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A three generation reproduction study with Sprague–Dawley rats consuming high-amylose transgenic rice

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ABSTRACT

The transgenic rice line (TRS) enriched with amylose and resistant starch (RS) was developed by antisense RNA inhibition of starch-branching enzymes. Cereal starch with high amylose has a great benefit on human health through its resistant starch. In order to evaluate the effect of transgenic rice on rats, the rats were fed diets containing 70% TRS rice flour, its near-isogenic rice flour or the standard diet as the control through three generations. In the present study, clinical performance, reproductive capacity and pathological responses including body weight, food consumption, reproductive data, hematological parameters, serum chemistry components, organ relative weights and histopathology were examined. Some statistically significant differences were observed in rats consuming the high amylose rice diet when compared to rats fed the near-isogenic control rice diet or the conventional (non-rice) standard diet. These differences were generally of small magnitude, appeared to be random in nature, and were within normal limits for the strain of rat used, and were therefore not considered to be biologically meaningful or treatment related.

1. Introduction

Rice is one of the most important cereal crops and staple foods for over half of the population in the world. The starch of rice is a major source of energy for many human communities, and consists of two major components such as a linear amylose moiety and a highly-branched amylopectin moiety (Gallant et al., 1997; Sharma and Yadav, 2008). The amylose is more resistant to digestion than other starch molecules and is an important form of resistant starch (RS). RS is an important raw material for the food industry (Simsek et al., 2009) and has been confirmed to possess potential health benefits and functional properties when consumed by humans. RS can improve large bowel health and prevent bowel inflammatory diseases and colorectal cancer (Topping et al., 2003). RS also can control glucose release, reduce insulin response, and decrease serum total cholesterol and triglycerides levels (Nugent, 2005; Sajilata et al., 2006). In addition, RS can accelerate the oxidation of postprandial lipids, thus reducing long-term accumulation of fat (Higgins et al., 2004). Therefore, the RS-enriched rice can reduce the risk for the development of Type-2 diabetes, obesity, and cardiovascular diseases. Although rice has the highest starch content among these major grain crops, its RS content is relatively low. In order to address this problem, high-amylose rice breeding is a valuable approach. In this study, the transgenic rice line (TRS) with high amylose was generated from the *Indica* rice cultivar TeQing (TQ) after transgenic inhibition of two starch-branching enzymes (SBEs) (SBE I and SBE IIb) through antisense RNA technique so that TRS held the homozygous transgene and revealed identical growth behavior and plant type of its near-isogenic TQ with the exceptions of grain compositions and starch structure (Wei et al., 2009; Zhu, 2009).

The use of recombinant DNA technology can greatly shorten the process to develop improved crop varieties (Parrott et al., 2010). A robust food safety assessment process has been developed to insure that these improved crops are as-safe-as crop varieties developed using conventional breeding techniques (CODEX, 2009; ILSI, 2004). However, the food safety of GM crops still remains a contentious issue for some world regions, and safety assessment procedures for transgenic crops are not fully accepted by the general population. The primary safety assessment of foods prepared by GM crops is based on the concept of substantial equivalence. This concept considers compositional analysis when comparing a transgenic crop with its closest genetically-related counterpart such as rice (Gayen et al.,

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Table 1
Composition of diets for rats in different test groups.

Ingredient (%)	TQ group	TRS group
Non-transgenic rice	70	–
Transgenic rice	–	70
Bean pulp	1.8	0.7
Wheat flour	–	3.2
Fishmeal	17.0	15.0
Grass powder	5.0	5.0
Yeast powder	2.0	2.0
Vegetable oil	1.9	1.4
Additive	1.0	1.0
Limestone	1.1	1.2
$CaHPO_4$	–	0.2
Salt	0.2	0.3

The control diet is made from corn, fish meal, soybean meal, milk powder, lysine, $CaHPO_4$, yeast powder, limestone, vegetable oil, salt, $CuSO_4$, $ZnSO_4$, $FeSO_4$, vitamin A, vitamin E, etc. Product license: SCXK (Beijing) 2009-0012.

2012; Li et al., 2007; Oberdoerfer et al., 2005), corn (Drury et al., 2008; George et al., 2004; McCann et al., 2007), soybean (Lundry et al., 2008) and wheat (Baker et al., 2006). The TRS rice has already undergone a compositional assessment in accordance with the principle of substantial equivalence. The results have revealed that the RS content in transgenic rice is significantly higher than the near-isogenic rice, while the insertion of the SBE gene does not affect its other nutrition constituents (Li et al., 2009). In some cases, 90-day feeding of rodent animals can be recommended to assess the potential for adverse effects of the foods prepared by GM crops, and the 90-day rodent feeding study on TRS rice has been conducted (Zhou et al., 2011). Many other GM crops including rice (Poulsen et al., 2007a, 2007b; Schrøder et al., 2007; Wang et al., 2002), soybean (Appenzeller et al., 2008; Chukwudebe et al., 2012; Delaney et al., 2008) and maize grain (Appenzeller et al., 2009a, 2009b; Hammond et al., 2006a, 2006b; Zhu et al., 2012b) have also undergone such studies. The regulatory accepted maximum duration safety study for GMO foods in the EU and USA is usually a 90 day rodent feeding study, although some critics indicate that longer studies may be appropriate. Therefore, long-term studies, or multigenerational studies, have been suggested for evaluating whether unintended effects can be observed for individual GM events (Haryu et al., 2009; Kılıç and Akay, 2008; Krzyżowska et al., 2010). The major goal of this study is to determine whether transgenic TRS rice have some detrimental effects through a three-generation study in rats with the consumption of transgenic TRS rice.

2. Materials and methods

2.1. Preparation of diet plant material

Transgenic rice line (TRS) and near-isogenic non-GM rice line (TQ) were provided by the Yangzhou University (Yangzhou, China) and both were cultivated in the adjoining plots of the experiment field under identical climate conditions. After harvest, the seeds were milled and prepared as complete feed, representing 70% of dry weight. Both rice diets were supplemented with vitamins and trace minerals to ensure an adequate supply of nutrients comparable to the standard diet. The rice diets were designed and processed by Ke Ao Xie Li Feed Co. Ltd. (Beijing, China), according the standards of the People's Republic of China, and were vacuum-packed with polyethylene bags and sterilized by ^{60}Co. Compositions of TRS diet and TQ diet are listed in Table 1 while nutritional compositions of the diets are listed in Table 2.

2.2. Animals

Sixty female and thirty male Sprague–Dawley rats were supplied and housed in the laboratory of animal research center of Jiangsu University (Zhenjiang, China) with license numbers SCXK(SU)2009-0002 and SYXK(SU)2008-0024. The selected animals were approximately 6 weeks old and acclimatized for a week prior to treatment. Rats were randomly divided into 3 groups following a computerized randomization scheme based on body weight and provided the corresponding diet. Two rats were housed in each polycarbonate cage with stainless steel cover. All rats were housed with ad libitum access to water and feed. Animal conditions were maintained at the environment with the temperature of 22 ± 2 °C, relative humidity of 40–60%, artificial illumination (fluorescent light) with a 12 h light/12 h dark cycle and air exchange of 18 times/h. However, female rats were housed individually with their litter in cages during the gestation and lactation periods. After F3 weaning, the selected offspring were housed individually with the corresponding diets and observed for 13 weeks until necropsy.

Table 2
Nutritional composition of diets for rats.

Components	Content (%)		
	Control group	TQ group	TRS group
Moisture	10	10	10
Crude protein	20	19.6	19.7
Crude fibre	2.9	2.1	2.0
Total calcium	1.1	1.1	1.2
Phosphorus	0.71	0.75	0.76
Crude fat	4.0	4.0	4.0
Crude ash	5.0	5.2	5.1

2.3. Experimental design

Sixty female SD rats (twenty rats/each group) were mated with thirty male rats (ten rats/each group) overnight with one male for two female rats. The mating periods for animals were 2 weeks. During each mating period, daily vaginal smears were examined for the presence of sperm. The presence of sperm in the vaginal smear and/or a vaginal plug was considered as the evidence for successful mating. The day of successful mating was designated as Day 0 of pregnancy. The male rats (F0) that were used for mating were terminated. The female rats were checked daily on days 20–24 for pregnancy to determine the time of delivery. On postnatal day (PND) 4, litters were randomly adjusted to eight pups comprising of four males and four females. At weaning on PND 21, 20 weanlings/female/group and 10 weanlings/male/group were randomly selected as F1 generation. F2 generation was acquired by using the same procedures described above. The offspring of different dams in a group from each generation were mated among themselves at Week 9 after weaning. During the mating, the cohabitation of siblings was avoided. Dams and their offspring were fed with corresponding diets during the periods of mating, gestation, lactation, offspring care and pubescence. In addition, vaginal smearing for the females from F1-F3 generations was performed to determine the stage of estrus prior to pairing with the male rats. On the day of scheduled terminal sacrifice from F1-F3 male rats, the left cauda epididymis was excised and weighed for sperm analysis. The sperm were counted by using a hemacytometer under a light microscope. A sperm count per gram of epididymal tissue was obtained. The sperm was stained with eosin and smeared on a slide glass, abnormal sperm were recorded from a differential count of 1000 spermatozoa in each sample, and the percentage of morphologically abnormal sperm was calculated. At weaning, F3 generation rats were chosen, whereby 10 rats/sex/group were provided with the corresponding diets and observed for 13 weeks. On the day of scheduled terminal sacrifice from F3 rats, the left testis of male rats was excised for cell cycle analysis and the left ovary of female rats was excised for antioxidant index analysis. The experiment design was the same as described in a previous study (Zhou et al., 2012). The rats fed TRS or TQ rice diet and the standard diet were housed in the same environment as previously described (Zhou et al., 2012).

2.4. Body weight gain and food utilization

The mortality and clinical signs of toxicity from all animals were observed daily. Body weights and food intake of these animals were measured once a week.

2.5. Hematology, serum chemistry, serum sex hormone levels and urinalysis

Rats were fasted overnight for at least 12 h, and blood samples were collected from the rats' orbital sinus under anesthesia. One part of blood samples in the presence of anticoagulant was used for the analysis of hematological parameters such as white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDW), which were measured by a BC3000 Hematology Analyzer (Mindray Inc., China). The other part without anticoagulant was used for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALKP), lactate dehydrogenase (LDH) and creatine phosphokinase (CK) activities, and total protein (TP), albumin (ALB), globulin (GLOB), urea nitrogen (BUN), creatinine (CREA), glucose (GLUC), triglycerides (TRIG), cholesterol (CHOL), high-density lipoproteins cholesterol (HDLC), low-density lipoproteins cholesterol (LDLC), potassium (K), sodium (NA), chloride (CL) and calcium (CALC)