REVIEW OF RESEARCH
IMPACT FACTOR : 5.7631(UIF) UGC APPROVED JOURNAL NO. 48514 ISSN: 2249-894X
VOLUME - 8 | ISSUE - 3 | DECEMBER - 2018

EXTRACTION OF ARBUSCULAR MYCORRHIZAL CHLAMYDOSPORES

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INTRODUCTION

a) Isolation of spores - Wet Sieving and Decanting method (Gerdemann and Nicolson, 1963):

Number of workers from time to time have evolved techniques for spores' separation but those techniques which remained nearly close to wet sieving and decanting (Gerdemann and Nicolson, 1963) were only found successful in the recent time. Therefore, spore extraction was done by wet sieving and decanting technique, which involved three steps – wet sieving, flotation and sedimentation. The steps involved in the procedure are:

1) Air dried rhizospheric soil was sieved through a series of sieves of mesh size 1-2 mm so as to remove unwanted stones and other organic debris from the soil sample.
2) Mixed a known volume or weight of soil (50g) with four times volume of water, stirred well, allowed heavier particles to settle and then decanted through a series of sieves arranged one above other in descending order having mesh sizes of 500µm, 300µm, 250µm, 125µm, 105µm and 45µm. The procedure was repeated until a statistically constant number of spores were removed from the sample. The number of repetitions varied with the soil type and volume of the sample.
3) Individually material from each mesh was washed into separate beakers. Then from each beaker, a small amount of the extract is transferred into separate petri dishes for its examination under a dissecting microscope for identifying and counting spores.

KEYWORDS: evolved techniques , organic debris.

INTRODUCTION :

b) Estimation of spores (Gaur and Adholeya, 1994) and Calculation of Spore Density:

Material required: Whatman No. 1 (size 11 cm diameter) filter paper, beakers, each containing the soil solution of respective sieve, Petri plate.

Procedure:

1) Whatman No. 1 filter paper was taken and folded into two equal halves. This was followed by a second fold, resulting in four equal quadrants. The paper was then reopened and two lines were drawn along the two folds in such a way that the filter paper was divided in four equal quadrants.
2) Vertical lines were drawn on one half of the filter paper, approximately 15 columns with each column about 0.5cm apart. Each column was numbered and the direction of counting was marked with an arrow mark.
3) The filter paper was then folded in such a manner that the marked portion became the receiving surface for the sample during filtration. Thus, the spores were received only on the marked surface of the filter paper and the rest of the filter paper was retained without spores.
4) This filter paper with spores laden on its marked surface was carefully spread in a petri plate (120 mm in diameter).

5) Under a stereo-zoom binocular microscope column by column in a vertical direction (i.e. in the direction of arrow) the filter paper was scanned for spore count by moving the petri plate in vertical direction column by column.
6) The spore density was then calculated and expressed in units per gram of dry soil.

c) Mounting of spores on glass slides (Schenck and Perez, 1990):

The procedure was mainly based on mountant PVLG (Polyvinyl lacto-Glycerol) (Koske and Tessier, 1983). However, Melzer's reagent was also used along with PVLG mountant, both were known to affect certain spore characteristics, such as wall features (Morton, 1988) and spore colour.

1) Spores collected on filter paper were selected under the stereo-zoom binocular microscope with the help of HPLC capillary of 50µm diameter. The spore was then dropped in the cavity of the cavity slide.
2) Here the spore was treated with surfactant i.e. 0.2% Chloramine-T, for a few minutes followed by 0.5% Streptomycin solution.
3) The spore was then rinsed with distilled water so as to remove the excess of surfactant.
4) The spore was again lifted with the capillary and was placed in the centre of a slide and a drop of Melzer's reagent was dropped on it.
5) Two drops of mountant PVLG, one on either side of the spore were placed.
6) This was followed by careful placing of cover slip with gentle pressure laid with the help of a tip of pencil or needle.
7) The spores were examined under the compound microscope and labeled for generic name, species name, date, site of collection.
8) These slides were later photographed and were used for arbuscular mycorrhizal fungal identification by referring "Manual for the identification of VA mycorrhizal fungi." (Schenck and Perez, 1990) and recommendations by other workers (Walker and Trappe, 1993; Morton and Redecker 2001).
9) Nikon FX – 35 WA microscope with camera attachment was used for microscopy and microphotography.

d) Classification of AM fungi Genera:

On the basis of natural relationship of AM fungi and the related fungi, recognized a new fungal Phylum Glomeromycota with a single class Glomeromycetes circumscribed for the phylum, containing four orders and more than 150 described species (Schüßler *et al.,* 2001). Oehl et al. (2011a) proposed a classification which was based on combined genetic and morphological characters. The genetic characters include partial sequences of β-tubulin, and SSU and LSU rRNA and morphological characters associated with color, shape and thickness, pore closure of subtending hyphae etc.

In their classification, they divided the Phylum Glomeromycota into three classes namely *Glomeromycetes*, *Archaeosporomycetes* and *Paraglomeromycetes*. Representative tree of the phylum *Glomeromycota* based on molecular (SSU, ITS region, partial LSU of the rRNA gene, and partial β-tubulin gene) and morphological analyses (spore wall structures, structures of the spore bases and subtending hyphae, germination, and germination shield structures). Adapted from (Oehl *et al.* 2008, 2011a–d). The drawings (Chart-1) in the central columns show the spore formation types of the genera, and the typical germination shields for those genera which form persistent shields already during spore formation.

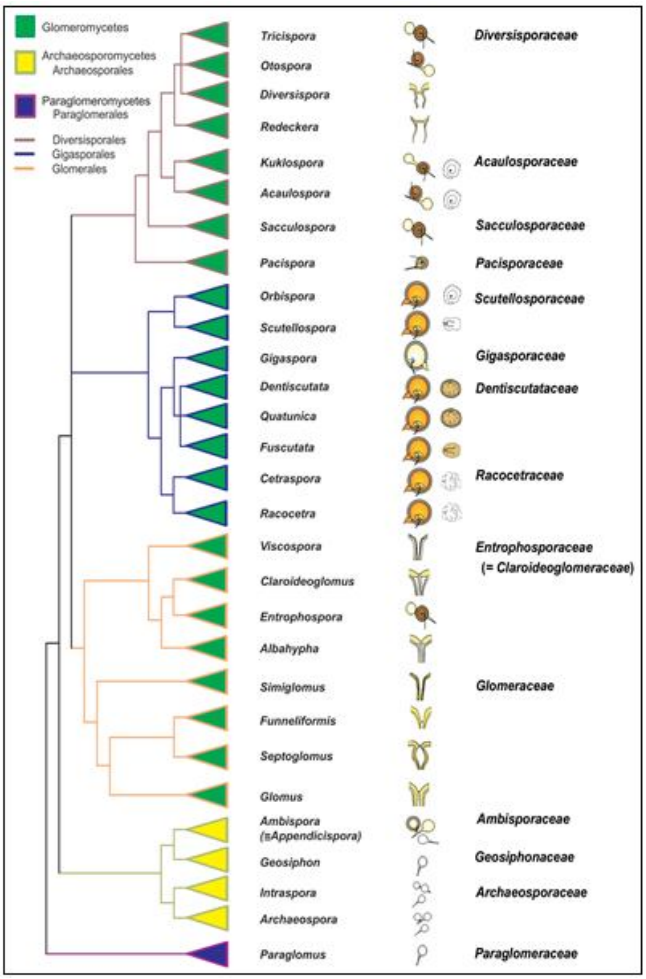

Chart 1: Tree of the phylum Glomeromycota based on molecular & morphological analysis

e) Key for Identification of Genera (Schenck and Perez, 1990):

A dichotomous key for separation of genera with certain modifications (Morton and Redecker, 2001) is as below:

1a. Spores produced as Chlamydospores formed singly or in loose clusters……..
……**ARCHAEOSPORA**

1b. Spores not produced as Chlamydospores……..

2a. Spores formed singly in soil or in sporocarps; if in sporocarps, spores not radiating from a central core of hyphae……**GLOMUS**

3a. Azygospores formed near or below a swollen hyphal tip……..

3b. Azygospores formed on a swollen hyphal tip……

4a. Spores formed laterally on hypha, below a swollen hyphal tip…… ……
……**ACAULOSPORA**

4b. Spores formed within the hypha, below a swollen hyphal tip……
……**ENTROPHOSPORA**

5a. Spores with two or more wall groups, the inner containing a coriaceous or membranous wall……
……**SCUTELLOSPORA**

5b. Spores of only one wall group, auxiliary cells echinulate or finely papilliate…
……**GIGASPORA**

f) Species Code for VA Mycorrhizal Fungi (Schenck and Perez, 1990):

A unique mnemonic four-letter species code was proposed for each described species of VA mycorrhizal fungi. The first letter of the four-letter species code designates the genus, with the initial letter of the genus usually used. Since there are two pairs of genera beginning with the same letter of the alphabet (i.e. *Acaulospora* and *Archaeospora*; *Gigaspora* and *Glomus*; *Sclerocystis* and *Scutellospora*), alphabetically, the first of each pair of genera beginning with the same letter was assigned the initial letter. Another letter was chosen to represent the second genus in the pair. *Acaulospora* was assigned **A,** and **R** was chosen to represent *Archaeospora*. Similarly, **G** was assigned to *Gigaspora*, while because of the strong **L** sound from the consonant combination, **L** was assigned to *Glomus*. **S** was assigned for *Sclerocystis* and **C** was chosen to represent *Scutellospora*. Once a letter is assigned to represent a genus, it should not be changed (Table-3).

Table No. 3
Letters to designate AM fungal genera in species code

Acaulospora	=	A
Archaeospora	=	R
Entrophospora	=	E
Gigaspora	=	G
Glomus	=	L
Sclerocystis	=	S
Scutellospora	=	C

The latter three letters of 'four-letter species code' are assigned from the species epithet. The second letter of the species code is invariably the initial letter of the species epithet. The third and fourth letters are consonants, whenever possible, usually from the second or third syllable of the species epithet. There is some variation in how the third and fourth letters are chosen because each species code must be unique. One must assure that the proposed species code has not been used previously. Due to the limited number of vowels in the alphabet, the use of the vowel for the third or fourth letter of the species code should occur only when no consonants remain in the species epithet. E.g. *Gigaspora rosea*= GRSA and *Glomus hoi*=LHOI.

CONCLUSION

Thus, the spores were received only on the marked surface of the filter paper and the rest of the filter paper was retained without spores.

Under a stereo-zoom binocular microscope column by column in a vertical direction the filter paper was scanned for spore count by moving the petri plate in vertical direction column by column.

The drawings in the central columns show the spore formation types of the genera, and the typical germination shields for those genera which form persistent shields already during spore formation.

Spores formed singly in soil or in sporocarps; if in sporocarps, spores not radiating from a central core of hyphae……GLOMUS

The latter three letters of 'four-letter species code' are assigned from the species epithet. The second letter of the species code is invariably the initial letter of the species epithet. The third and fourth letters are consonants, whenever possible, usually from the second or third syllable of the species epithet. There is some variation in how the third and fourth letters are chosen because each species code must be unique.

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