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Studies On The Diversity Of Mycorrhiza Associated With Some Bryophytes Of Kolhapur District

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Abstract: This Mycorrhizae, which are beneficial relationships among plants and soil fungus, are among the most significant terrestrial symbioses. Approximately 500 million years ago, these alliances are believed to have accelerated plant territoriality; now, these are crucial to the health of ecosystems. Fungal symbionts, recently discovered as belong to the three basic fungal groups Glomeromycotina, Ascomycota, and Basidiomycota, have long been recognized to be present in the living tissues of bryophytes. It has been demonstrated that every studied bryophyte extract is effective against every kind of fungal examined. When compared to extracts derived from naturally occurring materials, those cultivated under laboratory (in vitro) environments often have greater antifungal activity. Certain fungi that were studied responded similar to both extracts.

Index Terms - Mycorrhizae, Glomeromycotina, Bryophyte, Ecosystem, Fungal Lineages, Soil Fungi, Plants.

I. INTRODUCTION

The word "bryophyte" comes from the Greek terms "Bryon," which refers to moss, and "the Python," which means plant [Crandall-Stotler, B., 2009]. The bryophytes are the second biggest group of terrestrial plants. Because they need freshwater for every stage of their life cycle, even though they are terrestrial, they are referred to as "Amphibians of Plants Kingdom." They have a minimum of 400 million years of history [Hollowell, V. and Magill, R. 2001].

They are thought to be the oldest terrestrial plants still in existence. They may be discovered on rocks, dirt, and trees all over the world, from the tropical rain forests of the Amazon to the dry Australian desert, as well as the coast of Patagonia to the freezing tundra of the northern hemisphere [Delaux, P.M., Radhakrishnan, G.V., 2015]. Despite their small size, they play a significant role in many ecosystems both terrestrial and aquatic and are a key part of the earth's ecosystem. In the bryophyte life cycle, the gametophyte phase is long and dominating, whereas the stage of sporophyte phase is brief. Since they need water to germinate, they are mostly found in humid environments. They can reproduce both sexually and asexually.

For more than a century after Gottsche's discovery of them in 1843, they had been just briefly explained and shown by line drawings of a variety of liverworts, with their true nature remaining entirely hypothetical [Edwards, E., Cherns, L. 2015]. Then, using an electron microscope, research on both the liverwort and hornworts showed a variety of fungal species. The presence of healthy the hyphae in the host cells suggested that the relationships were probably mutualistic [Engel, J.J. 2005]. On the other hand, no evidence of potential mutually beneficial interactions has been found in mosses.

More recently, molecular research has shown that the fungi living in the thalli and/or rhizoids of the liverwort and the hornworts belong to the three main clades of mycorrhizal fungi, namely Ascomycota, [Fehrer, J., Reblova, M., 2005], Basidiomycota, and Glomeromycotina. Because bryophytes lack true roots, it was assumed that these associations between fungi and bryophytes were mycorrhizal-like, mutually advantageous symbiotic with bidirectional resource exchange between partners, based solely on cytological evidence and lacking confirmation from physiological studies [Field, K.J., 2012].

The study of various plant biological activities has attracted a great deal of attention from scientists in recent years since it is believed that plant extracts may find use in both agriculture and biopharmaceuticals. Vascular plant species are given a lot of attention in these studies, whereas other groups of organisms, such as bryophytes, are ignored. They are identified as the land plants basal or initial diverging lineage. They differ in both morphology and biochemistry.

The chemical breakdown of the varied group known as bryophytes is still unknown, despite the fact that several novel chemicals, mostly from liverworts, have been characterized for scientific use. Compared to vascular plants, they are used very seldom in ethno medicine, and certain conventional medicinal systems are known to use them sparingly.

Fig. 1 Axenic M. polymorpha sp. Ruderalis in vitro culture.

Bryophytes were highlighted as novel sources of agents because to the discovery of novel and intriguing chemicals, some of which have the potential to be highly active in pressing a, b. Larger yields of bryophyte biomass are required for any kind of extensive applications and research [Pejin B,2011]. But they are still issues with setting up axenic and in vitro cultures to resolve.

The distinctive popular characteristics of bryophytes are shown in fig. 1.1 below. They may be roughly categorized into three groups: mosses, which come in thalloid and foliose forms, liverworts, and hornworts. Since they are divided into three divisions/phylla, namely *Marchantiophyta*, *Anthocerophyta*, and bryophyte, in accordance with the most current bryophyte classification scheme.

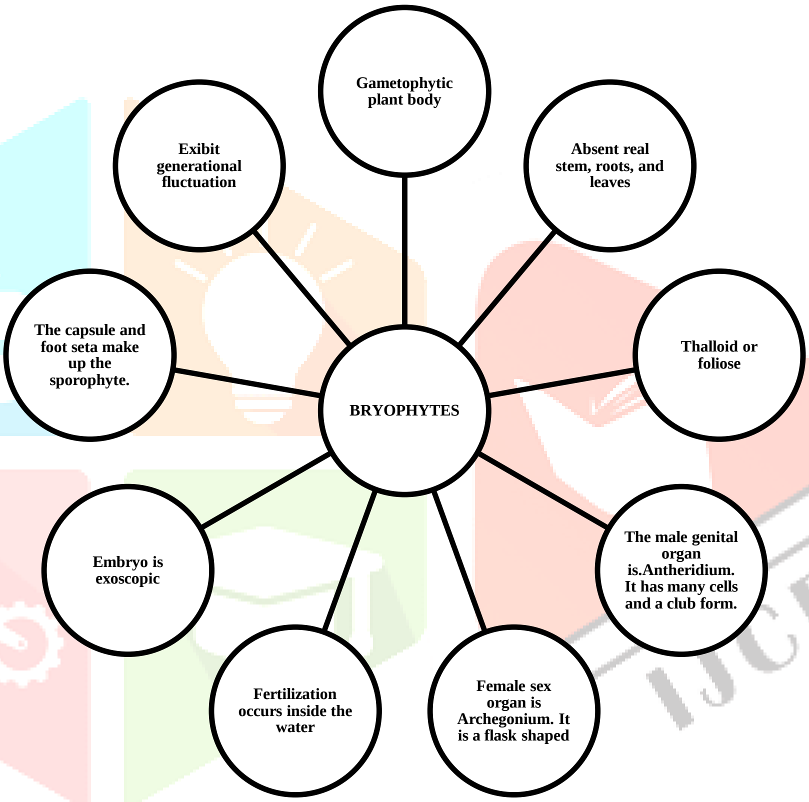

Fig. 2 Key Characteristics of Bryophytes.

1.1 Researched bryophytes' use in conventional medicine

Marchantiaceae plants are well-known traditionally Chinese medicinal herbs that are widely used to cure hepatitis, protect the liver, heal cutaneous tumefaction, and function as an antipyretic. The Chinese Guangxi Zhuang autonomy area is home to a large number of marchantiaceae plants, including *Mercanti polymorphic*, *Mercanti convolute*, and *Mercanti palaces*, which are utilized by the locals. These species coexist in groups and have similar morphologies, making it difficult to tell one from the other [Pryce RJ (1971)].

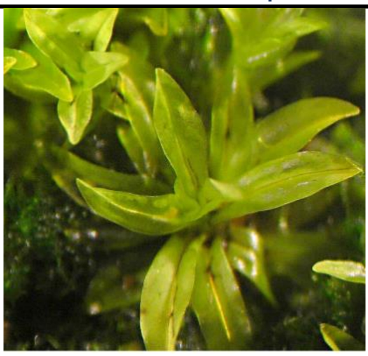

Fig. 3 Axenic in *A.undulates* in vitro culture.

Fig. 4 Axenic P. patens in vitro culture.

1.2 Formerly distinct types of components in mosses under study

It is well known that M. polymorphic, the liverwort, is multifunctional. But until quite just lately, it was considered as a complex of species with this designation; it's unclear whether there is also a chemical difference.

1.3 Objectives of the study

- Analyse the ecological role that mycorrhizae play in bryophyte development, keeping in mind the importance of nutrient intake, water absorption, and overall plant health.
- Create and refine techniques to investigate mycorrhizal relationships in bryophytes, advancing the science of plant-fungal interactions.

II. LITERATURE REVIEW

[Valdes, F. E., Peralta, D. F., 2023] The Devonian epoch is when Arbuscular Mycorrhizal Fungal (AMF) and ombrophytes first came into being as a symbiotic partnership. Previous investigations into the ecology and physiology of liverworts and hornworts have reported the existence of features that are thought to be missing in mosses, such as vesicles, spores, coils, and arbuscular, as well as intracellular and intercellular hyphae. The purpose of this research was to document the occurrence of AMF in a group of bryophytes, or liverworts and mosses, from the Punta Lara Naturally Reserve in Argentina. After staining senescent and green portions of gametophytes, AMF formations were visible under a microscope. We discovered spores, podocarps, vesicle and internal hyphae connected to the thallus and rhizoids of liverworts, mosses, and senescent moss Califia.

[Chen, K. H., 2022] Plant health is influenced by both the state of the plant and the microbiota, or varied population of bacteria that live inside it. Similar to the more well-researched angiosperms, bryophytes, which include moss, liverworts, and hornworts, are home to a variety of microbial eukaryotes such as bacteria, archaea, and fungus. Increasing proof points to bryophytes as crucial models for understanding the physiology, growth, evolution, and symbiotic relationships of plants. Recent work is adopting a more comprehensive approach, recognizing the presence of multiple microbial communities in bryophytes. A large portion of the study on bryophyte microbiota in previous years concentrated on certain symbiont species for each bryophyte group.

[Brundrett, M. C., 2018] Arbuscular mycorrhizal (AM) accounts for 72% of vascular plants, ectomycorrhizal (EcM) for 2.0%, ericoid mycorrhizal (1.5%), and orchid mycorrhizal (or 10%). Only 8% have no mycorrhizal (NM) at all, and 7% have sporadic connections among NM and AM. The majority of NM and NM-AM plants are either habitat or nutrition specialists, such as hydrophytes and epiphytes, or carnivores and parasites. In most families, mycorrhizal relationships are stable, but there are certain exceptions with intricate origins. Three distinct phases of mycorrhizal development are identified: the first wave began with AM in early plant communities on land, and the second wave included various novel NM and EcM linages, ericoid, and orchid Mycorrhyzae in the Cretaceous. In biodiversity hotspots, the latest wave of diversity, which is now occurring, has led to a connection between root complexity and fast plant diversification.

[Rimington, W. R., 2020] Mycorrhizal study requires a precise grasp of the variety as well as distribution of fungal symbiotic in terrestrial plants. Here, we update the original work by gathering data on the state of fungal symbioses in the liverworts, hornworts, and lycophytes by reviewing the available literature. Early-diverging land plants lineages were noticeably under-represented in their study, and here is our long-overdue opportunity to rectify that. Our analysis generates a list of a minimum of 591 species having known fungal symbiotic position, 180 of which have been included in the report of Mucoromycotina connections across these lineages, based on data from 84 papers, included recent, post-2006 studies.

[Oh, S. Y., Park, K. H., 2021] The variety of endophytic fungi that originate from ferns is infrequently researched, despite the fact that ferns are the basal category of vascular plant species and have been shown to have fungal relationships with arbuscular mycorrhizal fungi. Furthermore, since the majority of investigations have relied on microscopic or culture-dependent methods, it is possible that the variety of fungal species connected to ferns has been underestimated. In this work, we used a met barcoding technique to compare the endophytic fungal community found in the root system and sporophore of a threatened Korean ferns (Mankyua chejuense) to that of fungi in the nearby soil. Numerous Ascomycota-dominated endophytic fungi were found, and there were notable differences in the abundance and composition of the fungus across different environments. Analysing indicator species revealed that the ecological traits of endophytic fungi are comparable to those of fungal species found on other terrestrial plants.

III. METHOD AND MATERIAL

3.1 Plant material and production of extracts

Gametophytes of A. undulated and polymorphic were cultivated on MS medium that was treated without 0.1 M sucrose, and gametophytes of P. patens were produced on BCD media that was enhanced with 0.1 M glucose [Pryce RJ (1972)]. To explore the ways in which environmental factors impact bryophyte associations with different species, the plants were cultivated on either liquid or solid MS/BCD media, as shown in Table 1.

Before the growth media were autoclaved for 25 minutes at 114°C, their pH was brought to 5.8. The cultures were grown at 25 ± 2°C under a pair of circumstances: a prolonged day (16/8 hours of sunshine to darkness) and dark environment (24 hours of darkness), as shown in Table 1. The lighting was supplied by cool-white fluorescent bulbs with a radiation fluency of 47 mol/m. Cultures existed for four to six weeks in cultures. The BEOU bryophyte collections now contains voucher samples of A. undulated, the species M. polymorphic sp. ruderals, and P. patens (No. 4463, 4556, and 4509).

3.2 Conduct an antifungal activity test

The bioassays used five fungi: *Aspergillus fumigatus* (ATCC 9142), *Penicillium funiculosum* (ATCC 36839), *Trichoderma variable* (IAM 5061), and *Aspergillus* the colour versicolor is (ATCC 11730). The microorganisms used in the experiments came from Belgrade, Serbia's Mycological Laboratory, Division of Plant Physiology, and Centre for Biomedical Research. The micromycetes' cultures were maintained on Malt Agar (MA), maintained at +4°C, and subculture once a month [Rieck A, 1997].

3.3 Method of microdilution

The modified microdilution method was used to examine the extracts' antifungal properties. The plate's agar surfaces were treated with sterile 0.85% saline and 0.1% Tween 80 (v/v) to eliminate the fungus spores. A sterilised saline solution with a dose of around one x 10^-5 was used to regulate the spore dispersion in a final volume of 100 l per well. To be used later, the sprinkle was kept cold, at 4°C. Introduced dilutions have been cultivated on solid MA in order to confirm the lack of contamination and assess the authenticity of the inoculums. The serial dilution approach was used to measure Minimum Injurious Concentrations (MICs) using microtiter plate plates with 96 wells [Rowntree J K, 2010].

IV. RESULTS AND DISCUSSION

Table 1 reports the tested extracts for antifungal properties. It was clear that the extracts antifungal properties exceeded their antibacterial ones. The extracts' MIC and MFC varied between 0.1 to 1.0 mg/ml and 0.5 to 2.0 mg/ml, respectively [Sabovljevic A, 2008]. The highest antifungal effectiveness was seen in extracts of M. polymorphic grown on MS liquid medium, A. undulated developing on MS stable medium-sized, and P. patens created on BCD solid media, with MICs of 0.1 to one milligram me per millilitre and MFCs of 0.5 to 1 mg/ml [Chobot V, 2008].

Table 1 Minimal Concentrations of the chemicals tested that were fungicidal and inhibiting.

Fungi	*M. polymorphic*				*A. undulatum*			*P. patens*	Bifonzole	Ketoconazole
	MS solid medium light,	MS liquid medium, light,	MS solid medium, dark,	Nature	MS solid medium, light,	MS solid, medium, dark,	Nature	BCD solid medium, light, nature		
T. viride	0.16	0.4	0.1	0.5	0.49	0.14	1.0	0.87	0.1	0.67
	0.26	0.25	1.0	0.9	0.25	0.85	0.79	0.9	0.9	0.49
P. Funiculosum	0.39	0.99	1.0	0.1	0.9	0.97	0.49	0.16	1.0	0.16
	0.1	0.46	0.6	1.0	0.49	0.98	0.2	0.6	0.44	0.72
P. ochrochloron	0.7	0.2	1.0	0.6	0.79	0.47	0.6	0.0	0.97	0.97
	1.2	0.6	0.55	0.8	0.58	0.54	0.4	0.44	0.16	0.49
A. fumigatus	1.0	0.1	0.79	0.9	0.67	0.97	0.1	0.69	0.97	0.49
	0.9	1.0	0.49	0.4	0.97	0.58	0.5	0.49	0.14	0.72
A. Versicolor	0.4	0.3	0.65	0.77	0.58	0.97	0.6	0.79	0.57	0.69
	0.8	0.8	0.1	0.1	0.2	1.0	1.0	2.0	0.3	0.6

The data provided here indicate that some axenic cultures were more successful than the fungi under test. This finding may be explained by the fact that certain of the elements of the most successful extract are produced more effectively under artificial than under natural settings [Karatas H, 2010]. To validate this theory for this purpose, much more biomass is required, and further chemical research is required.

Bryophytes that were cultivated organically shown some promise for use in biotechnological operations. Given that the examined bryophyte extracts demonstrated a range of associations with specific fungi, when extensively used, the species being targeted and conditions for growth should be changed to reach the maximum.

In addition to providing adequate material of these small plants, the biomass produced by biotechnological techniques additionally stopped extinction and might be used to ex-situ conservation [Markham KR, 1974]. Therefore, bryophyte axenic culture offers a technique to get enough concentrations of natural medications that may be utilised to treat plant fungal infections in a manner that is safe for the environment and does not harm other living things.

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