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Studies on Morphometric Evaluation of Symbiotic Cyanobiont in two Species of Azolla Fern

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ABSTRACT

Azolla is very known biofertilizer for rice cultivation it can enrich soil N_2 content and for the soil fertility. In view of this, an invitro study was carried out on morphometric studies on Anabaena azollae present in two species of Azolla fern (A.microphylla, and A. fulicolides). The selected Azolla species were grown in standard medium prepared with garden soil, cow dung, super phosphate and its combinations in separate containers. The matured Azolla cultures were observed for its growth profile viz. number of leaflets per plant and fresh & dry weight of whole plant on 15d and 21d. Followed by, the morphometric study on symbiotic cyanobacteria present in the leaf cavities of two species of Azolla was determined through observing average number of cyanobaterial filament in the leaf cavity of azolla, length of each cyanobacterial filament, number of vegetative cells and number & position of heterocyst in each cyanobacterial filament.

Introduction

The ever increasing world population and shrinking agriculture land call for boosting agriculture productivity. Two broad options to satisfy this demand are use of high yielding crop varieties (HYVs), and judicious use of agrochemicals. Modern agriculture practices are resorting to increasing use of agrochemicals for augmenting productivity but compelling to compromise with their hazardous impacts on environment and non-target organisms. In view of this, deploying nitrogen fixing microorganisms as biofertilizers has emerged as eco-friendly option to increase crop yield (Brouers M *et al.*,1987; Mahalingam *et.al*.,2014).

Azolla (Salviniaceae) is a genus of heterosporous water ferns which contain an endophytic cyanobacterium, Anabaena azollae belongs to the family of Nostocaceae (Moore, 1969). In This photosynthetic associations Azolla fix CO2 by the Calvin cycle (Ray et al., 1979) and the *Anabaena* can supply their total N requirement by the fixation of atmospheric N2 (Peters and Mayne, 1974a,b; Ray et al., 1978; Peters, Ray et al., 1980; Peters, Toia et al., 1980). The potential of these symbiotic associations as an alternative nitrogen source in rice culture was documented (Watanabe et al., 1977; Talley and Rains, 1980)

The sporophyte of *Azolla* is 10-40 mm in diameter (10-25 mm in the case of *Azolla filiculoides*) and multi branched. Each branch includes a stem with bilobed leaves and adventious roots. Abscission of a branch or root allows fragmentation of plants and facilitates vegetative propagation (Rao 1935; Konar and Kapoor 1972; Addicott1 982; Peters and Calvert 1983).

The leaves of *Azolla* are sessile, alternate, often imbricate, in two ranks along upper side of the stem, 0.6–2 mm wide. Each leaf has an emerged, thick, greenish or reddish, and photosynthetic dorsal lobe and a very thin, immersed hyaline ventral lobe. The dorsal lobe has an ellipsoid cavity, measuring approximately 0.15 × 0.30 mm, that opens to the external environment through a pore, surrounded by two cell layers, located in the adaxial epidermis of the leaf cavity (Braun-Howland and Nierzwicki-Bauer, 1990).

The leaf cavity, an extracellular compartment formed by an in folding of the adaxial epidermis during development, contains an endosymbiotic community composed of a heterocyst-forming and N2-fixing filamentous cyanobacterium – *Anabaena azollae* Strasburger – (first described by Strasburger (Peters and Meeks, 1989; Adams, 2000).

The agronomic importance of *Azolla* is related to its ability to grow very successfully in habitats where little or no combined nitrogen is available (Macale & Vlek 2004). *Azolla* is an excellent biofertilizer and green manure having global distribution. Ability of *Azolla-Anabaena* system to fix atmospheric nitrogen at faster rates makes it an outstanding agronomic choice for the cultivation of rice under tropical conditions. Nitrogen fixation potential of the *Azolla-Anabaena* system has been estimated to be 1.1 kg N ha–1 day–1 and one crop of *Azolla* provided 20-40 kg N ha–1 to the rice crop in about 20- 25 days (Watanabe *et al*., 1977; Peter and meeks, 1989).

Therefore, the present study was aimed of morphometric identification of symbiotic cyanobionts in two species of Azolla cultures.

Materials and Methods

Collection and maintenance of *Azolla* culture

Two Azolla cultures used for the study ie., Azolla microphylla and A. filiculoides were collected from the Tamilnadu Agricultural University, Coimbatore, and maintained as pre culture in the Department of Biology, Gandhigram Rural Institute-Deemed University, Gandhigram, Tamil Nadu, India.

Mass cultivation of *Azolla* culture (Thangam and Mahalingam., 2010)

The *A. microphylla* and *A.filiculoides* were mass cultured individually in the large plastic containers containing 1 Kg of fine red soil had been filled and slurry was made by mixing 2kg of cow dung, 15gm of super phosphate in 5 liters of water.

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Then, *Azolla* of ½ kg spreaded over the prepared bed. The culture is allowed to grow for 21 days. The optimum temperature of about 25^{o} C and pH of 5.0 to 5.7 was maintained for 21 days.

Study on growth parameters of two species of Azolla

Two species of Azolla cultures grown in various treatments were separately collected and analysed for various growth parameters such as number of leaf lets per plant and fresh & dry weight of whole plant using standard procedures.

Number of leaflets

The leaf lets in each plant of A. microphylla and A.fuliculoides grown various treatments were physically counted at 15 & 21 days and the results were recorded.

Total fresh weight and dry weight of plants

The fresh weight of two different species of Azolla, A. microphylla and A.filiculoides grown in various treatments were estimated using physical balance and record the value at 15 & 21 days. Followed by, the two Azolla cultures were collected and dried under oven at 50oC by gradually increasing the temperature up to 120o C for every two hrs for 24hrs with dry biomass was weight using physical balance and record the dry biomass at 15 & 21 days.

Morphometric Evalution of symbiotic cyanobionts *Anabaena azolla*e in the leaf cavity of two species *Azolla* fern

A leaf let portion of two different *Azolla* plant species of was carefully dissected from the main stem, and transferred to clean slide. The leaf let sample was crushed and observed under light microscope. The symbiotic cyanobacteria present in the leaf cavity of *Azolla* were studied through various morphometric characteristics viz, number of cyanobacterial filament, the length of each filament and heterocyst characteristics (both number & position).

Statistical Analysis of Selected Parameters

All the experimental data were statistically analysed using MS Excel Software (2007) and Mean & Standard Deviation (SD).

Results and Discussion

Recent anatomical investigations have revealed that multicellular branched or unbranched secretory or glandular hairs are present in the leaf cavities of *Azolla* whenever *Anabaena* occurs (Konar and kapoor, 1972; Peters and mayne, 1974a).

The ubiquitous occurrence of *A.azollae* Strasburger, in cavities, in dorsal lobes of the leaves of the heterosporous water fern *Azolla*, appears to be the only well documented example of a symbiotic relationship between a fern and a blue-green alga. Physiological studies, have shown that the *Anabaena* fixes atmospheric nitrogen which is probably transferred to the fern (Fogg et al., 1973; Peters and Mayne, 1974 a).

The present study was focused on morphometric analysis of *Anabaena* strain present in two species of *Azolla* i.e., the *A.microphylla* and *A.filiculoides.* The two *Azolla* culture used for the study grown in various treatments were separately collected and analysed for various growth parameters such as number of leaf lets, and fresh & dry weight of whole plant and the results are recorded in Table 1.Between the two azolla culture, the growth characteristic was found insignificance.

The various morphometric characteristic of symbiotic cyanobacteria, *anabaena azollae* present in two species of *Azolla* cultures were analysed through observing average number of *Anabaena* filament in each leaf cavity of *Azolla*, length of each cyanobaterial filament, number of Vegetative and heterocyst cells present in each cyanobiant filament and the results and recorded in (Table 2 and 3; Plate 1 & 2). Between the strains of Anabaena, the morphometric characteristic were found as insignificant.

The ability to fix atmospheric nitrogen at substantially higher rates has led to the exploitation of the organism as bio-fertilizer. *Azolla* in rice paddy fields has a positive role in improving the soil fertility index (Peter and Meeks, 1989). *Azolla* has been used extensively and effectively in Asia for green manure in rice fields instead of chemical fertilizer

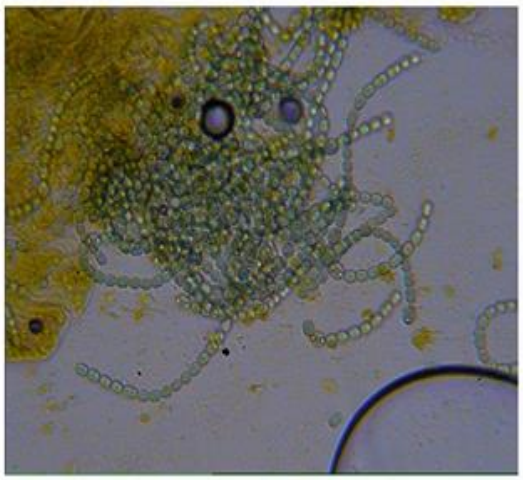

Plate 1. Light Microscopic view of Anabaena azollae strain 1 from leaf cavity of Azolla microphylla.

Plate 2. Light Microscopic view of Anabaena azollae strain 2 from leaf cavity of Azolla filiculoides.

Table 1. Growth characteristics on two species of *Azolla* fern grown on 15th d and 21st d. Values are mean of three replicates ± Standard deviation.

S.No.	Growth parameters	Number of branches in different Intervals		Number of Leaflets in different Intervals		Total fresh weight in different Intervals		Total fresh weight in different Intervals	
		15d	21d	15d	21d	15d	21d	15d	21d
1	A.microphylla	2.36 ± 0.20	4.16 ± 0.05	5.36 ± 0.2	6.30 ± 0.03	2.65 ± 0.29	3.37 ± 0.27	0.53 ± 0.01	0.67 ± 0.01
2	A.filiculoides	1.26 ± 0.15	1.26 ± 0.1	3.23 ± 0.14	2.4 ± 0.34	1.45 ± 0.03	2.33 ± 0.41	0.81 ± 0.005	0.24 ± 0.01

Table 2. Micrometry analysis of *Anabaena* Strains in two species of *Azolla* on 15th d and 21st d. Values are mean of three replicates ± Standard deviation.

Name of the Anabaena	*Azolla* source	Micrometry analysis of *Azolla* species (in µm)	
		15 th d	21st d
Anabaena Strains 1	Azolla microphylla	45 ± 1.0	55 ± 0.57
Anabaena Strains 2	Azolla filiculoides	35 ± 0.57	48 ± 0.57

Table 3. Micrometry analysis of *Anabaena* Strains in two species of *Azolla* on 15th d and 21st d. Values are mean of six replicates ± Standard deviation.

S.No.	Name of cyanobiant	No. of filament		No. of Vegetative cells		No. of heterocyst	
		15d	21d	15d	21d	15d	21d
1	Anabaena strain 1	7.99 ± 0.91	9.71 ± 0.86	46.38 ±0.83	46.16 ± 0.77	2.51 ±0.44	3.14 ±0.43
2	Anabaena strain 2	6.66 ± 0.65	8.38 ± 0.81	41.21 ±0.83	39.94 ±0.68	2.71 ±0.44	2.71 ±0.57

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