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A Study of Arbuscular Mycorrhizal Association in Three Species of *Asterella* (Aytoniaceae, Marchantiophyta) from Western Himalayan Region, India

Estudio de la asociación micorrícica arbuscular en tres especies de *Asterella* (Aytoniacaea, Marchantiophyta) de la región del Himalaya occidental, India

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Abstract

Most plant species including bryophytes and pteridophytes, develop mutualistic connections with arbuscular mycorrhizal (AM) fungi that facilitate greater water and nutrient uptake in plants and provide enhanced resistance against various biotic and abiotic stresses. The present study explores the diversity of AM associated with the thalloid liverwort *Asterella* sp. Three species of *Asterella* (*A. khasyana, A. blumeana,* and *A. wallichiana*) were studied to determine their association with AM fungi. The fungal association present in both types of rhizoids (smooth-walled and tuberculate) shows a higher degree of colonization in the smooth walled rhizoids as compared to the tuberculate rhizoids, validating the differential role of absorption performed by both types of rhizoids. *A. khasyana* and *A. blumeana* show the presence of both septate and aseptate fungal hyphae in their rhizoids, confirming the association of more than one fungus. Apart from rhizoids, the storage and midrib regions of the thallus show intense fungal colonization. Various AM structures, such as arbuscules and vesicles, are also observed in both

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rhizoids and the thallus. Spores of nine fungal species belonging to two genera, *Glomus* and *Gigaspora* were isolated from the rhizosphere soil of *A. Khasyana* and *A. wallichiana*.

Keywords: AM; arbuscules; mycorrhizae; liverworts; *Asterella*; rhizosphere; Glomeromycotina.

Resumen

La mayoría de las especies de plantas, incluidas las briófitas y las pteridofitas, desarrollan conexiones mutualistas con hongos micorrízicos arbusculares (MA) que facilitan una mayor absorción de agua y nutrientes en las plantas y proporcionan una mayor resistencia ante diversos estreses bióticos y abióticos. El presente estudio explora la diversidad de micorrizas arbusculares asociadas con las hepáticas taloides Asterella sp. Se estudiaron tres especies de *Asterella* (*A. khasyana, A. blumeana, A. wallichiana*) para determinar la asociación con MA. Asociación fúngica presente en ambos tipos de rizoides (de paredes lisas y tuberculados), se encuentra un alto grado de colonización en los rizoides de paredes lisas en comparación con los rizoides tuberculados, lo que valida el papel diferencial de absorción realizado por ambos tipos de rizoides. *A. Khasyana* y *A. blumeana* muestran la presencia de hifas fúngicas tanto septadas como aseptadas en rizoides, lo que confirma la asociación de más de un hongo. Además de los rizoides, la región de almacenamiento y de la nervadura central del talo muestra una intensa colonización fúngica. También se observan varias estructuras de MA como arbúsculos y vesículas tanto en rizoides como en talo. Se aislaron esporas de nueve especies de hongos pertenecientes a dos géneros *Glomus* y *Gigaspora* del suelo de la rizosfera de *A. Khasyana* y *A. wallichiana*.

Palabras clave: AM; arbúsculos; micorrizas; hepáticas; Asterella; rizósfera; Glomeromycotina.

INTRODUCTION

Bryophytes, the group of earliest land plants, invaded the terrestrial system and played a significant role in the habitat establishment. They constitute a significant component of some ecosystem eg. forest ecosystem. Microorganisms form intricate associations with bryophytes and play a pivotal role in establishment, promoting plant growth and facilitating nutrient cycling, contributing to the ecosystem functioning (Rousk *et al*., 2017; Xiao and Bowker, 2020; Glime, 2020). Mycorrhiza is known to be the key innovation of the terrestrial plant system and played a very significant role in the establishment and survival of plants under various abiotic conditions. (Field and Pressel, 2018). This mutualistic association leads to bi-directional exchange of photosynthates and minerals. Photosynthetically fixed host plant carbon and lipids (van der Heijden *et al*., 2015; Jiang *et al*., 2017) are utilized by fungal symbiont and in return fungi provide host plant with phosphorus and nitrogen (Leake and Read, 2016; van der Heijden *et al*., 2017).

Simple morphological structure of bryophytes with weak nutrient absorbing rhizoids facilitate symbiotic association with soil fungi to acquire water and nutrition from soil. AM association is frequently found in thalloid bryophytes such as liverworts and hornworts but lacks in mosses is likely due to their unique multicellular rhizoids removing the need for symbiosis (Pressel *et al*., 2010; Field *et al*., 2015). In bryophytes due to its lack of true root system, true mycorrhizal association is not considered by many workers (Humphreys *et al*., 2010), however many studies confirmed mutualistic exchange of nutrient-for-carbon in early diverging liverworts (Marchantiopsida, Haplomitriopsida, and Jungermanniopsida) with fungi belongs to Glomeromycotina, Mucoromycotina and Ascomycota, indicating the possible mycorrhizal-like association (Field *et al*., 2012, 2015, 2016; Kowal *et al*., 2018). Liverworts are frequently found associated with ancient AM Fungi (AMF) of Glomeromycotina (Rimington *et al*. 2018).

Asterella, a thalloid liverwort belonging to the family Aytoniaceae, with 48 species shows world-wide distribution (Bischler, 1998; Long, 2006). *Asterella* species are found to grow mainly in the exposed and nutrient poor sites. Many species of *Asterella* such as *A australis, A. bachmannii, A. bolanderi, A. chilensis, A. californica, A. khasyana, A. musicola, A. pringlei, A. tenera and A. wilmsii* are found to be associated with fungi specifically of Mucoromycotina/Glomeromycotina or both (Ligrone *et al.* 2007; Rimington *et al.,* 2020; Cottet and Messuti 2022).

A handful of information is available about fungal associated early land plants and their nutritional mutualisms. Himalaya is home to many diverse bryophytes, however a scanty information is available about the bryophyte-fungal association (Kumari *et al*., 2015; Sharma and Langer 2017; Verma *et al.*, 2022). The fungal association with bryophyte can provide unique opportunities to unravel the origin and evolution of land plants on terrestrial systems. The present study explores the extent of occurrence of association of AMF with three species of *Asterella* (*A. blumeana, A. khasyana, and A. Wallichiana*).

MATERIALS AND METHODS

Collection of material

The samples of the *Asterella blumeana, A. khasyana, and A. Wallichiana*, growing on exposed and dry soil were collected from Pithoragarh (29.4° to 30.3° N and 80° to 81° E, Western Himalayan region, India) during the month of April. The site is located at an altitude of 1645 m and the average air temperature was around 25°C. The collection site has sub-tropical type of vegetation and collection was done from the exposed dry soil from the road sides.

Clearing and staining of samples

To observe associated AM structures, the thalli and rhizoids were washed several times with tap water to remove all unwanted debris. Clearing was done by using 0.01% KOH for 24 hours. After clearing, thallus and rhizoids were stained with 0.05% Trypan Blue stain (25% lactic acid, 25% phenol, 25% distilled water and a pinch of Trypan blue stain). Stained whole rhizoid and transverse section of thallus were mounted in glycerine and observed for AM structures (fungal hyphae, arbuscules and vesicles) and photographed under the microscope (Olympus CX2li).

Isolation of AM fungal spores

AM fungal spores were isolated by a technique standardized by Gerdemann and Nicolson (1963). 5g of air dried rhizosphere soil was taken in a beaker and a suspension was made in 500 ml of water. This suspension was then stirred for a few minutes, following which the coarse particles were allowed to settle down. The soil solution was passed through the sieves of mesh numbers 50, 200 and 300 micron placed in decreasing mesh size. The contents of each mesh were washed and suspended in water in a separate beaker. Spores being lighter floated on the water surface and have a strong tendency to stick to the glass surface. Therefore, the spore suspension was gradually stirred and then filtered through Whatman paper No.1. The fungal spores form a distinct ring on the filter paper. The spore ring was then observed under a stereo microscope. AM spores are easily distinguished from the soil particles by their characteristic hyaline to coloured subtending hyphae and the wall. These spores were picked up and mounted in Hoyer's medium (distilled water 50 ml, gum Arabic 30 gm, chloral hydrate 200 gm and glycerol 20 ml) and observed under microscope. Morphological identification of the fungal spores based on spore colour, spore size, wall layers and fungal hyphal attachments was done by using suitable references e.g. Schenck *et al.* (1990) and INVAM (2025).

Quantification of mycorrhizal association

The colonization of AM was determined by the presence of arbuscules, inter/intracellular hyphae and vesicles within rhizoids and colonization of fungus in the thallus. The degree of AM colonization was determined by method given by Nicolson (1955) as follows:

$$\textit{Degree of AM colonization} = \frac{\textit{No. of rhizoids colonized by mycorrhiza} \times 100}{\textit{Total no. of rhizoids scanned}}$$

It was classified into five groups as follows (Kormanic and McGraw, 1982):

– Class I: up to 5%
– Class II: 6-25%
– Class III: 26-50%
– Class IV: 51-75%
– Class V: 76-100%

RESULT

Characteristics of fungal hyphae associated with different species of *Asterella*

Asterella blumeana (Nees) Kachroo

The AM association was present in rhizoids as well as in the thallus. Both types of rhizoids (smooth walled and tuberculate rhizoids) were found to have the AM (Fig. 1J, K). The degree of colonization of fungal hyphae in rhizoids was found to be high (76-100%) (Table 1) and specifically found higher in smooth walled rhizoids than that in tuberculate rhizoids. Two types of fungal hyphae (thin and thick) were recognized which were branched in some places. Thin hyphae were mainly present in tuberculate rhizoids. Both types, septate and aseptate hyphae were found with usually one or two hyphae strands across the length of the rhizoids. Rhizoids showed the mycelium invasion throughout the entire length. Vesicles are also formed within the rhizoids, which were darkly stained and cylindrical in shape (Fig. 1J). Fungal hyphae observed to be present throughout the thallus (Fig. 1D). Cells of the storage zone of the thallus showed extensive fungal hyphae. Vesicles of different shapes (round, cylindrical and elongated) were also observed in rhizoids and thallus. Lightly stained round vesicles were also observed in the thallus (Fig. 1G).

Asterella khasyana (Griff.) Pande, K.P. Srivat. et Sultan Khan.

The AM association was present in rhizoids as well as in thallus. Fungal hyphae were mainly present in smooth walled rhizoids and two to three fungal hyphae found running together, showing the intense invasion throughout the rhizoid. Rhizoids showed both thick and thin hyphae and some were branched (Fig. 1L, M). Hyphae showed formation of H-connection. The degree of colonization of hyphae in rhizoids was quite high (51- 75%) (Table 1). Hyphae showed differential stanning with some darkly stained and some lightly stained (Fig. 1L). The hyphae were found to be aseptate type only. Cylindrical vesicles were observed in the rhizoids (Fig. 1M). Storage zone and midrib region of the thallus was also found to be associated with intense fungal invasion (Fig. E). Arbuscule-like structures were also observed

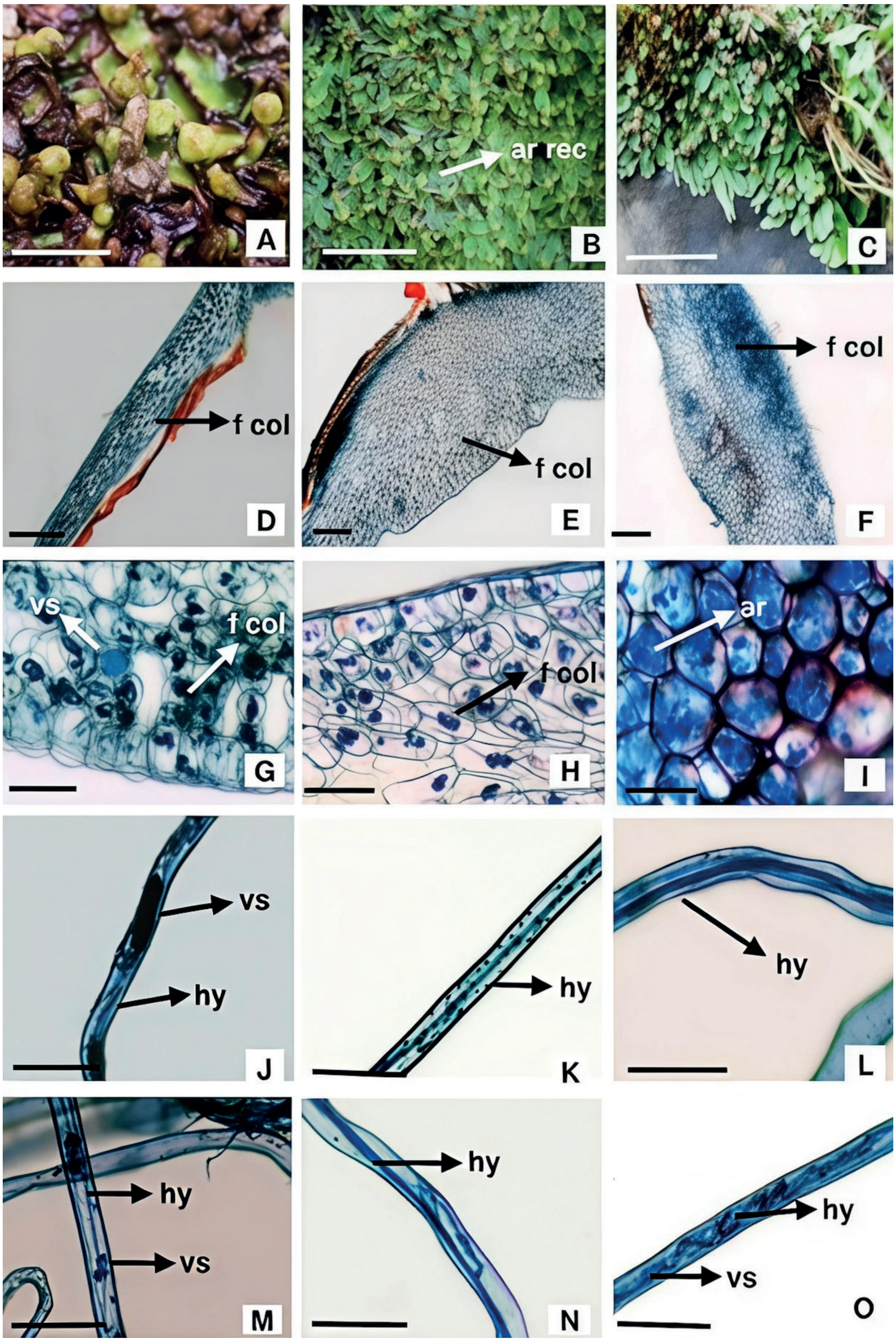

Fig. 1. A) Thallus of *A. blumeana* growing on dry soil. B) Thalli of *A. khasyana* growing on dry soil, showing the archegonial receptacle. C) Thallus of *A. wallichiana* growing on an exposed rock. D) Vertical section (V:S.) of the *A. blumeana* thallus showing fungal colonization (f col) in all zones. E) V.S. of the *A. khasyana* thallus showing greater fungal association (f end) in the storage zone, only a few cells show fungal association (f col). F) V.S. of the *A. wallichiana thallus* showing fungal association in the storage zone, with intense hyphal invasion (f col) observed in the upper region of the thallus. G) Enlarged view of *the A. blumeana* thallus, with cells showing fungal endophyte (f col). H) Enlarged section of the *A. khasyana storage zone* showing fungal colonization (f col). I) Section

of the *A. wallichiana* thallus midrib showing arbuscules (ar). J) Smooth-walled rhizoid of *A. blumeana* showing cylindrical vesicles. K) Fungal hyphae (hy) within a tuberculate rhizoid of *A. blumeana.* L) Rhizoid showing parallel running hyphae (hy) in *A. khasyana.* M) Rhizoid of *A. khasyana* showing disintegrating vesicles (vs). N) Rhizoid of *A. wallichiana* showing Y-sphaped connection formed by fungal hyphae (hy). O) Rhizoid of *A. wallichiana* showing hyphae (hy) and an oval vesicle (vs). Scale bars: Figs. A, B, and C = 3mm; D, E, and F= 50μm; G, H and I = 25μm; J-O = 100μm.

Fig. 1. A) Talo de *Asterella. blumeana* creciendo en suelo seco. B) Talos de *A. khasyana* creciendo en suelo seco mostrando el receptáculo arquegonial. C) Talo de *A. wallichiana* creciendo en roca expuesta. D) Corte vertical (V.S.) del talo de *A. blumeana* mostrando colonización fúngica (f col) en todas las zonas. E) Corte vertical (V.S.) del talo de *A. khasyana* mostrando mayor asociación fúngica (f end) en la zona de almacenamiento, solo unas pocas células muestran asociación fúngica (f col). F) Corte vertical (V.S.) del talo de *A. wallichiana* mostrando asociación fúngica en la zona de almacenamiento del talo, con una intensa invasión hifal (f col) observada en la zona superior del talo. G) Vista ampliada del talo de *A. blumeana*, células mostrando endófito fúngico (f col). H) Sección ampliada de la zona de almacenamiento de *A. khasyana* mostrando colonización fúngica (f col). I) Sección del nervio medio del talo de *A. wallichiana* mostrando arbúsculos (ar). J) Rizoide de paredes lisas de *A. blumeana* que muestran vesículas cilíndricas. K) Hifas fúngicas (hy) dentro del rizoide tuberculado de *A. Blumeana.* L) Rizoide que muestra hifas paralelas (hy) en *A. Khasyana.* M) rizoide de *A. khasyana* que muestra vesículas desintegrables (vs). N) Rizoide de *A. wallichiana* que muestra conexión Y formada por hifas fúngicas (hy). O) rizoide de *A. wallichiana* que muestra hifas (hy) y vesícula ovalada (vs). Barras de escala: Fig. A, B y C = 3 mm; D, E y F= 50μm; G, H e I = 25 μm; J-O = 100 μm.

in the cells of the storage zone (Fig. 1H). Fungal spores of *Glomus citricola, G. fulvum, G. fasciculatum, G. macrocarpum and G. microcarpum* were isolated from rhizosphere soil of *A. khasyana* (Fig 2A, B,C,D,F,H).

Asterella wallichiana St.

The fungal hyphae were found in smooth walled rhizoids (Fig. 1N,O). The degree of colonization of fungal hyphae in rhizoids was estimated to be 51-75% (Table 1). The thick-walled and branched aseptate hyphae were found in rhizoids. Few darkly stained oval and cylindrical vesicles (Fig. 1O) and Y-connection of hyphae (Fig. 1N) were also recorded. In the thalli the hyphae were restricted to the midrib region, which showed many arbuscule-like structures (Fig. 1F, I). Fungal spores of eight species *Gigaspora rosea*, *Glomus citricola, G. fasciculatum, G. geospermum, G. macrocarpum, G. maculosum, G. microcarpum and G. monosporum* were isolated from rhizosphere soil of *A. wallichiana.*

Characteristics of fungal spores associated with different species of *Asterella*

Gigaspora rosea Nicolson & Schenck

Isolated from the rhizosphere soil of *A. wallichiana*. The colour observed from pale yellow to brown and shape varies from globose to subglobose

Table 1. List of three *Asterella* species and the fungal structures formed by arbuscular mycorrhizal fungi.

Tabla 1. Lista de tres especies de *Asterella* y estructuras fúngicas formadas por hongos micorrícicos arbusculares.

Species N°	Taxa	Habitat	Degree of colonization	Presence of Hyphae in Rhizoids		Hyphae location	Characteristics of Hypae	Arbuscules	Shape of vesicles	Fungal spore isolated from Rhizosphere soil
				SW	TUB					
1	*Asterella blumeana*	Dry soil	V	+	+	Rhizoids, thallus Rhizoids, thallus	Thick, thin, branched, aseptate, septate	+	Round, cylindrical, elongated	NR
2	*Asterella khasyana*	Dry soil	IV	+	-	Rhizoids, thallus	Thick, branched, septate, aseptate	+	Cylindrical	*Glomus citricola, G. fulvum, G. fasciculatum, G. macrocarpum* and *G. microcarpum*
3	*Asterella wallichiana*	On rocks, dry exposed places	IV	+	-		Thick, branched, aseptate	+	Cylindrical, oval	*Gigaspora rosea, Glomus citricola, G. fasciculatum, G. geospermum, G. macrocarpum, G. microcarpum, G. maculosum, G. monosporum*

SW= smooth-walled rhizoids, TUB= tuberculate rhizoids, (+) = present, (-) = absent, NR= Not reported.

SW = rizoides de pared lisa, TUB = rizoides tuberculados, (+) = presente, (-) = ausente, NR = No reportado.

with diameter ranges from 160 to 230 μm. Spore wall is three layered, outer layer hyaline, inner layer is hyaline with sublayers (Fig 2A).

Glomus citricola Tang & Zang

Spores isolated from *A. khasyana* and *A. wallichiana* and present singly. Shape varies from globose to ovoid with 35 to 65 μm in size. Spore consists of two layers, the outer layer is hyaline, the inner layer is 0.8-2.0 μm thick (Fig 2B).

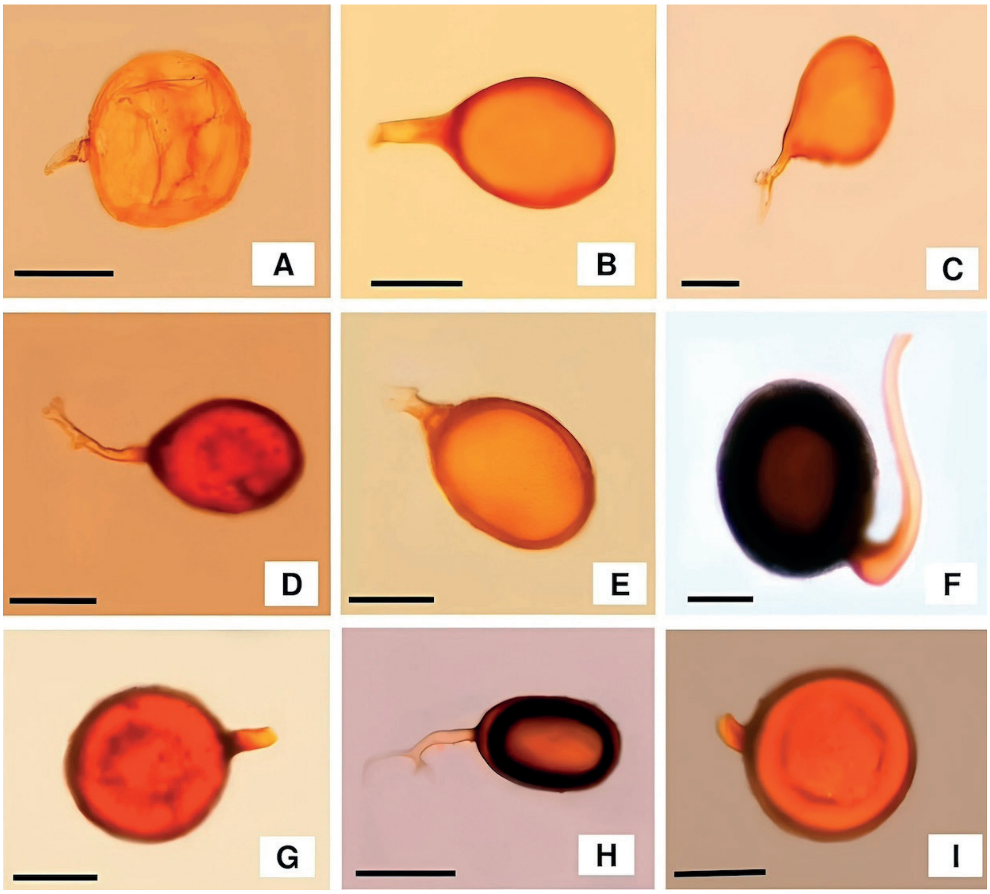

Fig. 2. Fungal spores isolated from the rhizosphere soil of *Asterella khasyana* and *A. wallichiana*. A) *Gigaspora rosea.* B) *Glomus citricola.* C) *G. fulvum.* D) *G. fasciculatum.* E) *G. geospermum.* F) *G. macrocarpum.* G) *G. maculosum.* H) *G. microcarpum.* I) *G. monosporum* Scale Bars: Figs. A-I = 20μm.

Fig. 2. Esporas de hongos aisladas del suelo de la rizosfera de *Asterella khasyana* y *A. Wallichiana.* A) *Gigaspora rosea.* B) *Glomus citricola.* C) *G. Fulvum.* D) *G. Fasciculatum.* E) *G. Geospermum.* F) *G. Macrocarpum.* G) *G. Maculosum.* H) *G. Microcarpum.* I) *G. Monosporum.* Barras de escala: Figs. A-I = 20 μm.

G. fasciculatum (Thaxt.) Gerdmann & Trappe. emend. C. Walker & Koske

Spores are 50 to 130 μm in size and are present singly with one subtending hyphae, isolated from the rhizosphere soil of *A. khasyana* and *A. wallichiana*. They are pale yellow in colour and shape varies from globose to sub-globose. Spore wall is three layered, the first layer is hyaline, second layer is smooth, pale yellow in colour and third layer is 0.5 μm thick and flexible (Fig 2D).

G. fulvum (Berkeley & Broome) Trappe & Gerdmann

Spores isolated from *A. khasyana*, are clavate in shape. Spore wall is single laminated, hyaline to pale brown in colour. Single subtending hyphae is present at the point of attachment (Fig. 2C).

G. geosporum Gerdemann & Trappe

Spores of *G. geosporum* were isolated from *A. wallichiana.* They were found singly in the soil. The colour ranges from yellow to orange and shape varies from globose to sub globose with a subtending hyphae at the point of attachment. Three layered spore wall is present. Outer layer is hyaline, middle layer is thick and yellow to orange in colour whereas, inner layer is semi rigid, pale yellow to orange coloured (Fig.2F).

G. macrocarpum Tulasnes & Tulasnes

Reddish coloured *G. macrocarpum* spores were isolated from rhizosphere soil of both *A. khasyana and A. wallichiana*. They were present in sporocarps consisting of 2-15 spores in a group with globose to subglobose shape. Spore size varies from 80-149 μm in diameter consisting of two layered wall, outer hyaline often absent in mature spores, inner layer thicker, dark reddish brown. Spore wall is found perforated at maturity (Fig 2E).

G. maculosum Miller & Walker

Spores of this species are found in the rhizosphere soil of *A. wallichiana*. They are globose to subglobes and borne singly in the soil with diameter 135 to 200 μm. They are hyaline, pale yellow coloured and become ochraceous at maturity with one to three subtending hyphae present. Spore wall is three layered, the outer layer is hyaline, the middle layer is pale straw to ochraceous coloured, and the inner layer is around 0.5-1.0 μm thick (Fig. 2G).

G. microcarpum Tulasnes & Tulasnes

Spores of this species were isolated from the rhizosphere soil of *A. khasyana, A. wallichiana.* Spores are present in groups, with diameter ranges from 22 to 55 μm. Colour varies from pale yellow to golden yellow, possessing globose to sub globose shape with subtending hyphae. Wall consists of two layers, the outer layer is hyaline and rough, the inner layer is smooth, pale yellow to golden yellow (Fig.2H).

G. monosporum Gerdemann & Trappe

Spores, isolated from rhizosphere soil of *A. wallichiana* were present singly, occasionally two or three. Spore shape varies from globose to sub globose. Wall of the spore is dull brown with single subtending hyphae (Fig.2I).

DISCUSSION

Present study confirms the presence of AM association with *Asterella blumeana*, *A. khasyana* and *A. wallichiana*. Arbuscules and hyphae were found in the internal parenchyma cells of the thallus in the midrib region. Rimington *et al*. (2018) confirmed AM association in *A. khasyana* showing association with fungi belonging to Mucoromycotina and Glomeromycotina. With respect to internal fungal colonization all three studied species of *Asterella* show association in both rhizoids as well as in the thallus. Frequent colonization observed at midrib region of thallus. Rimington *et al*. (2018) also reported fungal colonization at central midrib of liverwort thallus but its extent of fungal colonization varies from plants to plants. Bryophytes, being poikilohydric, absorb through the entire surface of the gametophyte. In present study it is observed that the mycorrhizal association in liverworts not only restricted to its absorptive organs (rhizoids) but also in midrib region and other parts of thallus. Presence of AM colonization apart from rhizoids might have helped in translocation and distribution of nutrients in absence of a well-developed conducting system in liverworts.

The primary role of rhizoids is the attachment to the substratum (Duckett *et al*., 1998; Crandall-Stotler *et al*., 2008; Goffinet *et al*., 2008). In present investigation fungal colonization is observed to be more in smooth walled rhizoids as compared to tuberculate rhizoids. *A. blumeana* shows association in both types of rhizoids. Tuberculate rhizoids are thick walled, have small diameter and peg-like tubercles. Whereas, smooth rhizoids are thin walled and large diameters and highly efficient in water absorption (Cao *et al*., 2014). McConaha (1941) stated that smooth-walled rhizoids normally grow forward, penetrate the soil, and help in absorption of water and minerals, whereas tuberculate rhizoids originate beneath the scales and prevent them from growing downwards. This acts as a capillary conducting system parallel to the thallus which helps in the distribution of water to all absorptive parts of the gametophyte. The variation in structure and distribution of the two types of rhizoids are related to its functions of anchoring, plant support and absorption (Cao *et al*., 2014). Presence of higher fungal colonization in smooth-walled rhizoids, compared to tuberculate rhizoids, confirms the specialized functional role of these structures. Smooth-walled rhizoids primarily enhance nutrient absorption directly from the soil, whereas tuberculate rhizoids are more involved in the internal distribution of water and nutrients, showcasing a sophisticated division of labour within the plant architecture that optimizes its survival efficiency.

Liverworts associated with Glomeromycotina fungi produce variable structures like aseptate hyphae, arbuscules and vesicles, which are very similar to those formed by the same fungi associated with vascular plants. All three studied species show presence of AM structures like aseptate hyphae, arbuscules and storing structure vesicles (Fig 1A, Table 1).

A. blumeana and *A. khasyana* show association with both septate and aseptate hyphae in the rhizoids (Fig 1A). Presence of two types of fungal hyphae confirms diverse association of fungi with bryophytes. Pressel *et al.* (2010), Bidartondo *et al.* (2011), Field *et al.* (2012, 2015) and Rimington *et al*. (2020) also reported the association of liverworts not only restricted with fungi belongs to Glomeromycotina but with other groups like Mucoromycotina, Basidiomycota or Ascomycota fungi are also show association. A considerable variation in vesicles shape (round, cylindrical, oval and elongated) is also observed in present study (Fig. 1 G,J,M,O). The variation in shape, size, position of vesicles and diverse pattern of colonization show presence of diverse AM fungi (Ligrone *et al*. 2007; Rimington *et al*., 2018). Marchantiopsida is reported to co-colonize with diverse AM fungi such as Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae along with Glomeraceae are reported in *Marchantia* (Rimington *et al*., 2018).

Nine fungal species belongs to two genera *Glomus* and *Gigaspora* were isolated from the rhizosphere soil of *A. khasyana* and *A. wallichiana* (Table 1, Fig. 2). Liverworts known to form association with fungi belong to Glomeromycotina, but it exhibits high specificity towards fungi belonging to Glomeraceae. It also commonly found to be associated with liverworts and hornworts (Rimington *et al*., 2018). In previous studies *Glomus* spp. are most commonly reported to form association with many bryophytes (Parke and Lindernam 1980; Turnau *et al*., 1999; Schüβler, 2000; Redecker *et al*., 2000). Present study also supports earlier observation of high affinity of liverworts towards AM fungi belonging to Glomeraceae.

CONCLUSION

AM colonization is present in both thallus as well as in rhizoids of the studied species of *Asterella*. More colonization in smooth walled rhizoids as compared to tuberculate confirms the function of differential labour of rhizoids. The main function of absorption is performed by smooth walled rhizoids and its association with fungi enhances the absorption capability. Presence of two types of fungal hyphae suggest that association of liverworts with more than one fungi which helped not only in nutrient translocation but also helped to survive in stressful conditions. Liverworts occupy a critical phylogenetic position in the evolution of land plants, serving as vital models for studying the origins of plant-fungi symbiosis. Despite their importance, research into AM associations in liverworts remains scarce, highlighting a significant gap in our understanding of plant evolution. Our findings of *A. blumeana, A. khasyana, and A. wallichiana* forming AM association help in adding more information on AM associated *Asterella* sp. and also our knowledge of fungal associations with liverworts.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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