International Food Research Journal 26(5): 1477-1484 (October 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Physicochemical characteristics and nutritional compositions of MR219 mutant rice and their effects on glycaemic responses in BALB/c mice

[1]Asma Ilyani, K., [1,2*]Rafii, M. Y., [3]Sobri, H., [4]Anna, L. P. K., [3]Rahim, A. H., [1]Mahmud, T. M. M., [1]Siti Zaharah, S., [5]Asfaliza, R. and [2]Oladosu, Y.

[1]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Division of Agrotechnology and Bioscience, Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia
[4]Department of Human Biology, International Medical University, 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Federal Territory of Kuala Lumpur, Malaysia
[5]Rice Research Centre, Malaysian Agriculture Research and Development Institute, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia

Article history

Received: 27 November, 2018
Received in revised form: 23 April, 2019
Accepted: 20 June, 2019

Keywords

Mutant MR219 rice
Amylose content
Glycaemic index
Glucose

Abstract

The awareness of the general public on healthy foods has been a major concern and people are looking for the right variety of rice for diabetic patients. High amylose content rice with low glycaemic index (GI), which is an indicator of sugar release in the blood, is beneficial for human health. The present work was aimed to determine the physicochemical characteristics and nutritional compositions of MR219 mutant rice, and the effects of amylose content to blood glucose response and glycaemic index in field condition. A total of 31 M_4 mutant lines (ML1 to ML31) were evaluated for physicochemical characteristics and nutritional compositions in comparison with the parental variety, MR219. In glycaemic response study, 48 female BALB/c mice were fed with glucose (a baseline), saline water, two check varieties (MR219 and MRQ74) and four selected mutant lines with different amylose contents. The physicochemical and proximate analysis revealed highly significant differences among the mutant lines. Some mutant lines improved amylose content and nutritional composition. Mutant ML3 had slightly higher amylose content than the parental variety and was recommended for glycaemic responses. However, the field experiment results showed two mutant lines namely; ML3 and ML30, having significantly lower glucose reading (5.49 mmol/L and 5.47 mmol/L, respectively) as compared to the parental variety and other mutant lines. The glucose level was found highest at 60 min after feeding but significantly dropped at 120 min. The normal glucose reading in ML3 and ML30 also resulted in moderate GI values (65% and 66%, respectively). As low and moderate GI foods are recommended for diabetic patients, ML3 and ML30 had high potential for their consumption, and can be suggested for further breeding program to develop low GI rice.

Introduction

The need for rice varieties with high nutrient content has become the main focus in new varietal development. One of the approaches to develop rice variety is through induced mutation. The induced mutation has been used in rice more than in any other crop as evidenced by the 815 rice mutant varieties listed in the FAO/IAEA Mutant Varieties Database (Oladosu *et al*., 2016). The awareness of the general public on healthy foods has been a major concern, and people are looking for the right variety of rice for diabetic patients. The prevalence of diabetes in Malaysia has increased drastically over the years, from 11.6% in 2006 to the current overall prevalence of 22.9%, from which 12.1% were newly diagnosed

*Corresponding author.
Email: mrafii@upm.edu.my

(Wan Nazaimoon *et al*., 2013). The Star (2013) reported that about 3.6 million adults are estimated to be affected by diabetes in Malaysia. It has put Malaysia as the number one country in Southeast Asia for having the highest number of diabetic patients. The dieticians and nutritionists nowadays face a challenging problem to supply a sufficient quantity of diet of the highest possible food value.

Rice is primarily composed of carbohydrate. Carbohydrate in rice is mainly in the form of starch. Starch is made up of long chains of glucose known as amylose and amylopectin, and it is the most common form of carbohydrates in foods. High-amylose varieties of rice, the less sticky long-grain rice, have a much lower glycaemic load, which could be beneficial for diabetics (Ohtsubo *et al*., 2016). The continuous increase in rice consumption leads to the increase in blood sugar levels. High blood sugar levels over a period of time can lead to major health problems. The end product of carbohydrate is glucose. Since the body stores glucose in the form of fat, excessive accumulation of fat blocks the blood vessel that leads to different life-threatening problems such as heart attack, stroke and circulatory crisis.

The effect of carbohydrates on blood responses is indicated by a parameter called Glycaemic Index (GI). The glycaemic index is a physiologically based measure of the effect of carbohydrates on blood glucose levels. It is a parameter of the blood glucose change after eating a certain food as compared to the change after eating a similar amount of glucose (Jenkins *et al*., 1981; Wolever *et al*., 1991). Health Care Asia (2013) stated that food with moderate or low GI resulted in a lower blood glucose levels when consumed. Wolever and Mehling (2002) recommended high carbohydrate with low GI diets for health benefits. Generally, rice is known as a high GI food, but some varieties also have low GI value. Previously, the GI of the most common carbohydrate foods have been determined, and among the foods that produced low GI values are legumes and whole cereal grains (Jenkins *et al*., 1988; Foster-Powell and Miller, 1995; Foster-Powell *et al*., 2002). Rice with high amylose content gives a lower GI than the low amylose content and waxy rice varieties (Miller *et al*., 1992).

The MR219 mutants rice were developed through mutation techniques utilising carbon-ion beam at National Institute of Quantum and Radiological Science and Technology, Japan and Malaysian Nuclear Agency, Bangi, Selangor, Malaysia (Oladosu *et al*., 2015). So far, there are no data on the GI levels recorded for this parental variety MR219 and its derived mutant lines. Therefore, the objectives of the present work were to determine the effects of amylose content on blood glucose response, and to evaluate the glycaemic responses in relation to diabetic condition.

Materials and methods

The MR219 seeds were irradiated with a carbon-ion beam (60 Gy) using AVF-Cyclotron in a collaborative effort by the National Institute of Quantum and Radiological Science and Technology, Japan and Malaysian Nuclear Agency, Bangi, Selangor. After several series of selection and fixation, 31 potential lines with the required adaptive traits were recovered at M_4 generation during the 2009 - 2012 seasons (M_0 - M_4). The M_4 seeds of these 31 mutant lines were evaluated including the parental variety MR219. Samples of rough rice were dehulled using a dehusking machine (Motion Smith Co., Singapore). The dehulled rice was passed through a 500 µm sieve screen on a Sample Mill (Cyclotec 1093, Foss analytical, Sweden) to obtain rice powder. Rice powder was used in all analyses except for the alkaline spreading value determination. All physicochemical and proximate analyses were carried out in a Randomised Complete Block Design and subjected to statistical analysis using SAS 9.4 Software.

Physicochemical characteristics and proximate analysis

The alkaline spreading value and gel consistency were determined following the method described by IRRI (2013), and amylose content was determined following the method described by Juliano (1971). The moisture was determined following the method described by SIRIM (1991). The ash and dietary fibre were determined following the method described by AOAC (2000). The protein, fat, and carbohydrate contents were determined following the method described by Persson (2000), FAO (1986) and Jeon (1995), respectively.

Evaluation of MR219 mutant lines for glycaemic responses through in vivo study

Female BALB/c mice of 8 w old were used in the present work and carried out in a Completely Randomised Design with the guidelines of Research and Ethics Committee of International Medical University (IMU). A total of 48 mice were divided into eight groups having six mice each. The study started after one week of mice arrival to allow them to adapt to the new environment. Normal diet was given to them throughout the period of adaptation.

Food samples preparation

A total of six rice genotypes consisting of two check varieties and four mutant rice lines were used in the present work. The selection of mutant rice lines was based on their ranking in amylose content and yield performance from the total 31 mutant rice lines evaluated. The three highest amylose content among the mutant lines: ML3 (23.38%), ML30 (22.70%) and ML18 (22.70%), and the best mutant line with great performance, ML21 (20.68%), were selected. A high amylose content rice MRQ74 (27.00%) and the parental variety MR219 (23.23%) were used as check varieties. Standard glucose (D-glucose, Sigma Aldrich) was used as a baseline, and saline water as a control. These totalled up to eight treatment groups. The rice samples were grounded to powder-like form (flour). The average weight of mice for each group was taken for food stock preparation. The maximum dosing volume is 10 mL/kg or 2 g/kg for mice (IACUC, 2011). Food stocks were made one day before oral feeding by dissolving the flour samples in distilled water.

Oral feeding and glucose monitoring of blood in mice

After the adaptation period, the mice were fasted (overnight fasting) for 12 h. However, distilled water was offered ad libitum. The blood samples were taken at time zero before given the test food to measure the glucose level. Blood collection was done following the standard procedure described by IACUC (2011). Blood sample was obtained by snipping not more than 1 mm of mice tail and gently milking the blood from the snipped tail. One droplet of blood was placed on a glucose test strip and was read using a glucometer (Accu-Check glucometer). Then the mice were given the test food samples by force feeding using the feeding tube. The size of the feeding tube used for feeding was 18 gauge. The mice were restrained by grabbing them by the scruff of their neck and grasping the skin over the shoulders with the thumb and middle fingers during force feeding. This extended the fore-legs to the side, thus keeping the front feet from pushing the gavage tube away. The blood glucose levels were determined again at 30, 60, 90 and 120 min after feeding. Data were subjected to statistical analysis such as analysis of variance (ANOVA) and mean comparisons using SAS 9.4 Software.

Estimation of Glycaemic Index

The percentage of GI was calculated using the formula prescribed by FAO-WHO (1998):

GI = [area under curve (test food) / area under curve (glucose)] × 100

where the area under curve was calculated based on Trapezoid technique in MS Excel to reflect the total rise of blood glucose levels after eating test foods.

Results and discussion

Physicochemical characteristics and proximate analysis

ANOVA for physicochemical characteristics and nutritional compositions (Table 1) showed that all traits (alkaline spreading value, gel consistency, amylose, moisture, ash, protein, fat, dietary fibre, carbohydrate, and energy) had high significant differences ($p < 0.01$) among the mutant lines. This result revealed that there was a high level of genetic differences among the mutant lines which could be exploited through the genotypic selection.

Mean comparisons of physicochemical characteristics and nutritional compositions among the mutant lines

Table 2 shows the mean values of all the physicochemical characteristics. Ten mutant lines namely; ML17, ML19, ML20, ML21, ML22, ML23, ML24, ML25, ML26 and ML29 were categorised as intermediate gelatinisation temperature group with alkaline spreading value of 3.00. Meanwhile, the parental line (MR219) and the remaining 21 mutant lines were categorised in low gelatinisation temperature group with the alkaline spreading value of 2.00. Yu and Wang (2007) found that the mutant Zhenong 1 had low alkaline spreading value which represented high gelatinisation temperature. However, the previous study by Wu *et al*. (2002) showed that there were no changes in gelatinisation temperature among irradiated rice cultivars. Starch

Table 1. Mean squares of ANOVA for physicochemical characteristics and nutritional compositions.

Source of variation	df	Alkaline spreading value	Gel consistency (mm)	Amylose (%)	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Dietary fibre (g/100 g)	Carbohydrate (%)	Energy (kcal/100 g)
Lines	31	0.854**	225.415**	4.804**	0.856**	0.031**	0.500**	0.668**	10.251**	3.109**	68.34**
Replications	3	0.010	1068.208**	1.510	0.007*	0.002	0.012	0.004	0.006	0.011	2.950
Error	93	0.032	80.660	2.091	0.003	0.001	0.007	0.004	0.004	0.016	1.118

Notes: **, * - Highly significant at $p \leq 0.01$ and significant at $p \leq 0.05$, respectively.

Table 2. Means (± SE) for physicochemical characteristics and nutritional compositions.

Lines	Alkaline spreading value	Gel consistency (mm)	Amylose (%)	Moisture (%)	Ash (%)	Crude protein (%)	Fat (%)	Dietary fibre (g/100g)	Carbohydrate (%)	Energy (kcal/100g)
MR 219	2.00 ± 0.00	65.50 ± 8.26	23.23 ± 0.43	10.33 ± 0.05	1.40 ± 0.04	7.80 ± 0.14	2.60 ± 0.07	4.35 ± 0.06	77.88 ± 0.16	374.80 ± 0.72
ML 1	2.00 ± 0.00	64.00 ± 3.56	20.48 ± 0.44	10.50 ± 0.04	1.60 ± 0.04	8.13 ± 0.09	2.40 ± 0.09	3.90 ± 0.08	77.38 ± 0.11	371.40 ± 0.75
ML 2	2.00 ± 0.00	73.50 ± 2.36	21.68 ± 1.32	10.50 ± 0.04	1.60 ± 0.05	7.93 ± 0.10	2.88 ± 0.03	2.83 ± 0.06	77.13 ± 0.16	371.73 ± 0.16
ML 3	2.00 ± 0.00	63.50 ± 10.50	23.38 ± 0.24	10.20 ± 0.04	1.52 ± 0.03	7.61 ± 0.07	2.52 ± 0.08	3.30 ± 0.06	78.20 ± 0.09	372.25 ± 0.42
ML 4	2.00 ± 0.00	71.50 ± 5.74	19.73 ± 0.44	10.68 ± 0.01	1.69 ± 0.02	8.64 ± 0.03	2.90 ± 0.03	2.14 ± 0.02	76.14 ± 0.05	369.07 ± 0.16
ML 5	2.00 ± 0.00	71.00 ± 5.92	20.40 ± 0.67	10.87 ± 0.02	1.62 ± 0.02	8.50 ± 0.03	2.50 ± 0.02	2.76 ± 0.02	76.50 ± 0.06	368.10 ± 0.10
ML 6	2.00 ± 0.00	84.00 ± 1.41	21.35 ± 0.74	10.20 ± 0.02	1.60 ± 0.02	8.60 ± 0.02	2.50 ± 0.01	3.60 ± 0.04	77.13 ± 0.03	372.40 ± 0.02
ML 7	2.00 ± 0.00	83.50 ± 2.50	19.78 ± 0.54	11.27 ± 0.02	1.51 ± 0.01	8.30 ± 0.02	2.60 ± 0.01	2.93 ± 0.04	76.34 ± 0.02	367.74 ± 0.16
ML 8	2.00 ± 0.00	63.50 ± 8.50	19.65 ± 0.30	9.64 ± 0.08	1.51 ± 0.01	8.00 ± 0.07	2.33 ± 0.02	3.75 ± 0.03	78.58 ± 0.15	374.57 ± 0.22
ML 9	2.00 ± 0.00	80.00 ± 4.55	19.60 ± 0.40	10.09 ± 0.04	1.50 ± 0.02	7.80 ± 0.01	2.00 ± 0.02	3.20 ± 0.02	78.64 ± 0.05	370.11 ± 0.10
ML 10	2.00 ± 0.00	77.00 ± 1.73	19.93 ± 0.42	10.51 ± 0.01	1.50 ± 0.01	8.20 ± 0.01	2.20 ± 0.03	8.20 ± 0.01	77.59 ± 0.04	379.37 ± 0.17
ML 11	2.00 ± 0.00	81.00 ± 4.93	20.90 ± 1.09	10.21 ± 0.02	1.46 ± 0.00	7.70 ± 0.02	2.10 ± 0.01	4.26 ± 0.03	78.54 ± 0.04	372.35 ± 0.05
ML 12	2.00 ± 0.00	85.00 ± 2.52	21.25 ± 1.46	9.78 ± 0.02	1.50 ± 0.01	7.80 ± 0.01	2.10 ± 0.02	4.20 ± 0.02	78.83 ± 0.03	373.78 ± 0.07
ML 13	2.00 ± 0.00	83.00 ± 2.65	20.20 ± 0.67	9.48 ± 0.02	1.60 ± 0.01	8.00 ± 0.05	2.30 ± 0.00	5.20 ± 0.02	78.64 ± 0.06	383.62 ± 1.98
ML 14	2.00 ± 0.00	77.50 ± 3.86	20.15 ± 0.23	10.78 ± 0.02	1.60 ± 0.01	8.00 ± 0.04	2.00 ± 0.01	4.70 ± 0.02	77.63 ± 0.07	369.90 ± 0.11
ML 15	2.00 ± 0.00	77.50 ± 4.99	20.93 ± 0.74	10.10 ± 0.01	1.48 ± 0.01	7.80 ± 0.02	2.10 ± 0.01	5.80 ± 0.02	78.52 ± 0.03	381.79 ± 1.95
ML 16	2.50 ± 0.29	82.00 ± 3.27	21.45 ± 1.35	10.87 ± 0.02	1.50 ± 0.01	7.90 ± 0.06	1.90 ± 0.04	6.00 ± 0.03	77.83 ± 0.06	372.03 ± 0.20
ML 17	3.00 ± 0.00	83.50 ± 4.03	20.18 ± 0.22	11.49 ± 0.02	1.50 ± 0.01	7.30 ± 0.01	2.10 ± 0.02	7.40 ± 0.02	77.63 ± 0.02	373.41 ± 0.18
ML 18	2.00 ± 0.00	85.00 ± 2.52	22.70 ± 0.51	10.88 ± 0.02	1.50 ± 0.01	7.30 ± 0.01	1.70 ± 0.02	3.70 ± 0.02	78.64 ± 0.04	366.42 ± 0.10
ML 19	3.00 ± 0.00	79.50 ± 2.63	20.30 ± 0.30	10.20 ± 0.01	1.50 ± 0.02	7.70 ± 0.01	1.70 ± 0.02	6.30 ± 0.02	78.92 ± 0.03	374.37 ± 0.08
ML 20	3.00 ± 0.00	71.00 ± 5.26	21.38 ± 0.48	10.01 ± 0.02	1.40 ± 0.02	7.70 ± 0.01	2.40 ± 0.01	4.80 ± 0.02	78.50 ± 0.02	375.92 ± 0.16
ML 21	3.00 ± 0.00	79.50 ± 4.50	20.68 ± 0.41	10.10 ± 0.02	1.70 ± 0.01	8.30 ± 0.02	2.90 ± 0.01	5.40 ± 0.01	77.00 ± 0.01	378.12 ± 0.17
ML 22	3.00 ± 0.00	73.50 ± 6.34	19.65 ± 0.10	10.70 ± 0.02	1.60 ± 0.01	8.20 ± 0.01	2.60 ± 0.01	7.10 ± 0.01	76.90 ± 0.02	378.01 ± 0.05
ML 23	3.00 ± 0.00	66.00 ± 3.27	20.93 ± 0.57	9.80 ± 0.02	1.50 ± 0.01	8.00 ± 0.03	2.60 ± 0.01	6.50 ± 0.02	78.10 ± 0.03	380.81 ± 0.12
ML 24	3.00 ± 0.00	69.50 ± 5.80	22.38 ± 1.49	10.21 ± 0.02	1.60 ± 0.01	8.30 ± 0.02	2.40 ± 0.01	4.10 ± 0.02	77.50 ± 0.04	372.92 ± 0.12
ML 25	3.00 ± 0.00	64.00 ± 9.20	21.08 ± 0.43	10.68 ± 0.01	1.50 ± 0.01	7.80 ± 0.01	2.30 ± 0.01	3.50 ± 0.02	77.72 ± 0.02	369.75 ± 0.12
ML 26	3.00 ± 0.00	85.00 ± 4.65	22.10 ± 0.37	10.51 ± 0.01	1.60 ± 0.01	8.20 ± 0.01	2.90 ± 0.03	1.60 ± 0.01	76.80 ± 0.04	369.28 ± 0.12
ML 27	2.00 ± 0.00	80.00 ± 3.74	22.18 ± 1.10	10.19 ± 0.01	1.50 ± 0.01	8.10 ± 0.01	2.30 ± 0.01	5.10 ± 0.01	77.92 ± 0.03	374.95 ± 0.02
ML 28	2.00 ± 0.00	73.00 ± 7.14	20.53 ± 0.59	10.79 ± 0.01	1.50 ± 0.01	8.00 ± 0.02	3.50 ± 0.01	2.90 ± 0.01	76.22 ± 0.02	374.18 ± 0.05
ML 29	3.00 ± 0.00	75.00 ± 4.51	20.15 ± 0.23	10.79 ± 0.01	1.50 ± 0.02	7.90 ± 0.02	3.30 ± 0.01	4.50 ± 0.01	76.53 ± 0.02	376.36 ± 0.11
ML 30	2.50 ± 0.29	61.50 ± 8.22	22.73 ± 0.86	9.88 ± 0.02	1.40 ± 0.02	7.60 ± 0.01	2.60 ± 0.02	2.00 ± 0.01	78.53 ± 0.04	371.87 ± 0.11
ML 31	2.50 ± 0.29	80.00 ± 3.74	19.90 ± 0.52	10.80 ± 0.02	1.80 ± 0.02	8.80 ± 0.00	2.60 ± 0.02	4.50 ± 0.02	76.02 ± 0.02	371.62 ± 0.20
Mean	2.36	75.28	20.96	10.41	1.54	8.00	2.43	4.39	77.64	373.53
Std. Dev.	0.48	11.80	1.66	0.46	0.09	0.36	0.41	1.58	0.9	4.19
Maximum	3.00	85.00	23.38	11.52	1.8	8.8	3.5	8.2	78.92	383.62
Minimum	2.00	61.50	19.60	9.44	1.4	7.3	1.7	1.6	76.02	367.74
LSD (α = 0.05)	0.25	12.61	2.03	0.07	0.05	0.12	0.09	0.09	0.18	1.48

is a polymer of glucose which contains a mixture of amylose and amylopectin. Rice starch has a semi-crystalline structure in its native state which is disrupted by cooking, transforming the starch into an edible, softer, gel-like material. Because it is correlated with the cooking time and texture of cooked rice, the temperature at which rice starch gelatinises is an important component of rice eating quality (Maniñgat and Juliano, 1979). Cooking time of the rice depends on the coarseness of the grains. The intermediate alkaline spreading value indicated the medium disintegration and classified as intermediate gelatinisation temperature which is highly desirable for quality grain (Bansal *et al*., 2006). The gel consistency test showed obvious variation in all lines. According to the mean, ML12, ML18 and ML26 had the highest value of 85 mm gel consistency, while ML30 had the lowest gel consistency of 61.5 mm. The gel consistency value for MR219 was 65.5 mm. Thus, those mutants and parental lines were categorised as soft rice. Soft and medium gel consistencies are preferable by consumers (Rohilla *et al*., 2000).

With regards to amylose content, the highest was found in ML3 (23.38%) while the lowest in ML9 (19.60%). However, the amylose content of the parental variety MR219 (23.23%) was slightly different from ML3. In another study on Pakistani mutant rice varieties, Shadab, Shua-92, Khushboo-95 and Sarshar had higher percentage of amylose content as compared to their parental varieties (Baloch *et al*., 2006). Conversely, according to Khin (2006), Manawthuka mutant (MNTK4-10) had a lower percentage of amylose content (22.42%) as compared to their parental variety (25.82%). Amylose content is an important index for appraising the quality of rice. It provides important scientific data for putting rice resources to rational use as well as selecting and breeding rice variety.

Mutant ML17 had significantly the highest moisture percentage (11.49%) as compared to the parent variety (10.33%). The mutant line ML31 had the highest ash percentage (1.80%), while the lowest was in ML20 and ML30 (1.40%). The ash of a foodstuff is the inorganic residue remaining after the foodstuff is ignited until it is carbon free (i.e. after the organic matter has been completely burnt off), usually at a temperature not exceeding red heat. The ash obtained is not necessarily of exactly the same composition as a mineral matter present in the original food as there may be losses due to volatilisation or some other interaction between constituents. Ash can be regarded as a general measure of quality and often is a useful indication of inorganic residue. The highest crude protein was observed in ML31 (8.80%) while the lowest was in ML17 and ML18 (7.30%). Meanwhile, the lowest fat content was recorded in two mutant lines namely; ML18 and ML19, with value of 1.70%. ML28 was observed to have the highest fat content which was 3.50%. The fat content in the parental line MR219 was 2.60%.

The dietary fibre was high in ML10 and low in ML26 with the values of 8.20 and 1.60 g/100 g, respectively. The dietary fibre content was 4.35 g/100 g in MR219. Other than that, ML31 had the lowest carbohydrate content which was 76.02%, while the highest content was recorded in ML19 with the value of 78.92%. The total carbohydrate content for the parental line was 77.88%. The energy content was highest in ML13 with the value of 383.62 kcal as compared to other mutant lines, while the lowest energy content was recorded in ML18 with the value of 366.42 kcal.

The evaluation of nutritional composition showed significant difference ($p \leq 0.01$) in all traits; moisture, ash, protein, fat, dietary fibre, carbohydrate and energy. The moisture content of all samples ranged from 9.48 to 11.49%. The moisture content of paddy rice should be 12 to14% to minimise grain fissures (Patindol, 2000). More than half tested mutant lines showed improvement in protein content as compared to parental variety MR219. The increased percentage of protein content was also found in Pakistani mutant rice variety namely; Shadab, Shua-92 and Sarshar as compared to their parents (Baloch *et al*., 2006). Increased protein content is one of the major breeding objectives around the world. However, lowering protein content has become one of the breeding objectives in Japan for kidney troubled patients. The mutant rice LGC-1 had low protein content and tested on patient with kidney disease, and was found to be very effective on the patients (Nishimura *et al*., 2000). Research on mutant rice nutrition is mostly focusing on their protein content. So far, no extensive study has been conducted on ash, fat, dietary fibre, carbohydrate and energy content in mutant rice.

Mean comparisons and blood glucose response among the treatments

The ANOVA revealed highly significant differences among the treatments, sampling time and also in the interaction of treatments with sampling time. Most of blood sugar level of food samples reached its maximum reading at 60 min after feeding and significantly dropped at 120 min (Table 3). The mice were recorded to have the highest blood glucose level at this particular time where the carbohydrates of the food were fully digested and reacted in blood glucose change. The extent and duration of the blood glucose response depend on the absorption rate which in turn depends on factors such as gastric emptying as well as the rate of hydrolysis and diffusion of nutrients in the gut (FAO-WHO, 1998).

Table 3. Glucose readings after given test samples.

Sampling time (minutes)	Mean (glucose reading, mmol/L)
0	4.97
30	5.99
60	6.41
90	6.37
120	5.78

Least Significance Difference (α = 0.05) = 0.21.

Table 4 shows the mean values for glucose reading among the treatments. Based on the results, the highest blood glucose reading was recorded in mice fed with standard glucose (8.11 mmol/L). Three food samples namely; ML18, ML21, and the parental line MR219 caused a pre-diagnosis of diabetes on the BALB/c mice. The glucose reading of the blood collected from mice fed with those three food samples were 5.96, 6.34 and 6.21 mmol/L, respectively. Meanwhile, two food samples namely; ML3 and ML30 showed normal glucose level with the values of 5.47 and 5.49 mmol/L, respectively. The low glucose reading was observed in blood collected from mice that were fed with the check variety MRQ74 and saline water (4.87 and 4.78 mmol/L, respectively).

Table 4. Mean glucose reading and glycaemic index of treatments.

Rice line / control	Mean (glucose reading, mmol/L)
Glucose	8.11(D)
Saline water	4.87 (N)
MRQ74	4.78 (N)
MR219	6.21 (PD)
ML3	5.47 (N)
ML18	5.96 (PD)
ML21	6.34 (PD)
ML30	5.49 (N)

Least Significance Difference (α = 0.05) = 0.27.

Glucose reading: (D) Diagnosis of diabetes = ≥ 7.0 mmol/L, (PD) Pre-diabetic condition = 6.0 – 6.9 mmol/L, (N) Normal glucose level = ≤ 5.9 mmol/L (ADA, 2012). Glycemic Index: 0 - 55 = low (L), 56 - 69 = moderate (M), 70 or more = high (H) (Brand-Miller *et al*., 2002).

The blood glucose response curve as shown in Figure 1 illustrates the interaction of glucose reading for every treatment with sampling time in every food samples.

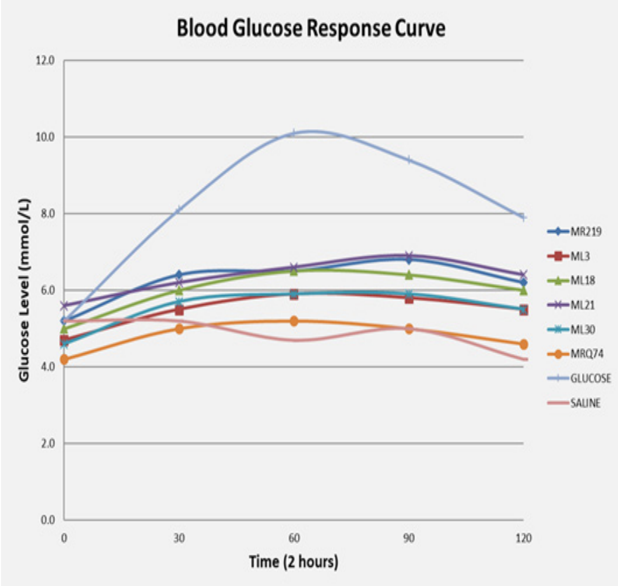

Figure 1. Blood glucose response curves of tested foods at 30 minutes intervals.

Glycaemic index

The glycaemic index (GI) is the incremental area under the blood glucose response curve, and is shown in Table 3. The higher the amylose content of each food samples, the incremental of the area under curve became smaller and resulted in a low GI. Although the relationship between rice starch and GI is complex, amylose content is generally accepted as being the principal determinant of GI in rice (Larsen *et al*., 2000). Here, ML3 and ML30 both had moderate GI rating with a value of 65.00% and 66.00%, respectively. Amylose content of ML3 was the highest among other mutant lines and parental variety, resulted in low GI as compared to others. Meanwhile, ML21 had the highest GI value (75.00%) because of its low amylose content (20.70%). Amylose is a tightly packed structure. Thus, it is more resistant to digestion. It breaks down more slowly, releasing glucose more gradually into the bloodstream and lowers the insulin demand. High amylose rice is less sticky, and have a much lower glycaemic load, which could be beneficial for diabetic patients. As a conclusion, high amylose content food should have a low GI and *vice versa*. Some researchers reported that low amylose content rice has high GI than intermediate and high amylose content rice (Panlasigui, 1989; Juliano *et al*., 1989a; 1989b). However, in the present work, the parental variety MR219 amylose content was higher than ML30 but it had high GI value as compared to ML30. This scenario might be due to different response of some variety or rice mutant lines crossed the sampling time or due to a delayed enzymatic hydrolysis as mentioned by O'dea *et al*.

(1980). Parvin *et al*. (2008) found that high amylose (29.00%) rice lower plasma glucose response and GI values as compared to low amylose (13.00%) rice in type 2 diabetic patients. In contrast, Miller *et al*. (1992) found high GI in a number of Australian rice varieties. These discrepancies could be due to the differences in the physicochemical characteristics, processing or cooking time.

Conclusion

Ion beam irradiation at 60 Gy significantly induced the genetic variability in physicochemical characteristics and nutritional compositions. The gel consistency test showed that all mutant lines had soft texture. One mutant line (ML3) was found to have the highest amylose content but not significantly different from the parent MR219. Some mutant rice lines such as ML10, ML18, ML19, ML21, ML22, and ML31 showed improvement in some particular nutrients. The estimation of glycaemic index revealed that two mutant lines could be consumed by diabetic patients. The two mutant lines (ML3 and ML30) were recorded to have normal glucose reading which was identified to have a moderate GI of 65 and 66, respectively. As low and moderate GI foods are recommended for diabetic patients, these two mutants (ML3 and ML30) have a high potential for their consumption. The findings also raise the value of knowing the GI in our food for awareness. Further study should be carried out on ML3 and ML30 by testing these mutant lines on diabetic patients.

Acknowledgement

The authors are grateful to the Malaysian Nuclear Agency, Universiti Putra Malaysia and Malaysian Agriculture Research and Development Institute for the facilities, support, and financial aids received in the completion of the present work. Appreciation also goes to the National Institute of Quantum and Radiological Science and Technology, Japan for providing the ion-beam facilities, and the Ministry of Science Technology and Innovation and the Ministry of Education, Malaysia for adequately funding the present work through ScFund MOSTI (06-03-01-SF0110) and Higher Institution Centre of Excellence (HICoE) Research Grants.

References

Association of Official Analytical Chemists (AOAC). 2000. Official Methods of Analysis. 17th ed. USA: AOAC.

Baloch, A. W., Soomro, A. M., Naqvi, M. H., Bughio, H. R. and Bughio, M. S. 2006. Sustainable enhancement of rice (*Oryza sativa* L.) production through the use of mutation breeding. Plant Mutation Reports 1(1): 40-42.

Bansal, U. K., Kaur, H. and Saini, R. G. 2006. Donors for quality characteristics in aromatic rice. Oryza 43(3): 197-202.

Food and Agriculture Organization (FAO) / World Health Organization (WHO). 1998. Carbohydrate in human nutrition (FAO Food and Nutrition Paper – 66). In Report of a Joint FAO/WHO Expert Consultation. Rome: FAO.

Food and Agriculture Organization (FAO). 1986. Manuals of food quality control - 7. food analysis: general techniques, additives, contaminants, and composition. Rome: FAO.

Foster-Powell, K. and Miller, J. B. 1995. International tables of glycemic index. The American Journal of Clinical Nutrition 62(4): 871S-893S.

Foster-Powell, K., Holt, S. H. A. and Brand-Miller, J. C. 2002. International table of glycemic index and glycemic load values. The American Journal of Clinical Nutrition 76: 5-56.

Health Care Asia. 2013. Diabetes: what to eat and how much to eat. Retrieved from Health Care Asia website: http://www.healthcareasia.org/2013/eat-well-diabetes-what-to-eat-and-how-much-to-eat/

Institutional Animal Care and Use Committee (IACUC). 2011. Guide for the care and use of laboratory animals. 8th ed. United States: National Academies Press.

International Rice Research Institute (IRRI). 2013. Standard evaluation system (SES) for rice. 5th ed. Philippines: IRRI.

Jenkins, D. J., Wolever, T. M., Buckley, G., Lam, K. Y., Giudici, S., Kalmusky, J., … and Wong, G. S. 1988. Low glycemic index starchy foods in the diabetic diet. The American Journal of Clinical Nutrition 48(2): 248-254.

Jenkins, D. J., Wolever, T. M., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., … and Goff, D. V. 1981. Glycemic index of foods: a physiological basis for carbohydrate exchange. The American Journal of Clinical Nutrition 34(3): 362-366.

Jeon, I. J. 1995. Carbohydrates and sugars. In: Jeon, I. J. and Ikins, W. G. (eds). Analyzing Food for Nutrition Labeling and Hazardous Contaminants, p. 87-107. United States: Marcel Dekker Inc.

Juliano, B. O. 1971. A simplified assay for milled rice amylose. Cereal Science Today 16(10): 334-340.

Juliano, B. O., Perez, C. M., Komindr, S. and Banphotkasem, S. 1989a. Properties of Thai cooked rice and noodles differing in glycemic index in noninsulin-dependent diabetics. Plant Foods for Human Nutrition 39(4): 369-374.

Juliano, B. O., Perez, C. M., Maranan, C. L., Abansi, C. L. and Duff, B. 1989b. Grain quality characteristics of rice in Philippines retail markets. The Philippine Agriculturist 72(2): 113-122.

Khin, T. N. 2006. Rice mutation breeding for varietal improvement in Myanmar. Plant Mutation Reports 1(1): 34-36.

Larsen, H. N., Rasmussen, O. W., Rasmussen, P. H., Alstrup, K. K., Biswas, S. K., Tetens, I., ... and Hermansen, K. 2000. Glycaemic index of parboiled rice depends on the severity of processing: study in type 2 diabetic subjects. European Journal of Clinical Nutrition 54: 380-385.

Maniñgat, C. C. and Juliano, B. O. 1979. Properties of lintnerized starch granules from rices differing in amylose content and gelatinization temperature. Starch 31(1): 5-10.

Miller, J. B., Pang, E. and Bramall, L. 1992. Rice: a high or a low glycemic index food. The American Journal of Clinical Nutrition 56(6): 1034-1036.

Nishimura, M., Horisue, N., Imbe, T., Sakai, M. and Kusaba, M. 2000. Using rice cultivar LGC-1 as a dietary food for patients with kidney disease. In Khush, G. S., Brar, D. S., Hardy, B. (eds). Advances in Rice Genetics, p. 83-85. Philippines: International Rice Research Institute (IRRI).

O'Dea, K., Nestal, P. J. and Antonoff, L. 1980. Physical factors influencing postprandial glucose and insulin responses to starch. The American Journal of Clinical Nutrition 33(4): 760-765.

Ohtsubo, K., Nakamura, S., Maeda, S., Kobayashi, A., Yamazaki, A. and Watanabe, S. 2016. Possibility of diabetes prevention by high-amylose rice and super hard rice. Journal of Diabetes and Obesity 3(1): 1-7.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A. … and Usman, M. 2016. Principle and application of plant mutagenesis in crop improvement: a review. Biotechnology and Biotechnological Equipment 30(1): 1-16.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Malek, M. A., Rahim, H. A., Hussin, G., … and Kareem, I. 2015. Genetic variability and diversity of mutant rice revealed by quantitative traits and molecular markers. Agrociencia 49: 249-266.

Panlasigui, L. N. 1989. Glycemic response to rice. Canada: University of Toronto, PhD Thesis.

Parvin, S., Hasan, Q., Knudsen, K. and Ali, L. 2008. Effects of parboiling and physico-chemical characteristics of rice on the glycemic and insulinemic indices in Type 2 diabetic subjects. Ibrahim Medical College Journal 2(1): 12-16.

Patindol, J. A. 2000. Methods and standards for rice grain quality assessment in the Philippines. In: Johnson, G. I., Le Van, T., Nguyen, D. D. and Webb, M. C. (eds). Quality Assurance in Agricultural Produce, No. 100. Canberra, Australia: Australian Centre for International Agricultural Research (ACIAR).

Persson, J.-Å. 2000. Handbook for Kjeldahl digestion: a recent review of the classical method with improvements developed by FOSS. 3rd ed. Höganäs: Foss Tecator.

Rohilla, R., Singh, V. P., Singh, U. S., Singh, R. K. and Khush, G. S. 2000. Crop husbandry and environmental factors affecting aroma and other quality traits. In Singh, R. K., Singh, U. S., Khush, G. S. (eds). Aromatic Rices, p. 201-216. India: Oxford & IBH Publishing Co. Pt. Ltd.

Standards and Industrial Research Institute of Malaysia (SIRIM). 1991. Malaysian Standard MS1191:1991 - specification for cereal-based snack foods. Malaysia: SIRIM.

The Star. 2013. Number of diabetics in Malaysia alarming. Retrieved from The Star website: https://www.thestar.com.my/news/nation/2013/06/14/number-of-diabetics-in-malaysia-alarming/

Wan Nazaimoon, W. M., Md Isa, S. H., Wan Mohamad, W. B., Khir, A. S., Kamaruddin, N. A., Kamarul, I. M., ... and Khalid, B. A. K. 2013. Prevalence of diabetes in Malaysia and usefulness of HbA1c as a diagnostic criterion. Diabetic Medicine 30(7): 825-828.

Wolever, T. M. and Mehling, C. 2002. High carbohydrate low glycemic index dietary advice improves glucose disposition index in subjects with impaired glucose tolerance. British Journal of Nutrition 87(5): 477-487.

Wolever, T. M., Jenkins, D. J., Jenkins, A. L. and Josse R. G. 1991. The glycemic index: methodology and clinical implications. The American Journal of Clinical Nutrition 54(5): 846-854.

Wu, D., Shu, Q., Wang, Z. and Xia, Y. 2002. Effect of gamma irradiation on starch viscosity and physicochemical properties of different rice. Radiation Physics and Chemistry 65(1): 79-86.

Yu, Y. and Wang, J. 2007. Effect of γ-irradiation on starch granule structure and physicochemical properties of rice. Food Research International 40(2): 297-303.