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RESEARCH ARTICLE

Studies on isolation and identification of AM fungi in association with lentil genotypes (*Lens esculenta* M.)

■ SENPON NGOMLE* AND B.N. PANJA

Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, COOCHBEHAR (W.B.) INDIA

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***Corresponding author:**
Email: sngomle29@gmail.com

ABSTRACT

An intensive survey at Kalyani Simanta seed farm of Nadia district under West Bengal was done to investigate the occurrence of AMF fungi in association with *Lens esculenta* and its identification. Different species of AMF were isolated from soil collected from 40 lentil genotypes. A total of 18 AMF spores were extracted. The 3 main genera identified were *viz.*, *Glomus*, *Entrophospora* and *Acaulospora*.

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INTRODUCTION

Mycorrhizal fungi differ from many plant–fungus associations because of their ability to create an interface for nutrient exchange which occurs within living cells of the plant (Brundrett, 2004). Mycorrhizal fungi interact with plants at different levels and can be grouped into obligately mycorrhizal, facultatively mycorrhizal and non-mycorrhizal plants (Brundrett, 2004).

Along with accessing soil nutrients, the hyphae of AMF fungi allow greater access to water through mechanisms such as stomatal regulations, increased root hydraulic conductivity, osmotic adjustments and maintenance of cellular water pressure and cell wall elasticity changes (Augé, 2000).

Studies have shown the capability of AM fungi to influence plant growth, crop quality and adaptability to stress conditions (Turnau *et al*., 1993).AMF fungi alleviate plant stunting caused by toxic metals by binding to these metals in the root zone with the aid of the extraradical mycelium and altering the plant cells ability to capture the metals. The polyphosphates produced by AM fungi are proposed to be the reason behind this sequestration though this has not been confirmed (Smith and Smith, 1997).The potential use of AM fungi has been recognized in detoxification of environments polluted with heavy metals and in phytoremediation (Khan, 2006). Lentil plant inoculated with *Glomus fasiculatum* enhances its yield potential as reported by Sattar and Gaur, 1989. A significant positive correlation is found between the percentage of AMF colonized roots and shoot dry matter and P concentration in lentil shoots (Germida and Talukdar, 1995). Dhingra *et al*. (1994) reported that lentils cv. LL 56 when sown untreated or seed inoculated with *G. fasciculatum*, the treated cultivar significantly increased the shoot dry matter and grain yield compared with the uninoculated control.

Lentil is an important pulse crop grown in the Indian sub-continent and many semi-arid regions of the tropics and sub-tropics. It ranks sixth and fifth in the world and India, respectively. Owing to the limited share in area and production in West Bengal, the study was carried out in order to make the venture of lentil production programme successful by considering arbuscular mycorrhizal fungi (AMF) which are viable and reliable option.

MATERIAL AND METHODS

During the period of experimentation, several materials were used and different methods were followed. Detailed descriptions of material used and methods followed are now described below.

Table A : Physico-chemical properties of alluvial soils

Soils	Mechanical composition (%)			pH	Organic carbon (%)	Total nitrogen (%)	Available P (ppm)
	Sand	Silt	Clay				
Alluvial	40.4	36.4	24.3	6.5	0.69	0.047	14.6

Location of experiment :

Experiments for the present studies were conducted using alluvial soil under field condition at Kalyani, Simanta District Seed Farm, Nadia District, West Bengal.

Experimental soils and their physico-chemical properties :

Alluvial soil samples from experimental plots of Kalyani Simanta District Seed Farm were collected separately for different genotypes and used for physico-chemical analyses. Sand, silt, clay, pH, organic carbon per cent, total nitrogen per cent and available P (ppm) of t were analyzed and presented in (Table A).

Collection of soil samples :

Soil samples required for the present investigation were collected from the lentil genotypes grown in experimental plots at Kalyani Simanta District Seed Farm. According to the objectives of the experiment, soil samples were collected at different times, air dried, processed and preserved in polyethylene packets at room temperature for future soil chemical and mycorrhizal analyses.

Isolation of arbuscular-mycorrhizal fungal (AMF) spores from soil :

AMF spores were isolated from soil by wet sieving and decanting method of Gerdemann and Nicolson (1963) using 500, 250, 150 and 45µ sieves. The isolated spores were suspended in thin layer of water in Petridish and counted under low power magnification by stereo-binocular microscope. For the convenience of spore counting, one sq. cm grids marked with black/ red/ blue ink of permanent marker were prepared on transparent sheet or white paper and then the marked sheet / paper was placed below the spore containing Petridish. All grids were examined, spores were counted and totaled. The total spores so obtained indicated the population of AMF in unit quantity of soil.

Determination of arbuscular – mycorrhizal species composition :

Different species of arbuscular - mycorrhizal fungi were identified as far as practicable from the metrical and other characters of azygospores or chlamydospores or sporocarp like spore colour, shape, surface ornamentation, spore contents and wall structures according to the standard description by Schenck and Perez (1988) and available in the INAMF website (*inAMF.caf.wvu.edu/fungi/taxonomy/speciesID.htm.*).

RESULTS AND DISCUSSION

Soil samples were collected from the rhizosphere of forty lentil genotypes grown at Simanta Seed district farm of West Bengal under field condition.

Morphological identification :

Spores isolated from soils were separated and identified using morphological characters including spore size, color, wall structures .

Spore types	Description	Name
T_{-1}	Spores globose or sub-globose, 205 - 210µm in diameter. The spore wall 15 µm thick, the thick appears laminated near the subtending hyphae. The subtending hypha 30 µm in diameter at the point of attachment and fades from dark brown or yellow near the spore to yellow or hyaline within approximately 100 um of the attachment. The large dark brown, globose or sub-globose chlamydospore with thick spore wall and wide and long subtending hypha distinguishes it from other AM-fungal spores.	*Glomus tenebrosum*
T_{-2}	Spore globose, measuring 182 - 282 µm in diameter, laminated wall with thickness of 5.3 µm in diameter, slightly brown in colour with rod like projection from wall. Subtending hyphae simple with 21 µm in width at the point of attachment.	*Glomus* sp. 1
T_{-3}	Chlymydospores 157 × 141 µm size, oval shaped. Having two wall layers, with composite wall thickness 3.6 µm – outer wall thick (2.5 µm) dark coloured, inner wall thin (1.1 µm) light yellow coloured. Spore surface smooth, content granulated and spore wall non laminated. Subtending hypha straight, short, 14 µm wide near the spore base, wall thickness 1.6 µm. The content of subtending hyphae is continuous with the content of spore through a narrow pore measuring 0.6 µm.	*Glomus* sp. 2
T_{-4}	Yellowish brown globose shaped spore measuring 199 - 205 µm in diameter, attached with funnel shape stalk of 46 µm in wide and 67 µm in long subtending hyphae. Spore is composite wall of 2 layered wall 3.8 µm in thick. Spore colour yellowish brown.	*Glomus* sp. *3*

Table Contd....

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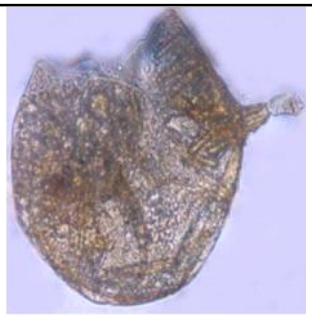
T_{-5}	Azygospores produced singly in soil, globose in shape, measuring 165 – 180 µm in diameter, composite wall thickness 6.5 µm with 2-3 separable walls.	*Entrophospora* sp. 1
T_{-6}	Spore globose to subglobose or broadly ellipsoid, measuring 150 - 225 µm in diameter. Spore wall composed of three layers measuring 5.6 µm in diameter. Spore pale yellow in colour, attached with sleeve like subtending hyphae at right angle. Subtending hyaline hyphae measure 197 µm long and 11.5 µm in wide.	*Glomus* sp.4
T_{-7}	Azygospores formed singly in soil and terminally on a bulbous suspensor like cell of 10.6 µm. Spore wall composed of three layers measuring 5.6 µm in diameter. The subtending hyphae is septate and measure 80 µm in diameter. Spore globose, measuring 97 - 102 µm in length. Spore consists of 2 layer measuring 1.7 µm in diameter. Spore wall darker than the spore colour.	*Glomus sp.*5
T_{-8}	Spore globose to broadly ellipsoid, 143 -168 µm, light brown in colour, content homogenous wall. Wall smooth, three layered and laminated. The colour of outer wall dark brown, middle wall light yellow and inner wall black. Inner wall separated from outer wall. Presence of a cicatrix of diameter 10.9 um.	*Acaulospora* 1
T_{-9}	Spores formed singly in soil on a bulbous suspensor like cell of 1.5 µm wall thickness, straw or reddish brown in colour, globose to subglobose in shape measuring 260-270 µm in diameter. Spore wall structure consists of two layers, with outer dark brown of 1.2 µm in diameter and inner brown colour of 4 µm in thick. Spore attached with subtending septate hyphae of 15.4 µm long.	*Scutellospora fulgida*
T_{-10}	Spore is yellowish brown, sub-globose with super composition of many broken walls. It measures 209 – 209 µm in diameter, two layered, with 4.79 µm wall thickness.	*Without attachment*
T_{-11}	Spores dimorphic, forming singly or in loose cluster in soil, spore yellow to reddish brown, globose to sub-globose, measuring 81.9 - 98.1 µm in diameter. Sporogenous hyphae of single spores is straight or slightly curved, light yellow to light brown in colour ,spore wall 5.1 µm thick and consisting of 1-3 walls.	*Glomus dimorpicum*

Table contd.…

Contd…. Table

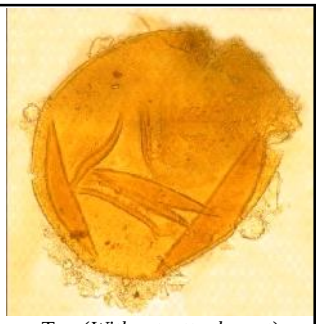
T_{-12}	Chlamydospores formed singly or in clusters in soil, spores globose, sub-globose measuring 272 - 275 µm in diameter. Composite wall thickness 7.5 µm, outer and inner wall separated occasionally in broken spores. Spore yellow to dark yellow in colour. Hypal attachment found, 13µm wide at the point of attachment, tapering to 3-4 µm thick a short distance from the spores.	*Glomus manihotis*
T_{-13}	Spores globose, sub-globose and consist of uniform guttules, occasionally irregular, 163 - 183 µm in diameter, pale yellow and brownish green at the periphery. Spore surface evenly pitted with depression of 4.9 µm in diameter. Spore wall consisting of four layers *i.e.* (1) Outer darker layer of 1.8 µm (2) Inner lighter layer of 1.8 µm (3) Inner darker layer of 1.3 µm (4) Inner lighter layer of 1.6 µm in wide. The thickness of spore wall 6.5 µm. Spore wall continuous except at the point of attachment.	*Acaulospora scrobiculata*
T_{-14}	Spores have three-layered walls with 9.5 µm thickness. They are yellowish brown to brown, globose to sub-globose 102 –135µm diameter. The outer layer hyaline to subhyaline, 4.5 µm thick and evanescent. The second layer yellowish brown, laminate and 3 µm thick, inner layer 2 µm thick.	*Entrophospora* sp. *2*
T_{-15}	Spores globose or sub-globose measuring 116 -125 µm in diameter. Spore wall consists of two distinct layers, outer layer thin and hyaline, inner layer yellow with a series of lamination occasionally visible. The spores bear straight subtending hyphae and the average width of the hyphae at the base of attachment 16 µm.	*Glomus macrocarpum*
T_{-16}	Spore globose or sub-globose in shape measuring 117-123 µm in diameter, yellowish brown in colour, wall 3 layered of 9.9 µm thickness. The outer layer hyaline to subhyaline, 5.1 µm thick. The second layer yellowish - brown, 3.2 µm thick. The inner layer hyaline 1.6 µm thick.	*Glomus* sp. *6*
T_{-17}	Spore globose in shape measuring 116 - 126 µm in diameter, light brown in colour with composite wall of 4 layered measuring 6.4 µm in width. Outré layer 1.9 µm in diameter followed by 1.7 µm, 1.6 µm and 1.1 µm width of other three consecutive layers.	*Glomus* sp.*7*
T_{-18}	Sporocarp varying from 630-1100×380-900 µm with loosely aggregated chlamydospores. Chlamydospores may be globose to subglobose to pyriform, single spore 70 - 100 µm pale yellow to yellow brown. Composite wall thickness 6 - 7 µm, two wall layers, outer 3 laminated. Subtending hypha straight, curved, constricted or swollen, 10 µm wide at base, wall 1.5 µm, pore closed by thin inner wall.	*Glomus aggregatum*

T-$_1$ (*Glomus tenebrosum*)

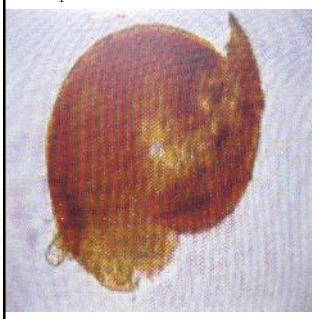


T-$_2$ (*Glomus* sp. *1*)

T-$_9$ (*Scutellospora fulgida*)



T-$_{10}$ (*Without attachment*)

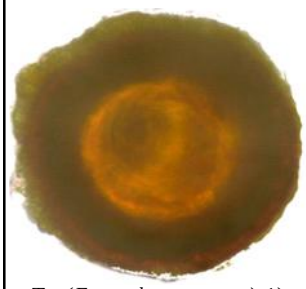


T-$_3$ (*Glomus* sp. *2*)

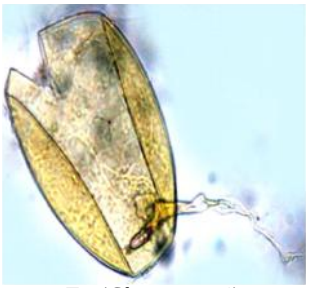
T-$_4$ (*Glomus* sp. *3*)

T-$_{11}$ (*Glomus dimorpicum*)

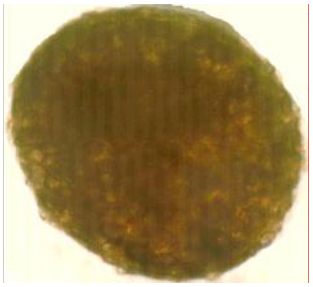
T-$_{12}$ (*Glomus manihotis*)



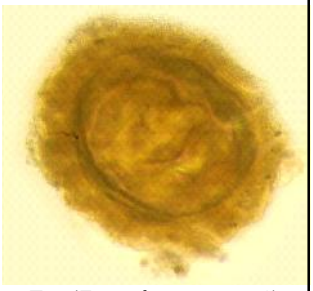
T-$_5$ (*Entrophospora* sp.*)* 1)



T-$_6$ (*Glomus* sp. 4)



T-$_{13}$ (*Acaulospora scrobiculata*)

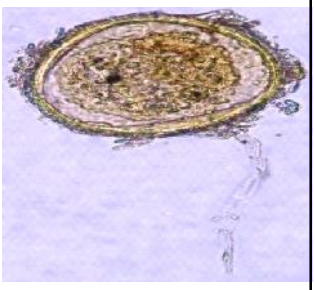


T-$_{14}$ (*Entrophospora* sp. *2*)

T-$_7$ (*Glomus* sp. *5*)

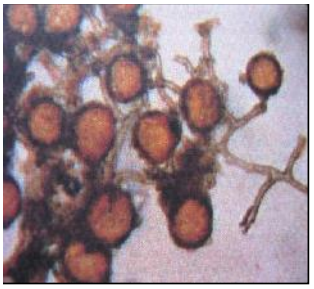
T-$_8$ (*Acaulospora 1*)

T-$_{15}$ (*Glomus macrocarpum*)



T-$_{16}$ (*Glomus* sp. *6*)

T-$_{17}$ (*Glomus* sp.*7*)



T-$_{18}$ (*Glomus aggregatum*)

Plate 1 : Different types of arbuscular mycorrhizal spores isolated

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