KAVAKA 59(4): 49-55 (2023) DOI: 10.36460/Kavaka/59/4/2023/49-55

Arbuscular Mycorrhizal Fungal Association in Bryophytes from Arunachal Pradesh: a First Report

Chunam Aniyam, Amanso Tayang, and Heikham Evelin*

Mycorrhizal Technology and Bryophytes Laboratory, Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh - 791 112, Arunachal Pradesh, India.
**Corresponding author Email:heikham.evelin@rgu.ac.in*

(Submitted on October 7, 2023; Accepted on November 5, 2023)

ABSTRACT

The present study evaluated arbuscular mycorrhizal fungal (AMF) association with bryophytes. Twenty bryophyte specimens were collected from different natural habitats of Tirap district in Arunachal Pradesh, India. Of the 20 specimens, 11 mosses and three liverwort species showed the presence of AMF structures, such as aseptate inter- and intra-cellular hyphae and vesicles. *Marchantia* sp. showed the highest percentage of AMF colonization (100%). Mosses, *Anomobryum auratum, Leptodontium handelii, Campylopus subgracilis, Ceratodon purpureus, Dicranella microspora,* and *Dicranodontium fleischerianum* showed no colonization. The study is the first report on bryo-mycorrhizal association from Arunachal Pradesh.

Keywords: Bryo-mycorrhizal association, Liverworts, Mosses

INTRODUCTION

The pioneer land plants, Bryophytes, occupy a significant position in the plant kingdom. The group is highly diverse, with approximately 28,000 species reported worldwide (Christenhusz and Byng, 2016). These groups of plants lack vascular systems and remain small-sized. However, they play an important role in the evolutionary history of land plants (Vanderpoorten and Goffinet, 2009), render various services to the ecosystem (Chimyang *et al.*, 2022), and possess immense medicinal importance (Mossang *et al.*, 2021).

AMF belong to the lineage Glomeromycotina and are the most widespread symbiotic partners of flowering plants (Rimington *et al.*, 2018). Despite the absence of true roots, Bryophytes have been reported to establish a symbiotic association with AMF as early as 1927 (Rayner, 1927). In addition, bryophytes are also known to harbour fungi belonging to Ascomycota and Basidiomycota (Pressel *et al.*, 2021). AM symbiosis with liverworts and hornworts is widely reported (Turnau *et al.*, 1999; Schüβler, 2000; Bidartondo and Duckett, 2010; Rimington *et al.*, 2018). The mosses are generally considered non-mycorrhizal (Vyas *et al.*, 2007; Rimington *et al.*, 2018). However, recent findings suggest the need to explore the moss-AMF relationship in greater detail. Liepina (2012) did not detect any AMF structures in 21 moss species collected from the boreo-nemoral zone in Latvia. Zhang and Guo (2007) and Vyas *et al.* (2007) reported the presence of AMF structures, spores, vesicles, hyphal coils, and intercellular non-septate hyphae in 24 moss species and *Funaria* sp., respectively. However, no arbuscules were detected. Thus, the information on bryo-mycorrhizal association is very limited. Therefore, the present study was undertaken to determine the presence of bryo-mycorrhizal associations in twenty bryophyte species collected from Tirap district, Arunachal Pradesh, India.

MATERIALS AND METHODS

In July 2022, 20 bryophyte species growing on soil (terricolous) and stone/rock (saxicolous) were randomly collected from four villages of Tirap district, Arunachal Pradesh. The district has an annual rainfall ranging from 50-800 cm, temperature from 6-34^{o}C, and relative humidity from 55-70%. The climate supports tropical and sub-tropical evergreen forests. Interspread grasslands and temperate forests are found in higher altitudes. The altitudes ranged from 200-4000 m amsl.

The villages considered for the study are Kheti (1220 m amsl), Thinsa (1707 m amsl), Barap (1298 m amsl), and Khonsa (1027 m amsl). Patches of the specimens were collected and brought to the laboratory. The identity of the specimens was determined by studying their morphological and anatomical features based on Gangulee (1969-1980), Chopra (1975), and Singh and Singh (2009).

To detect the presence of AMF structures, the samples were washed under running tap water to remove any soil particles. The samples were cleared and stained by following Cottet and Messuti (2019). Fifty green thalli (gametophytes) were immersed in 70% ethanol overnight at room temperature, followed by heating at 50^{o}C till the ethanol evaporated. The samples were then cleared in 1% potassium hydroxide for 20 min at 80°C, later acidified with 1% HCl for 10 min at 50°C, and stained for 20 minutes with 0.05% trypan blue. The tissue was then cut into about 1 cm long segments, and 50 such segments were mounted on slides in lactophenol. The slide was examined with a compound microscope (ZEISS, Lab. A1) at 10x,

40x, and 63x to detect the presence of AMF structures, i.e., aseptate inter-and intra-cellular hyphae, arbuscules, and vesicles. Colonization level was evaluated using the magnified intersection method (Mc Gonigle *et al.*, 1990). Fifty intersection points were considered for each sample. For *A. serpens f. fallax*, only 40 intersection points were considered due to less sample. The number of intersections cutting through the arbuscules, vesicles, or hyphae on the thallus was noted (**Table 1**). The intersections that do not pass any AMF structure were counted as negative. Colonization by arbuscules and vesicles was then calculated from the number of intersections cutting through arbuscules and vesicles by the total number of intersections, respectively. Hyphal colonization encompassed all the non-negative intersections. The colonization % was calculated as follows:

$$\text{Arbuscular colonization (\%)}: \frac{\text{Number of intersections where vertical hair cut through arbuscules}}{\text{Total intersections observed}} \text{ X } 100$$

$$\text{Vesicular colonization (\%)}: \frac{\text{Number of intersections where vertical hair cut through vesicles}}{\text{Total intersections observed}} \text{ X } 100$$

$$\text{Hyphal colonization (\%)}: \frac{\text{Total number of intersections observed - Number of negative intersections observed}}{\text{Total intersections observed}} \text{ X } 100$$

RESULTS AND DISCUSSION

The 20 species of bryophytes include three liverworts and 17 mosses belonging to 16 families (**Table 1**). In the present study, all three species of liverworts and 11 mosses were mycorrhizal (**Figure 1, Table 1**). Our study, for the first time reports the prevalence of AMF structures in growing tissues of 11 moss species: *Amblystegium serpens f. fallax*, *Barbella angustifolia*, *Eurhynchium muelleri*, *Hypnum macrogynum*, *Mnium lycopodiodes*, *Philonotis mollis*, *Pohlia flexuosa*, *Pogonatum junghuhnianum*, *P.aloides*, *P.stevensii*, and *Racopilum orthocarpum.* AMF structures, intercellular aseptate hyphae, and vesicles were detected in the parenchymatous cells of the phyllid and caulid, indicating AMF colonization. However, these structures were absent in the rhizoids, which may be due to their hollow nature. The arbuscules were not detected in any of the moss species. Our findings corroborate earlier reports by Zhang and Guo (2007) and Valdes *et al.* (2023). The absence of arbuscules may be explained by their ephemeral nature (Liepina, 2012) or lack of active symbiosis (Zhang and Guo, 2007). Six moss species, namely *Anomobryum auretum*, *Campylopus subgracolisus*, *Ceratodon purpureus*, *Dicranella microspora*, *Dicranodontium fleischerianum*, and *Leptodontium handelli* showed no AMF structures. *C. purpureus* was earlier reported as non-mycorrhizal by Liepina (2012). There is, however, no available literature on the other moss species.

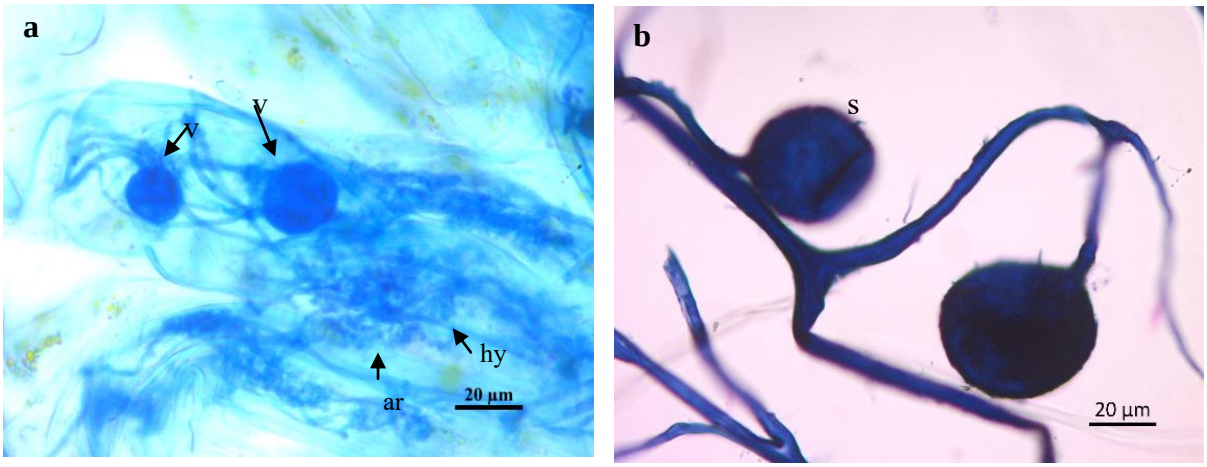

Figure 1: Arbuscular mycorrhizal fungi structures in bryophytes. a, arbuscules, intracellular hyphae and vesicles in thallus of *Dumortiera hirsuta*; b, spores of AMF in thallus of *Mnium lycopodiodes;* ar, arbuscules; hy, hyphae; v, vesicles; s, spore.

Table 1: Arbuscular mycorrhizal fungal (AMF) status of bryophyte species.

Bryophytes	Village	Location	Nature of habitat	Number of intersections					AMF colonization (%)		
				Negative	Arbuscules	Vesicles	Hyphae	Total	Arbuscular colonization	Vesicular colonization	Hyphal colonization
Bryophyta (Mosses)											
Amblystegium serpens f. fallax (Warnst.) Podp. (Amblystegiaceae)	Kheti	26° 55' 8.832" N 95° 30' 12.636"E	Terricolous	22	0	0	18	40	0	0	**45**
Barbella angustifolia Broth. ex Gangulee (Meteoriaceae)	Barap	26° 55' 8.832"N 95° 30 ' 12.636"E	Saxicolous	4	0	20	26	50	0	40	**92**
Eurhynchium muelleri (A. Jaegar) E. B. Batram (Brachytheciaceae)			Saxicolous	30	0	4	18	50	0	8	**40**
Hypnum macrogynum Besch. (Hypnaceae)			Terricolous	18	0	20	12	50	0	40	**64**
Mnium lycopodiodes Schwager. (Mniaceae)	Khonsa	26° 59' 34.35"N 95° 30' 4.96" E	Terricolous	11	0	4	35	50	0	8	**78**
Philonotis mollis (Dozy and Molk) Mitt. (Bartramiaceae)			Terricolous	25	0	1	24	50	0	2	**50**
Pogonatum aloides (Hedw.) P. Beauv. (Polytrichaceae)	Thinsa	26° 55' 52.68"N 95° 32' 4.2"E	Terricolous	11	0	0	39	50	0	0	**78**
Pogonatum junghuhnianum (Dozy and Molk.) Dozy & Molk. (Polytrichaceae)	Khonsa	26° 59' 34.35"N 95° 30' 4.96" E	Terricolous	15	0	0	35	50	0	0	**70**

Pogonatum stevensii Renauld & Cardot (Polytrichaceae)	Thinsa	26° 55' 52.68"N 95° 32' 4.2"E	Terricolous	18	0	0	32	50	0	0	**64**
Pohlia flexuosa Harv. (Mniaceae)	Kheti	26° 55' 8.832" N 95° 30' 12.636"E	Terricolous	24	0	0	26	50	0	0	**52**
Racopilum orthocarpum Wilson ex Mitt. (Racopilaceae)			Terricolous	20	0	0	30	50	0	0	**60**
Anomobryum auratum (Mitt.) A. Jaegar. (Bryaceae)	Barap	26° 55' 8.832"N 95° 30 ' 12.636"E	Saxicolous	50	0	0	0	50	0	0	0
Campylopus subgracilis Renauld & Cordot ex Gangulee (Dicranaceae)	Kheti	26° 55' 8.832" N 95° 30' 12.636"E	Saxicolous	50	0	0	0	50	0	0	0
Ceratodon purpureus (Hedw.) Brid. (Ditrichaceae)			Terricolous	50	0	0	0	50	0	0	0
Dicranella microspora (Dicranaceae)	Thinsa	26° 55' 52.68"N 95° 32' 4.2"E	Saxicolous	50	0	0	0	50	0	0	0
Dicranodontium fleischerianum W. Schultze- Motel (Leucobryaceae)	Khonsa	26° 59' 34.35"N 95° 30' 4.96" E	Terricolous	50	0	0	0	50	0	0	0
Leptodontium handelii Ther. (Pottiaceae)	Barap	26° 55' 8.832"N 95° 30 ' 12.636"E	Saxicolous	50	0	0	0	50	0	0	0
Marchantiophyta (Liverworts)											

Dumortiera hirsuta (Sw.) Nees (Dumortieraceae)	Thinsa	26° 55' 52.68"N 95° 32' 4.2"E	Terricolous	10	35	7	3	50	66	14	**80**
Jungermannia L. (Jungermanniaceae)	Khonsa	26° 59' 34.35"N 95° 30' 4.96" E	Terricolous	5	28	30	10	50	56	60	**90**
Marchantia L. (Marchantiaceae)			Terricolous	0	18	12	22	50	36	24	**100**

Negative: intersection in which no AMF structures were observed; Arbuscules: where vertical cross hair cut through the arbuscule; Vesicle: where vertical cross hair cut through the vesicle; Hyphae: where vertical cross hair cut through the hyphae

All three liverworts spp., *Marchantia* sp., *Dumortiera hirsuta*, and *Jungermannia* sp. **(Table 1)** showed the presence of hyphae, arbuscules, and vesicles in the parenchymatous tissue around the midrib, indicating active symbiosis. *Marchantia* sp. showed maximum AMF colonization (100%), followed by *Jungermannia* sp. (90%) and *Dumortiera hirsuta* (80%). Our finding is in accordance with previous studies (Vyas *et al.*, 2007; Liepina, 2012; Verma and Langer, 2014; Valdes *et al.*, 2023). AMF structures such as aseptate hyphae, arbuscules, and vesicles were reported in *Riccia* sp., *Conocephalum conicum*, *C. salebrosum*, *Fossombronia floevolata*, and *Pellia endiviifolia*, and *Marchantia nepalensis* (Vyas *et al.*, 2007; Liepina, 2012, Verma and Langer, 2014).

CONCLUSION

The study concluded that bryo-mycorrhizal associations are prevalent in moss spp., besides the well documented liverworts and hornworts. However, more studies must be conducted to determine the symbiotic nature of the association and the AMF species taking part in the colonization. Since not all mosses show AMF colonization, studies should be initiated to understand the physiology of these interactions.

ACKNOWLEDGEMENTS

The authors thank the Department of Botany, Rajiv Gandhi University, for providing laboratory facilities. The authors also thank Prof. P.L. Uniyal, Department of Botany, University of Delhi, for helping identify bryophytes. AT thanks the Ministry of Tribal Affairs for the National Fellowship for ST students.

REFERENCES

Bidartondo, M.I. and Duckett, J.G. 2010. Conservative ecological and evolutionary patterns in liverwort-fungal symbioses. *Proceedings of the Royal Society B: Biological Sciences*, **277(1680)**:485-492; doi: 10.1098/r spb.2009.1458.

Chimyang, N., Mossang, P., Shankar, V., *et al.*, 2022. Bryophytes in the ecosystem services: A review. *Journal of Bioresources*, **9(1)**:25-34; doi:10.5281/zenodo.8131443.

Chopra, R.S. 1975. Taxonomy of Indian Mosses. CSIR. Publication, New Delhi, India.

Christenhusz, M.J. and Byng, J.W. 2016. The number of known plants species in the world and its annual increase. *Phytotaxa*, **261(3)**:201-217; doi: 10. 11646/phytotaxa.261.3.1.

Cottet, A.C. and Messuti, M.I. 2019. New evidence about the interactions between liverworts in the genus *Symphyogyna* (Pallaviciniaceae) and Arbuscular Mycorrhizal Fungi. *Symbiosis*, **79(2)**:117-121; doi: 10.1007/ s13199-019-00634-2.

Gangulee, H.C. 1969-1980. Mosses of Eastern India and Adjacent Regions. Vols. I-III, New Central Books Agency Limited, Calcutta, India.

Liepiņa, L. 2012. Occurrence of fungal structures in bryophytes of the boreo-nemoral zone. *Environmental and Experimental Biology*, **10**:35-40.

McGonigle, T.P., Miller, M.H., Evans, D.G., *et al.*, 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular Mycorrhizal fungi. *New phytologist*, **115(3)**:495-501; doi: 10.1111/ j.1469-8137.1990.tb00476.x.

Mossang, P., Chimyang, N., Shankar, V., *et al.*, 2021. Bryophytes in medicine. *Journal of Bioresources*, **8(1)**:1-23; doi: 10.17605/ OSF.IO/VQ2TD.

Pressel, S., Bidartondo, M.I., Katie, J.F., *et al.*, 2021. Advances in understanding of mycorrhiza-like associations in bryophytes. *Bryophyte Diversity and Evolution*, **43(1)**:284-306; doi: 10.11646/bde.43.1.20.

Rayner, M.C. 1927. Mycorrhiza. *The New Phytologist*, **26(1)**:22-45.

Rimington, W.R., Pressel, S., Duckett, J.G., *et al.*, 2018. Ancient plants with ancient fungi: liverworts associate with early-diverging arbuscular mycorrhizal fungi. *Proceedings of the Royal Society B*, **285(1888)**:20181600; doi: 10.1098/rspb.2018.1600.

Schüßler, A. 2000. *Glomus claroideum* forms an Arbuscular mycorrhizae-like symbiosis with the hornwort *Anthoceros punctatus*. *Mycorrhiza*, **10**:15-21; doi: 10.1007/s005720 050282.

Singh, S.K. and Singh, D.K. 2009. Hepaticae and Anthocerotae of Great Himalayan National Park and its environs (HP), India. Botanical Survey of India, pp.398-400.

Turnau, K., Ronikier, M., Unrug, J. 1999. Role of mycorrhizal links between plants in establishment of liverworts thalli in natural habitats. *Acta Societatis Botanicorum Poloniae*, **68(1)**:63-68; doi: 10.5586/asbp.1999.011.

Valdés, F.E., Peralta, D.F., Velázquez, M.S., *et al.*, 2023. On the occurrence of arbuscular mycorrhizal fungi in a bryophyte

community of Puntal Lara Natural Reserve, Buenos Aires, Argentina. *Diversity*, **15(3)**:442; doi: 10.3390/d1503 0442.

Vanderpoorten, A. and Goffinet, B. 2009. Introduction to Bryophytes. Cambridge University Press, New York.

Verma, M. and Langer, A. 2014. Studies on AM associations in Marchantia nepalensis L. *Journal of Pharmacy and Biological Sciences*, **9**:26-29.

Vyas, D., Dubey, A., Soni, A., *et al.*, 2007. Arbuscular mycorrhizal fungi in early land plants. *Mycorrhiza News*, **19(2)**:22-25.

Zhang, Y. and Guo, L.D. 2007. Arbuscular mycorrhizal structure and fungi associated with mosses. *Mycorrhiza*, **17(4)**:319-325; doi: 10.1007/s0 0572-007-0107-8.