Effect of Soil Sulfur Deficiency on Sulfur Amino Acids and Elements in Brown Rice

BIENVENIDO O. JULIANO,[1] MARIA GRACIA B. IBABAO,[1] CONSUELO M. PEREZ,[1] RALPH B. CLARK,[2] JERRY W. MARANVILLE,[2] CEZAR P. MAMARIL,[3] NURUL H. CHOUDHURY,[4] CHRISTINE J. S. MOMUAT,[5] and IGMIDIO T. CORPUZ[5]

ABSTRACT

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The effect of soil sulfur deficiency and its amelioration on contents of sulfur amino acids and of other elements estimated by energy-dispersive X-ray fluorescence spectrometry was determined on brown rice produced in sulfur-deficient soils in Bangladesh, Indonesia, and the Philippines. Cysteine plus methionine levels in protein were normal for rice grown in control plots and comparable to those grown in treated plots in field experiments in Bangladesh and Indonesia. Low cysteine plus methionine levels in protein were observed only in pot experiments in brown rice with N:S ratios of 16 and 25. Amino acid sulfur accounted for a mean of 78% of total sulfur in the 32 brown rices investigated. Levels of elements in brown rice were not affected by sulfur treatment of the sulfur-deficient soils.

A shift in fertilizer production from ammonium sulfate to urea appears to have triggered an increased incidence of sulfur deficiency in wetland rice (Yoshida and Chaudhry 1979). Sulfur deficiency is now widespread in wetland rice in Indonesia and Bangladesh (Blair et al 1979, Islam and Ponnamperuma 1982). Sulfur deficiency results in yellowing of the youngest leaves, marked reduction in plant height and tiller number, and delayed maturity of the rice plant (Blair et al 1979, Islam and Ponnamperuma 1982). For optimum yield, the critical level of total sulfur in mature grains (rough rice) is reported to be 0.065%, and the critical N:S ratio is 26 (Islam and Ponnamperuma 1982). Sulfur deficiency has been reported to reduce the sulfur amino acids, cysteine and methionine, in seeds of wheat (Byers and Bolton 1979, Wrigley et al 1984) and legumes (Eppendorfer 1971). Cysteine and methionine are the major sulfur compounds in cereals and legumes (Byers and Bolton 1979, Jambunathan and Singh 1981, Maranville et al 1984). In pot experiments, protein sulfur content increased with sulfur amelioration without much increase in grain protein nitrogen (Ismunadji 1982). Preliminary studies on the cysteine content of brown rice from sulfur-deficient soils, using acid hydrolysis without prior performic acid oxidation, suggest that the cysteine content was reduced (Ismunadji and Miyaki 1980).

High cysteine and methionine levels in rice protein compensate for the limiting sulfur amino acids of legumes in rice-legume diets. The effect of soil sulfur deficiency and its amelioration on sulfur amino acids and the macro- and microelement contents of brown rice was investigated using samples from Bangladesh, Indonesia, and the Philippines.

MATERIALS AND METHODS

Samples of rough rice were obtained from field experiments at the Bangladesh Rice Research Institute (duplicate plots), Maros Research Institute for Food Crops in Indonesia, and from duplicate pot experiments of the Agronomy Department at the International Rice Research Institute (IRRI) in the Philippines. The rough rice was dehulled with a Satake THU 35 dehuller and the resulting brown rice examined. Duplicate samples of 100 brown rice grains were counted and weighed. Only samples with normal grain appearance and weight were selected for analysis. A portion was ground in a Udy cyclone mill with 40-mesh sieve. The flour samples were analyzed for crude protein using the micro Kjeldahl method. The flour was hydrolyzed in sealed tubes in 6*N* HCl for 23 hr at 110° C after N_2 flushing. It was then analyzed in a Beckman Spinco model 120C amino acid analyzer with AA-15 and PA-35 resin columns. An LKB model 4400 amino acid analyzer with Ultropac 8 resin column was used as an alternate. Cysteine was estimated by oxidation with performic acid before hydrolysis. Cysteic acid was analyzed in a Beckman Spinco 120C analyzer with an AA-15 resin column.

Selected flour samples were also analyzed by the Department of Agronomy, University of Nebraska, for sulfur (S), phosphorus (P), potassium (K), magnesium (Mg), silicon (Si), chlorine (Cl), aluminum (Al), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) using energy dispersive X-ray fluorescence spectrometry according to the method of Knudsen et al (1981). Variation for the determination, including both instrument and sampling variation, is about 5%.

RESULTS

The mean weight of brown rice based on 100-grain weight showed little or no increase with sulfur amelioration, although higher yields were observed (Table I). Neither crop of BR3 variety in Bangladesh showed any increase in protein, and only the 1984 crop showed higher cysteine plus methionine content with sulfur amelioration. However, the cysteine plus methionine content of the control samples was within the normal range. The 1984 crop had a lower cysteine plus methionine level in protein in the control, probably because of higher protein content. The same results were noted for lysine; both amino acids decreased with an increase in protein content (Eggum and Juliano 1973).

In the 1981 set of Indonesian brown rices, selected on the basis of similar protein contents in the range of 7–12%, cysteine plus methionine levels were slightly higher in the control (−S) than in the treated (+S) plots (Table I). However, for the 1985 set, mean values of cysteine plus methionine content were the same in both control and treated plots, although the range was wider in the treated ones. Protein content was more variable and yields were lower in the control plots. The lysine content of protein varied more in the treated plots, but the mean values remained the same.

In the potted plant experiments at IRRI in the Philippines, sulfur amelioration increased grain yield with a general decrease in protein content of brown rice (Table I). Cysteine plus methionine levels in protein in the control pots were lower than those in the treated pots and also lower than the levels in the control field plots in Bangladesh and Indonesia. Ammonium sulfate was used as the sulfur source in this experiment, and urea was added to the control pots to make nitrogen addition identical. The lower sulfur amino acid content in protein in the 1-ppm pots (2.9% vs. 5.2% in pots with 10 ppm S) cannot be explained alone by the higher protein content of brown rice, because the lysine content in protein was

[1]Cereal Chemistry Department, International Rice Research Institure (IRRI), Los Baños, Laguna, Philippines.
[2]Department of Agronomy, University of Nebraska, Lincoln 68583.
[3]Agronomy Department, IRRI.
[4]Rice Technology Division, Bangladesh Rice Research Institute, Joydebpur.
[5]Maros Research Institute for Food Crops, Ujung Pandang, South Sulawesi, Indonesia.

only about 10% lower in the control than in the treated pots (3.9 vs. 4.3%). Preliminary fractionation studies indicated that the grain with low sulfur content had more prolamin and glutelin than that with high sulfur content (IRRI, *unpublished data*).

Comparison of the contents of amino acid sulfur with total sulfur level by X-ray fluorescence spectrometry showed in Table II that amino acid sulfur accounted for 75–91% (mean 85%) of total sulfur in the 1981 set and 81–98% (mean, 92%) in the 1984 BR3 crops. In the Indonesian rices, amino acid sulfur accounted for 77–93% (mean, 85%) of total sulfur in the 1981 set, and 58–77% (mean 68%) in the 1985 crop. In the IRRI pot experiments, amino acid sulfur was 58–87% (mean 74%) of total sulfur. The overall weighted mean was 88% for BR3 and 75% for the Indonesian rices. The mean value for the 32 brown rice samples was 78% amino acid sulfur in total sulfur.

Mean N:S ratios in brown rice were 11.9 for the 1981 BR3 crop and 13.3 for the 1984 crop, giving an overall mean of 12.4 (Table I). The Indonesian brown rice had mean N:S ratios of 11.8 for the 1981 crop and 9.5 for the 1985 crop, with an overall mean of 10.5. In the IRRI pot experiments, the mean N:S ratio in brown rice was 13.1. Samples from two control pots showed higher N:S ratios than those from treated pots and had lower cysteine plus methionine levels: IR42, with an N:S ratio of 16.3, had 3.2% cysteine plus methionine in protein compared with 5.1% in the 10-ppm pot; similarly IR64, with an N:S ratio of 25.0, had 2.2% cysteine plus methionine in contrast to 5.0% in the 10-ppm pot. Syntha, with an N:S ratio of 9.0 for the control pot, had 4.4% cysteine plus methionine in protein compared with 5.1% in the 10-ppm pot.

Among the 18 Indonesian brown rices differing in protein content (Table II), amino acid sulfur content correlated positively (all at the 0.01 level of significance except where indicated) with total sulfur ($r = 0.63$), percent amino acid sulfur in total sulfur ($r = 0.64$), N ($r = 0.89$), and Cl ($r = 0.64$), and negatively with Ca ($r = -0.56$, 0.05 level). Nitrogen content correlated positively with Mg ($r = 0.49$, 0.05 level), and Cl ($r = 0.71$), and negatively with Ca ($r = -0.54$, 0.05 level). Ca and Mg contents were negatively correlated ($r = -0.48$, 0.05 level), whereas Al content correlated positively with Fe ($r = 0.66$) and Cu ($r = 0.85$). Ca content correlated positively with Mn ($r = 0.85$), Fe ($r = 0.67$), and Cu ($r = 0.70$) contents.

For all 32 brown rice samples, the amino acid sulfur level correlated positively with total sulfur ($r = 0.67$), N ($r = 0.47$), and Mg ($r = 0.49$). Total sulfur content correlated positively with P ($r = 0.50$) and Mg ($r = 0.49$). Total N content correlated positively with Mg ($r = 0.50$) and Cl ($r = 0.47$). Cl and Cu contents were positively correlated ($r = 0.44$) with each other.

DISCUSSION

Amelioration of sulfur-deficient soils in field experiments in Bangladesh and Indonesia improved yield but had little effect on cysteine plus methionine levels in brown rice protein, which were already at the normal level in the control plots (Table I). However, in the IRRI pot experiment samples, the 1-ppm sulfur (as $[NH_4]_2SO_4$) trial treated with urea showed low cysteine plus methionine values (2.2–3.2 g/16.8 g of N) in five of six varieties as compared with the 10-ppm $(NH_4)_2SO_4$-sulfur pots (5.0–6.3 g/16.8 g of N). The lower sulfur amino acid values in the 1-ppm S trial are attributed to the smaller loss of supplied nitrogen in pot experiments and the additional amount of sulfur supplied by natural sources and the atmosphere in the field experiments (Yoshida and Chaudhry 1979). Sulfur-free nitrogen fertilizers aggravate sulfur deficiency in pot experiments, as is the case with urea on rice (Yoshida and Chaudhry 1979) and ammonium nitrate-calcium nitrate on wheat (Byers and Bolton 1979). Low content of sulfur amino acids may be in part attributable to the increased level of prolamin, which has only 0.4% cysteine plus methionine (Mandac and Juliano 1978).

TABLE I
Weight, Protein Content, and Cysteine Plus Methionine and Lysine Contents of Protein of Brown Rice Obtained from Sulfur-Deficient Soils With and Without Sulfur Amelioration

Variety and Treatment		Mean Yield Rough Rice (t/ha)	Mean Wt Brown Rice (mg)	Protein Content (% wet basis)	N:S Ratio	Cysteine + Methionine Content (g/16.8 g of N)	Lysine Content (g/16.8 g of N)
Bangladesh (BR3)							
1981 crop							
Control		3.4	22	7.6	13.1	4.8	4.5
NPK (80:60:40)		6.4	23	7.2	12.1	5.4	4.4
NPK + $ZnSO_4$ (20 kg)		6.5	23	7.7	12.8	5.0	4.4
NPK + gypsum (200 kg 18% S)		6.2	23	7.2	10.2	5.1	4.5
NPK + $ZnSO_4$ + gypsum		6.1	23	6.9	11.2	5.1	4.4
1984 crop							
Control		2.2	21	9.5	14.7	4.6	3.5
NPK (120:80:60)		3.7	23	9.5	14.6	4.6	3.4
NPK + gypsum (20 kg 15% S)		6.6	22	9.2	10.6	5.6	3.4
Indonesia							
1981 set ($n = 4$)[a]							
−S	Range	···	17–27	7.2–12.1	10.6–12.7	5.0–5.7	···
	Mean	···	20	9.3	11.9	5.2	4.0
+S	Range	···	18–28	6.8–12.0	10.7–12.4	4.7–5.1	···
	Mean	···	21	9.2	11.6	4.9	4.4
1985 ($n = 8$)[b]							
−S	Range	3.2–4.8	15–20	6.0–9.6	8.6–10.1	4.4–5.5	4.0–4.4
	Mean	4.0	18	8.3	9.4	5.0	4.2
+50 kg/ha S	Range	5.4–7.9	17–20	7.2–9.8	8.3–11.7	3.7–5.5	3.5–4.9
	Mean	6.3	18	8.0	9.7	5.0	4.3
IRRI Philippines ($n = 6$)[c]							
1 ppm S (control)	Range	(39–105)[e]	14–20	6.8–11.8	9.0–25.0	2.2–4.4	3.6–4.5
	Mean	(68)[e]	17	10.4	16.8	2.9	3.9
10 ppm S	Range	(95–245)[e]	15–22	6.9–9.8	8.3–10.2	5.0–6.3	3.9–4.6
	Mean	(152)[e]	19	8.1	9.4	5.2	4.3
Pooled standard deviation[d]			±0.5	±0.33	±0.54	±0.28	±0.24

[a]Gati, Gemar, IR32, and IR44.
[b]Barito, Cisadane, IR30, IR36, IR54, IR56, IR9729-67-3, and IR13420-32-4. Only Barito, Cisadane, IR30, IR56, and IR9729-67-3 for N:S ratio data.
[c]IR42, IR54, IR56, IR60, IR64, and Syntha. S added as $(NH_4)_2SO_4$. N adjusted by urea addition. Only IR42, IR64, and Syntha for N:S ratio data.
[d]For BR3 representing duplicate plots per treatment.
[e]In g/hill.

TABLE II

Contents of Amino Acid Sulfur, Kjeldahl N, and Other Elements Estimated by X-ray Fluorescence Spectrometry in Brown Rice Obtained from Sulfur-Deficient Soils With and Without Sulfur Amelioration

Variety and Treatment	Range or Mean	Amino Acid S	Kjeldahl N	Total: % Dry Basis					Total: µg/g Dry Basis						
				S	P	K	Mg	Si	Cl	Al	Ca	Mn	Fe	Cu	Zn
Bangladesh (BR3)															
1981 crop															
Control	Mean	0.099	1.45	0.112	0.20	0.076	0.18	0.081	296	254	28	5.7	5.4	3.2	6.4
NPK (80:60:40)	Mean	0.104	1.36	0.114	0.24	0.084	0.20	0.093	236	264	27	6.8	4.8	2.6	8.3
NPK + $ZnSO_4$	Mean	0.104	1.47	0.115	0.23	0.070	0.20	0.086	256	334	16	5.4	2.6	2.6	8.4
NPK + gypsum	Mean	0.100	1.37	0.134	0.25	0.092	0.21	0.065	215	171	18	5.8	4.0	1.7	8.2
NPK + $ZnSO_4$ + gypsum	Mean	0.095	1.32	0.118	0.23	0.080	0.20	0.076	239	432	40	6.1	3.4	1.8	7.8
1984 crop															
Control	Mean	0.120	1.81	0.123	0.54	0.32	0.23	0.069	375	580	101	14.8	16.9	16.2	43.0
NPK(120:80:60)	Mean	0.117	1.81	0.125	0.52	0.32	0.22	0.159	412	1,040	124	16.6	12.8	11.0	39.7
NPK + gypsum	Mean	0.142	1.76	0.165	0.54	0.34	0.23	0.072	338	521	101	15.2	17.2	12.8	41.9
Indonesia															
1981 ($n = 4$)[a]															
−S	Range	0.111–0.169	1.38–2.31	0.129–0.182	0.33–0.43	0.11–0.13	0.24–0.27	0.069–0.088	193–560	178–253	26–63	4.5–5.3	3.3–5.6	0.8–2.1	7.4–12.1
	Mean	0.132	1.78	0.148	0.37	0.12	0.25	0.079	370	218	45	4.8	4.4	1.6	10.0
+S	Range	0.094–0.153	1.30–2.29	0.122–0.192	0.33–0.43	0.10–0.14	0.19–0.28	0.073–0.138	241–463 463	<100–349	23–52	3.1–4.8	5.4–10.2	1.7–2.1	9.2–11.4
	Mean	0.122	1.76	0.151	0.37	0.12	0.24	0.111	372	240	32	4.2	7.0	2.0	10.7
1985 ($n = 5$)[b]															
−S	Range	0.088–0.131	1.15–1.83	0.152–0.184	0.54–0.68	0.34–0.41	0.22–0.25	0.080–0.143	298–340	441–624	91–140	25.0–31.0	11.9–43.4	2.9–5.8	33.4–54.1
	Mean	0.110	1.59	0.169	0.64	0.38	0.23	0.118	321	544	114	28.4	20.9	4.3	45.9
+S	Range	0.104–0.130	1.38–1.87	0.144–0.169	0.51–0.66	0.33–0.39	0.22–0.24	0.080–0.104	254–330	485–788	50–129	23.3–46.8	14.0–17.3	3.3–6.2	42.8–47.4
	Mean	0.115	1.53	0.160	0.60	0.36	0.23	0.094	284	573	98	31.8	15.8	4.6	44.6
IRRI (Philippines) ($n = 3$)[c]															
1 ppm S (control)	Range	0.068–0.096	1.30–2.25	0.09–0.17	0.47–0.58	0.32–0.34	0.19–0.23	0.073–0.149	416–542	540–650	105–115	18.7–25.0	15.0–29.6	9.6–20.2	47.2–54.0
	Mean	0.085	1.99	0.13	0.53	0.33	0.22	0.108	493	585	108	21.8	20.9	14.4	49.7
10 ppm S	Range	0.100–0.111	1.32–1.87	0.12–0.15	0.47–0.53	0.30–0.40	0.20–0.23	0.063–0.119	396–608	522–562	84–94	21.4–21.9	16.6–20.4	5.5–24.2	37.7–47.8
	Mean	0.107	1.55	0.13	0.50	0.35	0.21	0.098	490	548	89	21.7	18.2	12.8	44.2
Pooled standard deviation[d]		±0.0067	±0.063	±0.0108	±0.035	±0.016	±0.027	±0.0094	±29.5	±153.2	±16.8	±2.16	±1.44	±1.78	±1.96

[a] Gati, Gemar, IR32, and IR44.

[b] Barito, Cisadane, IR30, IR56, and IR9729-67-3.

[c] IR42, IR64, and Syntha.

[d] For BR3 representing duplicate plots per treatment.

Sulfur amelioration had no consistent effect on the P, K, Mg, Si, Cl, Al, Ca, Mn, Fe, Cu, and Zn levels in brown rice (Table II). Considerable differences in levels of trace metals were noted in the two sets of samples of BR3. The Indonesian samples showed higher trace metal levels (except for Mg and Si) in the 1985 set. No increase in Zn level was evident from $ZnSO_4$ amelioration of BR3 in the 1981 trial.

Islam and Ponnamperuma (1982) postulated a critical total sulfur content of 0.065% for rough rice and a critical N:S ratio of 26 for rough rice yield. With our brown rice samples, levels of sulfur amino acids were normal for 0.10–0.16% sulfur and N:S ratios up to 14.7 but were low at N:S 16.3 and 25.0, corresponding to 0.136 and 0.087% sulfur. Presumably, the critical sulfur levels for brown rice (for protein quality of about 0.10% and N:S ratio of 15) are higher than those for rough rice (for yield). The N:S ratio was more discriminating than the sulfur content alone. Randall et al (1981) reported the critical values for optimum yield of wheat grain as 0.12% sulfur and an N:S ratio of 17. The values obtained for brown rice and wheat were similar.

Amino acid sulfur accounted for the major part of total sulfur content in brown rice, with mean values of 88% for BR3, 75% for the Indonesian rices, and 74% for the IRRI-grown rices (overall mean 78%). Byers and Bolton (1979) reported 75–85% of total sulfur as amino acid sulfur for wheat grain. Maranville et al (1984) reported that cysteine plus methionine sulfur correlated positively with total sulfur in corn, sorghum, wheat, and soybean seeds. Amino acid sulfur accounted for 54.8% of total sulfur in seeds of chickpea and 75.5% in pigeon pea (Jambunathan and Singh 1981).

CONCLUSIONS

Field samples of brown rices from sulfur-deficient soils in Bangladesh and Indonesia had normal cysteine plus methionine contents in protein as did those grown in sulfur-treated plots (N:S ratio 8–15). Low cysteine plus methionine levels in protein were observed only in pot experiments in brown rice with N:S ratios of 16 and 25. Amino acid sulfur accounted for a mean of 78% of total sulfur in the 32 brown rices investigated. Levels of elements in brown rice were not affected by sulfur treatment of the sulfur-deficient soils.

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