GROWTH AND NITROGEN FIXATION OF *Azolla pinnata* var. *africana*: ENVIRONMENTAL CONDITIONS AND FIELD PLOT INOCULATION ASSAYS

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Abstract

The growth and the nitrogen fixation of Azolla pinnata var. africana is discussed in regard to climatic conditions occuring in a dry tropical area. Optimum acetylene reducing activity (ARA) was observed at a light intensity of 60 klux, and both higher and lower light intensities reduced ARA. Optimum ARA is in the range of 25-35°C. The harmful effect of desiccation is slowed by adding alginate and storing at 6°C. Concentrations of nitrate-N and ammoniom-N have different effects on Azolla growth, and ARA. Plot assays show the importance of a residual effect on a second rice cycle.

Introduction

In order to measure the inoculation effect of Azolla on rice and on soil, one has first to determine the best conditions of growth and the best agronomical practices in a given area.

West Africa possess its own strain: Azolla pinnata var. africana which is recognized to occur in almost all the countries of the region. This region can be separated into two climatic areas (Charreau, 1974).

- a semi-humid tropical area with a rainy season ranging from five to seven months and conditions similar to Asiatic zones where Azolla grows;
- a dry tropical area with a rainy season ranging from two to five humid months, near 15° N latitude.

In the latter area solar radiation is high, so that potential Azolla productivity should be high. Unfortunately, water requirement for Azolla growth is also high and rainfall is often inadequate and varies widely from year to year, so that effective productivity is very low.

In this zone, adaptation of *Azolla* is a major problem. As *Azolla* could be very beneficial for the expected future nitrogen budget of these less developed countries, we have to consider its use under these extreme climatic conditions.

A. Ecological factors influencing N_2-fixation and Growth of *A. africana*.

I. LIGHT INTENSITY.

Experimental procedure: A tank was divided into five areas of 25 x 70 cm by floating partitions so that the culture medium added was identical in each area. Each area was shaded by a screen allowing transmission of either 100%, 60%, 36%, 22% or 7% of incident sunlight, which can reach easily a maximum of 90 klux at 1 p.m. in Dakar.

Each area was inoculated with 40g (fresh weight) of *A. africana*. After 8 and 15 days fresh weight was determined for each area. After 8 days the *Azolla* morphology was observed to be affected by high light intensities: leaves became red and roots were stunted. Under the lowest light intensity (Emax = 6.3 klux) *Azolla* morphology was not apparently affected but the productivity was low (Table 1.). The ratio chlorophyll/carotenoid decreased with increased incident sunlight.

After 15 days the ratio chlor./car. did not differ and productivity was proportional to incident sunlight intensity.

The Acetylene Reducing Activity (ARA) measured at 27°C in different sunlight intensities, is maximum when Emax. is 60 klux (300 n.moles $C_2H_4/h/cm^2$); higher and lower light intensities (1.7 klux) both reduce ARA (Roger and Reynaud, 1979), showing that an optimum energy input is required to obtain a high level of N_2-fixation.

II. TEMPERATURE.

In optimum light intensity, experiments on ARA were carried out in the range of 25°C-40°C; temperatures higher than 35°C increased ARA during the first hour of incubation but then inhibited it, at 40°C ARA is totally inhibited within 6 hours. Optimum ARA of *A. africana* is in the range of 25-35°C.

In the dry area of West Africa the growth of A. africana is very slow, and almost stops between December and April when the temperature range is between 15-28°C. In April, when the temperature increases, the doubling time is reduced to 3 days. This fact is markedly important as it is impossible to produce Azolla inocula between December and April, under these latitudes with the indigenous strain. However, we have tested A. caroliniana during January 1981, with a temperature range between 15-28°C, in a green house and have noticed that its doubling time is 3 fold less than that of A. africana. The use of this introduced strain could be indicated.

III. EFFECT OF DESICCATION.

During the dry season the relative humidity varies in 6 hours from 95% to 35%. If the fern is allowed to dry up, the time course of ARA falls to zero within 24 hours and remoistening in a proper growth medium does not restore ARA, and no more growth is observed (Reynaud, 1980). This harmful effect of desiccation can be slowed down by two methods summarized in Table 2.

1. Adding alginate (0.05% w/v) to the growth medium results in an ARA decrease of only 60% within 24 h during desiccation, and when the fern is remoistened, ARA is restored to its initial level.

2. Storing Azolla at 6°C under low humidity conditions preserved ARA for more than 48 hours.

We have successfully used these two methods for protecting Azolla during the shipment of inoculum to the field.

IV. EFFECT OF NATURE AND CONCENTRATION OF COMBINED NITROGEN.

In the Fleuve region, north of Senegal, amounts of nitrogen as high as 150 kg/ha^{-1} are required for rice cultivation so that Azolla has to be used along with chemical nitrogen fertilizer. Thus it is important to assess the effect of different forms and concentrations of chemical N on growth and nitrogen fixation of Azolla.

Azolla was grown in nutrient media containing 0, 8.8 and 88 ppm of ammonium-N or nitrate-N. In comparison to N-free medium, nitrate-N concentration had no effect on *Azolla* growth, but 88 ppm of NH_4-N had a negative effect and 8.8 ppm NH_4-N, a markedly positive effect (Table 3).

High nitrogen concentrations quickly depressed dinitrogen fixation. In the case of NO_3-N, it seems that this fixation is replaced by nitrate-N assimilation. On the other hand 88 ppm of NH_4-N stopped dinitrogen fixation of *Azolla*. With 8.8 ppm of combined-N, the acetylene reduction of the symbiotic system is depressed to about 40% after 12 days exposure.

A further experiment was conducted to examine the effects of a range of ammonium-N concentrations on *Azolla* (Table 4). Growth of the fern is positive in comparison to the growth in N-free medium with concentrations up to 44 ppm NH_4-N, but the ARA was depressed at all concentrations of NH_4-N. With 1.8 ppm, ARA was decreased to about one half after 6-12 days but recovered all its activity after 19 days of incubation.

These data show the limiting effect of low concentrations of combined-N on ARA and the fact that dinitrogen fixation and combined-N assimilation takes place simultaneously even at 44 ppm concentration of combined nitrogen. Utilization of nitrate seems preferable to the use of ammonium (Liu Chung Chu, 1979) as ammonium competes with dinitrogen in young fronds. (Watanabe et.al. 1981).

Our results are in good agreement with those of Yatazawa *et al.* (1980) for *A. pinnata* var. *imbricata* which is considered to be the Asiatic form of *A. pinnata.*

B. Plot Assays

Azolla pinnata var. *africana* trials were carried out in Senegal according to the recommendations of the International Network on Soil Fertility and Fertilizer Efficiency in Rice (INSFFER) for 1979. Four randomized plots of 2 square metres, each one containing

950 kg (dry weight) of sandy soil (N% = 0.140) covered in a plastic film to avoid N diffusion, were used for each treatment. The trials were carried out at the ORSTOM Station in Dakar (Senegal). Experiments to test the effects of Azolla inoculation were conducted during the wet season 1980 (August to November). Subsequently, the residual effect of Azolla was investigated during the following dry season 1981 (February to June) (Table 5).

During the first assay the climatic conditions were best for growth of Azolla i.e.: maximum light intensity: 70 klux, maximum relative humidity: 98%, temperature range: 22-37°C.

A tenth treatment was added to the nine treatments used in other INSFFER trials ; Azolla, previously dried at 60°C and ground, was incorporated in the soil 10 days before transplanting, at the rate of 60 kg/ha. In all treatments with N addition, urea was the sole nitrogen source; it was applied in three split applications: before transplanting, 20 days after transplanting and 40 days after transplanting. During the second cultivation cycle, only treatments 2 and 3 received urea-N (Table 6). All the treatments were always provided with tap water.

I. RESULTS ON THE FIRST TRIALS.

Inoculation with Azolla increased the grain and straw yield in all treatments (Table 5). Inoculation with Azolla without N fertilizer (treatments 4, 5, 6) increased the grain 38-40%, which is similar to the increase resulting from the addition of 30 kg urea-N/ha. The increase in the straw yield was higher when Azolla was incorporated (37%) than when it was not (28%). When no N fertilizer was added, the highest yield increase (54%) was obtained with two Azolla inoculations in succession, the first one before, and the second after transplanting (treatment 9). The combination of Azolla inoculation with 30 kg N/ha application (treatment 7,8) increased the yield more than did 60 kg urea-N/ha. The yield increases resulting from Azolla inoculation reported here are higher than the average yield increases observed in INSFFER (1980) trials conducted in Asia, due to the small plot area.

Incorporation of previously dried _Azolla_ (treat. 10), was significantly less favourable than other types of _Azolla_ incorporation. A comparison between treatment 3 (60 kg urea-N/ha) and treatment 10 (60 kg N/ha as organic N in the form of _Azolla_ powder) shows that the second form of N is less available to rice than the first one.

The growth of _Azolla_, expressed as total N from _Azolla_ per ha (Table 6) shows that the development of _Azolla_ was significantly better before (treatment 4,7) than after transplanting (treatment 5,6,8). Incorporation was always done 15 days after inoculation (0.15 kg _Azolla_ fresh weight/square metre). The growth of _Azolla_ was significantly increased with application of urea (treatment 7,8) at the rate of 30 kg N/ha.

II. RESIDUAL EFFECT.

During the dry season 1981, rice IR 1529 was replaced by KN1H300. This variety is assumed to grow better than IR 1529 with cold (15-30^{o}C) and dry climatic conditions, although its average yield during the dry season is lower than that of IR 1529. Its straw/grain ratio is also significantly higher (IR 1529: 0.8 and KN1H300: 1.3). With the exception of treatment 9, whose low yield remains unexplicable, the grain yields and the total N in the soil after two cultivation cycles were higher in treatments with _Azolla_ than in treatments with urea. Thus it follows than an _Azolla_ inoculation-incorporation treatment brings to the rice, during two cultivation cycles, an equivalent value of 90-120 kg urea-N per hectare. As the total N is higher in _Azolla_ treatment it seems possible that the residual effect of _Azolla_ inoculation could continue during 2nd rice cultivation cycle.

The most significant event during the second cycle was the good growth of rice in treatment 10. The yield was about the same as in treatments 3,7 and 8, which means that, during the first cultivation cycle, the majority of N in dried _Azolla_ was not available for rice. We can explain this by the fact that there is, after incorporation of dried _Azolla_ in the wet soil, an aerobic mineralisation of only a small fraction of the _Azolla_ material (20% of total nitrogen is mineralized in 15 days). But as the first 15 centimeters of soil became quickly anaerobic (Loyer et al. 1982), denitrification occurred and this mineralized Azolla-N was lost and not assimilable for rice. Anaerobic fermentation transforms Azolla-N into a form that is easily mineralisable under the aerobic conditions which occurred during ploughing for the second rice culture. The poor availability of Azolla-N, during the first rice cycle, can be enhanced if Azolla is composted and incorporated just before transplanting.

CONCLUSIONS.

In regard to these data:

The best growth of A.africana occurs during the short wet season.

Viability of fresh Azolla inoculum during transport can be improved by soaking the fronds previously in alginate (0,05%) and then draining and storing up to 48 h at 6^{o}C.

Nitrate has no effect on Azolla growth in nutrient media, but in the field, urea at the rate of 30 kg N/ha, has a positive effect.

Inoculation of Azolla always had a positive effect on rice yield.

A residual effect of Azolla was found on a second rice culture.

The degradation of the fern in the soil is not elucidated and suppositions must be confirmed by ^{15}N assay.

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Table 1 : Effect of shading on A. Africana biomass production and morphology

% sunlight	100	60	36	22	7
Fresh weight after 8 days in g from 40 g of inoculum	115	126	125	148	91
Leaves' coloration	green border red	green border yellow	green border yellow	light green	bright green
Chlorophyll a:% o dry weight	2.71	-	3.65	-	6.57
Fresh weight after 15 days of g from 40 g of inoculum	264	250	189	215	132
Leaves' coloration	pink	pink	light green	light green	bright green
Chlorophyll a:%o dry weigth	5.83	-	7.1	-	7.8

Table 2 : Effect of temperature and alginate (0.05 %, $10^{'}$) on fresh weight and Acetylene Reducing Activity of Azolla pinnata var. africana. Each value is the mean of triplicates.

Treatments on fresh Azolla 25°C	24 h		48 h	
	% fresh weight	% ARA	% fresh weight	% ARA
R.H. : 98% 25°C	94	95	92	90
R.H. : 30% 25°C	75	0	47	0
R.H. : 30% 0.05 % alginate 25°C	92	45	76	0
R.H. : 98% 6°C	97	100	96	88
R.H. : 39%, 6°C	97	90	94	65

R.H. : relative humidity obtained with K_2SO_4 (98%) and $CaCl_2$-$6H_2O$ (30–40%).

Table 3 : Effect of chemical form and concentration of N-nitrogen on the growth and the Acetylene Reducing Activity of Azolla pinnata var. africana, each value is the mean of triplicates.

Treatments	Fresh weight g.			% ARA		
	4 days	8 days	12 days	4 days	8 days	12 days
N-free medium	15	18	20	100	100	100
8.8 ppm NH_4^+	16	19	25	65	110	60
88 ppm NH_4^+	14	15	15	60	35	10
8.8 ppm NO_3^-	15	17	19	85	90	60
88 ppm NO_3^-	15	18	20	55	60	15

Table 4 : Effect of combined nitrogen ($(NH_4)_2SO_4$) on the growth and Acetylene Reducing Activity in *Azolla pinnata* var. *africana*.

Culture solution	% fresh weight 6 days	% fresh weight 12 days	% fresh weight 19 days	% ARA 6 days	% ARA 12 days	% ARA 19 days
Control - N	100	100	100	100	100	100
NH_4-N : 1.8 ppm	110	108	100	50	45	95
NH_4-N : 8.8 ppm	112	118	99	45	25	55
NH_4-N : 44 ppm	105	115	50	35	20	5
NH_4-N : 88 ppm	90	80	35	25	12	3
NH_4-N : 175 ppm	85	60	35	20	10	0

Table 5 : Effect of Azolla and urea on rice yield and straw conduct as proposed by INSFFER on a wet season and residual effect on the next dry season in Dakar (Senegal) (rice variety a: IR1529, b: KN1H300)

	Yields : (a) 1980 wet season, (b): residual effect dry season 1981							
	Grain				Straw			
	a T/ha	Control %	b T/ha	Control %	a T/ha	Control %	b T/ha	Control %
1. Control	3.7	100	2.4	100	3.2	100	3.3	100
2. 30 kg N/ha	5.1	138	2.8	117	3.9	122	4.6	139
3. 60 kg N/ha	5.5	149	2.7	115	4.4	137	4	121
4. Azolla inc. before transplanting	5.1	138	3.8	161	4.4	137	4.4	133
5. Azolla grown after transp. then inc.	5.2	140	3.5	146	4.6	144	4	121
6. Azolla grown after transp. no inc.	5.1	138	2.7	112	4.1	128	3.8	115
7. 30 kg N/ha + Azolla inc. before transp.	5.9	159	3.3	140	4.4	137	3.7	112
8. 30 kg N/ha + Azolla after transp. inc.	5.7	154	3	125	4.5	141	3.6	109
9. Azolla grown before and after transp. and inc.	5.7	154	2.4	100	4.3	134	3.3	100
10. 60°C dried Azolla, inc. as 60 kg N/ha	4.7	127	3.1	130	3.5	109	4.2	127

inc. = incorporation ; transp. = transplanting

Treatments	N imported as urea kg.ha^{-1}			N from *Azolla* kg.ha^{-1}	N exported with the yield kg.ha^{-1}			N‰ in the soil after the two cultures
	a	b	Total	a	a	b	total	
1 Control	0	0	0	0	45	34	79	0,135
2 30 kg N/ha	30	30	60	0	60	42	102	0,145
3 60 kg N/ha	60	60	120	0	66	40	106	0,149
4 *Azolla* inc. before transplanting	0	0	0	20	62	51	113	0,160
5 *Azolla* grown after transp. then inc.	0	0	0	7,5	64	46	110	0,140
6 *Azolla* grown after transp. no inc.	0	0	0	12,4	61	44	105	0,153
7 30 kg N/ha + *Azolla* inc. before transp.	30	0	30	34	69	44	113	0,133
8 30 kg N/ha + *Azolla* after transp. inc.	30	0	30	15	68	40	108	0,164
9 *Azolla* grown before and after transp. then inc.	0	0	0	35,8	67	34	101	0,149
10 60°C dried *Azolla*, inc. as 60 kg N/ha	0	0	0	60	55	43	98	0,156

inc. : incorporation, transp. : transplanting

a : rice culture during 1980 wet season, b: residual effect on a second rice culture during 1981 dry season.

Table 6. Nitrogen budget on two rice cultivation : effect of urea and *Azolla*.