



## Nutritional quality analyses of traditional varieties of rice (*Oryza sativa*) collected from tribal areas in eastern India

JALLI RADHAMANI<sup>1</sup>, ZEBA KHAN<sup>2</sup>, SUMAN SAHAI<sup>3</sup> and RISHI K TYAGI<sup>4\*</sup>

National Bureau of Plant Genetic Resources, New Delhi 110 012

Received: 17 February 2014; Revised accepted: 31 July 2014

### ABSTRACT

Traditional varieties of rice (*Oryza sativa* L.) having withstood the rigors of time (tolerating both abiotic and biotic stresses) are grown by Indian farmers in rural and tribal parts of India for their subsistence. Seeds of 100 traditional varieties were accessed from the Gene Campaign collection of rice from eastern India and evaluated for nutritional traits (protein, amino acid, starch, amylose and amylopectin) to identify (i) nutritionally superior cultivars for improving livelihood of farmers and tribal communities and (ii) trait-specific donor types which can be used in rice improvement programmes. Seeds of tested cultivars exhibited wide variation for nutritional traits. Higher amylose content ( $\geq 20\%$ ) were recorded in 16% cultivars. Amongst the 100 cultivars tested two cultivars, namely, GC/JH/2007/187 and GC/OR/2007/168 were identified with very high values for protein and amylose contents. Amylose content was positively correlated with starch and negatively correlated with amylopectin. Several superior cultivars were identified with better combination of two or more nutritional traits which can be recommended for cultivation by the farmers and tribal communities for their livelihood. Such cultivars can be used as donors in rice crop improvement programme, therefore, also need attention for their long-term conservation in field level seed banks and the National Genebank, for future use.

**Key words** Amylopectin, Amylose, Proteins, Rice, Starch, Traditional varieties

Rice (*Oryza sativa* L.) is rich in genetic diversity and thousands of varieties grown throughout the world. Plant genetic resources constitute a priceless reservoir of genes, we also depend on the genetic resources available in the form of farmers' varieties/landraces (Bawa 1993). In India, Jharkhand and Odisha states are considered as part of the birthplace of rice and are rich in its genetic diversity. However, highest levels of malnutrition statistics have been recorded in these states along with the tribal belt of Madhya Pradesh ([www.jharkhandnews.org/health.html](http://www.jharkhandnews.org/health.html)). Medicinal rices include Myanmar variety, black rice of China and Navara of India. Black rice has a body-strengthening fraction and pharmaceutical value known as "blood strengthening rice" or "drug rice" (Li and Lai 1989). Navara variety is believed to have medicinal properties and has some anti-neurotic properties when impure (Chopra 1933). Parboiled rice or rice powder gruel (Molla *et al.* 1985), rice water (Rivera *et al.* 1983) and extrusion-cooked rice (Tribelhorn *et al.* 1986) have all been effectively used for the treatment of non-infectious diarrhoea. Starch is the primary component of rice flour (Coffman and Juliano 1987) and consequently

plays an important role as a determinant of the food product quality.

In the present study, a total of 100 traditional rice varieties received from Gene Campaign and evaluated for nutritional traits to identify the nutritionally superior cultivars for improving the livelihood and nutritional status of farmers' families and other tribal communities by promoting their cultivation and conservation.

### MATERIALS AND METHODS

A total of 100 traditionally cultivated varieties of rice (hereafterward referred to as cultivars) accessed from Gene Campaign's collection of germplasm from eastern India. The accessions included traditional cultivated varieties from Jharkhand (66), Bihar (19), Odisha (11), West Bengal (2), Uttar Pradesh (1) and Chhattisgarh (1) in India, were evaluated nutritionally important traits. Seed sorting was done manually by visually observing the seed samples and separating fully mature seed from the immature/unfilled and deteriorated seed. Healthy seeds were separated and used for biochemical evaluation for various traits.

The study was conducted at Division of Germplasm Conservation, National Bureau of Plant Genetic Resources (NBPGR), New Delhi during 2012-13. Un-hulled approximately 2g seeds were dried in oven at 103°C for 1h and used for biochemical evaluation. Dried seeds were

<sup>1</sup>Principal Scientist (email: radhamani@nbpgr.ernet.in),

<sup>2</sup>Senior Project Officer (email:khanzeba02@gmail.com),

<sup>4</sup>Principal Scientist and Head (email: rktyagi@nbpgr.ernet.in, tyaginbpgr@gmail.com), Division of Germplasm Conservation;

<sup>3</sup>Chairperson, Gene Campaign (email: suman@genecampaign.org)

homogenously powdered using grinder (Maharaja, Whiteline, India) and stored in an air-tight container in refrigerator at 10°C until use. The protein content was estimated in all the samples by Lowry's Method (Lowry *et al.* 1951). The intensity of the blue colour was measured colorimetrically at 660 nm using spectrophotometer (LABOMED, UV/VIS Dual Beam 2700) and the amount of protein in the sample was calculated and expressed as percentage. The total amino acid content was determined colorimetrically using ninhydrin (Moore and Stein 1948). Estimation of starch was carried out by Anthrone reagent method (Van Handel 1968), where the samples were treated with 80% alcohol to remove sugars and then starch was extracted using perchloric acid. Starch was hydrolyzed to glucose and was estimated by the method described by Hodge and Hofreiter (1962). Both amylose, a linear polymer of D-glucose and amylopectin content were estimated by the method described by Sadasivam and Manickam (1992).

The experiment was carried out in a completely randomized block design. Biochemical evaluation was carried out in two replicates; each replicate comprised 25 seeds. Data were analyzed statistically using SPSS 16.0 statistical software (SPSS Inc., 233 South Wacker Drive, Chicago) for various traits studied.

## RESULTS AND DISCUSSION

Seeds of tested cultivars exhibited wide variation for nutritional traits (percentages of total soluble proteins, amino acids, starch, amylose and amylopectin). The results of descriptive statistical analysis of tested nutritional traits, viz. protein, amino acid, starch, amylose and amylopectin of cultivars are presented in Table 1. Amongst the various traits studied, starch and amylopectin showed the highest range of variability with highest level of variance. The mean protein content of the tested cultivars was 6.53%; about 44% of the cultivars showed higher values of protein content than that of the mean value. Amino acid content ranged from 6.62 to 21.68% and about 45% of the cultivar registered higher amino acid content than that of the mean value (12.13%). About 21% of the cultivars showed both proteins and amino acid with higher values than their respective mean values (Table 1).

Starch is most important component of rice grain; 42% of cultivars had higher starch content than that of the mean value (34.77%). Starch is positively correlated with amylose

content and the mean value of amylose was recorded as 17.04%. Based on the values prescribed in rice descriptors (IRRI-IBPGR 1980), the cultivars with >17% and ≥20% amylose content were categorized under the intermediate and high amylose containing categories, respectively. Amongst the 100 cultivars tested, 40 cultivars showed ≥17% amylose content and 16 cultivars showed ≥20% amylose content. Wide range of variation was recorded in amylopectin content (1.2 to 36%). Amongst the tested cultivars 44% contained higher amylopectin than that of the mean value (19.11%). Results of the ANOVA revealed that there was significant difference ( $P \leq 1\%$ ) among the various nutritional traits studied, i.e. protein ( $F=7.07$ ), amino acid ( $F=15.44$ ), starch ( $F=141.2$ ), amylose ( $F=17.82$ ) and amylopectin ( $F=138.42$ ).

Some 56 cultivars with ≥17% of amylose content were further re-grouped. Euclidean distance was estimated and the dendrogram was drawn based on paired group method (Fig 1). Total five distinct clusters were formed based on nutritional traits, i.e. cultivars with: (I) high amylopectin and with low starch (4 cultivars), (II) with low amylopectin, low starch and high protein (22 cultivars), (III) high amylopectin, high starch and high protein (23 cultivars), (IV) high amylopectin and high starch contents (3 cultivars) and (V) low amylopectin and low starch (4 cultivars). Group II and III contained almost 80% of the total 56 cultivars (having ≥17% amylose) while other groups I, IV and V contained only 4, 3 and 4 cultivars, respectively, with different combinations of nutritional traits (Fig 1).



Fig 1 Seed variability in rice cultivars (1) GC/JH/2006/144, (2) GC/JH/2004/14, (3) GC/JH/2006/67, (4) GC/BH/2004/22, (5) GC/BH/2004/20, (6) GC/JH/2007/185, (7) GC/BH/2007/253, (8) GC/JH/2004/17, (9) GC/BH/2006/151, (10) GC/JH/2007/186.

Table 1 Descriptive analysis of nutritional traits of rice germplasm

Trait	Range(Min-Max)	Mean $\pm$ SD	SEm	Variance	Confidence level (95%)	Varieties (%) with more than mean values
Protein (%)	3.62-11.22	6.53 $\pm$ 1.87	0.187	3.516	0.37	44
Amino acid (%)	6.62-21.68	12.13 $\pm$ 2.78	0.277	7.718	0.55	45
Starch (%)	13.8-52.85	34.77 $\pm$ 8.34	0.834	69.56	1.65	42
Amylose (%)	9.97-24.3	17.04 $\pm$ 2.98	0.298	8.91	0.59	60
Amylopectin (%)	1.28-36.02	19.11 $\pm$ 8.31	0.831	69.21	1.65	44

\*Significant at 5% level; SD-Standard deviation; SEm-Standard error of means

Table 2 Cultivars with relatively higher values for nutritional traits

Cultivar	Protein (>9%)	Amino acid (>16%)	Starch (>46%)	Amy- lose (>20%)	Amylo- pectin (>33%)
GC/JH/2007/187	11.22	*	*	20.53	*
GC/OR/2007/168	9.5	*	*	20.95	*
GC/JH/2006/120	9.41	17.37	*	*	*
GC/JH/2007/197	*	16.79	*	20.58	*
GC/JH/2006/98	*	17.06	*	24.3	*
GC/JH/2006/95	*	*	46.49	*	33.24
GC/JH/2007/202	*	*	51.98	*	36.03
GC/JH/2006/86	*	*	50.49	*	34.79
GC/JH/2006/89	*	*	50.31	*	36.03
GC/JH/2006/83	*	*	48.29	*	33.94
GC/JH/2007/228	9.23	*	52.85	*	34.92
GC/JH/2006/109	*	*	49.12	*	33.92
GC/BH/2007/249	*	17.48	*	*	33.94

\*Lower value recorded than that of mentioned in parenthesis for each trait in top row

For each nutritional trait evaluated, 10 top-ranking cultivars were identified and amongst these we attempted to identify the common cultivar(s) having relatively higher values for two or more nutritional traits (Table 2). The cultivars with higher amylose were recorded with either higher protein or amino acids contents. More than 33% amylopectin was recorded in eight cultivars collected from Jharkhand (7) and Bihar (1). Generally, cultivars with higher amylopectin (33%) content showed the higher starch (>46%) also. One cultivar collected from Jharkhand (GC/JH 2007/228) recorded notably higher protein (9.22%), starch (52.85%) and amylopectin (34.92%). Similarly, two cultivars, namely, GC/JH/2007/187 and GC/OR/2007/168 were identified with highest 100-seed weight, protein and amylose contents.

The Pearson correlation coefficient for relationship among nutritional traits are presented in Table 3. The starch was significantly positively correlated with amylose content. Contrastingly, amylose and amylopectin were correlated negatively.

Rice is rich in genetic diversity and varies in seed colour including brown, red, purple and black. Cooking quality of rice mainly depends on amylose content and gelatinization temperature. Amylose contents determine the texture of cooked rice. Rice varieties with higher amylose content absorb more water and have a fluffy texture after cooking (Frei and Becker 2003). Feeding with cooked rice with high amylose may be effective to control serum blood glucose and lipids (Magdy *et al.* 2010). Cristiane *et al.* (2007) also reported that serum triglyceride and cholesterol levels significantly decreased after consumption of a diet rich in amylose compared to a diet rich in amylopectin (low amylose). Shin *et al.* (2007) reported that the high resistant starch (HRS) in rice reduced serum cholesterol and low density lipoprotein (LDL) cholesterol. Based on the amylose

Table 3 Correlation coefficients amongst nutritional traits of rice cultivars

Trait	Starch	Amylose	Amylo- pectin	Protein	Amino acids
Starch	1				
Amylose	0.600**	1			
Amylopectin	0.023	-0.271**	1		
Protein	0.123	0.07	0.083	1	
Amino acids	-0.034	0.098	0.046	-0.047	1

\*Significant 1% level

content rice has been graded as waxy-glutinous (<3%), very low ((~9%), low (<17%), intermediate (~17%), high (~20%) and very high (>25%) (Bioversity International-IRRI, 2009). In the present study, 40% of the rice cultivars can be categorized under intermediate and 16% under high category with 20-25% amylose. The amylose content of starches usually ranges from 15% to 35%. High amylose content rice shows high volume expansion (not necessarily elongation) and high degree of flakiness. High amylose containing kernels cook dry, are less tender, and become hard upon cooling. Rice varieties with a greater proportion of starch in the form of amylose tend to have a lower glycemic index (GI). Starchy varieties with high amylose level are associated with lower blood glucose level and slower emptying of the human gastrointestinal tract compared to those with low levels of amylose (Coffman and Juliano 1987, Frei and Becker 2003). Amylose content of milled rice has been found to be positively correlated with hardness values of cooked rice and negatively with stickiness values (Perez *et al.* 1987). Amylose and amylopectin are significantly correlated negatively which is in confirmation with the earlier report (Oko *et al.* 2012).

Rice varieties with a greater proportion of starch in the form of amylopectin, is composed of glucose molecules with branched links is less resistant to digestion and tends to have a higher GI. The starch of waxy rice varieties consists of amylopectin only. These varieties absorb less water upon cooking and have a sticky texture (Frei and Becker 2003). In principle, foods with a higher GI are more quickly digested and rapid starch digestion is regarded as unfavorable, because fast digestion can cause a sensation of hunger shortly after the ingestion of rice, and the energy released is quickly used. The digestibility of amylose and amylopectin can be explained on the basis of molecular structure. Amylose, having an essentially linear and packed chain is more compact in the granule, which makes the access of digestive enzymes difficult while amylopectin molecule, with branched chain shows greater access to enzymes. Thus, amylose might not be totally digested by enzymes in the gastrointestinal tract (Behall *et al.* 1989, Goddard *et al.* 1984).

Generally, long kernel, high protein, amino acids and amylose content are preferred traits. Amylose content is generally used to evaluate some properties of product consumption, can aid in the choice of selecting the grain

quality for diets controlling some biologically relevant parameters such as blood glucose, GI and triglycerides is a potential valuable trait for use in the present scenario. On the other hand, the varieties with fast and easy digestion (with high amylopectin and non-resistant starch) can be recommended for children and patients with digestion problems. A total of 22 cultivars of (cluster II, Fig 1) having lower percentage of amylopectin ( $\leq 10$ ) in combination with higher percentage of amylose and high protein content could maintain relatively lower GI. These cultivars can be recommended as appropriate diet for farmers in tribal areas, laborers and diabetic patients. Cultivar GC/JH/2007/187 and GC/OR/2007/168 had very high 100-seed weight, protein and amylose which was found most suitable to be cultivated and consumed by rural and tribal communities as high amylase is associated with slower emptying of the human gastrointestinal tract and can provide energy to farm workers for longer duration. Such valuable cultivars can be used in crop improvement programs and need to be conserved in genebank for posterity.

#### ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support provided by Department of Science and Technology, New Delhi, India for above research. The authors are also thankful to the Director, NBPGR, New Delhi, for his support. Valuable contributions rendered by other project staff in NBPGR and in Gene Campaign are also acknowledged.

#### REFERENCES

- Bawa K S. 1993. Conservation of biodiversity in the Himalaya: Concluding remarks and an agenda for action. (In) *Himalayan Biodiversity Conservation Strategies*. pp 529–35. Dhar U (ed). Gyanodaya Prakashan, Almorá.
- Behall K M, Scholfield D J, Yuhaniak I and Canary J. 1989. Diets containing high amylose vs amylopectin starch: Effects on metabolic variables in human subjects. *American Journal of Clinical Nutrition* **49**:337–44.
- Chopra R N. 1933. *Indigenous Drugs of India: Their Medical and Economic Aspects*, p 655. The Art Press, Calcutta.
- Coffman W R and Juliano B O. 1987. Rice. (In) *Nutritional quality of cereal grains: genetic and agronomic improvement*. Olson R A and Frey K J (Ed). Agronomy Monograph No. 28, Madison: American Society of Agronomy, pp 101–31.
- Cristiane C D, Melissa W, Leila P S, Gabriele D S and Carlos A F. 2007. Effect of amylose content of rice varieties on glycemic metabolism and biological responses in rats. *Food Chemistry* **105**:1 474–9.
- Culliney K. 2011. Breeding rice for India: Quality, quantity and regional tastes: www.food navigator - asia.com.
- Frei M, Siddhuraju P and Becker K. 2003. Studies on the in vitro starch digestibility and glycemic index of six different indigenous rice cultivars from the Philippines. *Food Chemistry* **83**:395–402.
- Goddard M S, Young G and Marcus R. 1984. The effect of amylose content on insulin and glucose responses to ingested rice. *American Journal of Clinical Nutrition* **39**:388–92.
- Hodge J E and Hofreiter B T. 1962. Determination of reducing sugars and carbohydrates. (In) *Methods in carbohydrate chemistry*, Vol I, pp 380–94. Whistler R L and Wolfrom J L (Ed). Academic Press, New York.
- Hu P, Zhao H, Duan Z, Linlin Z and Wu D. 2004. Starch digestibility and the estimated glycemic score of different types of rice differing in amylose contents. *Journal of Cereal Sciences* **40**:231–7.
- IBPGR-IRRI. 1980. Descriptors for Rice, *Oryza sativa* L. The International Rice Research Institute, Manila, Philippines.
- FAO. 2004. International Year of Rice 2004: Rice and human nutrition. <http://www.fao.org/rice2004/en/f-sheet/factsheet3.pdf>.
- Li B J and Lai L Z. 1989. The study on the breeding of “black superior rice” by using biotechniques. (In) *Breeding research: the key to survival of the earth*. Iyama E and Takeda G (Ed). Proceedings of Sixth International Congress, SABRAO, Tsukuba, Japan, pp 289–91
- Lowry O H, Rosebrough N J, Farr A L and Randall R J. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**:265–75.
- Magdy S A, Beltagi E A, Hossam S, Mona A M, Amera T M and Sohir N A. 2010. Effect of amylose content and pre-germinated brown rice on serum blood glucose and lipids in experimental animals. *Australian Journal of Basic and Applied Sciences* **4**:114–21.
- Molla A M, Ahmed S M and Greenough W B. 1985. Rice based oral rehydration solution decreases the stool volume in acute diarrhea. *Bulletin of World Health Organization* **63**:751–6.
- Moore S and Stein W H. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry* **176**:367–88
- Oko A O, Ubi B E and Dambaba N. 2012. Rice cooking quality and physico-chemical characteristics: a comparative analysis of selected local and newly introduced rice varieties in Ebonyi State, Nigeria. *Food and Public Health* **2**:43–9.
- Otegbayo B O, Samuel F O and Fashakin J B. 2001. Effect of Parboiling on physico-chemical qualities of two local varieties in Nigeria. *Journal of Food Technology in Africa* **6**:130–2.
- Perez C M, Juliano B O, Pascual C G and Novenario V G. 1987. Extracted lipids and carbohydrates during washing and boiling of milled rice. *Starch* **39**: 386–90.
- Rivera E F, Aligui G L, Taaca A, Juliano B O and Perez C M. 1983. The use of “am” (rice water) among cases of acute gastroenteritis in children. *Philippine Journal of Pediatrics* **32**: 22–9.
- Sadasivam S and Manickam. 1992. *Standard Methods of Biochemical Analysis*. Thimmaiah S R (Ed). Kalyani Publishers, New Delhi.
- Shin C K, Chen S H, Hou W C and Cheng H H. 2007. A high-resistance-starch rice reduces glycosylated hemoglobin levels and improves the antioxidant status in diabetic rats. *Food Research International* **40**:842–7.
- Tribelhorn R E, O’Deen L A, Harper J M and Fellers D A. 1986. Investigation on extrusion for ORT samples. Colorado State University Research Foundation, Boulder, CO, USA.
- Tyagi A K, Khurana J P, Khurana P, Raghuvanshi S, Gaur A, Kapur A, Gupta V, Kumar D, Ravi V, Vij S, Khurana P and Sharma S. 2004. Structural and functional analysis of rice genome. *Journal of Genetics* **83**: 79–99.
- Van Handel E. 1968. Direct micro-determination of sucrose. *Analytical Chemistry* **22**:280–3.
- Zhou Z, Robards K, Helliwell S and Blanchard C. 2002. Composition and functional properties of rice. *International Journal of Food Science and Technology* **37**:849–68.