Glutamate Dehydrogenase Activity and Isoenzymes, Protein, and Soluble Amino Acids in Developing Grains of High and Low Protein Rice

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ABSTRACT

Protein, soluble amino acids, glutamate dehydrogenase (GDH) activity, isoenzymes, and dry matter accumulation were studied during kernel development of high and low protein rice cultivars from anthesis to maturity. Maximum grain filling occurred within 15 days after the date of anthesis. Free amino acids (11.18, 6.98 μg per kernel), protein (146.06, 83.77 mg per gram of kernels, dry weight), and GDH activity (31.5, 17.33 units) in rice kernels were significantly higher in high protein rice (HP 8) than in low protein rice (Ratna). The electrophoretic patterns of GDH showed two bands with R_p 0.20 and 0.17.

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The crude protein content of rice grain ranges from 5 to 17% on a dry weight basis (Juliano 1966, Juliano et al 1968). The wide variation in protein content in rice cultivars suggests that the protein content could be improved. Although the yield of rice has been enhanced, protein content correlated negatively with yield (Ghosh et al 1971, Nanda et al 1976, Gangadharan et al 1978). Efforts have been made to study the factors associated with the accumulation of protein in the rice kernel (Mitra et al 1976, Mandal et al 1979). To determine the biochemical factors responsible for the high protein content in certain rice varieties, we compared the protein content, dry weight, soluble amino acids, glutamate dehydrogenase (GDH) activity, and GDH isoenzymes during kernel development from anthesis to maturity in a high and a low protein rice.

MATERIALS AND METHODS

Rice varieties HP 8 (high protein) and Ratna (low protein) were grown under uniform fertilizer level (N at 100 kg/ha) at the Central Rice Research Institute, Cuttack, India, during the wet season of 1977. Tillers were carefully examined and tagged at anthesis. Random grain samples were collected in duplicate at five-day intervals from anthesis to maturity (30 days, six stages). Grains were collected from the center of the panicle to avoid differences due to age and translocation of metabolites. They were placed in an ice bath immediately after sampling. The samples were carefully dehulled by hand and stored at −20°C for further analysis.

Nitrogen in 10-15 grains was determined by the microKjeldahl method (AOAC 1970). Protein (N × 5.95) was determined.

Developing grains were homogenized with 80% ethanol and centrifuged at 12,000 × g for 15 min. The pellets were extracted three times with 80% ethanol and the supernatants evaporated to dryness in a bath of boiling water. The residue was dissolved in 2 ml of 0.1*M* citrate buffer (pH 2.2) and 0.5 ml of aliquot assayed for amino nitrogen content by the ninhydrin method (Moore and Stein 1954).

GDH activity (aminating) of developing grains was estimated as described by Kanamori et al (1972) and assayed in duplicate samples spectrophotometrically by measuring the progress of NADH oxidation at 30°C in a Specord-UV at 340 nm. Enzyme activity was expressed as the change in absorbance per minute per grain.

Protein (150 μg), as estimated by the Folin method (Lowry et al 1951), was analyzed by polyacrylamide gel electrophoresis using 10% polyacrylamide gels and a 3-mA tube (Davis 1964). After a 3-hr run, the gels were incubated with the substrate. Isoenzyme activities were visualized after electrophoresis (Kadam et al 1973).

RESULTS AND DISCUSSION

The dry weight of dehulled grains in both HP 8 and Ratna rice varieties increased progressively till maturity (Table I). However, HP 8 accumulated more dry matter than did Ratna in all stages except at five days after anthesis. The patterns of dry matter accumulation were similar for HP 8 and Ratna during

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TABLE I
Dry Weight, Protein Content, Soluble Amino Nitrogen, and Glutamate Dehydrogenase (GDH) Activity in Rice Kernels During Development

Days After Anthesis	Dry Weight (mg/kernel)			Protein Content (mg/g of kernels, dry weight)			Soluble Amino Nitrogen (μg/kernel)			GDH Activity (absorbance × 10^{-5}/kernel)		
	HP 8	Ratna	Mean	HP 8	Ratna	Mean	HP 8	Ratna	Mean	HP 8	Ratna	Mean
5	3.23	3.76	3.50	340.55	172.87	256.72	10.6	8.5	9.55	30	21	25.50
10	14.20	12.79	13.50	123.94	71.93	97.94	22.5	9.3	15.90	45	25	35.00
15	22.10	16.94	19.52	103.62	59.62	81.62	11.1	6.8	8.95	59	26	42.50
20	23.05	16.99	20.02	101.95	66.50	84.29	10.4	6.0	8.20	29	18	23.50
25	23.62	17.03	20.32	102.03	66.94	84.49	7.5	6.6	7.05	17	10	13.50
30 (maturity)	24.36	17.94	21.15	104.26	64.65	84.47	5.0	4.7	4.85	9	4	6.50
Mean	18.43	14.24	16.33	146.06	83.77	114.92	11.183	6.983	9.083	31.50	17.33	24.417
Least significant difference												
Days after anthesis												
$P = 0.05$		0.07			3.13			0.41			3.01	
$P = 0.01$		0.10			4.38			0.57			4.22	
Variety												
$P = 0.05$		0.04			1.80			0.24			1.74	
$P = 0.01$		0.05			2.53			0.33			2.44	
Interaction												
$P = 0.05$		0.10			4.42			0.58			4.26	
$P = 0.01$		0.14			6.20			0.81			5.98	

TABLE II
Glutamate Dehydrogenase Isoenzymes in Rice Kernels During Development

Days After Anthesis	HP 8		Ratna	
	Protein Band R_p[a]	Intensity of Protein Band	Protein Band R_p	Intensity of Protein Band
5	0.20	**[b]	0.20	*
	0.17	****	0.17	**
10	0.20	***	0.20	**
	0.17	*****	0.17	****
15	0.20	***	0.20	**
	0.17	*****	0.17	*****
20	0.20	***	0.20	**
	0.17	***	0.17	*
25	0.20	**	0.20	*
	0.17	**	0.17	*
30 (maturity)	0.20	**	0.20	*
	0.17	*	0.17	*

[a] R = relative mobility, $_p$ = protein.
[b] * = pale, ***** = very bright.

grain development. Dry weight of the developing kernel increased rapidly up to 15th day after anthesis; thereafter, accumulation of dry matter was slow. Similar observations were made by Rosario et al (1968), Cruz et al (1970), and Mandal et al (1979).

Protein Content

The protein content was higher in HP 8 than in Ratna in all stages of grain development (Table I). Variation of protein content between HP 8 and Ratna (104.26 and 64.65 mg per g of kernels, dry weight, respectively) at maturity was highly significant. In both varieties, more protein accumulated during the early period of development. Low accumulation in the latter part of grain development may be due to rapid carbohydrate synthesis. Similar observations were made by Palmiano et al (1968) and Cruz et al (1970). Jennings and Morton (1963) reported similar protein accumulation in wheat endosperm during development. No significant difference was observed in the protein content of HP 8 from 15 days after anthesis to maturity or in Ratna from 20 days after anthesis to maturity. However, accumulated protein was higher in HP 8 than in Ratna during these periods. The large proportion of protein was synthesized earlier in development, indicating the involvement of protein during embryo formation (Paul et al 1971).

Soluble Amino Nitrogen

HP 8 contained more soluble amino acids than did Ratna in all stages of grain development (Table I). The content of soluble amino nitrogen increased, reached a maximum level 10 days after anthesis, and then dropped. The differences in the availability of free amino acids between high and low protein seeds are significant up to 20 days after anthesis. Rana et al (1984) reported differences in free amino acid levels between high and low protein wheat. Gary (1975) also observed a higher amount of free amino acids in embryos of high protein wheat.

Higher quantities of soluble amino acids may contribute to high protein accumulation in HP 8 (Mitra et al 1976), since free amino acids are the precursors of protein in the grain.

GDH Activity

HP 8 exhibited more GDH activity throughout kernel development than did Ratna (Table I). Maximum GDH activity was noticed 15 days after anthesis, at which time HP 8 had more than twice the concentration of GDH than did Ratna. GDH activity declined 15 days after anthesis in both rices. The amount of glutamic acid was higher in HP 8 than in Ratna grains (CRRI 1978). GDH catalyzes the conversion of ammoniacal or inorganic nitrogen to organic nitrogen. Ammonia induces the formation of GDH in the roots of the rice plant (Kanamori et al 1972). Cagampang et al (1971) reported that the major free amino acids in rice grain are aspartic acid, glutamic acid, histidine, alanine, and ornithine. Evidently, pronounced GDH activity in HP 8 and the resulting high levels of glutamic acid contributed to its high protein content.

Isoenzyme Patterns of GDH

The GDH of HP 8 and Ratna showed differences during grain development. Protein with R_p 0.20 and 0.17 bands were observed in both varieties (Table II). However, qualitative differences existed in the intensity of the protein bands. The intensity of the GDH band started diminishing 15 days after anthesis. The intensity of the protein band of HP 8 was greater than that of Ratna, especially at 20 days. The GDH band with R_p 0.17 was more prominent. The prominence of bands was consistent with the pattern of soluble amino acids, protein content, and GDH activity in the developing grain.

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