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EVALUATION OF WILD RICE MUTANTS WITH CULTIVARS BY SEED PROTEINS AND THEIR AMINO ACID PROFILES

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Seed proteins were quantified in two wild rices (Oryza *rufipogon* and *O. australiensis*), three cultivars (IR-8, Sona and Fugiminori) and three true breeding induced mutants (dwarf. early flowering and awnless mutants) isolated in *O. rufipogon* during operation of a DAE research project. Protein content was maximum in the wild species *O. australiensis* and has further improved in the three mutants over their control and suggest the possibility of improving this parameter through mutation breeding of wild species. Aminoacid profiles of the free as well as bound fractions of the dehusked seed were also analysed in the eight genotypes mentioned above using automatic amino acid analyser. Protein content was positively correlated with total amino acids (free + bound) but negatively with the essential amino acid lysine. Correlation of each kind of aminoacid (free + bound) with any of the other in the profile estimated for the 136 possible combinations revealed significant correlations only for 12 amino acid pairs. Some amino acids were very high in the wild species suggesting the high potentiality of the wild species for the synthesis of quality proteins and a possibility of improvement of cultivars through cross breeding. The three mutants were associated with enhanced quantities of most of the amino acids of the free as well as bound type but to a lesser extent of the latter. It appears that the mutant gene(s) have a control on storage proteins through an overall increase in the nitrogen that is present in both these fractions. The awnless mutant was found to be nearer to the cultivars in most of the attributes amply suggesting the possible origin of the present day cultivars from wild progenitors by way of mutations as a major tool. None of the essential amino acids in the profile is significantly correlated to any other amino acid there by paving way for obtaining mutants with amino acid profiles of desirable combinations.

Keywords : Amino acid profiles; Cultivars; Seed proteins; Wild rice mutants.

Introduction

Cereals are generally low in their seed protein quality and quantity as compared to pulses.The need for improving these parameters has been stressed on several occasions by many nutritionists and biochemists. Although rice is known for its higher digestibility and protein efficiency ratio, the main limitation is its low quantity accompained by a distribution in the peripheral zones of the grain rendering further loss during milling[1]. Extensive genotypic and phenotypic variability is already known to exist among and within rice varieties and cultivars[2-5]. But the wide variation in protein content even within the same genotype[6] stresses the need for devising methods of sampling minimising the variance[7-9] and the procedures of Juliano and Beachell[10] seem to be pertinent to certain extent and were followed to the possible extent in the present studies. The present study on seed proteins and amino acid profiles in wild rices, mutants and cultivars is attempted to have an insight on to their interrelationships and on the nature of changes in these parameters occurred during mobilization besides assessing the significance of wild rices in mutation breeding.

Materials and Methods

Seed proteins as well as individual amino acids in the free and bound fractions of the dehusked seed were studied in two wild species (*Oryza rufipogon* and *O. australiensis*) and three cultivars (Sona, IR-8 and Fugiminori). During the operation of a DAE rsearch project, several morphological mutants of breeding value were isolated in *O. rufipogon* and were studied. However, only three of them viz., dwarf, early flowering and awnless were found to be true breeding and were included in the present study.

1. Seed Proteins : Seed proteins were quantified following the standard Microkjeldahl method. Air dried, dehusked seeds were ground to a powder and about 100 mg of it was subjected to digestion and the liberated ammonia was collected in a conical flask containing 2 ml of boric acid-indicator mixture and was titrated against

0.01N HCI until pink colour reappears. The estimated nitrogen was converted into protein by multiplying with 5.95 as the conversion factor.

2. *Amino acid profiles* : Dry and dehusked seeds were ground to a fine powder and was used for analysing the free and bound amino acids.

a) Sample preparation for free amino acids : 500 mg of the seed powder was taken in a mortar and macerated with 10 ml of 80% ethanol. Then the slurry was centrifuged and the supernatant was reduced to dryness under vaccum below 40°C. It was dissolved in 2 ml of sample buffer (acetate buffer, pH 2.2).

b) Sample preparation for bound amino acids : The residue from the above after centrifugation was taken; 20 mg of the pellet was taken in an ampule and 2 ml of 6N HCI was added to it followed by bubbling nitrogen gas into it. Later it was evacuated, sealed and was subjected to hydrolysis of seed proteins in a hot air-oven at 110°C for 24 hours. The hydrolysate was filtered and the aliquot was reduced to dryness under vaccum below 40°C and was finally dissolved in 5 ml of the sample buffer.

In both cases described above, the amino acids were determined by injecting 0.5 ml of the sample into an LKB 4101 Automatic Amino Acid Analyser; norleucine was added to the sample to act as an internal standard. As regards to the constituents ot the buffers and quantification of the amino acids, the procedures given in the technical manual were followed.

Results and Discussion

Seed proteins and total amino acids in each genotype : Protein content ranged from 8.23 to 11.40 among the eight genotypes of the present study (Table 1). The two indicas (IR-8 and Sona) have about the same quantity but to a lesser extent than in the japonica variety (Fugiminori). Among the wild and cultivars, protein content was maximum in *O. australiensis*. Further, gamma irradiation has fairly improved the protein content with a maximum in the awnless mutant.

Total free amino acids (Table 1) remained almost the same in two of the cultivars (Sona and Fugiminori) while it was lesser in IR-8. Interestingly the species *O. australiensis* having higher protein content among the wild and cultivars was associated with a minimum of free amino acids and maximum of bound type. The free as well as the bound amino acids increased in the three mutants over control (*O. rutipogon*) but the latter being to a lesser extent (Table 1). Total of all amino acids (free + bound) was positively correlated to grain proteins. However, this relationship with protein content was not significant when the free and bound amino acids were considered separately suggesting an independent variation of these two parameters in the genotypes studied here.

Patterns of aminograms of the free fractions: A perusal of the amino acid patterns in the eight genotypes (Table 2) revealed that proline and glycine were altogether absent in Sona while phenylalanine and threonine were absent in IR-8. Leucine was in minute quantities in the three cultivars while aspartic acid was higher in the cultivars than in the wild species. The amino acid valine was very high in *O. rufipogon* compared to the remaining seven genotypes. The four amino acids phenylalanine, histidine, glutanic acid and serine increased in the mutants over control; further the increment was relative to their protein values.

Patterns of amino grams of the bound fractions: The amino acid arginine was higher in Sona not only over the other two cultivars but also among the eight genotypes investigated (Table 2). The two wild species differ in such a way that the amino acids aspartic acid, threonine,serine, glutamic acid, alanine and arginine were more in *O. australiensis* while proline, isoleucine, leucine and tyrosine were more in *O. rufipogon*. The amino acids serine, glycine and phenylalanine decreased in the three mutants over control the quantities

being proportionate to their seed protein quantities. Similarly alanine, cystine, isoleucine, leucine and tyrosine were also reduced in the mutants but to a variable extent independently of the protein content. Compared to control, proline was more in two of the mutants (dwarf and early flowering) but lesser in the awnless.

Correlation of each kind of amino acid (free + bound) with any other in the profile was estimated in all the 136 possible combinations but significant associations were evident only for 12 amino acid pairs five of them were positive while the remaining were negative (data not tabulated). In addition, studies on the relationship of each amino acid with grain protein content revealed that only two of the 17 amino acids of the profile (serine and lysine) were significantly associated with protein content.

Comparison of essential amino acids : The essential amino acid patterns in the combined fraction of the present eight genotypes were compared to that of FAO standard and human milk (Table 2). Amino acids leucine, phenylalanine and valine were significantly higher in all the eight genotypes as against the FAO standard while they reached to the level of human milk in atleast some of the genotypes. Compared to FAO standard, threonine and cystine were in high quantities in Sona and Fugiminori but not in IR-8. Similarly cystine and methionine were more in *O: rufipogon* while threonine was more in *O. australiensis*.

With the exception of leucine, phenylalanine and valine, the other essential amino acids were in low quantities in the three mutants as against both the standards. Nevertheless, the quantities of some of them appear to have improved in the mutants over their control.

Seed protein quantities of the eight genotypes mentioned here and of the many lines investigated (the data of which is not included) suggest that the wild species are better in grain proteins than most other lines. The content further increased in the three mutants of *O. rufipogon* indicating a possibility of improving seed protein levels by mutation breeding involving wild species. Some amino acids of the present study especially the essential amino acids like lysine and valine were higher in the wild species indicating that the wild species have the potentiality for the synthesis of high quality seed proteins. Further the variability of the amino acids in the wild and cultivars indicate the source of limiting amino acids in the cultivars and further improvement

Table 1. Protein percentages (gms/100 gms seed powder) and total amino acids of the free and bound fractions (gms/100 gms seed powder) in eight rice genotypes.

Genotype	Protein	Amino acids	
		Free	Bound
IR-8	8.23	0.104	1.466
Sona	8.31	0.122	1.463
Fugiminori	9.21	0.124	1.702
O. australiensis	10.87	0.061	2.049
O. rufipogon	9.20	0.150	1.610
Dwarf	10.20	0.265	1.665
Early flowering	10.80	0.307	1.763
Awnless	11.40	0.362	1.808

Table 2. Patterns of individual amino acids (gms/100 gms proteins) in the free (F) and bound (B) fractions in eight rice genotypes.

Amino acid	Genotype																FAO Standard	Human Milk
	IR-8		Sona		Fugiminori		*O. australiensis*		*O. rufipogon*		Dwarf		Early flowering		Awnless			
	F	B	F	B	F	B	F	B	F	B	F	B	F	B	F	B		
Aspartic acid	0.53	10.33	0.23	8.06	0.30	11.09	0.06	11.59	0.09	8.51	0.27	8.75	0.18	8.50	0.36	8.53		
Threonine*	--	2.61	0.12	2.97	0.09	2.93	0.06	3.61	0.09	2.33	--	2.22	0.25	2.15	0.15	2.29	2.80	4.40
Serine	0.65	3.34	2.27	3.17	1.01	4.45	0.36	4.50	1.09	3.05	2.99	2.92	3.78	2.87	4.34	2.72		
Glutamic acid	1.01	18.77	1.52	15.73	1.50	15.23	0.60	20.15	1.73	15.83	2.74	19.06	2.86	16.03	4.95	15.49		
Proline	0.62	6.86	--	5.01	0.71	5.49	--	4.73	--	5.48	--	6.15	--	6.19	0.06	5.05		
Glycine	0.10	3.95	--	3.39	0.02	3.57	0.09	4.03	--	3.78	--	3.41	--	3.28	0.28	3.01		
Alanine	0.07	5.83	0.27	4.94	0.31	3.81	0.55	5.16	--	4.79	0.02	3.53	--	3.76	2.10	3.91		
Cystine*	0.24	0.76	0.92	2.00	0.96	2.99	--	0.69	0.68	1.79	1.98	1.27	2.75	1.39	0.66	1.32	2.00	3.05
Valine*	0.08	5.59	0.23	4.74	0.29	8.28	0.18	4.96	3.08	4.94	2.25	3.71	1.30	3.28	0.31	4.15	4.20	6.60
Methionine*	1.08	0.43	0.03	1.44	0.09	0.72	--	0.91	0.31	0.27	0.95	0.89	0.80	0.45	0.06	1.01	2.20	1.50
Isoleucine*	0.13	3.52	0.01	3.99	0.04	4.38	0.03	3.66	0.07	4.26	0.25	2.49	0.14	3.05	0.15	3.11	4.20	5.65
Leucine*	0.01	9.58	0.02	10.00	0.03	6.58	0.03	8.36	0.02	10.63	0.03	8.76	0.11	9.00	0.13	9.41	4.80	11.35
Tyrosine	0.04	3.47	0.06	3.45	0.30	4.93	0.21	3.54	0.27	5.16	0.41	3.07	0.29	2.93	0.22	3.27		
Phenylalanine*	--	3.56	0.42	3.87	0.09	3.14	0.05	4.73	0.04	4.38	0.16	3.83	0.18	3.72	0.12	3.05	2.80	4.05
Histidine*	0.03	1.83	0.16	1.68	0.31	2.66	0.04	2.04	0.12	2.13	0.28	1.36	0.29	2.47	0.21	3.44	2.40	2.40
Lysine*	0.01	3.20	0.05	3.17	0.17	3.29	0.22	3.37	0.26	3.24	0.21	2.67	0.49	3.42	0.32	1.42	4.20	5.75
Arginine*	1.74	5.34	0.92	9.06	0.34	6.32	0.33	7.59	0.26	6.55	0.13	5.35	0.58	7.91	0.91	5.45	2.00	4.15

* Essential amino acids (F+B) comparable to that of FAO standards (1970) and human milk (Kuppuswamy *et al.* 1958)

through cross breeding/somatic cell hybridization. The amino acid arginine was higher in all the genotypes as against both the standards (Table 2). Similarly valine and phenylalanine were also higher in most of the genotypes. However, methionine and lysine seem to be the two limiting amino acids in all of them. The observed negative relationship between grain protein content and lysine is undesirable because it limits the simultaneous increment of these two parameters.

Total free amino acids were more in the three mutants over control although to a variable extent (Table 1) which was due to an increase in most of the individual amino acids of the profile (Table 2). Instances of such enhanced accumulation of free amino acids were known in chlorophyll mutants of higher plants[11,12], male steriles of cotton[13] and sorghum[14] and even in the endosperm mutants of maize[15] which were attributed to a partial or complete block in protein synthesis. But in the present study, there was an increase in the amino acids of both free as well as bound fractions in the mutants but to a lesser extent of the latter type. It is presumed that the mutant gene(s) have a control on storage proteins through an over all increase in the nitrogen that is present in both these fractions. A simultaneous increase in the grain yield as well as seed protein in the awnless mutant is quite encouraging. More over, the mutant was found to be nearer to the cultivated varieties in most of its attributes (data not presented) accounting for a possible origin of the present day cultivars from their wild progenitors by way of mutations as a major tool of evolution.

Comparison of the essential amino acid patterns with those of FAO standard and human milk revealed that leucine, phenylalanine and valine were significantly higher in all the eight genotypes of the present study. More over none of the essential amino acids in the profile is not significantly correlated (positive or negative) with others paving way for isolation of mutants with amino acid profiles of desirable combinations.

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