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Research Article

ISOLATION OF MYCORRHIZAL SPORES FROM IN AND AROUND BABINA FOREST, JHANSI (U.P.) INDIA

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A B S T R A C T

Soil is a habitat of large number of micro- organisms viz bacteria, fungi, actinomycetes, algae and protozoa etc. constituting biotic environment of soil micro-ecosystems. These micro-organisms play important role in numerous physiological activities including biogeochemical cycle. For this microbial community forms associations with other organisms such as saprophytic, parasitic and symbiotic associations. Some of the soil inhabiting rhizospheric fungi have been found as growth promoting while some are pathogenic. Several soil fungi have been found to have symbiotic association with roots of higher plants which are called as mycorrhizal associations. Mycorrhizal associations in plants are of two type ectomycorrhiza or extra matrical spore and endomycorrhiza. Extra matrical spores of mycorrhiza are quite common and can survive in environment of low fertility, drought, disease and temperature extremes, where alone they fail to survive. Present work has been carried out to isolate and identify mycorrhizal spores growing symbiotically with roots of trees in Babina forest range of Jhansi district of Uttar Pradesh. For this surveys have been carried and soil samples have been collected. Using wet sieving method spores have been isolated and identified on the basis of shape, size and colour. Spores belongs to genera are Acaulospora, Glomus, Enthrophospora, Gigaspora and Scutellospora out in different seasons such as winter summer and rainy from December 2017 to November 2018.

INTRODUCTION

Increasing anthropological activities have caused many environmental challenges. Increased use of chemical fertilizers, fungicides, herbicides and pesticides are making soil, as well as farm yield decreased and thus resistance has started from a sector of society against use of such organochemicals. This led agricultural scientist to think about some alternative practices such as use of biofertilizers based organic farming. Fungi are eukaryotic spore producing, achlorophilous, organisms with a huge range of nutritional range. Among various types of associations that fungi form with other organisms, some members from symbiotic associations e.g. Lichens and Mycorrhiza.

Mycorrhizal fungi form a symbiotic relationship with the roots of higher plants. In this relationship fungi provides water for plants in increased amount, while the plants provide the fungus with carbohydrates formed from photosynthesis. Mycorrhizal fungi play an important role in absorption of nutrients from soil (Smith & Read, 1997).The world wide occurrence of these non-destructive fungal associations with plants shows that they have an important role in plant survival (Barrow *et al.*, 1997).

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Mycorrhizal fungi could be the most important and poorly understood resource for nutrient accession and plant growth in agriculture. Colonization of mycorrhiza on host plant helps in the suppression of disease (Cardoso and Kuyper, 2006).The symbiosis between plant roots and fungi works as a bridge for the energy and matter between plants and soil (Cordon and Whitbeck, 2007). The fungal hyphae are much thinner than roots and are able to penetrate smaller pores (Allen, 2011).

There are two major groups of Mycorrhizal fungi-Ectomycorrhiza and Endomycorrhiza. In ectomycorrhiza which is predominant on trees in temperate forest, the fungal partner associates with the outside in of plant cells, whereas in endomycorrhiza, including orchid, ericoid and arbuscular mycorrhiza (AM) part of the fungal hyphae is inside. Endomycorrhiza (VAM) fungus can be recognised by the irregular coenocytic hyphae which penetrate directly through the cells of outer cortex of the roots. Coils and continuous loops are frequently produced by penetrating hyphae. When hyphae reach the inner cortex, their growth is mainly intercellular with hustorium-like structures called arbuscules forming with in cells. Terminal bodies called vesicles often form in the intercellular spaces in the cortex. (Gerdemann, 1955).

Butler (1939) described vesicles produced outside of roots and noted their resemblance to the so called chlamydospores of the Endogonaceae. More than 80% of all land plant families are thought to have a symbiotic relationship with AM fungi that belong to the Glomeromycota.

Only a few families and genera of plants do not generally form arbuscular mycorrhizas; these include Brassicaceae (Their roots exudates are possibly even toxic to AM fungi) (Glenn *et al.*, 1988). Phylum Glomeromycota includes more than 10 genera namely- *Glomus* A, *Glomus* B, *Gigaspora, Acaulospora, Archaeospora, Diversispora, Enthrophospora, Sclerocystis, Scutellospora, Paraglomus, Pasispora* (Robinson-Boyar *et al.,* 2009). The main advantage of mycorrhiza is its greater soil exploration and increasing uptake of P, N, K, Zn, Cu, S, Fe, Mg, Ca and Mn and the supply of these nutrients to the host roots (Javot *et al.*, 2007, Sundar *et al.*, 2010).

Increased diversity of AMF positively affects plant biodiversity, variability and productivity.(Van der Heijden *et al.*, 1998, O'connor *et al.*, 2002). AM fungi produce glycoprotein, glomalin that is deposited on their outer hyphal walls and on adjacent soil particles (Miller and Jastrow, 2000). Spores are the most important propagules for most AMF and the impact these organisms produce on their hosts will spend on the ability for fast spore germination and colonization (Tommerup, 1983).

MATERIALS AND METHODS

The rhizospheric soil samples were collected from the Babina forest range, Jhansi for the isolation were of Mycorrhizal fungi at the depth of about 10-15cm deep. Babina is located in Jhansi District between 25°15′0.00″ N and 78°28′12.00″ E. (Fig. 1a, 1b and 1c.). Collected samples were brought to the laboratory for isolation and morphological study of mycorrhizal spores.

Wet sieving of soil sample

50 g. of collected rhizospheric soil mixed with hot water (40-50°C), the suspension was stirred several times with glass rod and allowed heavier particles settle down. The upper residues of the solution decanted with the help of nelgene sieves of different pore size (250µm to 38µm) and washed several times under the tap water. The residues those left on the different pore size sieves collected and centrifuged twice at 4000 rpm for 5 minutes. Pellets were collected and placed in 5ml of 5% sucrose solution. After centrifugation collected pellets are then transferred in Ringer's solution and examined microscopically. Mycorrhizal spores were picked with the help of a needle and a thin painting brush and placed in a watch glass containing clear distilled water. Isolated spores are kept in Ringer's solution for future use. (Gerdemann and Nicolson,1963).

Fig 1 a

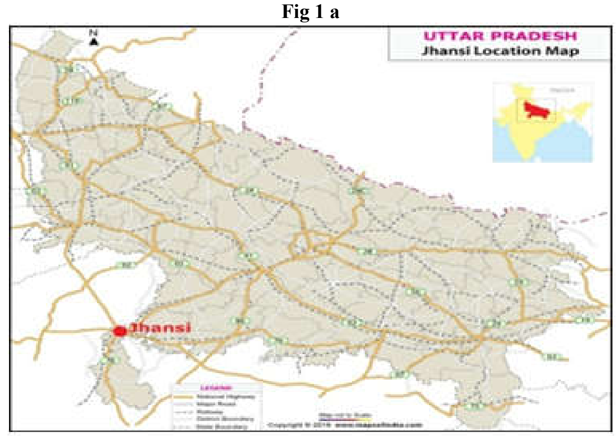

Fig 1b

Fig 1c

Description of research area

Ringer's solution

Chemical	Composition
NaCl	0.6g.
$CaCl_2$	0.01g.
KCl	0.01g
$MgCl_2$	0.01g.
Distilled water	100ml.

Size Measurement of spore

Mycorrhizal spores size measured by micrometry (Lawson, 1972). The occulometer was calibrated as-

One division on ocular micrometer in mm

$$= \frac{\text{known distance between two lines on stage micrometer}}{\text{number of divisions on ocular micrometer}}$$

RESULT AND DISCUSSION

Babina forests are dominated by angiospermic perennial trees. Dominant trees in the forest are

***Acaulospora brasiliensis* (A)**

These spores are a sparkling brownish yellow appearance in water under reflected light. These are approximately m 200 µm in size, globose, ovate in shape with roughened and pitted wall surface.

Khair (*Acacia catechu,* Fabaceae), Revanja (*Acacia leucophloea,* Fabaceae), Kardhai (*Anogeissus pendula,* Combretaceae), Dhak/palas (*Butea monosperma,* Fabaceae), Dhaman (*Grewia tiliaefolia,* Tiliaceae), Kaim (*Stephyegyne parvifolia,* Rubiaceae), Sagon (*Tectona grandis,* Lamiaceae), Dudhi (*Wrightia tinctoria,* Apocynaceae), Ber (*Zizyphus jujube,* Rhamnaceae), Ghont (*Zizyphus xylopyra,* Rhamnaceae), Gunj (*Abrus precatorius,* Fabaceae), Imali (*Tamarindus indica,* Fabaceae), Sheesham (*Dalbergia sissoo*, Fabaceae), Amaltas (*Cassia fistula,* Fabaceae), Neem (*Azadirachta indica,* Meliaceae), Menar (*Randia dumetorum*, Rubiaceae), Kaker (*Sterculia urens,* Malvaceae). Rhizospheric soil around trees of Babina forest have been collected in different seasons i.e. winter, summer and rainy season of 2018 have various types of mycorrhizal spores (Fig.2 A-S). It has been found during present study that maximum number of spores were found during winter season as compared to rainy and summer season. It has also been found that in summer season spore frequency is least.

Isolated Mycorrhizal spores have been identified with the help of keys suggested by- Gerdemenn & Trappe (1974), Becker and Hall (1976), Trappe (1977), Daniels and Trappe (1979), Trappe (1982), Trappe *et al.,*(1982), Schenck and Smith (1982), Walker (1982), Sieverding and Toro (1987),, Blaszkowaki (1988), Schwarzott *et al* (2001), Silva *et al.,* (2005), Kruger *et al*., (2011), Amutha and Shalini (2016).

***Acaulospora laevis* (B)**

Acaulospora laevis spore are large, generally globose, ellipsoid and thin walled. The spores were measured around 250 µm diameter. Surface of spores smooth or dull roughed.

***Acaulospora thommi* (C)**

Spores found singly in the soil and occasionally globose, subglobose, ellipsoid or irregular shaped, approximately 450 µm in diameter. Spores dark brown to orange yellow in colour. Spore wall thick and multilayered.

***Enthrophospora schenkii* (D)**

Spores hyaline, globose to subglobose, sometimes ovoid to pear shaped, 100 µm in diameter with smooth wall surface. Spores appear sparkling white when mature. Spores appear sparkling white when mature.

***Scutellospora nigra* (E)**

These spores are globose and ellipsoid with black colour, 300 µm in diameter and spore surface rough and pitted.

***Scutellospora scutata* (F)**

Spores hyaline/white to yellow brown, globose, subglobose and irregular in shape, 350 µm in diameter with roughened/smooth wall surface.

***Gigaspora albida* (G)**

Gigaspora albida formed singly in soil, dull white with a light yellow colour, occasionally ellipsoid and mostly spherical with about 250 µm diameter. Wall surface of spore may be smooth/roughened or pitted.

***Gigaspora gigantea* (H)**

These spores also formed singly in soil, about 400 µm in diameter, globose to ellipsoid with brownish yellow colour. Surface of spore are thick and smooth.

***Gigaspora margarita* (I)**

These are formed singly in soil, approximately 350 µm in diameter, yellowish brown to reddish brown in colour with smooth wall surface.

***Glomus constritum* (J)**

Spores of *Glomus constritum* are globose, ovate and ellipsoid in shape with 200 µm diameter. Colour of spore are black or brownish black and surface or spore are smooth.

***Glomus flavisporum* (K)**

These spores are globose and ellipsoid in shape around 175 µm diameters. Colours of spores are yellowish orange or brown and surface of spore may be smooth or dull roughened.

***Glomus fasciculatum* (L)**

Spores are globose, ovate and ellipsoid and around 75 µm in size and yellowish brown in colour. Surface of spore are smooth.

***Glomus magnicaulis* (M)**

Glomus magnicaulis spores are globose, subglobose and ovate in shape. Size of spore is about 125 µm. Colours of spore are brown with smooth wall surface.

***Glomus epigaeum* (N)**

Spores are mostly globose, subglobose and ovate in shape. Size of spores approximately 175 µm. Hyaline yellow/brown in colour with smooth wall surface.

***Glomus intraradices* (O)**

These spores are predominantly globose, subglobose, ellipsoid and yellow to grey brown in colour, 200 µm in diameter with smooth/dull roughened surface of spore.

***Glomus deserticola* (P)**

These spores are reddish brown in colour and mostly irregular and occasionally globose in shape. The surface of spores smooth/dull roughened and size of spore are 75 µm.

***Glomus mosseae* (Q)**

Glomus mosseae are globose to irregular in shape, pale yellow in colour, usually with typical funnel shaped hyphae. Spore approximately 150 µm in size and surface of spore are smooth.

Glomus occultum (R)

These spores are completely hyaline/whitish in colour and globose/ovate in shape. Size of spores 150 µm and surface of spore are roughened/ smooth.

Glomus sinuosum (S)

Glomus sinuosum are globose or pulvinate in shape with irregular surface due to protruding spore covered by a dense peridium. Colour of spores are dark brown or blackish brown and size of spores approximately 280 µm.

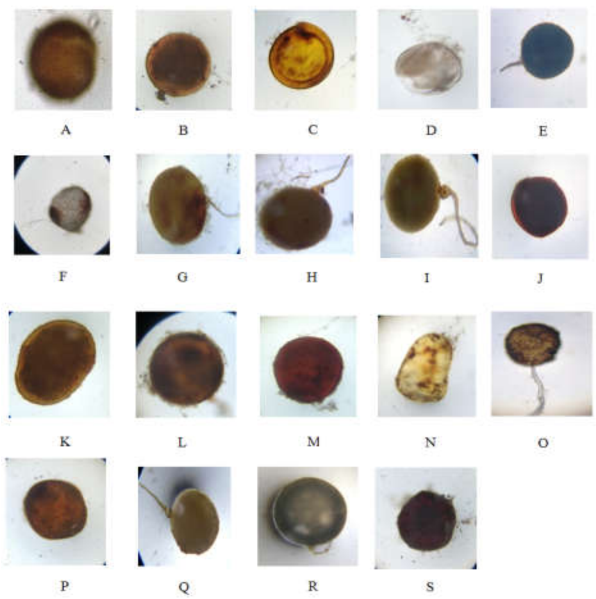

Fig 2 Isolated mycorrhizal spores: (A) *Acaulospora brasiliensis*, (B) *Acaulospora laevis* (C) *Acaulospora thommi*, (D) *Enthrophospora schenkii*, (E) *Scutellospora nigra*, (F) *Scutellospora scutata*, (G) *Gigaspora albida*, (H) *Gigaspora gigantia* (I) *Gigaspora margarita*, (J) *Glomus constritum*, (K) *Glomus flavisporum*, (L) *Glomus fasciculatum*, (M) *Glomus magnicaulis*, (N) *Glomus epigaeum*, (O) *Glomus intraradices*, (P) *Glomus deserticola*, (Q) *Glomus mosseae*, (R) *Glomus occultum*, (S) *Glomus sinuosum*.

Table 1 Morphological description of Mycorrhizal spores

S.N.	Spore type	Shape	Size (µm)	Colour	Wall surface
1.	*Acaulospora brasiliensis*	Globose, ovate	200 µm	Brownish yellow	Roughened, pitted
2.	*Acaulospora laevis*	Globose , ellipsoid	250 µm	Hyaline, brown	Smooth,dull roughened
3.	*Acaulospora thommi*	Globose, ellipsoid	450 µm	Yellowish orange	Smooth
4.	*Enthrophospora schenkii*	Globose, subglobose, ellipsoid, ovate	100 µm	Completely hyaline	Roughened, smooth
5.	*Scutellospora nigra*	Globose, ellipsoid	300 µm	Black	Roughened, pitted
6.	*Scutellospora scutata*	Globose, subgolobose, irregular	350 µm	Hyaline, whitish	Roughened, smooth
7.	*Gigaspora albida*	Globose	250 µm	Yellow, blackish brown	Smooth, pitted, roughened
8.	*Gigaspora gigantia*	Globose	400 µm	Yellow, brownish yellow	Smooth
9.	*Gigaspora margarita*	Globose, ovate	350 µm	Redish brown	Smooth
10.	*Glomus constritum*	Globose, ovate, ellipsoid	200µm	Brownish black, black	Smooth
11.	*Glomus flavisporum*	Globose, ellipsoid	175 µm	Orange, yellow brown	Smooth, dull roughened
12.	*Glomus fasciculatum*	Globose, ovate, ellipsoid	75 µm	Yellowish brown	Smooth
13.	*Glomus magnicaulis*	Globose, subglobose , ovate	125 µm	Brown	Smooth
14.	*Glomus epigaeum*	Globose, subglobose , ovate	175 µm	Hyaline yellow, brown	Smooth
15.	*Glomus intraradices*	Globose, ovate, ellipsoid	200 µm	Hyaline, inner yellow brown	Smooth, dull roughened
16.	*Glomus deserticola*	Globose, irregular	75 µm	Redish brown	Smooth, dull roughened
17.	*Glomus mosseae*	Globose, irregular, ovate	150 µm	Hyaline, light brown, yellowish	Smooth
18.	*Glomus occultum*	Globose, ovate	150 µm	Hyaline, whitish	Smooth, roughened
19.	*Glomus sinuosum*	Globose, pulvinate	280 µm	Dark brown	Irregular surface due to protruding spore covered by a dense peridium

Spores in the soil may be produced terminally laterally on subtending hyphae or on a single suspensor like cell. Characters of spores (colours, shape and size) may vary considerably depending on the development stage and environment conditions. Spore colour varies from hyaline to white to yellow, red, brown and black with all intermediate shades. The difference in colour may be due to pigmentation in spore wall or in spore content (Morton, 1988).

Morton (1988) suggested that variation in spore shape might be due to the result of environmental stress. Shape of spores is mainly governed by the genotype of the fungus and the substrate in which the spores are formed. Intraradical spores are mainly globose, subglobose to ellipsoid while the extraradical spores may be globose, subglobose, ellipsoidal, oblong ovate to highly irregular shaped (Muthukumar *et al*, 2009).

During microscopic examination spore were identified on the basis of morphological characters such as colour, shape, size and surface of spores. Total 5 genera of mycorrhizal spores are being reported from rhizospheric soil samples collected from the forest of Babina, Jhansi are- *Acaulospora, Glomus, Gigaspora, Scutellospora and Enthrophospora* with 19 species.

CONCLUSION

Babina forest is located in the district of Jhansi at 25°15´0.00″ N and 78°28´12.00″ E cardinal and has about 17 types of angiospermic trees. Soil samples have been collected randomly from the rhizoshpere and total 5 genera of mycorrhizal spores are being reported from rhizospheric soil samples collected from the forest of Babina, Jhansi. Genus *Glomus* isolated with 10 species, *Acaulospora* with 3 species, *Gigaspora* with 3 species, *Scutellospora* with 2 species *and Enthrophospora* with 1 species. Spores were identified on the basis of morphological characters such as colour, shape, size and surface. Spores which were collected in winter season were higher in number in comparison to any other seasons. The soil of Babina forest, have mycorrhizal spores in good quantity. As compared to other genus *Glomus* is more abundant in soil of Babina forest.

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