Megasporocarps of *Azolla* and their germination in varied paddy soils

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Abstract

Seven species of *Azolla, A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A . rubra, A. microphylla*, *A. nilotica* and *A. pinnata,* were cultivated in Khurda, Orissa (84?W to 85?E Long. and 19? to 20? N Lat.). Multi-branched tiny water fern *Azolla* sps. were grown in 4mx2m sized plots. The plots with *Azolla* were left for mat and sporocarp formation in January to March (2000-2002). At 30, 45, 60 and 75 days after inoculation (DAI), sporocarp count was made to record average per cent of sporocarp bearing plants and average number of plants giving 100 megasporocarps. The post harvest operations like collection, separation, up-gradation, cleaning, drying and storage management of megasporocarps were also carried out. The mature and full sized megasporocarps of *A. caroliniana, A. mexicana, A. microphylla, A.nilotica,* and *A. pinnata* were incubated in paddy soils with varied pH. The soil samples were collected from various places/states of India. Under Khurda condition, 5 species *(A. caroliniana*, *A. mexicana, A. microphylla, A. nilotica* and *A. pinnata)* produced a good number of sporocarps (52-76%) in prolonged mat–forming situation, but 2 other species (*A. filiculoides* and *A. rubra*) didn't produce any sporocarps. Soils of Thottapallikari, Kerala (pH, 4.3) and soils of Karnal, Haryana (pH, 8.3) were not suitable for emergence of plantlets from megasporocarps, whereas soil pH 6.1 to 7.6 of different States such as Orissa, West Bengal and Andhra Pradesh were favourable for emergence and establishment of young seedlings of *Azolla*.

Media Summary

Azolla is an ideal biofertilizer for rice crop and its biomass could be raised from megasporocarps as primary inoculum under adverse situation in coastal paddy soils of India.

Keywords

Vegetative fronds, sporulation, acidic soil, germination frequency, survival of young seedlings.

Introduction

The majority of tropical paddy soils of Asia are deficient in nitrogen and organic matter (Kawaguchi and Kyuma 1977). Role of organic matter in relation to increased rice production and soil fertility is well recognized (Tanaka 1978). The *Azolla–Anabaena* consortium, a promising biological system, is beneficial in contributing nitrogen and organic matter for lowland rice. As regards the biomass production, and quantity of nitrogen fixation and nutrient recycled, *Azolla* is highly efficient, cost effective and ecologically sound bio-fertiliser (Singh *et al* 1990; Watanabe and Liu 1992; Wagner 1997; Pabby *et al*. 2003). To produce *Azolla* inoculum in paddy fields, its vegetative fronds in large scale are required but there are several physical constraints in *Azolla* production and utilization. The thick wall of megasporocarp can withstand high temperature, drought condition and pest attack (Nayak and Singh 1988). Proper utilization of biological nitrogen fixation by the system depends on various factors like soil pH, temperature, light, etc. Hence, the objective of this study was to evaluate the sporocarp production at local condition and to determine the effect of soil pH on percent germination of megasporocarps and on survival of young seedlings using paddy soils of different pH collected from various states of India.

Materials and methods

Seven species *Azolla,* A*. caroliniana*, *A. filiculoides, A. mexicana, A. rubra, A. microphylla , A. nilotica, and* A*. pinnata,* collected from Central Rice Research Institute, Cuttack, Orissa having accession codes, were cultivated in P. N. College, Khurda, Orissa ($84^0$20′ West to $85^{0.}$42′ East Long. and $19^0$12′ to $20^0$10′ North Lat.) from 2000 to 2002. Seven numbers for each species were multiplied in an RBD (4m? 2m size multiplication plots) with 30 cm water tight bunds and interception of 50 cm water channel in a paddy field (soil haplaquept clay loam, pH, 6.4, organic C, 0.79%, total N, 0.09% and available P, 8.9 mg/kg). One hundred grams of vegetative fronds of each species were used for multiplication of *Azolla* in field condition, after sterilization with 0.01% mercuric chloride. Single super phosphate @ 3.5 kg/ha and furadon (Carbofuran, 3G) @ 2.5 kg/ha were applied. A standing water of 6 cm depth was maintained. Then multiplication plots with *Azolla* were allowed for mat formation and for sporulation without administering P fertilizer and pesticide for 75 days. Collection, separation, up-gradation, cleaning, drying and storage of megasporocarps were carried out. Undesirable materials like grass culms, empty husks and weed seeds, etc. were separated with test sieves. Unripe, chaffy, shrivelled, or unusually small-sized megasporocarps were discarded by winnowing to select the full-sized megasporocarps (Fig. 2 a,b). Stages of germination of megasporocarps were recorded (Fig. 2c,d,e and 3a,b). Under Khurda condition, average percent of sporocarp bearing fronds and average number of fronds giving 100 megasporocarps were recorded in mat forming condition at 30^{th}, 45^{th}, 60^{th} and 75^{th} days of inoculation. Megasporocarps were kept in field soils up to 3 years. The mature and full sized megasporocarps of *A. caroliniana, A. mexicana, A. microphylla* and *A. pinnata* were placed in earthen pots having different soil samples collected from the Division of Agricultural Soil Chemistry, Central Rice Research Institute, Cuttack. The soil belonged to different places/states of India, such as Thottapallikari , Kerala (pH, 4.3) Khopadan, Kerala (pH, 5.2), Burdwan , West Bengal (pH, 6.1), Baragarh, Orissa (pH , 6.3), Khurda , Orissa (pH , 6.4), CRRI, Cuttack , Orissa (pH, 6.5), Berhampur , Orissa (pH 7.1), Hyderabad , Andhra Pradesh (pH, 7.4) and Karnal, Haryana (pH, 8.3). At the cotyledonary leaf stage, young seedlings were placed in N-free sterile IRRI-nutrient media.

Results

Among the 7 species of *Azolla* grown in multiplication plots (Fig. 1a,b,c) under Khurda condition, *A. filiculoides* and *A. rubra* did not form sporocarps whereas a good number of sporocarps were observed in *A. caroliniana, A. mexicana, A. microphylla, A. nilotica* and *A. pinnata* in prolonged mats (Fig. 1d,e,f) at 60^{th} DAI during January to March (Table 1). Similarly, lower average numbers of fronds contributing 100 megasporocarps were observed in *A*. *microphylla* and *A. pinnata.* The average percentage of emergence of plantlets from megasporocarps was higher (70-80%) in Khurda (pH, 6.4) and CRRI, Cuttack (pH, 6.5) soils. In Bargarh (pH, 6.3) and Berhampur soils (pH, 7.1), the germination rates were above 60% (Table 2). In Hyderabad soil (pH, 7.6) germination rates were stagnant at 24%. In very acidic soil of Thottapallykari (pH, 4.3) and alkaline soil of Karnal (pH, 8.3) germination was nil. In Khopadan soil (pH, 5.2), germination was meagre and the young seedlings that were emerged did not survive. Nitrogen-free IRRI nutrient medium was suitable for nourishing young seedlings at the cotyledonary leaf stage (Fig. 2 d,e and 3c).

Table 1 :Record of sporulation in *Azolla* sps. (A–average percent of sporocarp bearing fronds; B-average no. of fronds giving 100 megasporocarps).

	No. of days after inoculation (DAI)							
Sps.	30 DAI		45 DAI		60 DAI		75 DAI	
	(A%)	(B)	(A%)	(B)	(A%)	(B)	(A%)	(B)
A. caroliniana	21.0	54.5	38.4	45.0	57.2	37.8	50.5	37.0

A. filiculoides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. mexicana	14.6	77.5	35.6	47.5	51.8	33.3	40.0	62.3
A. rubra	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. microphylla	24.0	63.6	48.5	41.2	76.3	22.8	61.0	25.6
A. nilotica	20.5	70.8	42.5	50.6	65.5	33.3	54.5	43.5
A. pinnata	22.8	61.6	46.8	48.3	70.5	25.8	60.0	28.5
CD (5%)	3.2	2.7	3.1	2.4	2.0	1.8	2.9	3.7

Table 2: Effect of soil pH on average percent of germination of megasporocarps (G) and average percent of survival of young seedlings of *Azolla* sps (S)

		Species							
Place/State	**pH**	***A. caroliniana***		***A. mexicana***		***A. microphylla***		***A. pinnata***	
		%G	*%S*	*%G*	*%S*	*%G*	*%S*	*%G*	*%S*
Khopadan, Kerala	5.2	0.0	-----	4.67	0.0	7.0	0.0	7.3	0.0
Burdwan, West Bengal	6.1	25.3	52.7	40.5	60.2	41.0	54.0	45.4	59.3
Khurda, Orissa	6.4	68.6	82.5	70.0	78.0	75.8	80.0	73.8	79.0
CRRI, Cuttack, Orissa	6.5	73.5	79.0	72.5	77.33	71.6	75.2	70.8	78.6
Berhampur, Orissa	7.1	61.2	63.6	62.5	65.0	70.4	62.0	65.0	62.8
Hyderabad, Andhra Pradesh	7.6	22.5	25.6	21.6	48.4	27.4	50.2	24.5	47.4

Karnal, Haryana	8.3	0.0	-----	0.0-	----	0.0	-----	0.0	-----

(a) Preparation of multiplication plots

(b) Growth of *Azolla* in multiplication plots

(c) Mat –forming fronds of *Azolla*

(d) Sporocarp formation in *A. pinnata*

(e) Sporocarp formation in *A.caroliniana*

(f) Sporocarp formation in *A. nilotica*

Figure 1 Growth and sporocarp formation of *Azolla* sps. in lowland paddy field.

(a) Processed megasporocarps

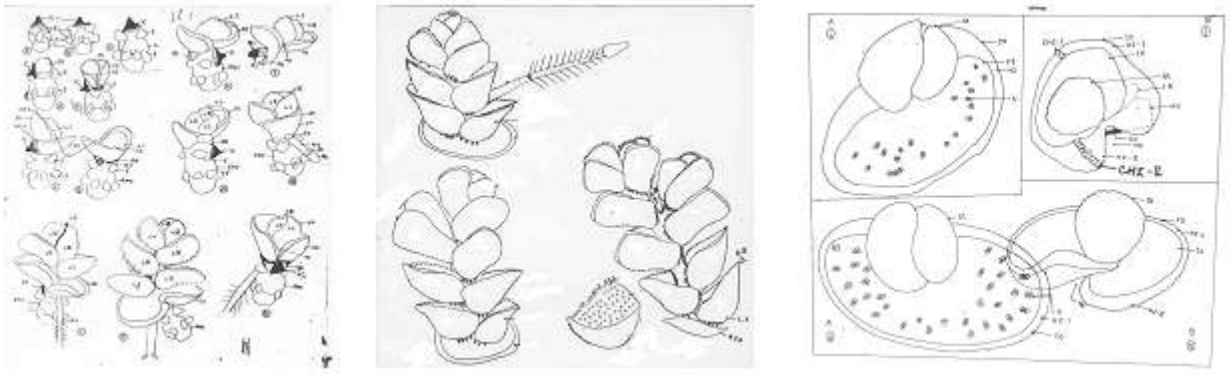
(b) A full size mature megasporocarp

(c) Emergence of a cotyledon

(d) First leaf inside the cotyledon

(e) Detached young plant

Figure 2 Megasporocarps and process of germination.



(a) Stages of germination of megasporocarp **(b) Stand establishment of young seedlings** **(c) Detached seedlings at cotyledonary stage**

Figure 3 Germination process and young seedlings of *Azolla* (Camera –lucida drawings).

Discussion

Sporocarp survey indicated that species like, *A. microphylla, A. nilotica, A. caroliniana and A. pinnata* were efficient sporocarp-producing species under Khurda condition. Prolonged mats in winter for 60 to 75 days resulted in higher yield of megasporocarps. Due to slow initial growth of young seedlings, mega sporocarps were not directly broadcast into a rice field (Singh et al. 2001). However, for the exceptional physical characters like thick wall and texture, megasporocarps were ideal for germplasm preservations and plantlets could be raised as primary inoculum in nursery-bed in adverse agro-ecological climates. Both the highly-acidic soil (pH, 4.3) and the alkaline soil (pH, 8.3) were observed to be deleterious for germination of megasporocarps. Soil pH of 6.0 to 7.6 belonging to coastal states of India, such as Orissa, West Bengal, and Andhra Pradesh were observed to be favourable for germination of megasporocarps and establishment of young seedlings.

Conclusion

From this experiment, it can be concluded that medium acidic paddy soils of coastal India are suitable for germination of megasporocarps of *Azolla*. The local species, *A. pinnata,* is also ideal for sporocarp yield and germination under local rice field condition.

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