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SUCCESSFUL GROWTH OF VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI ON SOME SYNTHETIC MEDIUM

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ABSTRACT

Vesicular arbuscular *Mycorrhizal (VAM) fungi* are an important group of soil fungi because they stimulate plant growth in soils with low levels of available phosphorus, and thus improve plantlet growth and nutrition. The symbiotic status is very important for plant mineral nutrition and health.

For the first time in Egypt under local conditions this study successed in developing VAM fungi *(Glomus Mosseae, and G. macrocarpum)* in two types of synthetic media modified M & S. However, in this media all spores vesicles, arbuscules and mycelium were obtained after one week on Perti dishes which were incubated at 35- 40°C.

INTRODUCTION

Vesicular-arbuscular mycorrhizol fungi are an important group of soil fungi because they stimulate plant growth in cultivated soils with low levels of available phosphorus (Abbott and Robson, 1984).

The beneficial effect of vesicular-arbuscular mycorrhizal (VAM) fungi on the growth, nutrition and health of host plant have been demonstrated by (Perrin, (1991) and Plenchette, 1991).

However, large scale use of vesicular-arbuscular endophytes still presents a greet challenge. Many different VAM species inhabiting agricultural soil (Hall, 1977). VA mycorrhizae obligate biotrophic generally developed on a large number of plant families from which host plant benefit in several aspects: improved acquisition of nutrients and water enables plant growth under unfavourable conditions, effects on the phytohormone balance which can stimulate flowering behaviour and fruit developments; and generally increased resistance towards soil borne pathogens (Schonbeck, *et al.*, 1994). No artificial medium has yet been developed that permits the culture and maintenance of these obligate symbionts with sufficient yield of mycelium and spores without loss of viability and infectivity (Janardhanan *et al.*, 1990).

Recently, Strullu and Romand (1986, 1987) described the reproducible in vitro production of VAM propagules using isolated vesicles, hyphae and endomycorriohzal roots as starting inoculum. The use of these inocula was facilitated by encapulation of intraradical forms of VAM fungi (Strullu *et al.*, 1991). But for the first time in Egypt under local conditions (Abdel Latif, 1999), can produce VA mycorrhizal fungus Glomus macrocarpum on medium modified after Murashige and Skoog (1962). She found that mycillium, vesicles and arbuscles can produced easily after 1-2 weeks at 40°C. She noticed that at a beginning of growth a few hyphae were formed with spores attached to their hayphae were branched in many ways.

Also, arbuscules were formed after one week and more spores were formed with huge amount of vesicles. This spores were germinated after 2 weeks also net work of hypae were formed in the plates with a large number of spores. Thes growth characters were observed in the two-tested method that used for inoculation on perti-dishes.

MATERIALS AND METHODS

I – Isolation of Endophytie of Mycorrhiza:- ***(Glomus mosseae* and *Glomus macrocarpum)***

1. From Soil

Spores of *G. mosseae and G. macrocarpum* were obtained from the soil by wet sieving (Gerdemann and Nicolson, 1963) from the farm of the Faculty, Of Agriculture at Moshtohor, Qualubaya.

2. From Host Plant

Roots and Root hairs of onions *(Allium cepa)* and faba been *(Vicia faba)* plants which grown in pots in the greenhouse, were taken three weeks after seed growing. The roots were cut to small pieces, washed with tap water several times. Specimens were then prepared for microscopic observation (Phillips and Hayman, 1970). These pieces were surface sterilized under a laminar flow hood by successive washes with 90% ethanol, 6% calcium hypochlorite (1 min.), chloramine T 20% puls two drops of Tlween 20 (10 min.), and rinsing for 10 min. in an antibiotic solution 200 mg L-1, as described method by (Diop *et al.*, 1994). Mycorrhizal root were cut into small pieces (1 cm) and then transferred to petri dishes.

II – Culture media

In this study five synthetic media were used, this media were modified after M & S Media various modifications of all media based on: nitrogen source (NH4) NO3 and KNO3 in various proportions at total N concentration from 2 to 5 mgl-1. Phosphours source KH2PO4 from 50 to 250 mgl-1. Na2EDTA from 10 to 50 mgl-1. pH was adjusted 6.7 - 7.2 while, percentage of sucrose ranged from 30 to 50 mgl-1. The chemical constituents of them are tabulated in Table (1).

All media were autocalved at 121°C for 30 min. The prepared media were poured in sterilized petri dishes (9 cm) as a rate of 10 ml in each. The sterilized segments were put over the media and incubated for 7 days at 35-40°C.

Table 1: Chemical structure of different medium.

Constituents (mg L-1)	Types of medium				
	(1)	(2)	(3)	(4)	(5)
$(NH_4)NO_3$	1.70	2.0	1	2.0	1.5
KNO_3	1.90	2.1	2.0	2.0	2.5
$CaCl2\text{-}2H_2O$	440	500	560	100	350
$MgSO_4\text{-}6H_2O$	370	400	350	100	150
KH_2PO_4	170	200	150	50	90
$(NH_4)H_2PO_4$	-	200	250	100	180
Na_2edta	33.60	20.0	40	10	12.0
$FeSO_4.7H_2O$	27.80	30.0	20	10	15.0
$MnSO_4.4H_2O$	22.30	-	20	0.5	0.338
$ZnSO_4.4H_2O$	8.60	9.00	10	0.5	0.172
H_3BO_3	6.20	7.00	10	0.5	0.129
KI	0.83	1.00	0.5	0.1	0.017
$Na_2MoO_4.2H2O$	0.25	0.01	-	0.1	0.005
$CuSO_4.5H_2O$	0.025	0.01	0.5	0.05	0.0005
$CoCl_2.6H_2O$	0.025	0.01	-	0.05	0.005
Glycine	2.00	4.00	5.0	10	3.0
Nicotinic acid	0.5	1.00	0.7	2.0	0.8
Pyridoxin-HCl	0.5	1.00	0.1	0.2	0.6
Sucrose	30	40	50	35	-
Agar	900	900	900	900	900

III - Reinfection to Allium sp. By VAM propagules produced on artificial media.

After two weeks VA mycorrhizal fungi produced on Petri dishes, vesicles, arbuscles and mycelium. Seeds of Allium were sterilized with sulfuric acid 0.1% (30 min), and washed with distilled water, then seeded in pots in green house. The grown seedling of onion which grown in pots for 1 week were inoculated with 60 *in vitro* produced spores and a mixture of other propagules (Mycelium). Four replicates were set up per treatment as follows:

1. Sterilized soil without inoculation as a control.
2. Soil inoculated with VAM fungi which grown on artificial media.
3. Soil inoculated with segments of naturally infected onion roots with VAM fungi.

*** Assessment of Variables:**

The establishment of VAM was regularly checked. Non destructive observation of extradical forms. Development of hyphae and multi germination of spores was carried out under the binocular microscope by the gridline - interect method (Giovannetti and mosse 1980).

RESULTS

In this study five media were tested for the growth of VA mycorrhizal fungi as mentioned in (Table 1). However, mycorrhizal fungi can grow well

after 1-2 weeks respectively. Hayphae spread out in all directions the main hyphae were often straight. At the medium number 2 and number 5 only, at the beginning mycelium was quickly covered the petri dishes and spread at all directions as observed (Fig. 1).

Fig. 1:Growth of mycelium of *(Glomus macrocarpm)* on synthetic medium after 2 weeks.

The main hyphae were often stright and bore numerous also, arbuscules produced after one week, vesicles produced after that, but little, mature spores were observed at all periods. Spores of *G. mosseae* were much bigger than *G. macrocarpum*. Also, number of arbuscles and vesicles for the *G. mosseae* were much abundant than *G. macrocarpum* as shown in Table (2) ; (3) and Fig. (2).

Table 2: Showed the number of vesicles and arbusculs after 2 weeks of *G. mosseae.*

Age of culture	Vesicles	Arbuscules
one week	19	29
Two weeks	51	39

V = Vesicles
A = Arbuscules

Table 3: Showed the number of vesicles and arbusculs after 2 weeks of *G. macrocarpum..*

Age of culture	Vesicles	Arbuscules
one week	12	19
Two weeks	26	29

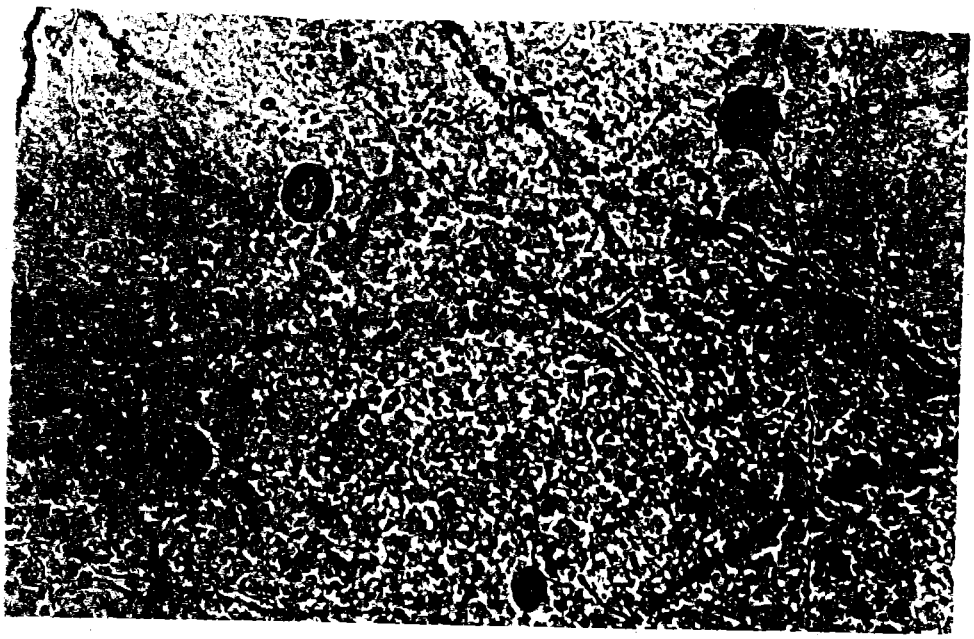

Fig. 2: Growth spores of mycorrhizae *(Glomus macrocarpum)* after 2 weeks on synthetic medium (X 200).

A = Penetration of arbsculus spores to mycelium.
B = Mycelium
C = Vesicles spores

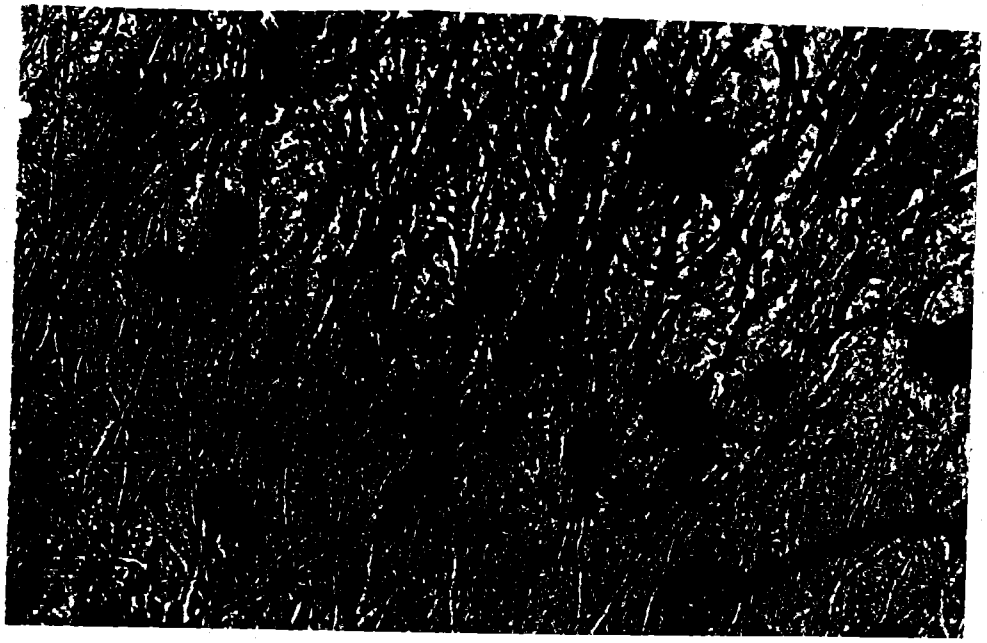

Fig. 3: Showed growth of mycorrhizae *(Glomus macrocarpum)* after 3 weeks on synthetic medium at (X 200).
A = Vesicles spores

Fig. 4: Growth of VA mycorrhizal fungi *(Glomus mosseae)* after 2 weeks (X 500).
Arbusculs attached with mycelium.
A = Arbusculs.
B = Mycelium.

Fig. 5: Growth of mycorrhizae *(Glomus mosseae)* after 2 weeks. This Fig. Show vesicles, attached to the mycelium (X 200).
A = Vesicles.
B = Mycelium

Reinfection of VAM fungi:

In this experiments roots and root hairs of infected plants were examined to calculate the number of VAM mycorrhizal fungi. The second treatment which included which infected VAM fungi which grow on artificial media and the third treatment which included soil infected with segments of roots which infected with VAM fungi gave the same number of vesicles, arbuscules and mycelium. So that, roots of Allium artificially infected with segments of root which infected mycorrhizal fungi isolated from soil gave results similar with VAM fungi which growth on artificial media as shown in table 4. So that spores, able to complete life circle successfully.

The roots of Allium were removed from pots, washed with tap-water and cut into 1 cm long pieces. Specimens were then prepared for microscopic observation (Phillips and Haufman, 1970).

Table 4: Showed the number of VAM fungi which grow on synthetic media and another which extracted from soil.

Treatment	Vesicles	Arbuscules	Mycelium
Sterillized soil without any inculation (Control)	0.0	0.0	0.0
Sterilized soil inculated with VAM grow on synthetic media	29	21	16
Sterilized soil inculated with VAM isolated from soil	30	20	16

V = Vesicles
A = Arbuscules
M = Mycelium

DISCUSSION

Mycorrhizal fungi grow on artificial media abundantly with a huge amount of hyphae were formed with spores attached with them and a huge amount of arbuscules also vesicles were formed after two weeks. A large net work of hyphae formed a large amount of arbuscules. This results came in agreement with Abdel Latif (1999).

This media were modified after M & S the most important determining factor on this media the concentration of nitrogen because when this media contained less than 0.2 mMN. The fungus died. But when the percentage ranged 1 from 5 mycelium strongly branched, saptate spread intercellularly and arbuscules, vesicles appeard clearly. Also when the phosphorus concentration less than 170 mgl-1 of KOH2PO4 VAM died. But when concentration 200 mgl-1 VAM fungi good growth. Also, Na2EDTA when percentage less than 10 mg L-1 VAM died but when ranged from 12-20 mgL-1 VAM growth well. While, the most important factor the adjusted of pH when less than 6VAM fungi died but when ranged from 6.7-7.2 VAM growth well.

Also, Strullu and Romand (1986, 1978) described the reproduction *in vitro* production of VAM propagules using isolated vesicles, hyphae and endomycorrhizal roots as starting inoculum. The intraradical forms of VAM fungi, genus Glomus of VAM fungi which were produced on artifical medium can be able to complete their life cycle *in vivo* and colonized root of *Allium cepiea* under green house condition after 2 weeks this results confirmed with Abdel Latif (1999) and Strallu *et al.* (1991).

This study aims to production of VA mycorrhizol fungi on artificial medium *in vitro*. Spores which were obtained from it, were very active to infect root and gave the same results similar to VAM fungi extracted from soil.

So that, we can produce VAM fungi abundantly on artificial media easily.

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تنمية فطريات الميكروريزا الحويصلية من جنس جلوماس على أنواع مختلفة مـــن البيئات الصناعية

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في الدراسات السابقة أجريت محاولات عديدة لتنمية فطريات الميكروريزا الداخلية علـــى البيئـــات الصناعية ولكن هذه المحاولات باءت بالفشل ولكن في هذه الدراسة تم اعداد خمس بيئات معدلـــة مـــن بيئـــة مورشينج وسكوج وقد روعي فيها نسب كل من الفوسفور والنيتروجين وذلك نظرا لأهميتهم لنمو فطريـــات الميكروريزا وكانت أفضل هذه البيئات هي البيئة الثانية والخامسة.

وقد نمت فطريات الميكروريزا عليها وبغذارة لأول مرة في جمهورية مصـــر العربيـــة وقـــد تـــم الحصول على هيئات الفطر والجراثيم بأعداد كبير وأمكن تميز كل نوع عن الاخر وكذلك تمـــيز كـــل مـــن الميسليموم والشبكة الكبيرة التي يكونها الفطر وكذلك الجراثيم الحويصلية بأطوارها المختلفة.

وتعتبر هذه الدراسة محاولة جيدة إذا تم استخدامها من الناحية التطبيقية حيث تمكننا من الحصـــول على هذه الفطريات فى صورة يمكن بها زيادة الكفاءة الانتاجية لبعض المحاصيل الهامة.