Asian Journal of Chemistry Vol. 21, No. 6 (2009), 4694-4702

Nutritional Parameters and Their Genetic Variability in Bold Grained Rice (*Oryza sativa* L.) Genotypes of Assam

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In Barak valley zone of Assam the physically hard working people prefer bold grained rice with good taste because of slow digestion and longer retention in the stomach. But most of the modern high yielding rice varieties is medium and fine grained. Keeping the need of rural people in mind, the present experiment was conducted on 47 bold grained rice genotypes with two recommended high yielding check varieties namely Ranjit and Monohar Sali of Barak Valley, Assam. The mean performances of 8 nutritional parameters of these 49 genotypes were evaluated. Crude protein content (%), total soluble protein content (g/100 g of oven dry sample), the amino acids lysine content and tryptophan content (g/16 g of N) varied from 7.09 to 13.10, 4.10 to 6.69, 3.20 to 4.45 and 0.56 to 1.10, respectively. The ash content (%) and phosphorus content (%) varied from 0.88 to 1.50 and 0.213 to 0.430, respectively. Calcium and iron content (mg/100 g) ranged from 16.59 to 37.98 and 1.11 to 3.92, respectively in the bold grained rice genotypes. The difference between phenotypic (PCV) and genotypic (GCV) coefficient of variations was very small for all the above nutritional parameters indicating greater role of genetic factors in their expression. In the present investigation, high heritability associated with high genetic advance was found in the characters *viz.*, calcium content and iron content. Breeding method based on progeny testing and mass selection could be useful in improving these traits. High heritability along with moderate genetic advance was observed in the characters crude protein content, total soluble protein content and ash content. Judicious application of pure line selection could be effective for improving these characters. Lastly high heritability with low genetic advance was recorded for the amino acids lysine and tryptophan content and phosphorus content. This indicated that these nutritional parameters were mostly governed by non-additive gene action (dominance and epistasis) in their inheritance.

Key Words: Nutritional parameters, Genetic variability, Bold grained rice.

INTRODUCTION

Barak valley zone comes under one of the six agro-climatic zones of Assam and lies between 24°15´ and 25°9´ N latitude and between 92°16´ and 93°15´ E longitude. The zone is characterized by an undulating topography with wide plain

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area and low lying waterlogged area. Physically hard working rural people of Barak valley zone of Assam consume bold grained rice because of slow digestion and longer retention in the stomach. This group of rice genotypes releases energy slowly and the consumers do not feel hunger for longer time while doing strenuous jobs like tillage, harvesting, carpentry, black smithy, road construction, *etc*. So the bold grained rice genotypes have been traditionally and widely grown in this zone since time immemorial. No literature is available on the genetic variation of nutritional parameters in bold grained rice. The present investigation was therefore undertaken to assess the nutritional parameters of 49 rice genotypes including two checks and to estimate their genetic variation for further genetic improvement. Rice is the prime source of carbohydrate and a major source of protein to the rural people of Barak valley despite the fact that protein content in rice is very low (7 to 14 %). Lysine and tryptophan are the limiting amino acids in rice and other cereal crops, hence the proportions of these two amino acids in protein molecule determine the quality of rice protein. The range of lysine content (g/16 g of N) of the 49 genotypes varied from 4.45 to 3.20. The genotypes G_{11} and G_{28} (4.45 g/16 g of N) showed the highest mean performance for this character. The lowest lysine content was registered by the genotype G_{25} (3.20 g/16 g of N). The range of tryptophan content (g/16 g of N) of the 49 genotypes varied from 1.10 to 0.56. The highest mean performance was recorded by the genotype G47 (1.1 g/16 g of N). The lowest mean performance for the character was registered by the genotype G_{43} (0.56 g/16 g of N). Ash content represents the total amount of mineral present in food. Rice ash like others contains several minerals of nutritional importance in varying proportion. The minerals have been identified with three main functions in the body, namely skeletal function as constituents of skeletal tissues, electrolyte function in body fluids and cells and cofactor function in enzyme and other proteins, which mediate body metabolism. Each mineral has its distinct functions and characteristic symptoms of deficiency. The minerals phosphorus and calcium are required as important structural components of the skeleton. The range of ash content (%) of the 49 genotypes varied from 1.50 to 0.88. The highest mean performance was recorded by the genotype G_{39} (1.50 %) followed by the genotypes G_{20}, G_{18}, G_{46}, G_{32}, *etc*. The lowest mean performance was showed by the genotype G_{36} (0.88 %) (Table-2). Thus, the genotypes G_{39}, G_{20}, G_{18}, G_{46} and G_{32} are the potential genotypes for mineral content profile improvement in breeding programme.

EXPERIMENTAL

The experimental material consisted of 47 bold grained rice genotypes collected from different parts of Barak valley zone, Assam, along with two recommended high yielding check varieties namely Ranjit and Monohar Sali. The experiment was conducted in randomized block design (RBD) with three replications during kharif season 2006. Freshly harvested seed samples from 49 rice genotypes were collected. The grains of each replicate were dehusked carefully before drying at 40 °C,

powdered and stored in a plastic bag sealed thoroughly before analysis of different nutritional characters. The different nutritional parameters were estimated as follows.

Crude protein content: It was determined by Micro-Kjeldahl's method AOAC[1]. The nitrogen in protein of the sample was converted to ammonium sulphate by sulphuric acid during digestion. This salt on steam distillation liberated ammonia and was collected in boric acid solution and titrated against standard acid (0.1 N HCl). Since 1 mL of 0.1 N acid was equivalent to 1.401 mg nitrogen; calculation was made to arrive at the nitrogen content of the sample.

The nitrogen content of the sample was calculated based on the formula:

$$\text{Total nitrogen (A)(g/100 g of sample)} = \frac{(a-b)\times \text{normality of HCl} \times 14.01}{\text{Weight of the sample (g)}}$$

where, a = volume of standard acid required for sample, b = volume of standard acid required for the blank.

The crude protein content in 100 g of sample was calculated by multiplying total nitrogen (A) with conversion factor 5.95. *i.e.*, crude protein content (g/100 g of sample) = A × 5.95

Total soluble protein content: Total soluble protein content from the grain sample was extracted by the following method. About 0.5 g of dried powder sample was taken in a centrifuge tube and to it, 5 mL of 0.1 N NaOH was added and stirred in cold condition for 15 min followed by centrifugation at 3000G for 10 min. The supernatant was decanted to a test tube and the residue was again treated with 5 mL of 0.1 N NaOH and stirred for 15 min followed by centrifugation as before. The supernatant was decanted in the previous test tube. The procedure was repeated four times. 2 mL of the above supernatant was mixed with 2 mL of 20 % tri-chloro acetic acid (TCA), kept in cold for 1 h for precipitation and again centrifuged at 3000G for 10 min. The residue was dissolved in 10 mL of 0.1 N NaOH. This solution was used for protein determination following Lowry *et al.*[2].

Lysine content: The protein in the grain sample was hydrolyzed with a proteolytic enzyme, papain. The α-amino groups of the derived amino acids were made to form a complex with copper. The ε-amino group of lysine would not couple with copper and it was made to form ε-dinitropyridyl derivative of lysine with 2-chloro-3,5-dinitropyridine. The excess pyridine was removed with ethyl acetate and the colour of ε-dinitropyridyl derivative was read at 390 nm wave length.

Calculation: A standard curve was prepared from the reading of the standard lysine. The absorbance of the blank was subtracted from that of the sample and the lysine content of the sample was calculated from the graph.

$$\text{Lysine content of the sample} = \frac{\text{Lysine value from graph in } \mu g \times 0.16}{\text{Per cent N in the sample}}$$

$$= \text{g per 16 g of N.}$$

Tryptophan content: It was determined by the method described in 'Biochemical Methods' by Sadasivam and Manickam[3]. The indole ring of tryptophan developed an orange-red colour with ferric chloride under strongly acidic condition. The colour intensity was measured in spectrophotometer at 545 nm wavelength.

Calculation: The absorbance value of the blank was subtracted from that of the sample and the tryptophan content was calculated from the standard curve.

$$\text{Tryptophan content in the grain sample} = \frac{\text{Tryptophan value from graph in } \mu g \times 0.096}{\text{Per cent N in the sample}}$$

= g/16 g of N.

Ash content: The ash content was determined following the method as described in the AOAC[4]. For this, 5 g powdered sample was taken in a silica crucible, charred in low Bunsen flame and finally ignited at 600 °C for 6 h in the muffle furnace.

$$\text{Ash content (g/100 g sample)} = \frac{\text{Weight of the ash} \times 100}{\text{Weight of the sample}}$$

The estimations were done in triplicate and their mean value was recorded as percentage of ash content in moisture free sample.

Preparation of mineral solution: The mineral solution was prepared according to the method described in AOAC[4]. For this purpose, ash was dissolved in HCl (1:1) on a water bath at 100 °C and the solution was evaporated to dryness. After that, 4 mL hydrochloric acid and 2 mL glass distilled water were added, warmed and the acid soluble portion obtained after filtration made up to 100 mL in a volumetric flask with glass distilled water. This solution was used for the estimation of minerals *viz.*, phosphorus, calcium and iron.

Phosphorus content: It was determined by the method of Fiske and Subbarow[5]: To an aliquot (0.1 mL) of the mineral solution, 1 mL of ammonium molybdate, 1 mL of hydroquinone and 1 mL of sodium sulfite (Na_2SO_3) solution were added and mixed well after each addition. The volume then was made up to 15 mL with water and the solution was mixed thoroughly. After 0.5 h, the optical density of the solution was measured in a spectrophotometer against a reagent blank at 660 nm wavelength.

The amount of the phosphorus was calculated out from the standard curve of phosphorus. The estimations were done in triplicate and the mean value was recorded as gram of phosphorus per 100 g of moisture free sample.

Calcium content: It was determined flame photometrically in a flame photometer, Systronics (Model-MK III) burner unit 121 at higher sensitivity.

The amount of calcium was calculated out from the standard curve prepared by using calcium solution of known strength. The estimations were done in triplicate and their mean value was recorded as mg of calcium per 100 g of moisture free sample.

Iron content: It was estimated by the method of Wong[6]: To an aliquot (6.5 mL) of the mineral solution, 1 mL of 30 % sulphuric acid and 1 mL of saturated potassium persulphate solution were added and mixed thoroughly. After 20 min, the optical

density of the solution was measured in the spectrophotometer at 540 nm wave length against a reagent blank. The amount of iron was calculated out from the standard curve, which was prepared by using iron solution of known strength. The estimations were done in triplicate and their mean value was recorded as mg of iron per 100 g of moisture free sample.

The analysis of variance for each nutritional parameter was calculated as per Panse and Sukhatme[7]. The phenotypic (PCV) and genotypic (GCV) coefficient of variation and heritability in broad sense (h_{bs}^2) for nutritional parameters were calculated as per Singh and Chaudhary[8]. Genetic advance as % of mean was computed following the method of Johnson *et al.*[9].

RESULTS AND DISCUSSION

The analyses of variance for different nutritional parameters are presented in the Table-1. It reflected significant genetic variation among the rice genotypes at P = 0.01 or 0.05.

TABLE-1
ANALYSIS OF VARIANCE FOR DIFFERENT NUTRITIONAL PARAMETERS OF BOLD GRAINED RICE

Source	d.f.	Mean sum of squares							
		Crude protein content % N × 5.95	Total soluble protein content (g/100g of oven dry sample)	Lysine content (g/16 g of N)	Tryptophan content (g/16 g of N)	Ash content (%)	Phosphorus content (%)	Calcium content (mg/100g)	Iron content (mg/100g)
Replication	2	3.02^{-2}‡	2.22‡	2.157^{-3}‡	2.007^{-3}‡	6.2^{-4}‡	5.61^{-5}	0.74	1.435^{-2}
Genotypes	48	7.31‡	1.34‡	0.418‡	4.524^{-2}‡	7.7^{-2}‡	8.742^{-3}‡	112.21‡	0.93‡
Error	96	1.61^{-3}	1.88^{-2}	2.791^{-4}	1.292^{-4}	6.53^{-5}	1.04^{-4}	1.02	5.1^{-3}

†Significant at 5 % probability level, ‡Significant at 1 % probability level.

The mean performance of 49 genotypes is presented in Table-2. From the Table, it revealed that the mean performance of all the nutritional parameters varied significantly in the bold grained rice genotypes. The crude protein content ranged from 7.09 to 13.10 % and total soluble protein content ranged from 4.10 to 6.69 (g/100 g of oven dry sample). The results conformed to several earlier reports[10-14]. The genotype Bar Madhava (G_{28}) recorded the maximum crude protein and total soluble protein content 13.10 and 6.69 %, respectively. Lysine and tryptophan are the two limiting amino acids in rice and other cereal crops, hence the proportions of these amino acids determine the quality of rice protein. The range of lysine content (g/16 g of N) of the 49 genotypes varied from 3.20 to 4.45. The genotypes Matonga (G_{11}) and Bar Madhava (G_{28}) recorded the highest lysine content. The range of tryptophan content (g/16 g of N) of 49 genotypes varied from 0.56 to 1.10. The highest tryptophan content was recorded by the genotype Malati (G_{47}). These results confirmed to the earlier report[15].

TABLE-2
MEAN PERFORMANCE OF DIFFERENT NUTRITIONAL PARAMETERS OF THE BOLD GRAINED GENOTYPES OF RICE

Genotypes	Crude protein content (%N × 5.95)	Total soluble protein content (g/100g of oven dry sample)	Amino acid Lysine content (g/16g of N)	Amino acid Tryptophan content (g/16g of N)	Ash content percentage (%)	Phosphorus content percentage (%)	Calcium content (mg/100g)	Iron content (mg/100g)
Soulpona (G_1)	11.90	5.84	4.06	0.88	1.06	0.313	29.88	1.31
Lati Sali (G_2)	12.11	6.45	4.11	0.91	1.10	0.330	33.24	2.69
Chuto Mula (G_3)	11.08	5.42	4.31	0.82	1.06	0.310	29.28	2.58
Kartic Kalma (G_4)	10.07	5.83	4.29	0.67	0.98	0.303	21.57	2.17
Basanta Bahar (G_5)	9.92	6.40	3.81	0.77	1.04	0.283	27.97	2.63
Karmi Sail (G_6)	11.24	5.19	3.36	0.72	1.12	0.347	22.82	2.28
Soularpona (G_7)	12.07	6.18	3.80	0.76	0.96	0.383	32.54	2.33
Chaku Sail (G_8)	8.97	5.96	3.53	0.72	1.29	0.403	21.99	2.45
Dhola Mula (G_9)	11.46	5.82	4.15	0.68	1.21	0.273	37.98‡	3.15
Kuiari Sali (G_{10})	11.96	5.52	3.87	0.82	1.28	0.233	21.79	3.57
Matonga (G_{11})	11.06	6.18	4.45‡	1.01	1.40	0.317	31.09	2.11
Chafa Sali (G_{12})	10.58	4.72	4.15	0.83	1.18	0.337	30.33	2.15
Methi Chikon (G_{13})	11.25	4.80	4.22	0.71	1.34	0.310	20.36	2.84
Probat Jeera (G_{14})	11.40	4.62	4.43	0.63	1.07	0.270	31.75	2.11
Kamal Bhog (G_{15})	8.32	6.09	3.98	0.62	1.35	0.297	18.50	3.10
Betguti Dhan (G_{16})	8.98	4.19	3.43	0.60	1.04	0.327	33.13	2.75
Heera Dhan (G_{17})	7.29	4.82	3.37	0.67	1.27	0.270	16.74	2.61
Baodun (G_{18})	11.09	5.65	4.12	0.76	1.44	0.260	18.46	2.16
Kapilee Dhan (G_{19})	7.58	5.14	3.27	0.72	1.31	0.383	30.30	2.42
Chandmoni (G_{20})	12.67	6.27	4.36	0.77	1.49	0.403	31.93	3.31
Gourarchor (G_{21})	12.86	6.16	4.40	0.95	1.01	0.320	19.05	2.22
George Sail (G_{22})	11.06	5.83	4.28	0.79	0.96	0.283	29.24	2.65
Herapowa (G_{23})	10.76	5.21	4.18	0.72	0.89	0.283	26.49	2.22
Dudh Mula (G_{24})	10.95	5.64	3.87	0.93	1.23	0.313	17.23	2.11
Gajep Sail (G_{25})	7.09†	5.34	3.20†	0.77	1.36	0.347	19.47	3.92‡
Kali Makuri (G_{26})	8.37	5.82	3.62	0.64	1.04	0.223	32.44	2.63
Bata Sail (G_{27})	8.28	6.25	4.04	0.70	1.27	0.240	29.78	2.45
Bar Madhava (G_{28})	13.10‡	6.69‡	4.45‡	0.87	1.38	0.350	33.05	3.60
Monohar Sali (G_{29})	11.93[c]	5.41[c]	4.11[c]	0.59[c]	1.09[c]	0.310[c]	18.55[c]	1.91[c]
Ranjit (G_{30})	9.73[c]	5.16[c]	4.21[c]	0.67[c]	1.16[c]	0.323[c]	16.67[c]	1.11*[c]
Hacha Lath (G_{31})	11.23	6.18	4.07	0.71	1.26	0.287	20.07	2.12
Samras (G_{32})	10.27	6.24	4.04	0.78	1.42	0.277	33.56	2.63
Atha Sail (G_{33})	10.71	6.25	4.16	0.65	1.08	0.270	21.67	2.02
Latha Sail (G_{34})	9.29	6.61	3.93	0.65	1.17	0.313	17.35	3.16
Haladhar Sali (G_{35})	9.14	6.17	3.82	0.93	1.26	0.373	30.39	1.86
Chatri Sail (G_{36})	9.38	5.31	4.03	0.70	0.88†	0.330	16.59†	2.28
Maghi Sail (G_{37})	8.93	4.52	3.46	0.69	1.13	0.387	33.38	2.33
Mala (G_{38})	8.57	5.17	3.29	0.74	1.34	0.410	18.92	2.42

Genotypes	Crude protein content (%N × 5.95)	Total soluble protein content (g/100g of oven dry sample)	Amino acid Lysine content (g/16g of N)	Amino acid Tryptophan content (g/16g of N)	Ash content percentage (%)	Phosphorus content percentage (%)	Calcium content (mg/100g)	Iron content (mg/100g)
Dome Sail (G_{39})	7.90	5.91	3.26	0.98	1.50‡	0.430‡	26.76	2.55
Agani Sali (G_{40})	7.27	5.73	3.28	0.61	1.17	0.310	30.58	3.44
Rashi (G_{41})	8.25	4.33	4.04	0.85	1.05	0.280	17.49	2.65
Hathi Sali (G_{42})	8.90	4.85	3.81	0.63	0.96	0.360	19.50	3.26
Shem Sail (G_{43})	10.03	5.33	4.06	0.56†	1.19	0.388	21.71	2.42
Daura Sail (G_{44})	9.18	5.44	3.30	0.82	1.22	0.213†	30.15	2.95
Chingra Sail (G_{45})	9.26	6.20	4.19	0.71	1.07	0.377	25.99	2.65
Khasi Dhan (G_{46})	9.18	6.15	4.21	0.58	1.43	0.413	21.49	2.67
Malati (G_{47})	10.39	6.23	3.45	1.10‡	1.22	0.267	26.25	2.74
Zoli (G_{48})	10.20	4.10†	4.16	0.93	1.08	0.373	30.35	2.25
Lalia Sail (G_{49})	11.26	4.64	4.08	0.70	1.31	0.403	32.05	3.70
$SE_{d\pm}$	0.033	0.11	0.014	0.0093	0.0066	0.00833	0.825	0.05814
$CD_{0.01}$	0.086	0.295	0.036	0.025	0.017	0.022	2.18	0.15

$SE_{d\pm}$ = Standard error of difference, $CD_{0.01}$= Critical difference at 1 % probability level,
†Minimum mean value, ‡Maximum mean value, [c]Mean values of check varieties

Rice ash contains several minerals of nutritional importance in varying proportions. The ash content (%) of the 49 rice genotypes varied from 0.88 to 1.50 %. The genotype Dome Sail (G_{39}) recorded the highest ash content (1.50 %). The phosphorus content (%) among the rice genotypes varied from 0.21 to 0.43 %. The highest phosphorus content was recorded by the genotype Dome Sail (G_{39}). The calcium content (mg/100 g) of 49 rice genotypes ranged from 16.59 to 37.98. The highest calcium content (37.98 mg/100 g) was recorded by the genotype Dhola Mula (G_9). Iron content (mg/100g) of the 49 rice genotypes varied from 1.11 to 3.92.

The highest iron content (3.92 mg/100 g) was observed in the genotype Gajep Sail (G_{25}). The results are in agreement with the earlier reports[10,14]. These results suggest that some bold grained rice genotypes are very rich in one or the other nutritional parameter in comparison to the modern HYVs *i.e.*, the two check varieties in the present study. So the superior bold grained rice genotypes for each nutritional parameter could be used as parents in hybridization programme to transfer the desirable gene or gene block for the specific parameter into the genetic background of future HYV of rice.

The comparison of characters as regards to the extent of genetic variation could be better judged by the estimation of genotypic coefficient of variation (GCV) in relation to their respective phenotypic coefficient of variation (PCV). The difference between PCV and GCV was very small for all the nutritional parameters (Table-3). This small difference between PCV and GCV for each parameter revealed the small influence of environment but the predominant role of genetic factors on the expression

of the nutritional parameters.

TABLE-3
ESTIMATION OF GENETIC PARAMETERS FOR DIFFERENT NUTRITIONAL CHARACTERS IN BOLD GRAINED RICE GENOTYPES

Characters (Nutritional parameters)	Range		Mean	Standard error of mean $SE_{m\pm}$	PCV (%)	GCV (%)	Heritability in broad sense h_{bs^2} (%)	GA As % of mean
	Max.	Min.						
Crude protein content (as %N × 5.95)	13.20	7.02	10.090	0.0200	15.47	15.46	99.92	31.85
Total soluble protein content (g/100g of oven dried sample)	6.72	4.05	5.600	0.0800	12.13	11.88	95.91	23.97
Lysine content (g/16 g of N)	4.45	3.20	3.920	0.0096	9.53	9.52	99.78	19.58
Tryptophan content (g/16g of N)	1.10	0.56	0.750	0.0066	16.29	16.16	99.15	5.43
Ash Content (%)	1.50	0.88	1.190	0.0047	13.50	13.48	99.74	27.73
Phosphorus content (%)	0.43	0.21	0.323	0.0059	16.91	16.61	96.52	5.89
Calcium content (mg/100g)	37.98	16.59	25.620	0.5830	24.09	23.76	97.32	48.29
Iron content (mg/100g)	3.92	1.11	2.540	0.0410	21.98	21.79	98.37	44.53

Heritability refers to the proportion of phenotypic variance that is attributed to genes. Heritability of metric characters is of great importance to the breeders as its magnitude indicates the accuracy with which a genotype can be recognized by its phenotypic expression and determines the generation in which selection can be profitable. The genetic advance in percentage of mean is the magnitude of improvement that can be made in a particular character by selecting a certain proportion of population in a definite direction.

In the present investigation, high heritability associated with high genetic advance was found for the characters *viz.*, calcium content and iron content. It indicated that these characters were mostly governed by additive gene action. This finding is in agreement with the earlier report[16]. Breeding method based on progeny testing and mass selection could be useful in improving these traits. High heritability along with moderate genetic advance was observed in the characters crude protein content, total soluble protein content and ash content. These results are consistent with the earlier reports[16,17]. Judicious application of pure line selection may be effective for improving the characters. In last, high heritability with low genetic advance was recorded for the amino acids lysine and tryptophan content and the mineral phosphorus content. These parameters were mostly governed by non-additive gene action (dominance and epistasis). This result confirmed to the earlier report[16].

Conclusion

The findings of the present investigation are very important for future breeding programme for quality improvement towards protein and mineral contents in bold grained rice. The genotype Bar Madhava (G_{28}), Matonga (G_{11}) and Malati (G_{47}) recorded the higher crude protein and total soluble protein content with high proportion of the two limiting amino acids lysine and tryptophan. Hence, these three bold grained genotypes could be used as parents in future hybridization programme to transfer the desirable gene or gene block into the genetic background of future HYV of rice. Similarly, the genotype Dome Sail (G_{39}) followed by the genotypes Chandmoni (G_{20}), Baodun (G_{18}), Khasi Dhan (G_{46}), Samras (G_{32}) recorded higher mean performance for mineral content profile and hence could be utilized as parents in successful breeding programme.

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(*Received*: 26 August 2008; *Accepted*: 25 March 2009) AJC-7382