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AZOLLA- A SYMBIOTIC NITROGEN FIXING FERN

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Azolla is a water fern belonging to the plant group Pteridophytes. It is a small free floating aquatic fern having an endosymbiotic community living in the dorsal lobe cavity of its leaf. The endosymbiotic community is composed of two type of prokaryotic organism: one is a nitrogen-fixing filamentous cyanobacteria – *Anabaena azollae* Strasb. and the second is a variety of bacteria that some identified as *Arthrobacter* sp. In this association, it is assumed that an exchange of metabolites, namely fixed nitrogen compounds, occurs from the cyanobiont to the host.

The existence of the two symbionts inside the *Azolla* leaf cavity and its relationship with the fern, namely the metabolites flow between the host and the symbionts, can be seen as a unique micro-ecosystem with own well established characteristics. This association is maintained during all the life cycle of the pteridophyte (Grilli and Forni 1999).

One of the most interesting features is the role played by the cyanobacterium in this association. Filaments of *Anabaena azollae* are localized in the cavity of the dorsal lobe of the fern leaves, where special conditions stimulate high heterocyst frequency and a vegetative cell differentiation during leaf development.

Anabaena colony associated with shoot apex lacks heterocysts and therefore, is unable to fix nitrogen. Anabaena filaments cease to grow in mature leaves, and differentiate heterocysts-the site of nitrogen fixation. Besides the cyanobacteria, a population of bacteria undergoes a pattern of infection identical to Anabaena and probably is the third partner of this symbiosis (Carrapico 2002). The prokaryotic colony –cyanobacteria and bacteria – are also present in the sexual structure (sporocarps) of the fern. The cyanobacterium is transferred from the sporophyte to the next generation via the megasporocarp. A cyanobacterium colony resides between the megasporocarp wall and the megasporium one and inoculates the newly emerging sporophyte plant. A colony of symbiotic cyanobacteria is formed near the shoot apex and thus enables symbiosis to be established within the developing leaf cavities. The presence of bacteria in the megasporocarps in the association with the cyanobacteria also suggest a behavior pattern similar to the cyanobionts. The presence of *Anabaena* throughout the life cycle of the fern favours the obligatory nature of the symbiosis and suggest a parallel phylogenetic evolution of both partners.

Environmental factors such as humidity, light intensity, photoperiod, salinity and temperature plays an important role in regulating the growth and metabolism of the association.

This symbiotic association is the only fern-cyanobacteria association that presents agricultural interest by the nitrogen input that this plant can introduce in the fields and for that reason it has been used in several tropical and subtropical countries in different continents. Historically, *Azolla* has been used as green manure for wetland rice in northern Vietnam and central to southern China for centuries (Wagner 1997). A single crop of azolla provides 20-40 kg nitrogen per hectare compensating approximately one third of nitrogen demand for crop. High temperature which generally prevails in northern parts of India has restricted the use of this water fern because activity of enzymes are inhibited at high temperature.

Only after the oil crisis in the 1970s the research and use of this type of association has been intensified because of the price increase of the chemical fertilizers and its negative impact in agriculture, namely in the countries of the third world. Meanwhile, since the introduction of a market economy system in those countries, the increase on the supply of the chemical fertilizers has reduced the traditional use of Azolla as green manure for rice cultivation, namely in China and Vietnam (Sapp 2003). Besides its use as a N-supplement in rice based ecosystems, it has also found limited use in crops such as taro, wheat, tomato and banana.

A problem associated with the use of chemical fertilizers is the adverse effect on long term soil fertility, soil productivity and environmental safety. A new strategy for increasing rice production, particular in developing countries should be taken in account for programs to utilize the biological fertilizers which will not only increase the rice productivity, but also improve the long term soil fertility.

For those reasons, *Azolla* use is yet a real option as a green fertilizer, especially in the developing countries that presents a low cost of labor force. In industrial countries, the potential

of *Azolla* as symbiotic N_2-fixing system should continue to be exploited for a more developing environmentally- friendly agricultural system, namely in particular segments of this important economic activity.

Other uses of the *Azolla-Anabaena* system are now in progress such as animal feed and bio-filter in industrial and domestical effluents.

Azolla is very rich in proteins, essential amino acids, vitamins (vitamin A, vitamin B_{12}, beta carotene), growth promoter intermediaries and minerals like calcium, phosphorus, potassium, ferrous, copper, magnesium etc. *Azolla*, on a dry weights basis, is constituted of 25-35% protein content, 10-15% mineral content and 7-10%, a combination of amino acids, bioactive substances and biopolymers. Carbohydrate and oil content in *Azolla* is very low thus, the bio-composition of *Azolla* makes it one of the most economic and efficient feed substitute for livestock. Moreover, *Azolla* can be easily digested by livestock, owing to its high protein and low lignin content.

Azolla possess great ability to absorb heavy metals like chromium, cadmium, copper, zinc etc. from the water. The metal polluted effluent from industries like chemical, leather, electroplating etc. and other different metals can be checked by cultivating *Azolla*.

Azolla is therefore, environment friendly and it is essential to harness the water fern to its full potential (Lumpkin 1980).

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