Hydrogen Sulfide: Effects on the Physiology of Rice Plants and Relation to Straighthead Disease

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ABSTRACT

Rapid techniques were used for measurements of oxygen release and nutrient uptake of several rice cultivars, and these variables were correlated with plant responses to straighthead and akagare diseases. Straighthead-resistant cultivars had higher oxygen release and lower nutrient absorption, whereas the susceptible cultivars had lower oxygen release and higher nutrient absorption. Oxygen release from three-week-old seedlings of six rice cultivars was inhibited by 0.2, 5.0, and 10.0 μg/ml H_2S. Straighthead-resistant cultivars released more oxygen than the straighthead-susceptible cultivars. Hydrogen sulfide at 0.05 μg/ml caused reductions in nutrient uptake of cultivars Dawn and Zenith, whereas cultivars Bluebelle and Saturn were significantly stimulated by this concentration and were unaffected by 0.1 and 0.2 μg/ml H_2S. Radiophosphorus uptake of the four cultivars was inhibited by pretreatment with 1.0, 5.0, and 10.0 μg/ml H_2S and differentiated in descending order of resistance: Bluebelle, Saturn, Dawn, and Zenith by 0.1 μg/ml H_2S. The sulfide theory of straighthead and mild sulfide disease is supported; nutrient uptake and oxygen release by rice seedlings are new tools for evaluating sulfide responses of rice breeding lines.

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Hydrogen sulfide inhibits respiration and oxidative power of rice roots, thus retarding the uptake of various elements (8, 10, 20, 21, 30, 36, 37). Mitsui and his coworkers (26) demonstrated that 0.07 μg/ml H_2S inhibited nutrient uptake and caused wilting of rice seedlings.

Until very recently, H_2S toxicity to rice plants was recognized only on iron-deficient soils, found mainly in Japan, where H_2S had been demonstrated to cause akiochi (autumn decline) disease (9, 39), and water disease of citrus in Florida (13, 23). Consequently, with respect to rice, the prevailing view was that rice-toxic levels of H_2S could not occur in iron-excess soils (33). However, both theoretical and experimental analyses indicated the presence of rice-toxic levels of H_2S in Louisiana rice fields (16, 17, 18). More recently, thermodynamic calculations and analyses of sulfide compound formation combined with sulfide-ion electrode measurements (3, 32), have confirmed the accumulation of rice-toxic levels of H_2S in iron-excess soils. Furthermore, these concentrations of H_2S found in iron-excess soils were found to inhibit respiration and activities of metallo-enzymes in rice roots (2). Consequently, an hypothesis was advanced (19) that the toxicant, H_2S, causes diseases such as the straighthead and mild sulfide diseases which occur in the Gulf Coast (U.S.A.) rice areas. Straighthead had long been recognized as a disease without a known cause.

Oxygen release from rice roots is a well-known phenomenon. Differences in oxygen release have been correlated with the resistance or susceptibility of rice cultivars to akagare (4), akiochi (14), and the straighthead and mild sulfide diseases (22). Oxidizing power facilitates the ability of roots to absorb nutrients and to detoxify toxicants (33). However, oxygen release from roots of rice cultivars has not been correlated previously with their ability to absorb nutrients.

In this paper, we describe a simple technique for the measurement of nutrient uptake by rice seedlings, and present evidence that responses of rice cultivars to H_2S with respect to both oxygen release and nutrient uptake are similar to their responses to straighthead, thus supporting the hypothesis that straighthead disease of rice is caused by H_2S. Furthermore, evidence is obtained for support of the existence of mild sulfide disease because of significant correlations between higher-yielding cultivars and their resistance to the effects of rice-toxic levels of H_2S on nutrient uptake and oxygen release.

MATERIALS AND METHODS.—*Effect of H_2S on oxygen release from rice seedlings.*—Three-week-old seedlings of rice cultivars Bluebelle, Dawn, Norin 22, Saturn, Yubae, and Zenith were treated by immersing their roots for a 12-hour period in flasks containing known concentrations of H_2S in water at 25 ± 2 C. Pretreated seedlings were washed thoroughly in tap water and oxygen release from individual seedlings was determined by the method of Joshi et al. (22).

Nutrient uptake by H_2S-pretreated seedlings.—Ten-day-old rice seedlings of uniform height and vigor were selected for H_2S treatments. Solutions of different H_2S concentrations were prepared by diluting a stock solution which was standardized by iodometric titration (15). Seedlings of each rice cultivar were grouped into 45 seedlings and the roots of each group were fully immersed in solutions of 0, 0.05, 0.1, 0.2, 1.0, and 2.0 μg/ml H_2S in 125-ml Erlenmeyer flasks for a 12-hour period at room temperature (25 ± 2 C).

Following pretreatment with H_2S, the seedling-roots were washed three times with deionized water and each group of 45 seedlings (which had been pretreated with a known concentration of H_2S) was subdivided into three groups of 15 seedlings each, in order to obtain three nutrient uptake replicates for each H_2S concentration. Each replicate of 15 seedlings was then transferred to a 20 × 2.5 cm test tube containing 25 ml of one-quarter-strength Hoagland and Snyder solution (41) and incubated at 25 ± 2 C under fluorescent light of 10,000 lux intensity. The volume of nutrient solution in each tube was maintained with deionized water.

Conductivity of the nutrient solution (ion uptake) was measured at 0 hours and at 80 hours of incubation. Nutrient uptake by the seedlings was obtained by subtracting the 80-hour reading from the 0-hour reading. A Barnstead PM-70 CB conductivity bridge (Barnstead Co., 225 Rivermoor St., Boston, Mass. 02132) with a Beckman CEL-VS 01 conductivity cell of 0.1 constant (Beckman Instruments, Inc., Cedar Grove, N. J. 07009) was used to measure conductivity of the nutrient solutions. This conductivity bridge has a scalar range of 1,199 nanomhos to 119.9 millimhos.

Effect of H_2S on radiophosphorus uptake of rice seedlings.—Seedlings of each rice cultivar were grown in vermiculite, in 946-ml polyethylene containers at 25 ± 2 C under fluorescent light of 10,000 lux intensity, timed for a 12-hour day. The vermiculite was saturated with deionized water at the time of planting and kept moist by adding quarter-strength Hoagland and Snyder solution after sprouting. Twelve-day-old seedlings of uniform vigor were grouped into 30 seedlings per group, and the roots of each group were fully immersed in solutions of 0, 1, 5, and 10 μg/ml H_2S concentrations in 125-ml Erlenmeyer flasks for a 12-hour period.

Pretreated seedlings of each group were divided into three groups (replicates) of 10 seedlings each, and each replicate was transferred to a 25-ml Erlenmeyer flask containing 10 ml of $KH_2{}^{32}PO_4$ solution (approximately 1.01×10^{-9} mg/ml) in deionized water. Samples (0.1 ml) were drawn from each flask at 0, 3, 6, and 9 hours and added to scintillation vials containing 10 ml of scintillation fluid (400 ml toluene + 400 ml dioxane + 240 ml ethanol + 80 g naphthalene + 5 g 2,5-diphenyloxazole). The radioactivity was measured with a Beckman LS-250 liquid scintillation counter. Counts were made at 95% efficiency. Radiophosphorus uptake by seedlings was obtained by subtracting counts made at 3, 6, and 9 hours from those at 0 hour and expressed as dpm/mg dry weight of the seedling.

Assay of different rice cultivars and selections for oxygen release and nutrient uptake.—Two-week-old seedlings were used for the oxygen assay and 10-day-old seedlings for the nutrient uptake studies. The methods used were those described above.

Data analyzed statistically, and the mean differences were evaluated with Duncan's new multiple range test (12).

RESULTS.—*Effect of H_2S on oxygen release from rice seedlings.*—A 12-hour pretreatment with 0.2, 5.0, and 10.0 μg/ml H_2S inhibited oxygen release of 3-week-old seedlings of all six cultivars tested. Yubae and Bluebelle had the highest oxygen release at each concentration of H_2S, with Dawn, Norin 22, and Saturn being lower and Zenith the lowest in oxygen release (Table 1).

Similar results were obtained when 2-week-old seedlings of these cultivars were treated with 0.05, 0.1, 0.2, 5.0, and 10 μg/ml H_2S with the exception of Saturn which showed greater resistance to H_2S than Dawn, Zenith, and Norin 22.

Effect of H_2S on nutrient uptake by rice seedlings.—Nontreated Dawn and Zenith absorbed two to three times more nutrients than Saturn and Bluebelle.

TABLE 1. Oxygen release from 3-week-old seedlings of six rice cultivars pretreated with different concentrations of H_2S for a 12-hour period

H_2S (μg/ml)	Oxygen release[z] (μliters/minute)					
	Bluebelle	Dawn	Saturn	Zenith	Yubae	Norin 22
0	.92 a	.62 a	.82 a	.59 a	1.25 a	.71 a
0.2	.73 b	.51 b	.47 b	.47 b	.66 b	.54 b
5.0	.61 c	.45 c	.33 c	.33 c	.66 b	.54 b
10.0	.54 c	.45 c	.24 c	.19 d	.66 b	.47 b

[z]Data are averages of six replicates. Means in each column which are followed by a letter in common, do not differ significantly, $P = 0.05$.

At 0.05 μg/ml H_2S, nutrient absorption of Dawn and Zenith was reduced 38 and 24% respectively, whereas that of Saturn and Bluebelle was stimulated 40 and 48%, respectively (Table 2). Reductions of 58% and 50%, respectively, occurred in the nutrient uptake of Dawn and Zenith at 0.1 μg/ml H_2S. As compared with nontreated seedlings, Bluebelle and Saturn nutrient uptake was unaffected at both 0.1 and 0.2 μg/ml. At 1.0 and 2.0 μg/ml H_2S, approximately 34, 51, 73, and 79% reduction occurred in nutrient uptake of Bluebelle, Saturn, Zenith, and Dawn, respectively.

Effect of H_2S on ^{32}P uptake by rice seedlings.—Uptake of ^{32}P by nontreated plants of all four cultivars increased with time of exposure. Dawn absorbed maximum ^{32}P followed by Saturn, Bluebelle, and Zenith (Table 3). Significant inhibition of ^{32}P uptake of all cultivars occurred as a result of pretreatment with 1.0 μg/ml H_2S. This inhibition increased at higher H_2S concentrations with the exception that Zenith showed less inhibition at 5.0 and 10.0 μg/ml H_2S. At 1.0 μg/ml H_2S, inhibition of ^{32}P uptake was highest in Zenith followed by Dawn, Saturn, and Bluebelle in decreasing order of inhibition.

Maximum inhibition of ^{32}P uptake of Bluebelle and Dawn by all three concentrations of H_2S occurred after 3 hours of uptake. No further reductions occurred at 6 and 9 hours. Saturn and Zenith, however, showed greater inhibition at 6 and 9 hours, than at 3 hours. All interactions except the cultivar × concentration × time interaction were significant (Table 4).

Assay of different rice cultivars and selections for oxygen release and nutrient uptake.—Twenty-eight rice cultivars and selections were assayed for their oxygen release, and all of these, except Yubae, Stg. 653570 and Fortuna were tested for nutrient uptake (Table 5). Their resistance or susceptibility to straighthead or akagare diseases were compared with their oxygen release and nutrient uptake wherever possible. In general, straighthead resistant cultivars (e.g., Bluebelle and Belle Patna) had a higher oxygen release and a lower nutrient uptake. Susceptible cultivars like Dawn, Zenith, Blue Rose, and Arkrose had lower oxygen release and higher nutrient absorption. Texas Patna, a cultivar with medium oxygen release, had a very low nutrient uptake, correlated with its resistance to straighthead. Moderately susceptible cultivars like Saturn and Cody had a medium oxygen release and a higher nutrient absorption. Cultivars with very low oxygen release were susceptible to straighthead.

Fortuna, a moderately resistant cultivar, had a low oxygen release; its nutrient uptake was not determined. Both Norin 22 and Norin 36 have been reported to be susceptible to akagare disease (4); however, they showed

TABLE 2. Nutrient (quarter-strength Hoagland and Snyder solution) uptake in 80 hours by 10-day-old seedlings of four rice cultivars pretreated with different concentrations of H_2S for a 12-hour period

H_2S (μg/ml)	Nutrient uptake[z] (mmhos conductivity)			
	Bluebelle	Dawn	Saturn	Zenith
0	17.60 ac	50.76 a	23.03 ac	44.70 a
0.05	26.00 b	31.33 b	32.33 b	33.08 b
0.10	19.16 a	21.36 c	28.53 bc	22.23 c
0.20	19.73 a	16.06 cd	19.16 a	24.93 c
1.00	12.00 c	11.16 d	11.53 d	12.06 d
2.00	11.43 c	10.76 d	11.13 d	11.23 d

[z]Averages of three replicates of 15 seedlings each. Means in each column which are followed by a letter in common do not differ significantly, $P = 0.01$.

TABLE 3. ^{32}P uptake (dpm/mg dry weight) at various times by 10-day-old seedlings of four rice cultivars pretreated with different H_2S concentrations for a 12-hour period[a]

Cultivar	Time (hours)	H_2S (μg/ml)			
		0	1.0	5.0	10.0
Blue-belle	3	48.76	17.66	12.56	8.66
	6	58.40	21.30	14.26	10.00
	9	61.63	27.60	15.36	13.13
Dawn	3	104.30	21.46	23.53	21.30
	6	120.16	31.13	28.90	22.63
	9	129.10	31.13	31.23	21.73
Saturn	3	51.56	15.70	18.63	10.00
	6	58.90	14.66	9.40	10.13
	9	60.00	17.96	17.96	13.16
Zenith	3	37.36	9.70	8.16	14.30
	6	48.60	6.26	14.40	12.76
	9	46.26	1.63	14.36	12.30

[a]Data are averages of three replicates of 10 seedlings each.

TABLE 4. Analysis of variance of ^{32}P uptake by four rice cultivars at 3, 6, and 9 hours after being pretreated with different H_2S concentrations (1.0, 5.0, 10.0 μg/ml)

Source of variation	d.f.	M.S.
Cultivar	3	6,298.38**[a]
Concentration	3	24,647.41**
Cultivar × Concentration	9	1,719.42**
Error (a)	32	69.39
Time	2	397.53**
Cultivar × Time	6	75.64**
Concentration × Time	6	111.56**
Cultivar × Concentration × Time	18	27.21
Error (b)	64	19.62

[a]** indicates significant difference, $P = 0.01$.

TABLE 5. Comparative measurements of oxygen release and nutrient uptake of different rice cultivars and their resistance or susceptibility to straighthead and akagare diseases[a]

Cultivar	C.I.[b] or P.I.[c] No.	Oxygen release (μliters/minute)	Nutrient uptake (mmhos conductivity)	Straighthead response	Akagare response
Yubae	P.I. 224938	1.25			
Norin 37	P.I. 224874	1.02	19.46		
Norin 36	P.I. 224873	1.01	17.27		S
Belle Patna	C.I. 9433	0.97	10.43	5	
Caloro	C.I. 1561-1	0.95	23.10	3 (2)	
Vista	C.I. 9628-2	0.92	13.37	1	
Stg. 653570		0.86		(5)	
Norin 32	P.I. 224870	0.85	18.73		R
Bluebelle	C.I. 9544	0.83	17.60	5 (3)	
Nova 66	C.I. 9481	0.81	17.67	3	
Norin 29	P.I. 162121	0.81	15.33		R
Bbt.× CP 231	C.I. 9402	0.71	19.30	4 (5)	
Saturn	C.I. 9540	0.67	23.03	3	
Norin 22	P.I. 294361	0.64	16.33		S
Labelle	C.I. 9708	0.62	16.13	4	
Nato	C.I. 8998	0.60	14.07	3	
Texas Patna	C.I. 8321	0.60	9.03	5	
Cody	C.I. 8621	0.58	22.86	3	
Starbonnet	C.I. 9584	0.56	17.16	4	
Fortuna	C.I. 1344	0.49		4	
Dawn	C.I. 9534	0.42	50.76	1	
TP 49	C.I. 8991	0.41	18.84	1	
Blue Rose	C.I. 2128	0.41	30.97	1	
Zenith	C.I. 7787	0.37	44.70	2	
Magnolia	C.I. 8318	0.32	14.34	2	
Arkrose	C.I. 8310	0.30	23.37	2	
Sunbonnet	C.I. 8989	0.28	19.57	1	
Della	C.I. 9483	0.19	22.03	1	

[a]Oxygen release data are averages of six 2-week-old seedlings. Nutrient uptake data are averages of three replicates of 15 seedlings (10-day-old) each. Straighthead response of cultivars: Highly Susceptible 1; Susceptible 2; Moderately Susceptible 3; Moderately Resistant 4; Resistant 5. Numbers without parenthesis indicate Texas ratings (Ref. 6; N. E. Jodon, *personal communication*) and numbers within parenthesis indicate Arkansas ratings (T. H. Johnston, *personal communication*) for straighthead. Akagare ratings: R = Resistant, S = Susceptible.

[b]C.I. No. = Cereal Investigation Number.

[c]P.I. No. = Plant Introduction Number.

high oxygen release and low nutrient absorption in the present study.

DISCUSSION.—Simple and rapid physiological tests were employed for evaluation of rice seedling lines for response to hydrogen sulfide. Intercultivar differences in oxygen release and nutrient uptake were correlated with the resistance/susceptibility status of several cultivars to sulfide and straighthead. Cultivars which were resistant to straighthead were more tolerant to the effects of H_2S than susceptible cultivars. Both straighthead and H_2S tolerant cultivars had higher O_2 release and lower nutrient uptake.

Growth of plant roots requires a supply of oxygen. Since aeration is greatly impeded in water-saturated soil, the rice plant makes an adaptation to the biochemically-reduced environment by secreting oxygen into the rhizosphere from which nutrients are taken (1, 11, 24, 25, 29, 34). The oxidizing power of rice roots causes hydrogen sulfide to be oxidized and to lose its toxic effect (7, 28, 38, 40). According to Armstrong (4), root oxidation is the result of simple diffusion of gaseous oxygen present in the normal intercellular spaces of the cortex, and the metabolic oxygen secretion proposed by Mitsui (27) is probably of little significance in oxygen evolution from the roots. Armstrong (5) demonstrated a 200% increase in the oxygen release rates of rice cultivars Norin 36, Norin 37, and Yubae when they were cooled from 23 to 3 C and concluded that higher respiratory rates must have been responsible for less oxygen secretion by cultivars with low oxidizing power and for the intercultivar differences at 23 C.

The remarkable inhibition of oxygen release, radiophosphorus absorption, and nutrient uptake by rice field levels of H_2S as well as the work antedating this study (2, 3, 16, 17, 18, 31, 32), all support the hypotheses (19) that: (i) H_2S is the primary incitant of straighthead disease of rice, (ii) soluble sulfides cause symptomless mild sulfide disease associated with yield reduction and visible late-season plant decline which is widespread in Gulf Coast rice areas. Straighthead is believed to be an acute (symptomatic) manifestation of a more general and underlying susceptibility of rice cultivars to soluble sulfides, called mild sulfide disease.

The existing evidence suggests that H_2S causes straighthead and mild sulfide diseases on iron-excess soils. Hydrogen sulfide toxicity occurs commonly on a variety of soils, including both iron-excess and iron-deficient soils throughout the world. Hydrogen sulfide has been reported as a causal factor (among others) in 12 of 27 physiological disorders of rice (35, 39).

The techniques for measurements of oxygen release and nutrient uptake provide tools for finding resistance to H_2S, and aid in the detection of H_2S effects on local rice germ plasm. Physiological tests enable comparisons of rice plant germ plasm from different geographical regions of the globe because the laboratory is free of the multiple and diverse environmental factors which compound and obscure responses to sulfides. Cultivars can be compared in field trials only by multiple replications of locations and years and only for certain responses. Unfortunately, disease responses such as straighthead response are not invariant or even relatively constant between locations, as evidenced by differences between Texas and Arkansas straighthead ratings of cultivars, therefore physiological tests in the laboratory should be accorded paramount consideration for preliminary sulfide-response ratings of rice germ plasm from other parts of the world.

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