Graded doses of the compounds were administered intraperitoneally in groups of four mice each. The dose of compound killing two animals out of four was taken as its ALD_{50}.

Gross behavioural effects:

The graded doses of the compounds were administered intraperitoneally in groups of five mice each. After the administration of a particular compound, the animals were observed for the resulting behavioural effects. If any like spontaneous motor activity (SMA), ataxia, loss of righting, pinna and corneal reflexes, straub tail and convulsions etc., in order to establish whether a particular compound had a stimulant effect, depressant effect or no effect on central nervous system.

Result And Discussion

The compounds recorded in table 1 were screened for the activity of their central nervous system. All the compounds were non-toxic. Their ALD_{50} ranged from 681 to > 1000 mg/kg, i.p. The compound Nos. 1, 2, 3, 4, 7, 8 and 10 increased the SMA and reactivity to external influences indicating the stimulant nature of these compounds. Compound Nos. 5, 6 and 9 were CNS-depressant. Compound Nos. 2 and 10 showed the presence of writhing (twisting of belly).

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NITROGEN FIXATION BY BLUE-GREEN ALGAE ASSOCIATED WITH DEEPWATER RICE

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ABSTRACT

A study of ^{15}N dilution and $^{15}N_2$ gas incorporation by deepwater rice demonstrated that blue green algae epiphytic on the aquatic tissues of deepwater rice could fix molecular nitrogen and a part of the fixed nitrogen was transferred to the aerial portions that were not exposed to $^{15}N_2$ gas.

Introduction

FARMERS growing deepwater or floating rice could neither derive the benefit from short-stature, high yielding rice varieties, nor apply chemical fertilizer. In comparison to irrigated rice or shalow-water rainfed rice, the improvement of rice varieties and cultural method has ben neglected. Yields as low as 1 ton per ha are fairly common; yields higher than 3 tons per ha are seldom obtained[1].

Because the ratio of straw weight to grain weight is four or more due to long stems and growth period, deepwater rice can accumulate considerable biomass despite low grain yield. Farmers have been growing deepwater rice without nitrogen fertilizer, but still considerable biomass production has been obtained. Little is known about the sources of nitrogen to support such biomass without nitrogen fertilizer.

From the nodes under water, clusters of roots grow. Often the roots in the soil are rotted. Aquatic roots are suspected to have the ability to absorb nitrogen from the floodwater, but the extent of this ability is not known; neither is the contribution of biological nitrogen fixation to its nitrogen nutrition.

Studies of epiphytic N_2-fixation in rice and weeds a shallow-water rice field by Roger *et al*[2] and Kulasoo-

riya *et al*[3] have indicated that epiphytic microorganisms make only a limited contribution of nitrogen in this ecosystem. This was related to the small amount of biomass offered to epiphytism. In deepwater rice, however, a large part of the plant remains under water and offers greater biomass for colonization by aquatic microorganisms.

Kulasooriya *et al*[4] reported on the population and N_2-fixing (acetylene reducing) activity by the blue-green algae (BGA) associated with deepwater rice. BGA, green algae, and diatoms were attached to the surface of the roots which spread to the water from the nodes (exposed root), the roots covered by leaf sheaths (inner root), the leaf sheaths and, to a lesser extent, the culms. BGA were found not only on the surface of the leaves, but also inside the air cavities of leaf sheaths and blades. No BGA were found within the host cells. The growth inside the air cavity was common within senescent or dead materials. Photodependent acetylene reduction activity per unit weight (specific ARA) of plant parts was higher on the leaf sheath, and on the aquatic root at the upper stems that were still under water than on other parts. At the lower stems, the leaf sheath supported higher specific ARA than other parts.

Stimulated by these findings, we studied nitrogen fixation by epiphytic BGA first by indirect ^{15}N dilution method, and then by $^{15}N_2$ method.

Materials And Methods

Deepwater rice (DW 6255) was grown at the International Rice Research Institute in pots containing ^{15}N-labelled (6.95 atmom % excess) amonium sulphate (500 mg N/pot). At 50 days old, the rice was transferred either in shallow water (5 cm) or deep water (110 cm). In the deepwater plot (14 × 38 m), water depth was increased 10 cm every other day to a final depth of 100 cm, which was maintained until maturity. To prevent algal growth, the stems under water were enclosed by a round metal support (20 cm in diameter and 90 cm tall) with black cloth wrapped around it. This treatment was compared with the control plant, whose stems under water were exposed to light. Five replications were set for each treatment. At maturity, the plants were harvested, separated into various plant parts, and analyzed for total N and ^{15}N abundance.

To demonstrate N_2 fixation directly and to measure the amount of the fixed nitrogen transferred to the aerial part, we conducted a $^{15}N_2$ gas feeding experiment with deepwater (110 cm) rice grown at IRRI's deepwater plot. The rice was grown with 600 mg N/pot of unlabelled ammouium salt. At the heading stage, the entire submerged portion plus 15 cm of the aerial part of the rice culms of a plant were enclosed with a soft, plastic chamber 25 cm in diameter. The upper opening of the chamber was tightly sealed around the culms with modeling clay. The upper part of the aerial shoot was exposed to the air and maintained above water. The chamber was completely filled with floodwater where the rice was grown and the assay chambers were submerged in the deepwater plot.

^{15}N-labelled N_2 gas (98 atom % excess) was washed by potassium permanganate solution and, subsequently, by acidic sodium sulfate[5]. One liter of ambient air and one liter of $^{15}N_2$ gas were replaced with water inside the chamber. The gas in the chamber was circulated through an air pump for 5 min to facilitate solubilization of gas in the floodwater. Isotope content in the gas phase was monitored daily by emission spectroscopy. Because ^{15}N content in N_2 gas was diluted to one third for 2 or 3 days, labelled gas phase was renewed every 2 or 3 days. Exposure of plant to $^{15}N_2$ was continued for 9 days with three times renewal of $^{15}N_2$ gas. Control plants were grown beside the ^{15}N-fed rice plant. There were 4 replications for each treatment. After the assay chamber was removed, the rice plants were grown in the deepwater plot until maturity. After harvest, aerial and submerged portions were separated. The submerged parts were divided into two parts—the horizontally floating part and the submerged lower part. ^{15}N abundance of the different plants components was determined by mass spectrometry. A ^{15}N analysis in the control-plant parts was also conducted.

Results And Discussion

N^{15}-dilution experiment

Plants grown in deepwater had a lower enrichment of ^{15}N than those in shallow water. In shallow-water rice, ^{15}N contributed 27% to the total N in the plant, including roots in soil. In deepwater rice, this value was 21%. If nitrogen uptake from soil was similar in both conditions, the lower ^{15}N content in deepwater rice would mean that N sources other than soil contributed to the nitrogen uptake of deepwater rice.

The shading of the submerged stems greatly inhibited the growth of the deepwater rice. Unexpectedly, the average ^{15}N content in the shaded deepwater rice was almost equal to that in the unshaded one (18% *vs* 21%). Nevertheless ^{15}N contents in aquatic plant parts—the exposed root, the inner root, and the leaf sheath, which are major sites for epiphytic BGA—were significantly higher in shaded than in unshaded condition (table 1). This finding indirectly supports the previous results on algal growth observation and ARA assays[4].

TABLE 1

Total N and ^{15}N atom % excess in various parts of rice grown in deep water (IRRI), 1979 wet season

Parts	Submerged portions not shaded[a]		Submerged portions shaded	
	Total N mg/pot	Atom % excess	Total N mg/pot	Atom % excess
Aerial parts				
Grain	346 ± 32	1.68 ± 0.08*[b]	234 ± 29	1.41 ± 0.14
Leaf blade	122 ± 23	1.60 ± 0.08*	65 ± 7	1.39 ± 0.08
Culm and leaf sheath	116 ± 9	1.67 ± 0.04*	105 ± 3	1.33 ± 0.11
Upper submerged parts				
Leaf sheath	58 ± 8	0.93 ± 0.07	21 ± 1	1.01 ± 0.09*
Culm	75 ± 14	1.71 ± 0.06*	43 ± 3	1.42 ± 0.11
Exposed root	20 ± 5	0.60 ± 0.33	27 ± 5	0.80 ± 0.10*
Inner root	4 ± 1	0.67 ± 0.06	3 ± 1	0.89 ± 0.09*
Lower submerged parts				
Culm	67 ± 10	1.94 ± 0.05*	15 ± 2	1.24 ± 0.07
Exposed root	130 ± 9	0.81 ± 0.06	97 ± 9	0.94 ± 0.10*
Root in soil	45 ± 7	1.58 ± 0.10*	7 ± 1	0.98 ± 0.07
Whole plant		1.84 ± 0.09		1.47 ± 0.11

[a]Average ± standard error of four plants.

[b]Atom % excess indicated with asterisk is significantly higher than that of other treatments.

TABLE 2

N_2-fixation by deepwater rice exposed to $^{15}N_2$ for 9 days (IRRI), 1980 wet season.

Plant parts	Total N mg N/pot	Atom % excess	Fixed N[a] µg N/plant
Aerial parts			
Grain	184 ± 33	0.04 ± 0.02	149
Leaf blade	75 ± 17	1.77 ± 0.88	2780
Leaf sheath	28 ± 3	0.30 ± 0.21	208
Culm	26 ± 4	0.11 ± 0.07	57
Floating parts			
Leaf sheath	36 ± 3	3.12 ± 1.14	2310
Culm	27 ± 1	0.48 ± 0.15	264
Root	6 ± 3	2.01 ± 0.77	267
Submerged parts			
Leaf sheath	29 ± 3	1.37 ± 0.46	831
Culm	28 ± 4	0.27 ± 0.07	153
Root	35 ± 3	0.66 ± 0.28	625
Root in soil	68 ± 6	0.23 ± 0.11	329
Whole plant	542 ± 51		7923
Submerged weed	41 ± 12	0.97 ± 0.48	823

[a]Assuming the average of 48.1 atom % excess $^{15}N_2$ during 9 days exposure.

$^{15}N_2$ gas experiment.

The results are shown in table 2. The ^{15}N enrichment was found in all parts of the deepwater rice and associated weeds exposed to ^{15}N. Among the parts under water, the leaf sheath had the highest ^{15}N enrichment, followed by aquatic roots, culms, and roots in the soil.

Atom % excess in various parts under water was roughly proportional to specific ARA previously reported[4]. This indicated that ^{15}N found in these parts was the product of N_2-fixation by epiphytic BGA.

Labelled N was also found in the aerial part not exposed to $^{15}N_2$. The ^{15}N abundance in the grain was significantly higher than that in control plant (0.005 ± 0.001 atom % excess). About 40% of the fixed nitrogen was found in the aerial parts, among which leaf blade acted as the sink.

N_2-fixation at the aerial part by the transported $^{15}N_2$ gas during exposure is unlikely, because the aerial part has negligible ARA. It is, therefore, reasonable to consider that ^{15}N found in the portion not exposed to $^{15}N_2$ was transported from nitrogen which was fixed under water.

Eight mg N per plant was fixed during 9 days. This value is higher than the values reported by Ito *et al*[6], Yoshida and Yoneyama[7], and Eskew *et al*[8] by shallow-water rice. ^{15}N abundance found in the submerged portion in this experiment is much higher than that found in the root of shallowater rice which was reported by the above-mentioned authors. High ^{15}N abundance in the acquatic parts of the deepwater rice is, in all likelihood, related to the biomass accumulation of epiphytic BGA.

Previous observations had been that epiphytic BGA preferentially developed on the submerged decaying tissues of the host[4]. The idea that N_2 fixation by epiphytic microorganisms results in the accumulation of nitrogen only in the decaying tissues where epiphytic BGA grow preferentially is not supported by this experiment. A part of the fixed nitrogen was utilized by growing rice. BGA growing on the aquatic root, which has the ability to absorb nutrient from the water[9], may play a role in providing fixed nitrogen to the host plant. The mechanism of nitrogen supply from the fixing BGA is not yet known.

If we extrapolate the observed N_2-fixing activity for 9 days to 100 days, which is assumed as the submerged growth period of deepwater rice, N_2 fixation explains about 15% of the total nitrogen in deep water at maturity (80 mg N over 550 mg N in total biomass of rice).

Because BGA grown in IRRI's deepwater plot grew much less profusely than BGA associated with deepwater rice in the deepwater rice area of Thailand (visual observation by authors), the role of BGA in nitrogen nutrition of deepwater rice field could be much greater than that of BGA found at IRRI.

This paper calls attention to the possibility of an associated relationship between photodependent N_2 fixation with deepwater rice and probably with other aquatic plants.

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ANNOUNCEMENT

OPTICS 1982 CONFERENCE

Optics 1982 conference is organised by the Optical Group of the Institute of Physics and will be held during 8–10 September 1982 at the University of Edinburgh, England.

The conference follows in the series of biennial meetings – Optics 1976 – York, Optics 1978 – Bath and Optics 1980 – Manchester and as at those meetings the aim will be to cover many aspects of pure and applied optics. It is intended at the conference to mark the centenary of the birth of Max Born. The conference will, as usual, be accompanied by an equipment exhibition.

Further details about the Conference can be had from: The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK.