would be quite serious.

Some scientists believe that the safety of transgenic crops is still uncertain mainly due to the questions about possible unintended effects may exert by the insertion of foreign genes (Janczyk et al., 2007; De Schrijver et al., 2007; Dona and Arvanitoyannis, 2009). The transgene expression may change when a transgene is placed in a different genetic background through transgenic technology. Unpredictable alterations and changes in the expression level of hundreds of genes may occur due to the insertion of the foreign gene (De Schrijver et al., 2007). There are suggestions regarding possible interaction of the 'foreign' DNA sequences with the activity of the existing genes of the plants may disrupt, modify or silence active genes or activate silent genes lead to disruption of metabolic and biochemical pathways in unpredictable ways, produce new toxic compounds or improve the content of the already

* Corresponding author at: National Center for Food Safety Risk Assessment, Beijing, China. Tel.: +86 67779019.

E-mail address: hbxu1231602@vip.sina.com (H.B. Xu).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.08.032

existing ones (Janczyk et al., 2007; Dona and Arvanitoyannis, 2009).

To assess the possible unintended effects of transgenic foods, 90-day rodent feeding studies have been recommended to assess the potential adverse effects of transgenic crops (FAO/WHO, 2000; EFSA, 2008). The sensitivity of animal feeding study to evaluate adverse effects of transgenic crops has been documented (EFSA, 2008). A large number of transgenic crops have undergone such studies with almost no harmful effects observed, including rice (Cao et al., 2011; Schrøder et al., 2007; Tang et al., 2012; Wang et al., 2002; Zhou et al., 2011), soybean (Appenzeller et al., 2008; Delaney et al., 2008) and maize (He et al., 2008, 2009; MacKenzie et al., 2007; Appenzeller et al., 2009; Juberg et al., 2009). However, the safety of transgenic crops to human and animals are far from certain. There have been some controversies regarding the safety assessment of transgenic crops. A small number of studies have reported apparently harmful effects. In the Vecchio study (Vecchio et al., 2004) the authors fed pregnant Swiss mice and male litters on a standard laboratory chow containing 14% GM soybean. Then, they focused their attention on Sertoli cells, spermatogonia and spermatocytes by means of immunoelectron microscopy at 2, 5 or 8 months of age. Their results indicated that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II is decreased in 2 and 5 month-old GM-fed mice, and is restored to normal at 8 months. Moreover, they found in GM-fed mice of all ages considered, the number of perichromatin granules was higher and the nuclear pore density lower. The author further found in GM-fed mice enlargements in the smooth endoplasmic reticulum of Sertoli cells. However, some scientists thought that the Vecchio study was flawed (Zhang and Shi, 2011). A three-generation study reported that there were some minimal histopathological changes in liver and kidney and some biological effects observed in F3 female offspring of rats fed a Bt maize (Kilic and Akay, 2008). The appearance of these apparently harmful effects inevitably aroused suspicion among people and cause public anxiety. A survey indicated that between 20% and 30% of the US public has a negative attitude toward products that contain GM crops, even though farmers in the US have raised crops for more than a decade (International Food Information Council (IFIC) Report, 2008). As we all know, consumer acceptance is the most important bottleneck for the development of transgenic technology.

Male reproduction is a complex process that involves the testes, epididymis, accessory sex glands and associated hormones (Yakubu, 2012). This complex process is strictly regulated by the hypothalamic-pituitary-testicular axis, which involves testosterone (T), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (D'Cruz et al., 2010). According to the Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) guidelines, the following parameters are considered to be the most predictive of fertilizing capacity of the male rats: sperm motility, sperm head counts and morphology (Terpsidis et al., 2009). The mammalian testis is a very sensitive organ that can be affected by many factors, when exposed to a toxicant molecular and cellular changes can be usually detected (Zhang and Shi, 2011; D'Cruz et al., 2010). Therefore, many research groups are concerned about whether the GM food exert negative effects on the male reproductive system in order to ensure the safety of GM food by a number of methods over many species of animals (Zhang and Shi, 2011). There has been speculation that transgenic foods may produce unintended outcomes that affect the reproductive function of human beings or animals (Nap et al., 2003; Zhang and Shi, 2011). The entire process of the sperm maturity action requires approximately 70 days in rats (OECD, 2001; Šimić et al., 2012). If unintended effects happen in the transgenic rice due to the insertion of the foreign gene, the male reproductive system of the consuming animals might be at risk after exposure to transgenic crops for 90 days.

Considering rice is a widely consumed staple food and serious debate continues on the safety evaluation of transgenic crops in order to protect large number of people from the risk of potential adverse effects of the transgenic rice, it would be better to close the debate by conducting extensive toxicological evaluations. Owing to this unclear situation with questions from consumers and regulatory authorities (Lu, 2010), it makes sense to evaluate the male reproduction toxicity of TT51 rice in this 90-day feeding study. This prompted us to investigate the toxicological effects of TT51 rice in growth performance and reproductive parameters of male Wistar Rats through this 90-day feeding study.

2. Material and methods

2.1. Plant materials

Transgenic Bt rice (TT51) and its none-transgenic counterpart (MingHui63) were cultivated in the experimental field of Central China Agricultural University (WuHan, China) in adjoining plots, under identical environmental conditions. After harvest, the seeds were dried, de-hulled, vacuum-packed and stored in a dry warehouse for use in the experiment. Additional common rice purchased at the supermarket was selected as an additional negative control.

2.2. Certification and compositional analysis of rice

Samples of the TT51 and MingHui63 rice were evaluated for the presence of the CryAb/CryAc gene using polymerase chain reaction (PCR) following standards of the Ministry of Agriculture of China (NY/T1193-3) and the Bt protein (encoded by CryAb/CryAc) was detected with an antibody specific enzyme linked immunosorbent assay (ELISA). The TT51 rice was found to be positive for the presence of CryAb/CryAc gene and protein while the MingHui63 rice was found to be negative.

Two random samples were selected from each kind of rice for compositional analysis. Analysis of crude nutrients: protein, fat, fiber, moisture and ash were determined in accordance with Chinese Standard methods (GB/T5009.5-2010, GB/T5009.6-2003, GB/T5009.3-2010, GB/T5009.88-2008, GB5009.3-2010 and GB5009.4-2010). Carbohydrate was determined using the following calculation carbohydrate% = 100% − (% protein + % fat + % ash + % moisture) as described by Han et al. (2005).

2.3. Diet formulation and compositional analysis

Based on the results of the compositional analysis, flours of MingHui63 and TT51 were formulated into rodent diets at concentration of 60% by mass. An additional AIN93G rice-based diet was included as negative control. All diets were produced following AIN93G guidelines (Reeves et al., 1993). All diets were vacuum-packed and sterilized by gamma irradiation using $_{60}$Co. All diets were produced by Hua Fu Kang Feed Co., Ltd., (Beijing, China). The composition of all diets is summarized in Table 1, and the nutritional composition of these diets is presented in Table 2.

2.4. Animals and housing

Twenty-four male Wistar Rats obtained from the experimental animal production center of Vital River Laboratories (VRL) Co., Ltd., (Beijing, China) were 4 weeks of age at the start of acclimation. The animals were housed singly in stainless steel covered cages in a room with a temperature of 20–23 °C, 40–55% relative humidity, a 12 h light/12 h dark cycle and air change of 10 times/h. The animals were provided with unlimited tap water throughout the study. All animals were acclimatized for 7 days prior the start of the study, during that time they were supplied

Table 1
Composition of diets for rats.

Ingredient (%)	Control	MingHui63	TT51
Rice	60.00	60.00	60.00
Casein	21.10	20.19	20.33
Cane sugar	1.20	1.20	1.20
Corn starch	5.19	10.36	8.72
Soybean oil	4.84	3.40	3.34
Cellulose	2.82	0.00	1.56
Minerals	3.50	3.50	3.50
Vitamins	1.00	1.00	1.00
Methionine	0.18	0.18	0.18
Choline choride	0.17	0.17	0.17