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THE AZOLLA-ANABAENA COMPLEX AND ITS USE IN RICE CULTURE

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ABSTRACT

More than 50 strains of 5 azolla species, collected from all over the world, are maintained at IRRI. Screening for high temperature-tolerant strains was done in growth chambers and in the field. No strain was satisfactorily tolerant of high temperature in the dry season. Preliminary studies on the conditions needed for azolla sporulation were made.

Differences in azolla's growth with limited phosphorus nutrition were observed among strains and species. The mineralization rate of nitrogen fixed by the Azolla-Anabaena complex also differed among species and strains. Azolla can supply 70 kg N/ha when grown 4-6 times/rice crop. That nitrogen promotes rice yields comparable to that obtained by 70-100 kg chemical fertilizer.

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THE AZOLLA-ANABAENA COMPLEX AND ITS USE IN RICE CULTURE

The rate of N_2-fixation in the Azolla-Anabaena symbiosis rivals that in the Rhizobium-legume symbiosis. In open paddies at IRRI, 22 crops of azolla/year fixed 450 kg N/ha (Watanabe 1978, Watanabe et al 1980). The use of azolla in rice production is widely practiced in many rice growing countries. We reported earlier results with azolla in this research paper series in 1977 (Watanabe et al 1977, Espinas et al 1977). This paper provides results of more recent research with the Azolla-Anabaena symbiosis and should be useful for planning azolla use in rice paddies of the tropics.

PHYSIOLOGY AND NUTRITION OF AZOLLA

More than 50 strains of Azolla pinnata, A. filiculoides, A. mexicana, A. caroliniana, and A. microphylla had been collected from all over the world by June 1981. The strains are described in Table 1.

The cut fronds of azolla at shoot tips are surface sterilized by Chlorox (2%) and polysorbate (Tween 80) (0.1%) solution to eliminate free-living algae and other pests. The fronds are then inoculated into the mineral solution and grown in 25/21°C (12 hour day/12 hour night) under 12 klux light intensity at 75% relative humidity. The strains are maintained in mineral nutrient solution in flasks as described earlier (Watanabe et al 1977). Azolla thus grown are maintained at 15°C under about 1 klux fluorescent light for 3 weeks, and thereafter transformed to new media and grown again under 25/21°C. Some azolla strains are grown in the greenhouse for further multiplication.

The strains at IRRI were compared with respect to temperature requirements, phosphorus requirement, sporulation ability, and growth in the rice field.

Temperature requirement

The use of azolla in the tropics is sometimes restricted by its low tolerance for high temperature. Temperature tolerance tests were completed for 27 strains -- A. pinnata from 1 to 23 except 7, A. filiculoides 101 to 103, A. mexicana 201, and A. caroliniana 301.

The ferns were grown at 3 different temperature levels (26/18°C day/night for average 22°C; 33/25°C for average 29°C, and 37/29°C for average 33°C) at 30 klux for 12 hours on their surface by fluorescent and incandescent lamps. The minimum relative humidity was 75%. Growth was in a 20-cm diameter dish containing 1 liter nitrogen-free mineral nutrient solution, which was changed every 2 days and the ferns were grown until saturation or initial sign of senescence and fresh weight was measured every week. Total nitrogen in the fern was measured at final harvest. The culture solutions were combined for every 8 days and their ammonium content determined. The results are summarized as follows:

- For most azolla, except for A. filiculoides, the relative growth rate was highest at the first week and this maximum growth rate was higher at 33°C than at 22°C. The highest value of maximum growth rate was 1.9 doubling days (days required to double fresh weight) by strain 8 at 33°C. As ferns grew, the growth slowed most severely at higher temperature.

- The maximum biomass (Fig. 1) was higher at 22°C than at 33°C in all strains. At 22°C, the period attaining the maximum biomass was 30-50 days, and highest value was 14 g N/m^2 or 320 g dry matter/m^2 by A. caroliniana 301. At 29°C, the maximum biomass was attained in 20-35 days and the highest value was 6.3 g N/m^2 and 150 g dry matter /m^2 by A. caroliniana 301. At 33°C most azolla yielded less than 4 g N/m^2; only 5 strains of A. pinnata yielded more. Most azolla had a maximum biomass after 13-23 days but some strains grew up to 30 days resulting in higher maximum biomass. The highest maximum biomass at 33°C was 5.5 g N/m^2 or 140 g dry matter/m^2 by A. pinnata 9.

- Azolla strains were divided into four major groups by growth performance at three temperatures. Group 1 consisted of major strains of A. pinnata and A. mexicana with average performance. Group 2 consisted of two A. filiculoides strains and one A. pinnata strain, characterized by poor growth at 33°C. Group 3 consisted of A. pinnata 2, 9, 14, 16, and 22, characterized by better growth at 3 temperature levels. Group 4 consisted of A. caroliniana, A. filiculoides 101, and A. pinnata 21, characterized by higher biomass at 22°C.

- Ammonia formation in the culture medium occurred mostly in crowded conditions. At 33°C, some strains of A. pinnata (2, 4, and 8) and A. mexicana released or formed ammonia at 0.3-0.8 ppm N during their initial exponential growth stage. This indicates that the major way to provide nitrogen to accompanying rice is through azolla decomposition, not through the excretion of ammonia.

Table 1. Strains of azolla in the collection at IRRI, May 1981.

Species	Strain[a]
A. pinnata[b]	1: Bicol, Philippines, 2: Malaysia, 3: Bogor, Indonesia, 4: Banaue, Philippines, 5: Bangkok, Thailand, 6: DAT (Department of Agriculture, Thailand) 15, 7: DAT 16, 8: DAT 17, 9: Cheng Mai, Thailand, 10: Sri Nagor, Bangladesh, 11: Tangail, Bangladesh, 12: Bharatpur, Nepal, 13: Khumaltar, Nepal, 14: Bicol 2, Philippines, 15: Apalit, Philippines, 16: Floridablanca I, Philippines, 17: Vietnam green I, 18: Vietnam green II, 19: Vietnam purple, 20: Vietnam wild, 21: Putian Zhu, China, 22: Tancheng, China, 23: Cuttack, India, 24: Floridablanca II, Philippines, 25: Ivory Coast, 26: Senegal, 27: Nagoya green, Japan, 28: Nagoya red, 29: Changsha, China, 30: Guandong, Xinhui, China, 31: Yi Ling, China, 32: Jianci, China, 33: Nanchun, China, 34: Majayjay, Philippines, 35: IITA, Nigeria, 36: Cagayan, Philippines, 37: Tieng Giang 2, Vietnam, 38: Tieng Giang 3, Vietnam, 39: MIA, Australia, 40-42: Garut 1-3, Indonesia
A. filiculoides	101: East Germany, 102: Hawaii, USA, 103: California, USA, 104: Japan, 105-6: Hamburg 1-2, Germany, 107: Walka Lake, N.V., USA, 108: Cranmore, USA
A. mexicana	201: California, USA, 202-4: Guyana 1-3
A. caroliniana	301: Ohio, USA, 302: Madison, USA
A. sp. (Unidentified)[c]	401-20: 20 strains from Paraguay

[a] Strain number and designation by country (if not described in the designation). [b] #25, 26, and 35 are morphologically different from other A. pinnata strains (A. africana). [c] Some collections were tentatively identified as A. microphylla.

Phosphorus requirement

The addition of phosphorus fertilizer is the most important cultural practice for successful growth of azolla in the paddy (Watanabe et al 1980). The minimum requirement of phosphorus in the culture solution and the azolla tissue to maintain normal growth was determined by batch culture and continuous flow culture. In batch culture, azolla growth was enhanced by changing the phosphorus concentration from 0 to 5 ppm. Above 5 ppm, biomass production was not enhanced despite increased phosphorus content in the fern. It appeared that the fern growth was not increased by phosphorus application when fern phosphorus content exceeded 0.3% (dry matter basis).

A device was developed to grow azolla in a tray with continuous flow of nutrient solution at the flow rate of 500 or 1,000 ml/hour. When phosphorus concentration was decreased from 1 to 0.12 ppm, and to 0.06 ppm, the fern did not show any phosphorus deficiency symptom. At 0.06 ppm, the fern contained 4.5% N and 0.1% P (dry matter basis). At 0.03 ppm P, the fern's biomass was lower than that at 0.06 ppm P, fern leaves turned brownish red and smaller, and roots elongated and became fragile. These are symptoms of phosphorus deficiency. The fern contained 3.3% N and 0.06% P. At 0 ppm P, the symptom of phosphorus deficiency was more severe. The experiment established the minimum level of phosphorus required by azolla at 0.06 ppm or 2 μM (Subudhi and Watanabe 1979, 1981).

To compare the differences in azolla growth where phosphorus is limited, species and strains of azolla were grown at 0.03 ppm P in flowing culture with A. pinnata 5 from Bangkok as the reference plant. The tests were in a greenhouse. The results are shown in Table 2.

Great difference among azolla strains in the ability to grow with limited phosphorus was found.

Species other than A. pinnata had poorest growth in phosphorus-limited culture. A. pinnata from Cuttack (23) was characterized by fragmented fronds when phosphorus deficient. A. pinnata Vietnam green (17) did not turn red, but its growth was retarded with limited phosphorus. Strain 17 is a mutant lacking in the ability of synthesizing anthocyanins.

From calculation of phosphorus uptake to the fern, it appeared that growth in phosphorus-limited condition was associated with ability to absorb phosphorus from the culture.

Nutrient content of azolla

To evaluate the nutrient status in azolla, the content of nutrients in the fern at sufficient and deficient conditions was determined.

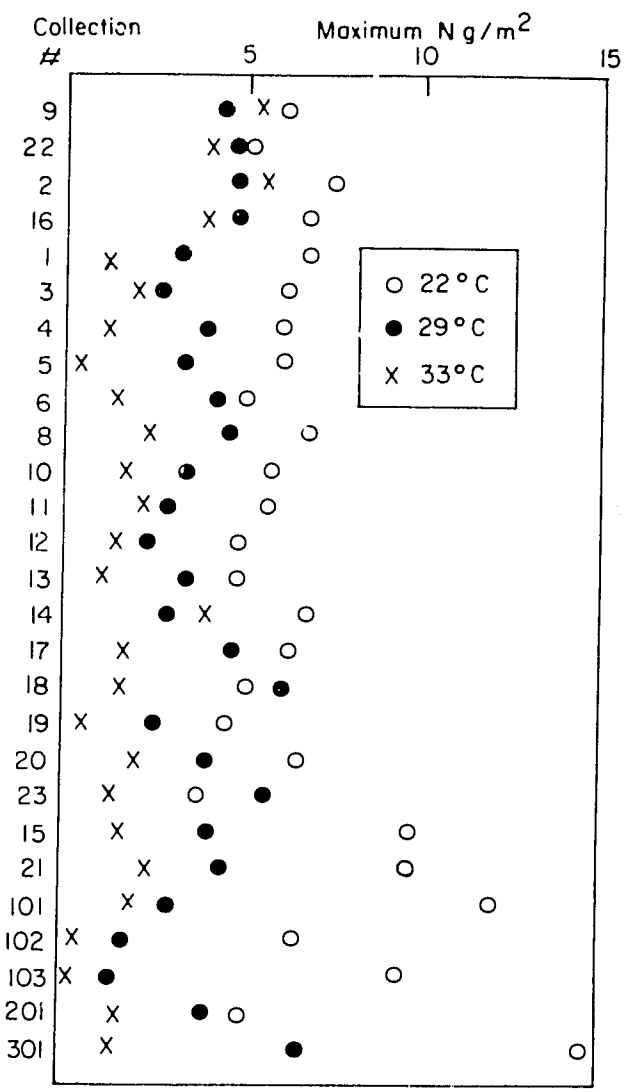

Fig. 1. Maximum biomass expressed as amount of nitrogen.

A. pinnata Bicol (1) was grown by batch culture in nutrient solutions lacking phosphorus, potassium, calcium, magnesium, and iron, respectively. After 12 days of growth, the dry weights of the fern expressed as percentages of those grown in complete nutrient solutions were 70% in K-deficient, 38% in Fe-deficient, 37% in P-deficient, 15% in Ca-deficient, and 6% in Mg-deficient solutions. Mineral contents of the fern grown in complete nutrient solution at 12 days growth were as follows: 4.9% N; 1.1% P; 3.2% K; 0.2% Ca; 0.7% Mg; and 0.09% Fe. In the ferns deficient in each nutrient, the nutrient contents were 0.08% P, 0.4% K, 0.18% Ca, and 0.016% Fe. Magnesium content in the magnesium-deficient fern was not determined because only a small amount of the fern was obtained.

Factors affecting sporulation

Azolla forms sporocarps and reproduces sexually. Little is known about the conditions needed to induce or develop the sporocarps. Sporocarps are more tolerant of adverse environments than vegetatively growing azolla sporophytes. Knowledge on sexual reproduction would open the way for germplasm collection as sporocarps and to sexual combination for azolla breeding. In preliminary experiments the sporulation of A. pinnata Tancheng (22), A. filiculoides (103) and A. mexicana (201) -- strains from temperate areas -- was repeatedly observed. Strains from tropical areas seldom produced sporocarps, which suggested that the capability of sporulation is determined hereditarily and reflects the environment where azolla survives naturally.

A. mexicana (201) was used to study environmental requirements for sporulation. Low temperature stimulated sporulation (Table 3). Day/night temperatures of 26/18°C gave the highest number of sporocarps. The 26/18°C temperature was also optimum for vegetative growth. As sporulation was stimulated, the ratio of microsporocarps (male) to megasporocarps (female) increased.

In the greenhouse where day/night temperatures were always higher than 25/20°C photoperiod combinations of 12 hours - 12 hours, 15 hours - 9 hours, and 8 hours -16 hours did not induce sporocarps, but where temperature was controlled at 25/20°C for 12 hours each, sporocarps were observed.

It is unlikely that photoperiod is the main factor for the sporulation Phosphorus deficiency stimulated sporulation, whereas iron deficiency and addition of ammonium sulfate (5 ppm N) depressed sporulation.

The addition of either soil water where A. mexicana has long been grown, or water that contained decomposing A. mexicana stimulated sporulation, suggesting the presence of antheridium-forming factors. The addition of the supernatant of azolla water extract at 0.2% in culture solution stimulated sporulation. The active fraction was dialyzable. The active substance remains to be identified.

Table 2. Fresh weight, doubl[illegible]me, frond size, phosphorus and nitrogen content, and acetylene reduction activities of azolla grown at .03 ppm P in continuous flow culture (after 11 days growth). IRRI, 1979.

Species	Strain number	Designation	Fresh matter (g/m^2)[a]	Doubling time (days)	Frond size (cm^2)	P (% in dry matter)	N (% in dry matter)	Acetylene reduction activity (µmol C_2H_4/g fresh azolla per h)
A. pinnata	5	Bangkok	750	2.78	0.47	0.064	3.7	0.58
	13	Khumaltar	640	2.92	0.39	0.05	4.1	0.62
	17	Vietnam green	480	3.31	0.41	-	-	-
	2	Malaysia	460	3.36	0.43	0.065	3.4	0.32
	23	Cuttack	380	3.69	0.07	0.053	2.5	0.05
	21	Putiang Zhu	340	3.88	0.40	0.073	3.4	0.30
	19	Vietnam wild	340	3.88	0.35	-	-	-
	10	Sri Nagor	310	4.09	0.28	-	-	-
	3	Bogor	270	4.38	0.23	-	-	-
A. mexicana	201	California	250	4.56	0.37	-	-	-
A. filiculoides	101	Hawaii	170	6.01	0.36	0.085	2.8	0.09
A. caroliniana	301	Ohio	110	9.01	0.26	0.09	2.4	0.23

[a] Inoculum was 47.1 g fresh weight/m^2.

Effect of combined nitrogen on N_2 fixation and nitrogen nutrition

In fields where a high amount of nitrogen fertilizer is required, azolla may be used with chemical nitrogen fertilizer. Fertilizer nitrogen depresses the nitrogen-fixing ability of all biological nitrogen-fixing systems. We examined the effect of fertilizer nitrogen on Azolla-Anabaena symbiosis.

Azolla was treated by varying concentrations of ammonium or nitrate. Acetylene reduction activity (after a 24-hour exposure of azolla to combined nitrogen) was depressed only slightly even at 10 mM concentration of nitrogen. Four days exposure to 1 mM combined nitrogen depressed acetylene reduction of the symbiotic system to about a half.

The simultaneous assimilation of dinitrogen and combined nitrogen was examined by an alternate labeling procedure. Either dinitrogen or combined nitrogen (ammonium or nitrate) was labeled by ^{15}N. In the presence of unlabeled nitrogen of another form. After 4 days growth at 1 mM NH_4-N, 47% of total azolla nitrogen was originated from both NH_4-N and dinitrogen. Nitrogen from dinitrogen explained 46% of this newly assimilated nitrogen. At 10 mM NH_4-N, 61% of azolla nitrogen was newly assimilated, and 30% of the assimilated nitrogen came from dinitrogen.

Table 3. Effect of temperature on the sporulation of A. mexicana (70% relative humidity, natural light, 2 weeks). IRRI, 1980.

Temperature (°C) day/night (12 hour/12 hour)	Mic:Meg[a] (%)	Mic/g f. w.[b] (no.)
36/27	6.5	9.7
29/21	31	65.0
26/18	50	82
20/15	36	68

[a] Percentage of number of microsporocarps to megasporocarps. [b] Number of microsporocarps per gram fresh weight of azolla. The values are the mean of three flasks.

These data show that dinitrogen fixation and ammonium assimilation from the azolla root take place simultaneously even at 10 mM concentration of combined nitrogen. Nitrate was less depressive to dinitrogen fixation than ammonium. Nitrate was preferably accumulated on the root, while ammonium was preferably transported to young fronds similarly as dinitrogen was transperted. This difference in the azolla transport pattern for ammonium and nitrate explains the less depressive effect of nitrate on nitrogen fixation.

Symbiotic nitrogen fixation by the Azolla-Anabaena complex is, therefore, protected from depressive action of combined nitrogen by unknown factors.

USE OF AZOLLA IN PADDY FIELDS

Growth performance of various azolla collections in the field

To identify azolla strains with best growth, azolla growth was tested 4 times a year at IRRI in open and 50% shaded fields in 1979. No difference in maximum biomass nitrogen was observed among four strains of A. pinnata (Table 4). The period to achieve maximum biomass was shortest for A. pinnata Bogor (3). From April to May, when solar radiation and temperature were highest, azolla growth was poorest. Growth of azolla other than A. pinnata was poor. Shading depressed azolla growth, except in the hottest period but even their growth in shade was inferior to the growth in other periods.

In 1981, other azolla strains were examined during slightly different periods. A. pinnata Bangkok (5) was used as a reference strain. Two levels of phosphorus (3.75 and 7.5 kg P_2O_5/ha) application were compared. Superphosphate was split 5 times and applied every 3 days for 2 weeks. Shading was not done.

The effect of phosphorus was observed only in the hottest period (April-May). Among five azolla strains that grew four times, there was no difference in the maximum biomass nitrogen (Table 5). A. caroliniana Ohio (301) grew well.

Table 4. Maximum fresh weight and N content of azolla strains[a/] grown in nonshaded plots for 4 seasons, and meteorological data. Inoculum size was 100 g/m^2. IRRI, 1979.

Species and strain	May-Jun			Sep-Oct			Dec-Feb			Apr-May		
	Fresh wt (kg/m^2)	N content[b/] (g/m^2)		Fresh wt (kg/m^2)		N content (g/m^2)	Fresh wt (kg/m^2)		N content (g/m^2)	Fresh wt (kg/m^2)		N content (g/m^2)
A.p. Banaue (4)	1.3 b	(23)[c/]	3.7	1.5 c	(25)	4.0 b	1.4 b	(25)	4.1 a	0.9 c	(25)	2.7 a
A.p. Bicol (1)	1.6 ab	(30)	4.6	1.6 c	(20)	4.7 ab	1.4 b	(25)	4.1 a	1.0 bc	(25)	2.8 a
A.p. Bangkok (5)	1.8 a	(21)	3.4	2.4 a	(42)	4.8 a	1.9 a	(25)	4.7 a	1.4 a	(35)	2.9 a
A.p. Malaysia (2)	1.6 ab	(30)	4.4	1.9 b	(35)	4.7 ab	1.3 b	(23)	3.9 a	1.0 bc	(25)	3.0 a
A.p. Bogor (3)	1.2 b	(14)	3.7	1.7 bc	(25)	4.5 ab	1.4 b	(23)	4.4 a	1.1 b	(21)	3.5 a
A.f. Hawaii (102)	0.8 c	(40)	1.9	-			0.9 c	(21)	2.7 b	0.2 e	(19)	0.5 c
A.f. California (103)	-			-			0.6 e	(23)	2.7 b	0.2 e	(14)	0.4 c
A.m. California (301)	-			0.50 d	(23)	1.2 c	0.7 c	(21)	2.0 b	0.5 c	(21)	1.6 b
Meteorological data (daily av for 6 wk)												
Solar radiation (cal/m^2)	454			395			418			521		
Air temp (°C) max	31.8			30.9			28.8			32.3		
min	24.3			23.2			21.6			23.9		
Sunshine duration (hours)	5.6			4.3			7.2			8.2		
Rainfall (mm)	4.6			18.1			0.2			8.6		

a/ In a column, means followed by a common letter are not significantly different from each other at 5% level.
b/ Estimated from the average N percent from 3 other seasons. c/ The numbers in parenthesis indicate the days when maximum biomass was obtained.

Table 5. Maximum N content of azolla strains grown in field plots, IRRI, 1980.

Species and strain	Sep-Nov N content[a] (g/m^2) High P	Jun-Feb N content (g/m^2) High P	Apr-May N content (g/m^2) High P	Apr-May N content (g/m^2) Low P	Jul-Aug N content (g/m^2) High P
A. pinnata					
Bangkok (5)	4.5 a (21)	4.5 a (30)	1.1 bc (35)	0.3 c (9)	2.9 abc (14)
Cuttack (23)	2.3 d (16)	3.3 b (28)	1.6 ab (28)	1.5 abc (33)	3.6 a (28)
Vietnam green (17)	3.6 abc (21)	3.7 ab (28)	1.6 ab (30)	0.3 c (9)	3.5 ab (14)
Khumaltar (13)	3.6 abc (19)	-	2.6 a (30)	2.7 a (30)	2.3 c (21)
DAT 15 (16)	3.4 bc (21)	3.1 b (23)	1.5 abc (35)	0.6 bc (14)	-
Apalit (15)	3.1 cd (21)	-	-	-	2.0 c (21)
Tangail (11)	4.4 a (21)	3.3 b (21)	1.6 ab (33)	1.7 ab (33)	2.7 bc (23)
A. caroliniana					
Ohio (301)	3.7 abc (23)	4.0 ab (26)	1.8 ab (35)	1.7 ab (33)	2.3 c (28)
Meteorological data (daily av for 5 wk)					
Solar radiation (cal/cm^2)	388	412	565		471
Air temp (°C) max	30.1	28.0	34.6		30.7
min	23.1	22.1	23.5		23.8
Sunshine duration (hours)	5.0	5.6	8.9		5.9
Rainfall (mm)	5.5	0.7	0.2		12.2

[a] Values in parenthesis are days to achieve maximum biomass nitrogen.

Weekly nitrogen-fixing rate per unit area for all strains was maximum in the second week except in the hottest period. A. pinnata Bangkok (5) and A. caroliniana Ohio (301) showed the highest nitrogen-fixing rate, amounting from 1.8 to 2.3 kg N/m^2 per week (2.6-3.3 kg N/ha per day). (Data are not presented.)

At the lower phosphate level in the hottest period, growth of A. pinnata Bangkok (5), Vietnam green (17), and DAT 15 (16) was poor. A. pinnata Cuttack (23) showed fragmented small fronds at the lower phosphate level, as observed earlier in water culture. But, strain 23 (Cuttack) persisted and recovered at the fourth week. We cannot explain the discrepancy between field and water culture azolla performance in a phosphate-deficient condition.

A. caroliniana Ohio (301) showed best growth at 29°C in the growth chamber and rivaled the growth of A. pinnata in the field. The species is, therefore, recommended in the tropics.

Decomposition of azolla and availability of azolla nitrogen to rice

Azolla nitrogen becomes available to rice after its mineralization. The mineralization time sequence was shown previously (Watanabe et al 1977). Mineralization may differ with carbon-nitrogen ratio or simply with nitrogen content in azolla.

Nitrogen mineralization from fresh azolla at 30°C in a flooded soil was examined to compare species and strains. Mineralization took place rapidly during the first week; after the third week mineralization slowed down. Based on the mineralized nitrogen of A. pinnata Malaysia (2) for 40 days as 100, the mineralized nitrogen was: A. pinnata Bicol (1) 97, A. pinnata Bangkok (5) 55, A. pinnata Banaue (4) 50, A. pinnata Bogor (3) 44, A. filiculoides California (103) 42, A. filiculoides Hawaii (102) 36, and A. mexicana Ohio (301) 16. Nitrogen content (dry matter) was 4.88, 4.97, 5.00, 4.67, 4.89, 4.54, 4.25, and 3.46%, respectively. This means that the lower the nitrogen in the azolla

body, the less mineralizable its nitrogen. Chemical components other than nitrogen may affect the availability of azolla nitrogen.

To examine the fate of azolla nitrogen in flooded soil, azolla was labeled by ^{15}N and placed in pots in different ways. When azolla was placed on the soil surface, highest losses, amounting to as much as 60% for 6 weeks, were observed. When floated on the water, losses were 50% for 6 weeks. When azolla was incorporated to soil, only 33% was lost. The availability of ^{15}N in azolla to rice growth in the pot was 53% when azolla was incorporated and only 10% when azolla was floated on the water.

In a field experiment (1-m^2 plots), ^{15}N-labeled azolla was either incorporated, mixed only on the soil surface, or floated on the water. Azolla was introduced either 30, 53, or 78 days after transplanting of rice and uptake of ^{15}N by rice was determined after harvest. ^{15}N recovery to the following rice crop was also determined. Results are shown in Table 6. Highest recovery was obtained from incorporated azolla. When azolla was applied at later stage, the availability of its nitrogen decreased.

In the second rice crop, little difference in ^{15}N recovery to rice was observed among treatments. Lower nitrogen recovery by the first crop when azolla was applied later was not compensated by the higher recovery by the second crop. The availability to the second crop was decreased to about one-third or one-fifth of that to the first crop. It is concluded that a residual effect of azolla nitrogen should not be expected.

Both experiments showed that soil incorporation of azolla is necessary to make the most nitrogen in azolla available to rice.

Growth of azolla with rice and its effect on rice yield

Azolla has an ability to fix N_2 as much as 1.5 kg N/ha daily or 500 kg N/ha annually in a paddy (Watanabe et al 1980). This amount exceeds the nitrogen requirement for rice.

In the rice field, azolla growth is fast at the rice crop's early stage, but declines later because of shading by the rice plants. Widening the distance between rice plant rows to allow cultivation of azolla during the whole growth of wetland rice was tried experimentally in China.

At IRRI, we grew rice in a 35-m^2 plot in a wide-narrow row spacing. Rows spaced 53 cm were alternated with rows 13.6 cm apart. The distance between hills within 1 row was 6.6 cm for a density of 50 hills/m^2. Fresh azolla (*A. pinnata* Bangkok (5)) was added at 13-18 kg/plot. A total of 53 kg P_2O_5 as superphosphate/ha was applied -- half before transplanting and the rest at the time of azolla inoculation was added to all. Forty-nine g of carbofuran/plot was applied weekly. After 15-20

Table 6. Availability of azolla ^{15}N to rice crops. IRRI, 1978-79.

Inoculation time (days after transplanting)[a]	Treatment	Recovery(%) of applied ^{15}N 1st crop	2d crop[b]
30	Floated on water	13	4.8
	Placed on soil sample	14	4.5
	Mixed with soil	26	5.1
53	Floated on water	14	3.6
	Placed on soil surface	15	4.1
	Mixed with soil	28	5.4
78	Floated on water	11	4.3
	Placed on soil surface	8	4.0
	Mixed with soil	14	4.8

[a] Amounts of N applied were 41.3 kg/ha at 30 days after transplanting (DT), 32.2 kg/ha at 53 DT, and 41.1 kg/ha at 78 DT. [b] N contents in grain, straw, and root were included.

days, when azolla covered the plot surface, the plot was drained for 2-3 days to make the azolla adhere to the soil surface. The azolla was incorporated with the soil by feet, hand weeder, or rake. After incorporation, fresh inoculum was applied with superphosphate and insecticide (1 g carbofuran 3% a.i./m^2).

The growth of azolla is shown in Table 7. In the first trial, azolla was grown 6 times. Because azolla growth near rice heading was poor, 4 crops of azolla were grown from the second trial. For the fourth and fifth rice crops, one crop of azolla was grown before transplanting.

To study the effect of azolla incorporation, three treatments -- no nitrogen, with chemical nitrogen, and with azolla -- were compared (Table 8). Ammonium sulfate was applied before transplanting and incorporated with the soil. The response of the first rice crop to azolla incorporation was apparent only at late stages of growth. No yield increase from azolla incorporation was observed probably because soil fertility was high. In the second crop rice growth in the early stage was better in azolla-treated plots than in no-nitrogen plots because of the carry-over of nitrogen from the previous 6 crops of azolla in this experiment.

In the third rice crop, some plots were severely affected by boron toxicity, especially in chemical nitrogen plots, and yields were lower than in azolla-treated plots (Table 8). Azolla-treated plots generally showed less boron toxicity. In the fourth trial, plot size was changed to 49 m^2. Distance between hills in a row was 13 cm.

The average for 5 trials indicated that growing several crops of azolla with rice in wide rows and incorporating azolla can give about 1 t/ha yield increase over a control with no azolla or nitrogen fertilizer added.

Nitrogen uptake during rice crop was determined (data were not presented). Except for the first crop in which nitrogen uptake in the nitrogen-fertilizer treatment was always higher than in the azolla plots, nitrogen uptake in azolla plots exceeded that of chemical nitrogen treatment only 70 days after transplanting. This suggests that nitrogen from azolla was more slowly absorbed than nitrogen from chemical fertilizer.

FUTURE RESEARCH NEEDS

The series of studies mentioned above provided basic information to azolla use in tropical rice culture. From that research we feel that future research needs are:

- Screen azolla for tolerance for high temperature. Azolla likes temperature near 25°C and its growth deteriorates at 30°C or higher.
- Cooperate with experts in cryptogamic botany to increase knowledge of sporulating factors in azolla. Sporulation is stimulated by low temperature optimum for vegetative growth.
- Screen azolla strains for ability to grow in phosphorus-limited conditions. Phosphorus is a critical factor for better growth of azolla.

Table 7. Growth of azolla together with rice, 1978-80.

Rice crop sequence	Cropping duration (transplanting to harvest)		Days of harvest (after transplanting) and azolla yield (kg N/ha)						Total N produced by azolla (kg/ha)
1st	Dec 1978-Apr 1979 (121 days)	Days	23	40	57	74	92	112	
		N content	27	15	20	19	16	7	104
2d	May-Aug 1979 (124 days)	Days	24	44	76	100			
		N content	21	20	14	13			68
3d	Nov 1979-Mar 1980 (124 days)	Days	21	43	64	80			
		N content	23	20	12	16			70
4th	Feb-Jun 1980 (121 days)	Days	0	37	60	83			
		N content	26	12	14	11			63
5th	Jul-Nov 1980 (106 days)	Days	0	21	59	80			
		N content	7	20	21	11			59

Table 8. Comparison of grain yields of azolla-treated plots and of plots with and without chemical nitrogen. IRRI, 1978-80.

Trial sequence		Chemical nitrogen applied (kg/ha)	Grain yield (t/ha)[a]		
			Chemical nitrogen	Azolla	0 nitrogen
1st	IR26	107	8.3 a	6.7 a	6.3 a
2d	IR42	60	4.5 b	5.7 a	3.7 b
3d	IR42	100	2.2 b	3.6 a	2.7 b
4th[b]	IR42	60	6.2 a	6.2 a	5.2 b
5th[b]	IR42	60	4.7 a	5.0 a	3.3 b

[a] Any 2 row means followed by a common letter are not significantly different from each other at the 5% level.
[b] Field is different from the previous one. One crop of azolla was grown before transplanting. Plant density was half that in the previous trials.

- Introduce New World azolla species like A. caroliniana to tropical South Asia. Start international collaboration to test various strains useful to national programs. Current taxonomy of azolla needs to be re-examined.

- Tests of nitrogen yield and availability of azolla are needed. Availability of nitrogen to rice is different among azolla strains and species.

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