Azolla-Anabaena Association and Its Significance In Supportable Agriculture

Azolla-Anabaena İlişkisi ve Dayanıklı Tarımdaki Önemi

Minireview

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ABSTRACT

The *Azolla-Anabaena* association is important agronomically owing to its capacity to fix atmospheric nitrogen at cheaper and faster rates and making it available to crop plants. Nitrogen fixing capacity of the system is due to the presence of a symbiotic cyanobacterium *Anabaena azollae* which inhabits the dorsal lobe of the leaves. The system is important as it helps in enrichment and maintaining soil fertility and thus offers sound ecological sustainability on a long term basis. Owing to its several other uses as green manure, water purifier, animal feed etc. The system is referred to as "green gold mine". However, it is still an underutilized association with tremendous potential for exploitation.

Key Words
Azolla-Aanabaena system, Nitrogen fixation, Bioinoculant, Ecological sustainability.

ÖZET

Azolla-Anabaena ilişkisinin, atmosferik azotu daha ucuz ve hızlı bir şekilde temin etme kapasitesi ve azotu bitkilerin hasatı için ulaşılabilir hale getirmesi tarımsal açıdan oldukça önemlidir. Sistemin azot temin etme kapasitesi yaprakların sırt kısımlarında konaklayan simbiyotik siyano bakterilerden kaynaklanır. Sistem toprak verimliliğini artırmada ve korumada yardımcı olduğu ve dolayısıyla uzun vadede ekolojik sürdürebilirliği sağladığı için önemlidir. Sistem yeşil gübre, su arıtıcı, hayvan yemi vb. gibi diğer kullanım alanlarından dolayı "yeşil altın madeni" olarak anılmaktadır. Bununla birlikte muazzam potansiyeline rağmen bu birlikteliğin kullanımı hala yetersizdir.

Anahtar Kelimler
Azolla-Aanabaena sistemi, Azot özümseme, Biyoaşılayıcı, Ekolojik sürdürebilirlik

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INTRODUCTION

Azolla is a free floating water fern that floats in the water and fixes atmospheric nitrogen because of its association with the nitrogen fixing cyanobacterium Anabaena [1]. It is widely distributed both in temperate and tropical regions. An *Azolla-Anabena* system is ideal for the cultivation of rice under tropical conditions because of its ability to fix atmospheric nitrogen and capacity to multiply at faster rates. The relatively quick decomposition of the biomass and rapid availability of its nitrogen to the standing crop makes it agronomically outstanding. *Azolla* is eco-friendly as it never leads to contamination of the environment. It also does not compete with the rice plants for photosynthesis or nutrition. Such biological systems are able to provide 1.5-2.0 million tonnes of nitrogen for crop production in India whereas at least 3.3-4.4 million tonnes of urea will be required to give similar amounts of nitrogen. The use of *Azolla* is quite popular among the farmers of Vietnam and China. In fact the initial reports of using *Azolla* as a cheap source of nitrogen was realized by the Chinese farmers and there were several records on the use of *Azolla* as manure in the beginning of 17th century in China and Vietnam. They described this plant as miniature nitrogen fertilizer factories. The book written by Jia Ssu Hsieh in 540 A.D describes the cultivation and use of *Azolla* in rice fields. In India, Singh and co-workers popularized the use of *Azolla* in rice cultivation [2-6]. At present many progressive farmers and non-governmental organizations are employing *Azolla* as an invaluable input in agriculture and some of them are actively engaged in its popularization as biofertilizer, green manure, poultry feed and fodder. However, certain key scientific issues need to be addressed to make the system more compatible with the present day need and demand.

The benefits of *Azolla* application in the rice field are following:

- Basal application of green manure @ 10-12 tones/hectare increases soil nitrogen by 50-60 kg/ha and reduces 30-35 kg of nitrogenous fertilizer requirement of rice crop.
- Under low land conditions a thick mat does not allow weeds to grow in rice field thus *Azolla* suppress the weed growth and creates congenial condition for rice production. Additionally, *Azolla* reduces evaporation of surface water thus increases water use efficiency in rice.
- *Azolla* may be used for the production of hydrogen fuel and biogas, control of weeds and mosquitoes, and the reduction of ammonia volatilization that accompanies the application of chemical nitrogen fertilizer.

SPECIES AND DISTRIBUTION

The genus *Azolla* was placed in the family Salviniaceae in the order Salviniales. However, *Azolla* has been placed in the monotypic family *Azollaceae* [7]. There are seven extinct and twenty five fossil species of *Azolla* [8]. The most commonly found forms of *Azolla* are *A. pinnata*, *A. filiculoides, A. rubra, A. microphylla, A. imbricata* and *A. caroliniana*. *Azolla pinnata* is the most widely distributed species in India and throughout the world in both tropical and temperate regions. It is generally found to inhabit in paddy fields, canals, ditches and rivers. In India *A. pinnata* has been found to be distributed widely in stagnant and shallow waters. The growth is luxuriant during the months of August to March and it dies in summer due to increase in the temperature.

MORPHOLOGY AND REPRODUCTION

Azolla is a tiny plant of with a diameter of 1-2.5 cm generally. Plants are triangular or polygonal in shape and consist of floating branched rhizome bearing small alternate overlapping leaves with simple roots. The main rhizome bears several alternate branches with attached lateral branches and at the point of attachment each branch has an abscission layer which helps in the vegetative propagation. The bilobed leaf consists of a dorsal and ventral lobe. The dorsal lobe is green or purple in colour and has a central cavity within it containing the symbiont. The white or ventral lobe is relatively thin and always remains partially submerged in water and helps the plant to float in water. Roots occur at branch nodes on the ventral surface of the stem.

The main mode of multiplication is vegetative and lateral branches detach from the main rhizome due to formation of abscission layer at their base [7].

However, sexual reproduction occurs during limited periods of the year. Sexual reproduction is carried out through structures known as sporocarps which occur in pairs in either same sex or opposite sex at the lateral branch axils on the ventral side of the rhizome. The microsporocarps are large and globular whereas the megasporocarps are relatively small and ovoid and are referred to as the male and female reproductive structures, respectively. Each microsporocarp contains 5-2 stalked microsporangia and each microsporangium forms nearly 64 microspores and each megasporangium produces one megaspore. The microsporangium is partitioned to pseudo-cellular mass structure known as massulae. Mature sporocarps detach from the plant and sink to the bottom. Antheridia develop within the massula and produces antherozoids which escape through the mucilage of massula. The megaspore divides and forms the prothallus which produces archegonia with one egg cell in each archegonium. After fertilization, the zygote divides and sporophytes are formed.

TAXONOMY

Azolla has been categorized into two subgenera viz. *EuAzolla* and *Rhizosperma* on the basis of the number of megaspore floats [9]. The subgenus *EuAzolla* is characterized by the presence of three megaspore floats and consists of species such as *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. rubra* and *A. microphylla*. In contrast the subgenus *Rhizosperma* consists of nine megaspore floats. *A. pinnata* and *A. nilotica* belong to this subgenus. The basic chromosome number in all species of the section as well as the section Rhisosperma is n = 22 except in *A. nilotica* where n = 26. The taxonomic assignment of *Azolla* is difficult because many accessions do not form sporocarps under culture conditions. Precise identification of the members of section *Azolla* is cumbersome due to similarity in vegetative characters. However, it is comparatively easy in *Rhizosperma* because the members are distinct from each other in this group [10]. In order to solve the complexity in the identification of species many attempts have been made on the basis of cytology [11], fingerprints [12], DNA probes [13], DAF [14], RAPD markers [15], and GC content [16,17]. Difficulty in the identification of *Azolla* strains is a major stumbling block in the successful exploitation of this organism as a bioinoculant for rice paddy fields. All these techniques would ultimately help in the clear differentiation and identification of specific strains. Further usefulness of the system and technology development for biofertilizer depends greatly upon the identification of right strains adapted to a particular habitat. Therefore, molecular tools including PCR-RAPD techniques are expected to play a significant role in understanding the diversity and genetic variability among species. Better understanding of the variability will help us in enhancing its usefulness as an effective biofertilizer as well as to exploit it for a host of alternate uses for which this underutilized system is well known.

SYMBIONT AND NITROGEN FIXATION POTENTIAL

The nitrogen fixing capacity of the system is due to the presence of a symbiotic cyanobacterium Anabaena *azolla*e which inhabits the dorsal lobe of the leaves. The average daily N_2 fixing rate is reported to vary between 1.0 to 2.6 Kg N/ha [18]. It has been reported that *A. pinnata* fixed 75 kg N/g dry weight/day and produced a biomass of 347 ton fresh weight/ha in a year [5]. There is wide variability in the nitrogen fixing capacity among *Azolla* species [19]. However, environmental conditions, nutrient availability, rate and time of inoculation of the strains have an influence on the growth and nitrogen fixing potential of *Azolla* [6].

EFFECTS OF AZOLLA INOCULATION

Azolla grows well in water logged paddy fields along with rice. Fresh *Azolla* plants can be inoculated in the field at the rate 0.5 to 1.0 t/ha and make nitrogen available to plants in around 20 days after inoculation. There are reports 14-40% increases in rice yield due to *Azolla* dual cropping [1, 20]. *Azolla* incorporation was found to be comparable to application of 30 kg N/ha when used as a green manure and dual crop and has resulted in increased crop yields [21]. The same study also showed that the response of *Azolla* green manure was better in dry season than in wet season and short duration rice varieties responded better than the late duration varieties. Increase in the straw yield, number of panicles and number of grains due to *Azolla* application was observed [19]. Maximum rice yield increase with an output ratio recovery of 2.07 with incorporation of 7.5 tonne fresh *Azolla* per hectare along with 90 kg nitrogen per hectare was reported [22]. Studies at Centre for Conservation and Utilization of Cyanobacteria, Indian Agricultural Research Institute, New Delhi revealed that addition of four organic amendments such as *Azolla*, blue-green algae, farmyard manure and vermicompost could give optimum yield in organic Basmati rice in addition to improving grain and soil quality [23].

The application of *Azolla* in rice paddy fields has a positive role in improving the soil fertility index. Inoculation of *Azolla* has been reported to enhance the soil biological health in addition to sustaining rice yields. *Azolla* decomposes rapidly in soil and supply nitrogen to the crop plant. The biological health of the soil due to application of *Azolla* has resulted in improving mineralization and consequent increase microbial status of the soil. Soil fertility is also influenced by the humic substances formed during the decomposition of *Azolla* [24]. In Tamil Nadu Agricultural University, India significant contributions have been made by Dr. Kannaiyan on *Azolla* biofertilizer technology and have also screened some strains of *Azolla microphylla* highly tolerant to high temperature and salinity (personal communication). Some of the strains screened were found to produce high biomass also. His group was able to develop a hybrid through sexual hybridization and this hybrid was found to fix high amount of nitrogen. Maximum population of bacteria, fungi and actinomycetes and high urease and dehydrogenase activities was observed due to organic farming using *Azolla* as one component [25]. Significant and steady enhancement in the soil biological health related parameters due to bio-inoculants including *Azolla* was also observed [23]. Soil enzyme activity is considered as an index of microbial activity and fertility of the soil. The results pointed out the role of biological input of *Azolla* in maintaining the ecological and soil conditions to sustain crop productivity. They observed that application of *Azolla* in dry form results in increase in soil organic matter and triggers the growth and multiplication of soil microorganisms. These reports emphasize the potential of using *Azolla* in other crops.

OTHER USES OF *AZOLLA*

Azolla has also multidimensional uses and recently it has also gained importance as poultry feed and fodder. *Azolla* has many uses such as use as human food, animal feed, medicine, production of biogas, hydrogen fuel, water purifier, weed control, reduction of ammonia volatilization and because of the multifaceted uses it has been aptly referred to as "green gold mine" [26]. The potential of *Azolla* as meal for chicken was also reported [27]. They emphasized the importance of this unconventional feed as an effective alternative to feed chicken. *Azolla* is used to feed pigs and is a good source of minerals and essential amino acids [28]. In the recent times *Azolla* is gaining tremendous importance in phytoremediation of polluted waters. *A.microphylla* biomass was successfully cultivated in secondary treated Municipal waters of Delhi [29]. Phytoremediation potential of three different species of *Azolla* to chromium has also been carried out [30]. These results show that *Azolla* may be successfully employed in phytoremedation of polluted water bodies. The botanicals derived from *Azolla*-Anabaena system was shown to possess bioactivity. It is also an excellent composting material. Experiments conducted at IRRI, Philippines showed that conversion of compost from *Azolla* was rather quick but further research is required to evaluate the exact potential [31]. Fresh *Azolla* collected from fields and ponds were used in compost preparation and since it has an excellent carbon nitrogen ratio it decomposes rapidly and accelerates the decomposition of other organic residues inside the compost pit [32].

CONSTRAINTS IN USAGE

There are several constraints that hamper the successful use of *Azolla* as biofertilizer. Temperature is a deterrent for growth because the optimum temperature for *Azolla* growth is 25-30°C. There are strains of *Azolla* which can grow even at higher temperatures (45°C). Higher temperature leads to pest infestation whereas at low temperature growth is retarded. It prefers to grow in partially shaded conditions. Bright sunlight coupled with high temperature affects the growth of *Azolla* adversely in summer months.

The preferred pH for the growth of *Azolla* is 4.5 to 8.0 with an optimum range of 5.0- 7.0. Since it requires water to grow a minimum level of water is to be maintained always for its growth. The growth and multiplication is related to the phosphorous level. At least 25 ppm of phosphorous is needed for good growth. *Azolla* is highly prone to attack by insects and pests. These problems can be controlled by the application of pesticides such as furadon and carbofuran. Botanicals such as neem are also useful in controlling the pests. However, a good management practice will minimize the incidence of pests to some extent. Stress factors such as high salinity, alkalinity, heavy metals, and incidence of UV-B etc. is also a growth limiting factor in the successful exploitation of *Azolla-Anabena* system.

PERSPECTIVES FOR THE FUTURE

Traditionally, the *Azolla-Anabena* association is an excellent biofertilizer for lowland rice cultivation. However, no attention has directed on its utility for other crops with an emphasis on plant growth promotion. It produces a large quantity of biomass rich in nutrients. There are few reports on their use as an composting material, its incorporation has been reported to trigger growth and increase the soil microbial activity. Moreover, it has an excellent C/N ratio and thus, its integration as compost and its efficacy in the reclamation of degraded agricultural waste lands also need to be addressed. There have been no systematic studies on the exact phytochemical composition of these plants despite its importance as a source of food for cattle and human beings. Some reports are available on the chemical composition but the exact biological activity has not been explored. Further, studies on their biological activity as antibacterial, antifungal and anti-insecticidal agents need to be evaluated. Cutting edge technologies in the field of molecular biology must be employed to understand the nature of the symbiosis and attempts could be made to revalidate the symbiosis. Attempts to improve the frequency of sporulation will lead to development of desirable hybrids for abiotic stress tolerance. Increasing soil salinity is a serious threat to agriculture worldwide and therefore, screening and development of salinity tolerant strains need further emphasis. In the absence of concerted efforts and lack of proper extension activities *Azolla-Anabaena* association continues to remain as an underutilized despite its vast potential.

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