The Utilization of Azolla in the Philippines: Biological Problems and Solutions

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ABSTRACT

The nationwide utilization of azolla as a green manure for irrigated lowland rice fields was launched in 1982. Sixty eight provincial nurseries and 3,000 community propagation ponds were established to service a target areas of 300,000 ha. By 1988, only about 30% of the target area is using azolla as a green manure and as feed supplement for livestock.

Although the field data incontrovertibly indicate that azolla provides consequential benefits to rice farmers, its adaptation has been limited due to biological constraints. The technical assistance grant from the Asian Development Bank financed the establishment of the Azolla Varietal Improvement Laboratory at the Institute of Biological Sciences, University of the Philippines Los Baños, in cooperation with the Bureau of Soils and Water Management, Southeast Asian Regional Center for Graduate Study and Research in Agriculture, and the National Azolla Action Program. Hybridization work is currently undertaken to develop genetically superior hybrid lines.

INTRODUCTION

The rising cost of chemical nitrogen fertilizer and the imperative to increase rice production prompted the Philippine Government to launch the National Azolla Action Program (NAAP) in 1982. The NAAP was mandated to develop appropriate farm-based technology for the utilization of azolla as an organic fertilizer for irrigated lowland ricefields. Although the NAAP was based at the University of the Philippines Los Banos, its operation was in close coordination with the Bureau of Soils and Water Management, Department of Agriculture.

The objectives of the Program are four-fold.

One, to establish a National Inoculum Center (NIC) at UPLB and subcenters at selected agricultural schools throughout the country The NIC conducts physiological screening of various azolla varieties for adaptation to local condition. It is also responsible for the formulation of appropriate technology for the infarm utilization of azolla. The subcenters are in-charge of field verification trials and serve as the venue for training of government extension workers and farmer leaders.

Two, to establish Regional Propagation Centers (RPC) in the 12 administrative regions of the country. The RPC serves as the inoculum source for provincial and community nurseries that will be established.

Three, to prepare an area operation plan for each region to identify, classify, and prioritize wetland rice growing areas suitable for azolla cultivation.

Four, to produce information and extension materials.

In 1984, the United Azolla Program (UAP) was organized by the Department of Agriculture to hasten technology dissemination to the countryside. With this development, NAAP relinquished extension activities and concentrated on the management of the regional provincial and community nurseries. The UAP established 68 provincial nurseries and 3000 community nurseries to service a target area of 300,000 (Mabbayad, 1987).

By 1988, only about 30% of the target areas have adapted the azolla technology. In spite of this initial setback, during the National Symposium on Bio-Organic Fertilizer held on 9-12 October 1989 at the University of the Philippines Los Baños, the participants singled out azolla as still the most preferred farm-grown organic fertilizer for lowland rice fields. The delegates unanimously felt that the technology for in-farm utilization of azolla is already proven and available, the problem though is the lack of azolla strains that are better adapted to the prevailing agro-climatological condition in the country.

BIOLOGY OF AZOLLA

Taxonomy and Geographic Distribution

Azolla Lam. is a genus of aquatic fern with a worldwide distribution. There are six living species namely, *A. filiculoides* Lam., *A . caroliniana* Wild, *A. microphylla* Kaulf, *A. mexicana* Presl., *A. pinnata* R. Brown and *A. nilotica* Descaine ex Mettenius. It grows in ponds, ditches, and paddy fields. Prior to its dispersal by man, the species are endemic to the following areas: eastern N. America, southern S. America through western N. America, tropical and subtropical America, northern S. America through western America, upper reaches of the Nile to Sudan, most of Asia and the coast of tropical Africa. In most of these areas, the fern is regarded as a weed (Moore, 1969).

In the Philippines, there are sporadic collections of *A. pinnata* in Bontok and Lanao (Copoland, 1960) and in Palo, Leyte (herbarium record in the Museum of Natural History, UPLB).

Some of the common names of azolla are: water velvet, mosquito fern, duckweed (English); algen fern, wasserfarme (German); helechito del agua and helecho acuatico (Spanish); lu ping, ho ping, man chiang hung shu (Chinese); akaukikusa, koakaukkikusa, oakaukikusa (Japanese); cehak posknabey, chak pos kra bey, chak krahen (Khmer); nae harnghern (Laos); beo hoa dau, beo giau, beo dau (Vietnamese); and pako sa tubig (Philippines (Khan, 1988).

Life Cycle

Azolla is a heterogenous fern. It follows a heteromorphic-diplobiontic type of life cycle where an autotrophic diploid sporophyte generation alternates with an endosporic male and female haploid gametophyte generation. The Azolla plant represent the sporophyte generation (Fig. 1). Its body consists of a floating stem, usually dichotomously branching, with tiny alternately arranged fronds (or leaves). Simple roots hanging in the water occur at the nodes (Tan, *et.al.*, 1986).

At some stage during the growth of an Azolla plant, the branches break. The fragments then grow into individual plantlets. The fragmentation process results in rapid vegetative multiplication of the fern population. A population of *Azolla* double its biomass in 3-5 days under optimal condition.

The embryology of *A. pinnata* was described by Konar and Kapoor (1972). The sporophyte differentiate sporocarps at the

Archegoni are borne by the mega-gametophyte, while anteridia are borne by the micro-gametophyte. The egg cell develops within the archegonium while antherozoids are liberated from the antheridia. Fertilization is accomplished resulting in the development of embryonic sporophyte. The sporophyte floats to the surface of the pond after 1 or 2 leaves have differentiated.

An azolla plant produce two kinds of spores, microspores (commonly referred to as "male" spores) and megaspores (the ``female" spores). In the Philippines, sporulation occur during the cold season, from about November up to February of the following year. In some cases, sporulation may commence as early as September and last until May (Table 1). The intensity of sporulation is typically at its maximum during November up to December (Payawal an Paderon, 1986). The ratio between the number of megasporocarps and microsporocarps per plant is exceedingly variable. It varies between species and even monthly for a given population (Table 2). Naces (1989) have shown that the sporocarp ratio is altered by growth hormone treatments (Table 3). The addition of 0.1 to 100 ppm GA_3 to the culture medium tends to inhibit megasporangium development thereby inducing microsporangia development. The fertilized megaspore is very resilient and can withstand extreme desiccation (Xiao, et al 1987).

Table 1. Sporulation index[a] of azolla species under lowland field conditions.

SPECIES	MONTH											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep	Oct.	Nov.	Dec.
A-microphyla	57	30	31	17	12	0	0	0	28	28	51	48
A-caroliniana	0	0	0	0	0	0	0	0	59	70	53	43
A-mexicana	0	0	0	0	0	0	0	0	0	0	45	18
A-pinnata (Bangkok)	43	12	35	12	0	0	0	0	0	0	0	0
A-pinnata (Australia)	35	17	4	1	1	0	0	0	29	54	74	68

[a] $\text{Sporulation Index} = \frac{\text{number of plants bearing spores}}{100} \times 100$

The Azolla-Anabaena Symbiosis

The usefulness of azolla is on the fact that it is the only aquatic fern that fixes atmospheric nitrogen and converts it into a form useful to the rice plant. Azolla harbors with its leaf cavity colonies of *N-fixing cyannobacteria, Anabaena azollae* Strasburger. Nitrogen fixation occurs within special cells of the cyanobacteria's filament - the heterocysts. The number of heterocysts in a filament therefore directly determine the proficiency of N-fixation. In a healthy Azolla plant, heterocysts are developed only in large number from the 11th to the 20th leaf of a branch. The rate of N-fixation varies between fern species and under different growth conditions (Lumpkin and Plucknett, 1982). Moore (1969) reported that on the average, nitrogen fixation is 222 mg/day/gram dry weight. Twenty two continuous cropping of *A. pinnata* in one year under IRRI field condition yielded 8 t dry matter per hectare and 465 kg N/ha. The growth rate and N-fixing activity however fluctuated between the seasons (Watanabe, et al., 1980). In India, an annual gain of 800 kg N/ha was reported (Singh, 1979).

Table 2a. Sporocarp ratio of azolla species in La Trinidad, Benguet.

SPECIES	MONTH June Mega/ Micro	 July Mega/ Micro	 Aug. Mega/ Micro	 Sep. Mega/ Micro	 Oct. Mega/ Micro	 Nov. Mega/ Micro
A. pinnata (0038)	1/7	1/2	1/1	1/9	1/2	1/4
A. pinnata (7004)	5/1	0/1	0/1	0/1	0/1	0/1
A. filiculoides (1013)	1/1	1/1	1/3	1/3	1/3	
A. mexicana (2002)	-	-	-	-	-	2/1
A. caroliniana (3005)	1/1	-	-	1/1	2/1	1/1
A. microphylla (4014)	1/1	1/1	3/1	1/1	1/5	-
A. nilotica (5001)	2/1	-	-	-	-	1/1

Table 2b. Sporocarp ratio of azolla species in Los Banos, Laguna.

SPECIES	Jan. Me/ Mi	Feb. Me/ Mi	Mar. Me/ Mi	Apr. Me/ Mi	May Me/ Mi	June Me/ Mi	July Me/ Mi	Aug. Me/ Mi	Sep. Me/ Mi	Oct. Me/ Mi	Nov. Me/ Mi
A. pinnata (0038)	1/2	2/1	1/1	-	-	-	-		-	-	-
A. pinnata (7004)	-	-	-	-	-	-	-	-	-	-	1/35
A. filiculoides (1013)	1/1	1/1	-	-	-	-	-	-	-	-	-
A. mexicana (2002)	-	5/1	-	-	-	-	-	-	-	-	-
A. microphylla (4014)	-	1/1	1/1	2/1	-	-	-	-	-	-	-

[a]Me = Megasporocarp; Mi - Microsporocarp

Table 3. The effect of growth hormones on the sporocarp ratio fo UPLB-1 hybrid.

CONCENTRATION(ppm)	GA_3 Mega/Micro	IAA Mega/Micro
0.0	2/1	2/1
0.000	1/1	2/1
0.1	1/1	1/1
10.0	1/3	1/1
100.0	1/2	0/0

Infusion of the leaf cavity with the cyano-bacteria occurs during leaf initiation in the apical meristem. Thus, prior to the 11th leaf, the *Anabaena* filaments are in a state of vegetative development, and it is only at the 11th leaf that the colony begin to differentiate thick-walled heterocysts. From the 20th leaf, the *Anabaena* filaments enter the period of senescene -- so are the azolla fronds. The embryo sporophyte is infected with the cyanobacteria's spores during its emergence from the megasporal body. As the shoot pushes away the megasporal cap, it is inoculated with the spores of the cyano-bacteria (Chang, 1987).

Other Uses of Azolla

In addition to its utility as N-organic fertilizer for irrigated lowland ricefields azolla has other uses. Azolla can gainfully be used as feed supplement for poultry, ducks, and swine (Querubin, 1988). Research shows that azolla meal can be used up to 10% in swine starter ration, 20% in swine grower ration, and 15% in broiler ration without significantly affecting the performance of the test animals (Alcantara, 1985). Egg production and egg size were not significantly affected when fresh azolla was fed to mallard ducks up to 20% of the feed ration (Alejar, et al, 1984).

The following uses of azolla have been suggested or investigated (Alejar, et al, 1984).

- producing compost for upland crops;
- using *Azolla* to suppress weeds in rice paddies;
- extracting phosphorus from eutropicated water,with *Azolla* green manure or compost as a by-product;
- absorbing heavy metals from polluted water; and
- suppressing mosquito breeding.

AZOLLA VARIETAL IMPROVEMENT

When the azolla program of the government was launched in 1982, eight varieties of azolla were identified which are tolerant to high light intensity and high air temperature. These two climatic factors were recognized as the most fundamental constraints in the cultivation of the fern in the lowland. The designated strains were distributed to the different regional centers for field evaluation and eventual dissemination to provincial and community propagation ponds.

It has been shown incontrovertibly that azolla provides consequential benefits to lowland rice farmers. It has also been confirmed that its utilization nationwide had been very limited due to same environmental constraints (Watanabe, 1989; San Valentin 1988). That is to say that, the exotic azolla varieties that has been recommended have some biological imperfections *viz a viz* the local agroclimatic environment. Because azolla has a worldwide distribution, it is possible that the taxon has a wide latitude of genetic variability. Therefore, it is possible to generate improved hybrid lines that would be more tolerant than the exotics to environmental constraints.

The following are the identified biological constraints to the adaptation of the azolla technology.

1. Sporadic sporulation of the exotic varieties in the farmer's field.

   Azolla produces spore during the cold months-November, December, January, and February. These are dry months when there are no azolla in the rice field. Therefore, the fern's spores will not accumulate in the paddy soil. Thus, every planting season, thee farmer is compelled to get the live inoculants from regional or community propagation ponds, a task that is grossly inconvenient. Furthermore, the propagation ponds are very expensive to maintain. This is one of the major drawbacks that frustrate the initial attempt of the government to introduce the use of azolla as an organic fertilizer.
2. Susceptibility of the exotic varieties to endemic microbial pathogens and insect pests.
3. Narrow range of tolerance to sailinity and acid/alkaline condition.
4. Low tolerance to herbicide residue and low available phosphorus.

Realizing these constraints, a proposal for technical assistance was submitted to the Asian Development Bank (ADB) to finance the establishment of an Azolla Varietal Improvement Laboratory (AVIL) at the Institute of Biologicl Sciences, UPLB, in cooperation with the Bureau of Soils and Water Management, Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA), and the National Azolla Action Program (NAAP) of UPLB. The AVIL's objective is to develop genetically improved Azolla lines that will be tolerant to the various environmental constraints identified. In other words, the Laboratory will endeavor to develop agronomically viable azolla hybrids.

In-Vitro Fertilization Method

Because under lowland condition, azolla sporulates sporadically, then the first major problem was to establish a field station where the different varieties of the fern will regularly produce both

Fig. 2a. The immature phase of megaspore development. Note the megasporangium epidermis (1) completely enclosing the megaspore.

Fig. 2b. The mature-unfertilized phase of megaspore development. Note that the megasporangium epidermis has already been ruptured with the apical segment becoming a 'cap-like' appendage (1).

Fig. 2c. The mature-fertlizee phase of megaspore development. Note the cap (1), the extended floats (2), and the cluster of microspores adhering to the basal portion of megaspore.

Fig. 2d. The sprouting phase of megaspore development. Note the first leaf (1) emerging from the megaspore.

kinds of spores. Payawal and Paderon (1986) reported that low temperature is required for sporocarp induction. Thus, a field station solely for spore production was established at the Benguet State University, La Trinidad.

Azolla is a heterosporous nonflowering vascular plant. Therefore, the second problem was to develop an in-vitro method of hybridization. To successfully hybridize azolla, it is necessary to distinguish morphologically between the different developmental stages of the megaspore. This is because an azolla plant produces the microspores simultaneously. According to our observation, the megaspore passes through four distinct developmental stages, namely: (i) the immature phase, (ii) the mature-unfertilized phase, (iii) the mature-fertilized phase, and (iv) the sprouting phase.

Immature phase. The magaspore is fully enclosed by the megasporium's epidermis (Fig. 2a).

Mature-unfertilized phase. The megasporangium has grown in lenght and in breadth thereby rupturing the megasporangium's epidermis at the equatorial portion. The apical segment becomes a cap-like sructure separated from the basal portion which is commonly firmly attached to the basal part of the megaspore. The floats, which developed on the apical portion of the megaspore, are visibly thickened but not fully expanded (Fig. 2b).

Mature-fertilized phase. The fiscals of the megaspore are fully extended. The cap is still firmly attached to the apex. One to several microspores are firmly adhering to the basal part of the megaspore. The megaspore may show some signs of greening (Fig. 2c).

Sprouting phase. The cap is now displaced by the emerging first leaf. The leaf is fan-shaped and may or may not be chlorophyllous (Fig. 2d). At this stage, the embryonic sporophyte is still firmly enclosed by the megaspore. This phase is followed by the full emergence of a free living Azolla sporophyte.

To facilitate in-vitro fertilization, mature-fertilized megaspores are collected and placed in a petri dish with distilled water or culture solution. The mature microsporocarps are collected and dried resulting in the rupturing of the microspores. The megaspores and the microspores are then mixed together in the peri dish. Within a month, if fertilization is successful, embryo sporophyte will be emerging.

The UPLB Azolla Hybrid Lines

Twelve lines of azolla hybrid has been produced to date (Table 4). Five of the hybrid strains have been analyzed for their isozyme patterns. The result conclusively indicates that they are true hybrids (Baculod, 1988). These are (1) UPLB-4 line 1, (2) UPLB-4 line 3, (3) UPLB-5 line1, (4) UPLB-5 line 3, (6) UPLB-6 line 3. (Complementary analysis of isozyme patterns conducted by the Soil Microbiology Laboratory, International Rice Research Institute of the putative hybrid lines have so far confirmed that UPLB-1 is a true hybrid).

Table 4. Putative azolla hybrids produced at the Institute of Biological Sciences, UPLB.

HYBRID	PARENT (Female)		PARENT (Male)
UPLB-1 (1 line)	*A. microphylla* 94018)	x	*A. mexicana* (2001)
UPLB-2 (7 lines)	*A. mexicana* (2002)	x	UPLB 1
UPLB-3 (8 lines)	*A. microphylla* (4018)	x	UPLB-1
UPLB-4 (3 lines)	*A. microphylla* (4018)	x	*A. filiculoides* (1009)
UPLB-5 (3 lines)	*A. microphylla* (4003)	x	*A. caroliniana* (3004)
UPLB-6 (4 lines)	*A. microphylla* (4003)	x	*A. caroliniana* (3004)
UPLB-7 (14 lines)	*A. microphylla* (4007)	x	UPLB-1
UPLB-8 (2 lines)	*A. microphylla* (4003)	x	UPLB-1
UPLB-9 (1 line)	UPLB-1	x	*A. microphylla* (4003)
UPLB-10 (1 line)	UPLB-1	x	*A. microphylla* (4018)
UPLB-11 (1 line)	*A. microphylla* (4007)	x	*A. microphylla* (4018)
UPLB-12 (1 line)	*A. microphylla* (4007)	x	*A. microphylla* (4003)

The first hybrid (UPLB-1) is a cross between *A. microphylla* 4018 and *A. mexicana* 2001.UPLB -1 hybrid is not temperature dependent to sporulate, thus, it is capable of producing microspore and megaspore all year round (Table 5). This is the only known strain of azolla with this superior characteristic. Spores of UPLB-1 that accumulated in pond soil after a year of continuous cultivatation germinated even after the soil samples were air dried for months. The megaspores buried in the soil retain their viability and within a week of re-hydration, the megaspores germinated. This implies that continuous cultivation of UPLB-1 in ricefields for at least a year will result in the persistence of the fern population . The need to maintain a propagation pond in the farmer's field, or for the farmers to transport sacks of planting materials every planting season can be averted.

(Samples of UPLB-1 are presently distributed to the different NAAP Regional Centers for evaluation and eventual distribution. Materials were provided to the Department of Botany, Burdwan University, West Bengal, India, for evaluation and eventual dissemination. Personal communicarition from Dr. D.P. Kushhari indicates that the hybrid is doing very well and will soon be used as one of the varietal materials for field dissemination.)

Table 5. Sporulation index and the megasporocarp to microsporocarp ration in UPLB-1 hybrid under lowland condition (1989).

MONTH	MEGAS-POROCARP	MICROS-POROCARP	SPORULA-LATION INDEX[a]
January	1	0	25
February	2	1	82
March	2	1	66
April	4	1	88
May	1	1	92
June	6	1	21
July	1	1	188
August	1	1	188
September	1	2	76
October	1	1	52
November	1	1	188
December	1	1	188

[a]Sporulation Index = $\frac{\text{number of plants bearing spores}}{100} \times 100$

The sporulation index for the other putative azolla hybrids is given in Table 6 while the megaspore to microspore ratio is presented in Table 7.

Screening of UPLB-2 hybrid lines (UPLB-1 x *A. mexicana* 2002) by Dr. Rosalinda P. Garcia indicates improved resistance to infestation of the three most virulent pathogen of azolla, namely, *Sclerotium* sp., "Rhizoctonia-like" isolate-2 and isolate-8 (Fig 3,4, and 5).

The webworm *Ephestiopsis vishnu* is one of the most serious pest of azolla in the Philippines (Baculod, 1988). Screening of the hybrid lines by Dr. Venus Calilung indicates that some UPLB-2 hybrid lines have developed resistance to this pest (Table 8).

On going germination experiments under varying pH condition (from pH 3 to pH 9) involving soil samples containing fertilized megaspores of UPLB-1 hybrid indicate that the pH of the water medicine does not affect the germination of spores. The seedlings are presently maintained under the same growth condition to screen for acid/alkaline tolerant hybrid lines.

Table 6. Sporulation index for the other putative UPLB-hybrids under lowland condition (1989).[a]

HYBRID	MONTH										
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
UPLB-2_1	-	-	22	33	-	-	-	-	-	-	-
UPLB-2_2	-	-	56	27	-	-	58	-	-	-	-
UPLB-2_3	18	22	78	23	-	7	-	-	-	-	-
UPLB-2_6	18	-	96	70	4	-	-	-	-	-	15
UPLB-2_7	5	-	42	63		-	-		-	-	-
UPLB-3_2	-	-	54	53	-	-	-	-	-	-	-
UPLB-3_4	-	28	57	-	-	-	-	-	-	-	-
UPLB-3_8	-	-	-	93	-	97	-	-	-	-	-
UPLB-3_9	-	-	-	93	-	-	-	-	-	-	20
UPLB-4_7	-	-	97	60	-	19	63	45	-	48	80
UPLB-5_2	-	-	-	17	-	-	-	-	-	-	-
UPLB-5_3	15	-	100	97	-	100	57	100	50	68	100
UPLB-6_1	-	-	-	-	-	-	-	-	-	-	48
UPLB-6_2	54	72	50	87	-	-	-	-	-	30	-
UPLB-6_3	-	-	87	37	-	-	-	-	-	-	-
UPLB-7_1	-	-	80	87	-	-	-	-	-	-	-
UPLB-7_3	-	-	-	-	-	-	-	-		-	40
UPLB-7_4	-	-	-	-	-	100	56	45	-	-	35
UPLB-7_5	-	-	13	37	-	-	-	-	-	-	30
UPLB-7_6	-	-	56	-	-	-	-	-	-	-	-
UPLB-7_8	-	-	-	98	60	37	60	-	-	-	-
UPLB-7_9	-	-	-	80	17	-	-	55	-	-	90
UPLB-7_{10}	-	-	-	87	40	-	-	-	-	-	-
UPLB-7_{12}	-	-	-	20	-	-	-	-	-	-	-
UPLB-10	38	-	50	37	-	-	-	-	-	-	-

[a] The following Azolla hybrids did not sporulate: UPLB-3_1, UPLB-3_3, UPLB-3_5, UPLB-3_7, UPLB-4_1, UPLB-4_3, UPLB-5_1, UPLB-6_4, UPLB-7_2, UPLB-7_7, UPLB-7_{11}, UPLB-$_{13}$, UPLB-7_{14}, UPLB-8_1, UPLB-8_2, UPLB-$_9$, UPLB-$_{11}$, and UPLB-$_{12}$.

$$\text{Sporulation Index} = \frac{\text{number of plants bearing spores}}{100} \times 100$$

Complementary to the azolla breeding work, the technique to replace the native cyano-bacteria symbiont of a given azolla variety with another strain to potentially enhance its N-fixing capability is being attempted. Presently, the laboratory researchers are already successful in developing an Anabaena-free azolla plant. This is a preliminary step towards the development of a new Azolla-Anabaena symbiont.

Table 7. Sporocarp ratio of the other putative UPLB-hybrids under Los Baños condition[a]

HYBRID	MONTH										
	Jan.	Feb.	Mar.	Apr	May	June	July	Aug.	Sep.	Oct.	Nov.
	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi	Me/ Mi
UPLB-2_1	-	-	1/1	0/1	-	.	.	.	.		-
UPLB-2_2	-	-	2/1	6/1	-		1/1	-			-
UPLB-2_3	2/1	3/1	1/1	1/1	-	1/0	-	-	-	-	
UPLB-2_6	1/1	-	2/1	1/1	1/1	.	-	.	-	-	1/1
UPLB-2_7	2/1	-	2/1	2/1	.	.	-	.	-	-	.
UPLB-3_2	-	-	1/2	1/2	-	-	-	-	-	-	-
UPLB-3_4	-	3/1	2/1	.	-	.	.	.	.	-	.
UPLB-3_6	-	-	-	2/1-	2/1	-	-	-	-	-	-
UPLB-3_8	-	-	2/1	-	-	-	-	-	.	-	3/1
UPLB-4_2	-	-	1/1	2/1	-	2/1	4/1	1/1	-	2/1	.
UPLB-5_2	-	-	-	1/0	-	-	-	-	.	-	.
UPLB-5_3	3/0	.	3/1	2/1	-	2/1	3/1	1/1	1/1	2/1	2/1
UPLB-6_1	-	-	.	-	-	-	-	-	.	-	12/1
UPLB-6_2	2/1	2/1	2/1	3/1	-	-	-	-	-	2/1	-
UPLB-6_3	-	-	4/1	4/1	-	-	-	-	.	.	-
UPLB-7_1	-	-	2/1	4/1	-	-	-	-	.	.	-
UPLB-7_3	.	.	-	-	-	-	-	-	-	.	4/1
UPLB-7_4	.	.	-	-	-	3/1	5/1	4/1	.	.	4/1
UPLB-7_5	-	-	1/1	-	-	-	-	-	-	-	-
UPLB-7_7	-	-	-	2/1	-	-	-	-	.	-	-
UPLB-7_8	-	-	-	2/1	2/1	3/1	4/1	-	-	.	-
UPLB-7_9	-	-	-	4/1	5/1	-	-	3/1	-	-	1/1
UPLB-7_{10}	-	-	-	4/1	4/1	-	-	-	-	-	-
UPLB-7_{12}	-	-	-	1/0	-	-	-	-	-	.	-
UPLB-7_{10}	3/1	-	2/1	4/1	-	-	-	-	-	-	-

[a]The following Azolla hybrids did not sporulate: UPLB-3_1, UPLB-3_3, UPLB-3_5, UPLB-3_7, UPLB-4_1, UPLB-4_3, UPLB-5_1, UPLB-6_4, UPLB-7_2, UPLB-7_7, UPLB-7_{11}, UPLB-7_{13}, UPLB-7_{14}, UPLB-8_1, UPLB-8_2, UPLB-9, UPLE-11, and UPLE-12.
Me = megasporocarp; Mi = mcirosporcarp

Table 8. Fresh weight of azolla UPLB-2 hybrid populations after 10 days of infestation (DAI) of mixed larval instars of the webworm *Ephestiopsis vishnu* (Initial weight = 15 g).

STRAIN	NONINFESTED CONTROL	INFESTED			MEAN WT. (10 DAI)	PER CENT WEIGHT LOSS
		1	2	3		
UPLB-21	17.2	15.9	15.2	10.5	13.9	7.5[a]
UPLB-22	21.1	5.8	8.5	5.3	6.5	56.5
UPLB-23	18.34	13.2	7.8	11.9	11.0	26.9
UPLB-24	25.8	15.8	12.6	14.1	14.2	5.5[a]
UPLB-25	15.4	11.5	13.7	6.6	10.6	29.3
UPLB-26	18.9	3.1	10.5	10.2	7.9	47.1
UPLB-27	20.4	11.7	18.5	8.9	13.0	13.3[a]

[a]Resistant to webworm infestation.

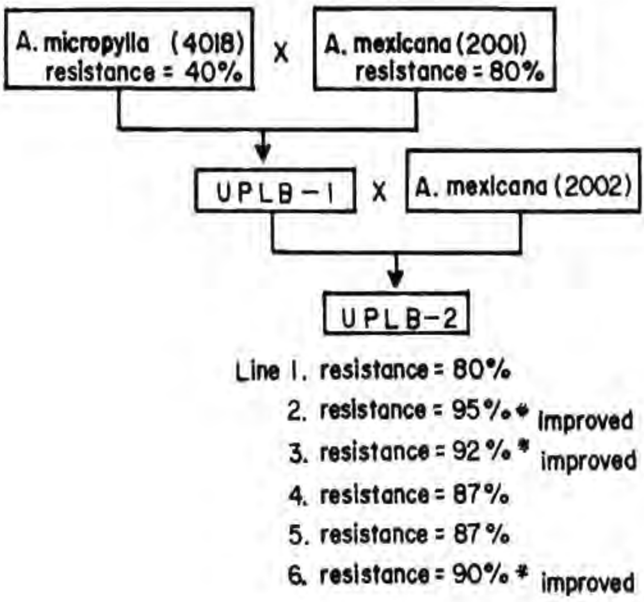

Fig. 3. Schematic diagram illustrating the development of resistance to velerotium sp. by hybridization.

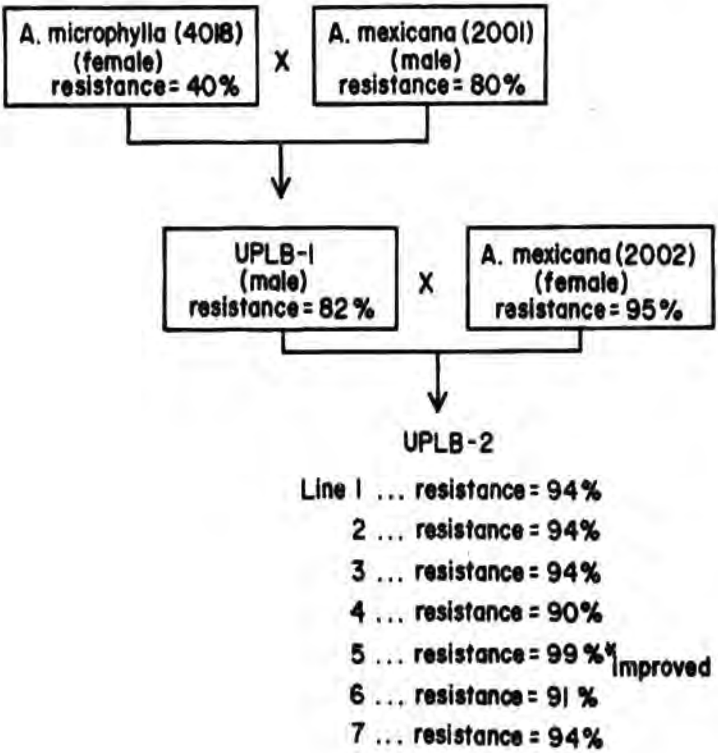

Fig. 4. Schematic diagram illustrating the development of resistance to "Rhizoctonia-like" isolate # 2 through hybridization.

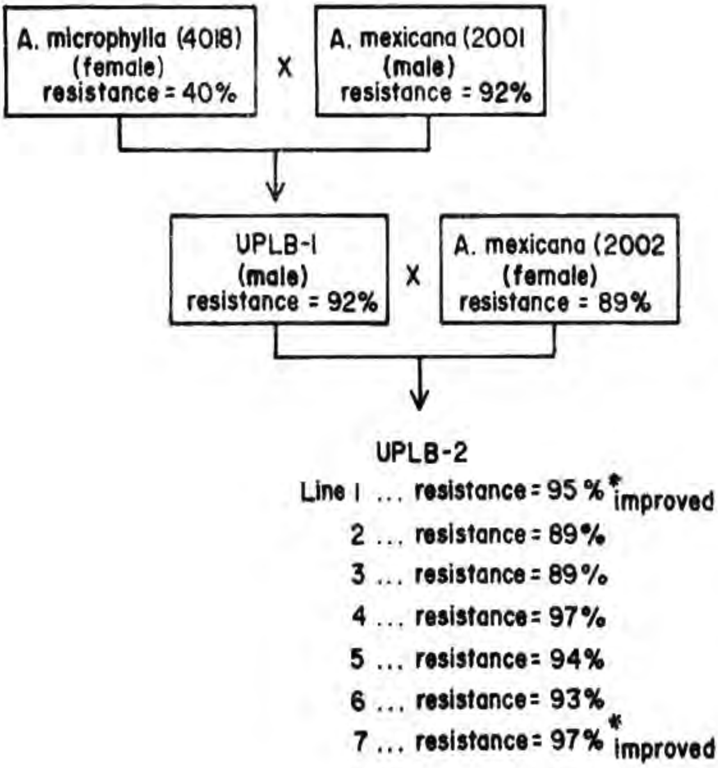

Fig. 5. Schematic diagram illustrating the development of resistance to "Rhizoctonia-like" isolate # 8 through hybridization.

SUMMARY

The in-vitro breeding technique that was developed was employed to generate azolla hybrid lines utilizing four species of azolla, namely, *A. microphylla* x *A. mexicana, A. microphylla* x *A. filiculoides, A. microphylla* x *A. caroliniana* and *A. filiculoides* x *A. pinnata*. All of the attempted crosses except the last one were successful. The different hybrid populations are being screened for desirable agronomic characteristics.

Some of the hybrids have already shown useful characteristics such as the capacity to sporulate all year round, resistance to pest and diseases, and potential tolerance to extremely acid or alkaline conditions.

REFERENCES

Alcantara, P.F. 1985. Feeding value of azolla meal for poultry and swine. Paper presented during the Agricultural Policy Workshop on Organic and Inorganic Fertilizer. 18 January 1985, University of the Philippines at Los Banos, Philippines. 12pp.

Alejar, A.S., B. B. Mabbayad, and **R.P. Escobin.** 1984. Riceduck integrated farming system Mimeographed progress report to the National Azolla Action Program. 11 pp.

Baculod, P. 1988. Electrophoretic analysis of soluble proteins and enzymes from selected of azolla and their hybrids. M.S. Thesis, University of the Philippines at Los Banos.

Copeland, E. D. 1960. Fern flora of the Philippines. Bureau of Printing, Manila 557 pp.

Khan, M. M. 1988. Azolla agronomy. SEAMEO Regional Center for Graduate Study and Research in Agriculture (SEARCA) Publication. 153 pp.

Konar, R. N. and R. K. Kapoor. 1972. Anatomical studies on Azolla pinnata. Phytomorphology. 22: 221-223.

Lin Chang, 1987. Study on the association between Anabaena azollae Strasburger and Azolla microphylla Kaulfuss during generation of megasporoscarps. Ph. D. Dissertation. University of the Philippines at Los Banos. 91 pp

Lumpkin, T. A. and D. L. Plucknett. 1982. Azolla as a green manure use and management in crop production. Westview Press., Boulder, Colorado, pp.25-30.

Mabbayad, B. B. 1987. The azolla program of the Philippines. In: Proceeding of the Workshop on Azolla Use. Fuzhou, Fujian, China. 31 March-5 April, 1985. pp. 101-108.

Moore, A. W. 1969. Azolla: biology and agronomic significance. Bot. Rew. 35:17-34.

Naces, J, L. 1989. The effect of hormones on the pattern of sporangial development and morphology of the UPLB Azolla hybrid 1.B.S. Biology Thesis. University of the Philippines at Los Baños. 71 pp.

Payawal, P. C. an E.M. Paderon. 1986. Sporocarp formation and spore germination of Azolla strains in thePhilippines. Phil. Agr. 69: 633-643.

Querubin, L. J. 1988. Research on azolla as livestock feed in the Philippines. *In:* Proceedings Regional Workshop on Azolla Esearch and Development. 9-13 May 1988, SEARCA, Los Baños, Laguna. pp. 117-119.

San Valentin, C. O. 1988. Azolla research in the Philippines under the National Azolla Action Program. *In:* Proceedings Regional Workshop on Azolla Research and Development. 9-13 May, 1988, SEARCA, Los Baños, Laguna. pp. 77-80.

Scharpenseel, M. W. and K. Knuth. 1987. Use and importance of Azolla-Anabaena in industrial countries *In*: Proceedings of the Workshop on Azolla Use. Fuzhou, Fujian, China, 31 March-5 , April1985. pp. 153-167.

Singh, P. K. 1979. The use of Azolla in rice production in India. *In:* Nitrogen and Rice. IRRI,Los Baños, Laguna. 409-418 pp.

Tan, B. C., P. C. Payawal, I. Watanabe, N. Lacdan and **C. Ramirez.** 1986. Modern taxonomy of azolla: a review. Phil. Agr. 69: 491-512.

Watanabe, I. 1989. Physiology and agronomy of Azolla-Anabaena symbiosis. *In*: Proceedings Regional Workshop on Azolla Research and Development. 9-13 May 1988, SEARCA, Los Baños, Laguna. pp. 109-116.

Watanabe, I. , S. Berja, and D. J. de Rosario. 1980. Growth of Azolla in paddy field as affected by phophorous fertilization. Soil Sci. Plant Nutri. 26: 301-307.

Xiao Qing-yuen, Shi Yan Cuang-Li, and Peng Ke-lin. 1987. Germination of Azolla filiculoides Lam. sporocarps and factors affecting their growth. In: Proceedings of the Workshop on Azolla Use. Fuzhou, Fujian, China. 31 March- 5 April, 1985. pp. 31-38.