

## Sulphur application and amino acid content of brown rice

Sulphur, an essential element for plant growth, has been rather neglected in rice culture. This is understandable, since traditional fertilizers, ordinary superphosphate, ammonium sulphate and low grade potash salts, contain sulphur.

Recently it was found that sulphur deficiency seems to be widespread in rice areas in Indonesia. Sulphur deficiency was found in Java, Bali, North Sumatra, West Sumatra and South Sulawesi<sup>4,5,6,7 10</sup>. It is not unlikely that more areas suffer from sulphur deficiency. Sulphur applications increased grain yields by 12 to 45% in South Sulawesi under field conditions<sup>10</sup>. Five out of eight sites examined were responsive to sulphur application. Sulphur deficiency in rice was reported also in Brazil<sup>13</sup>, India<sup>1</sup>, the Philippines<sup>9</sup> and Pakistan<sup>2</sup>. The introduction of high yielding rice varieties and the use of high analysis sulphur free (or low sulphur) fertilizers seem to aggravate sulphur deficiency. It covers a wide range of soil types, from the light sandy regosol to the heavy grumusol.

Sulphur deficiency in rice is easily overcome by the use of elemental sulphur or sulphur containing fertilizers, like ammonium sulphate, potassium sulphate and gypsum. Recent findings indicated that the application of sulphur increased not only grain yield, but also the methionine content of brown rice<sup>4,5,6</sup>.

In the 1976-77 wet season a pot experiment (Table 1) was conducted at CRIA to study the effect of sulphur on the yield and the amino acid content of brown rice, using the soil low in sulphur content sampled from Ngawi, East Java. The soil type was grumusol. Sources of sulphur supply were elemental sulphur, ammonium sulphate, potassium sulphate or gypsum.

The elemental sulphur was applied at 1, 2 or 3 weeks before transplanting to the soil which was kept either at the moisture content of the field capacity or at the submerged condi-

Table 1. Sulphur treatments, using four different sources of sulphur

Treatment*	N	P	K	S	S-source
A	2	0.4	4	—	—
B	2	0.4	4	0.5	Elemental S (ES)
C	2	0.4	4	0.5	Elemental S (ES)
D	2	0.4	4	0.5	Elemental S (ES)
E	2	0.4	4	0.5	Elemental S (ES)
F	2	0.4	4	0.5	Elemental S (ES)
G	2	0.4	4	0.5	Elemental S (ES)
H	2	0.4	4	0.5	Ammonium sulphate
I	2	0.4	4	0.5	Potassium sulphate
J	2	0.4	4	0.5	Gypsum

- \* A No sulphur applied.  
 B ES applied 3 weeks before transplanting, field capacity.  
 C ES applied 2 weeks before transplanting, field capacity.  
 D ES applied 1 week before transplanting, field capacity.  
 E ES applied 3 weeks before transplanting, submerged.  
 F ES applied 2 weeks before transplanting, submerged.  
 G ES applied 1 week before transplanting, submerged.  
 H Ammonium sulphate applied 1 day before transplanting  
 I Potassium sulphate applied 1 day before transplanting  
 J Gypsum applied 1 day before transplanting. The fertilizers used, i.e. urea, ammonium sulphate, triple superphosphate, potassium chloride, potassium sulphate and elemental sulphur were applied by mixing with the soil.

tion, to know the rapidity of the dynamic change of elemental sulphur to sulphate sulphur as affected by the time of application and different water regimes under the tropical condition. After rice seedlings of 2 week old were planted, all pots were kept submerged under 2 cm of water until one week prior to the harvest. Rainwater was used for watering. After the harvest, filled grains were separated from empty grains, and were unhusked. The brown rice obtained was ground and used for amino acid analyses.

To 50 mg of brown rice powder taken into

a vacuum reaction tube, 15 ml of 6 N HCl was added. The mixture was cooled in acetone plus dry ice, subjected to a vacuum to remove dissolved air, and hydrolyzed at 110°C for 16 hrs in an Al-block heater (Pierce Reacti-Therm Heating Module). The solution was evaporated to dryness in a vacuum vibrating waterbath evaporator at 45°C. After adding 1 ml of 0.01 N NaOH, the residue was allowed to stand for 4 hrs. Then 1 ml of 0.1 N HCl followed by 3 ml of IPH-DL was added. IPH-DL is a sample diluter, consisted of 0.4 l distilled water, 19.7 g sodium citrate, 16.5 ml conc. HCl, 0.1 ml caprylic acid, 20 ml thiodi-glycol, and 4 ml BRIJ-35, with a total volume of 1 litre, Na concentration of 0.2 N and pH at 2.2. The solution was centrifuged at 3000 RPM for 10 min and the amino acid content was determined by the Hitachi KLA-5 amino acid analyzer.

Table 2 indicates that sulphur application increased grain yield to about two to three

times that of the non-sulphur treatment. Elemental sulphur applied 3 weeks before transplanting at field capacity gave the highest yield. Application of elemental sulphur at field capacity gave higher yields than the application under a submerged condition. Field capacity condition seemed to stimulate the oxidation of elemental sulphur into sulphates, which can readily be absorbed by rice roots.

In addition to increasing the grain yield, sulphur application increased the methionine content of brown rice to 1.7 to 2.5 times that of the non-sulphur treatment. The methionine content was higher with elemental sulphur applied at field capacity than with that applied under submerged conditions. In the former case, the methionine content was the same irrespective of the time of application, while in the latter case, application at one week before transplanting gave the lowest content. The application of elemental sulphur at the field capacity increased the content of most

Table 2. Amino acid content of brown rice of plants treated with and without sulphur

Amino acid % (dry weight basis)	Treatment									
	A	B	C	D	E	F	G	H	I	J
Lysine	0.48	0.51	0.55	0.57	0.43	0.43	0.51	0.55	0.20	0.43
Histidine	0.29	0.36	0.38	0.40	0.33	0.32	0.36	0.42	0.15	0.31
Arginine	1.07	1.16	1.22	0.29	0.96	1.04	1.19	1.27	0.49	1.01
Asparted acid	1.19	0.93	1.11	1.05	1.07	1.11	0.78	0.95	0.86	1.02
Threonine	0.36	0.37	0.46	0.44	0.40	0.42	0.27	0.32	0.29	0.41
Serine	0.50	0.48	0.43	0.58	0.52	0.55	0.27	0.34	0.29	0.53
Glutamic acid	1.86	1.85	2.12	2.06	2.01	2.10	1.49	1.82	1.62	1.98
Proline	0.38	0.44	0.47	0.42	0.59	0.58	0.44	0.52	0.48	0.54
Glycine	0.47	0.47	0.52	0.49	0.50	0.53	0.40	0.47	0.43	0.51
Alanine	0.61	0.59	0.66	0.66	0.59	0.63	0.52	0.63	0.56	0.62
Cystine	0.06	0.17	0.16	0.18	tr	0.17	tr	tr	tr	0.16
Valine	0.58	0.60	0.62	0.61	0.65	0.69	0.54	0.66	0.58	0.68
Methionine	0.13	0.30	0.31	0.30	0.28	0.31	0.22	0.25	0.23	0.32
Isoleucine	0.52	0.54	0.54	0.54	0.41	0.45	0.36	0.44	0.39	0.45
Leucine	1.22	1.17	1.18	1.15	0.86	0.92	0.65	0.80	0.70	0.90
Tyrosine	0.82	0.78	0.79	0.71	0.51	0.58	0.38	0.48	0.41	0.60
Phenyl-alanine	0.81	0.80	0.82	0.79	0.56	0.62	0.45	0.54	0.35	0.22
Grain yield (g/pot)	13.1	43.3	37.9	39.8	26.4	28.0	34.0	28.0	29.0	36.2

tr: trace

treatment: see Table 1.

of the other amino acids too, whereas other treatments including applications of ammonium sulphate, potassium sulphate and gypsum gave inconsistent effects on the amino acid composition, i.e., some amino acids were increased while others were reduced, although the methionine content was increased in all cases.

The above result indicates that the sulphur application can increase grain yields and improve the nutritive value of rice in areas with sulphur deficient soils.

As rice is the staple food for most Asian people, an increase of its nutritive value may have a significant implication to the human nutrition in Asia.

Sulphur deficiency is widespread in the world<sup>3,11)</sup> and it seems to affect the quality of animal feeds too. It was reported that sulphur application caused increased concentrations of S-amino acids as well as all other amino acids examined in oat clippings<sup>8)</sup>. An increase in methionine and cystine content due to the application of sulphur was also reported in alfalfa<sup>12)</sup>.

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- 3) Anon.: Sulphur—The essential plant food element. The Sulphur Institute, London, Washington (1967).
- 4) Ismunadji, M.: Sulphur deficiency in lowland rice in Indonesia. Proc. Internat'l Seminar on Soil Environment and Fertility Management in Intensive Agriculture, Tokyo, (1977).
- 5) Ismunadji, M.: The role and distribution of sulphur deficiency in lowland rice in Java. First Symposium on the Role of Research Findings in Agricultural Development, Maros, Indonesia (1977) [In Indonesian].
- 6) Ismunadji, M. & Makarim, A. K.: The role of sulphur in increasing rice production. Second National Soil Sci. Congress, Yogyakarta, Indonesia (1977) [In Indonesian].
- 7) Ismunadji, M., Zulkarnaini, I. & Miyake, M.: Sulphur deficiency in lowland rice in Java. *Contr. Centr. Res. Inst. Agr. Bogor*, 14, 1-17 (1975).
- 8) Jordan, H. V.: Sulphur as a plant nutrition in the Southern United States, *U.S. Dept. Agr. Tech. Bull.*, No. 1297 (1964).
- 9) Lockard, R. G., Ballaux, J. O. & Liougson, E. A.: Response of rice plants grown in three potted Luzon soils to addition of boron, sulphur and zinc. *Agron. J.*, 64, 444-447 (1972).
- 10) Mamaril, C. P. et al.: Sulphur response of lowland rice in South Sulawesi, Indonesia. *Contr. Centr. Res. Inst. Agr. Bogor*, 22, 1-11 (1976).
- 11) Tisdale, S. L.: Sulphur in agriculture—17 years of research. *Sulphur in Agr.*, 1, 2-3 (1977).
- 12) Tisdale, S. L. et al.: Methionine and cystine content of two strains of alfalfa as influenced by different concentrations of sulphate ion. *Agron. J.*, 42(5), 221-225 (1950).
- 13) Wang, C. B.: Sulphur fertilization of rice. In: The fertility of paddy soils and fertilizer application for rice. ASPAC Food and Fertilizer Technology Center, Taipei, Taiwan (1976).

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- 1) Aiyar, S. P.: A chlorosis of paddy (*Oryza sativa*) due to sulphate deficiency. *Current Sci. (India)*, 74, 10-11 (1945).
- 2) Alam, S. M. & Karim, M.: Effect of sulphur on S-uptake and dry matter yield of rice plant with respect to soil sulphur. *Pakistan J. Scientific Res.*, 24, 222-226 (1972).