

HORDEUM VULGARE: A SUITABLE HOST FOR MASS PRODUCTION OF ARBUSCULAR MYCORRHIZAL FUNGI FROM NATURAL SOIL.

B. CHAURASIA* – P.K. KHARE

*e-mail: bhaskarchaurasia@rediffmail.com

EPB, GB Pant Institute of Himalayan Environment and Development,
Kosi-Katarmal, ALMORA (UA) 263463, India

*Corresponding author

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Abstract. Four plant species viz. *Hordeum vulgare*, *Triticum aestivum*, *Phaseolus vulgaris* and *Phaseolus mungo* were examined for mass production of consortium of AM fungi present in the rhizosphere soil. Rhizosphere soil were collected from teak dominated forest and was used as a source of AM inoculum. Surface sterilized seeds of all the four selected plant species were raised in a mixture of sterilized soil and sand in 3:1 ratio. Mass production of AM fungi was observed in terms of (%) AM colonization, AM spore population, and effectiveness of AM consortia was recorded in terms of height and dry weight of inoculated and uninoculated plants. Out of four test species *H. vulgare* responded as the most suitable host. After 60 days, *H. vulgare* showed the highest colonization (92%) and 74 spores per 25 g soil. It was observed that plants having higher AM colonization showed higher AM spore production showing a positive correlation, however, it was not found in *T. aestivum*. Study indicates that *H. vulgare* is suitable for early colonization and mass production of AM fungi. After 90 days of pot culture, pot soil from all host were analyzed for AM flora. In all, eleven AM species were identified from the consortium. Maximum numbers of AM species (8 species) were found associated with *H. vulgare*. Three species of AM fungi i.e. *Glomus aggregatum*, *G. fasciculatum* and *Sclerocystis pakistanica* were found to dominate in the soil of *H. vulgare*.

Keywords. AM fungi, AM colonization, *Glomus aggregatum*, *G. fasciculatum*, *Sclerocystis pakistanica*, mass production

Introduction

Ubiquitous occurrence and importance of AM fungi for plant growth is now a well-established fact. Distribution and abundance of AM fungi vary greatly among different sites i.e. natural and manmade ecosystems [7, 9]. Investigation on the distribution of AM fungi is still required to be taken up with particular reference to tropical forests. Natural soil offers consortium of indigenous mycorrhizal fungi and often used as source of inoculum. Since isolation and selection of AM species (effective for growth promotion) and raising of pure culture of these species is difficult, a suitable host is required to maintain the AM culture. The beneficial use of AM inoculum in agriculture and raising nurseries has been reported [15, 22].

Approximately 155 species of AM fungi have been described so far [13]. Some AM species are broadly effective while others, though they colonize many host species, may promote the growth of only a few [6, 23]. Therefore, the first consideration in inoculum production is the choice of the fungal isolates which are capable of growth promotion of a

given plant. Thus preliminary trails on a variety of isolates are required to find out the most effective ones. Another biological consideration in the production of inoculum is the host plant upon which the fungus will grow [17]. The degree of importance of this consideration also depends upon the growth system (pot culture, hydroponics, etc.). Mass propagation of AM fungi varies greatly on root structure, and habitat of host plant [5].

The objectives of present study were (i) selection of suitable host for mass production, and (ii) effectiveness of AM consortium present in the rhizosphere soil on selected host species, namely *Triticum aestivum*, *Hordeum vulgare*, *Phaseolus vulgaris* and *P. mungo*.

Materials and methods

Study location and collection of soil samples

The present study was carried in teak (*Tectona grandis*) dominated tropical dry-deciduous forests around Sagar in Central India. Sagar district is situated a few kilometres north of Tropic of Cancer between 23°05'–24°25'N and 78°10'–79°15'E on an average altitude of 517 m above mean sea level. The soil samples were collected randomly after removing 5–10 cm of top layer, from teak dominated forest. Samples were thoroughly mixed and used, treated as inoculum.

Selection of host

Four-host plant species, viz. *Triticum aestivum*, *Hordeum vulgare*, *Phaseolus vulgaris* and *P. mungo* were tried for selection of the suitable host for mass production of AM spores. All the four species were selected on the basis of their (i) suitability to the agroclimatic conditions of the area; (ii) having thick root system for sizeable sporulation and colonization; and (iii) annual growth habit.

Pot and potting mixture

Open pot culture was used for the mass production of AM fungi. A layer of 100 g of collected soil samples (inoculum) was spread over pot mixture (sterilized sand : soil = 1 : 3; about 3 kg) in earthen pots (30 cm height and 25 cm diameter). These pots were used for the experimentation.

Surface disinfection of seeds

There will be enough number of microbes alongwith AM population in indigenous soil samples (inoculum) so there is no need of surface disinfection of seeds, but in order to decrease microbial contamination and to achieve healthy seedlings, seeds of *Hordeum vulgare*, *Triticum aestivum*, *Phaseolus vulgaris* and *Phaseolus mungo* were surface disinfected with 0.01% (w/v) HgCl₂ for 2 minutes and washed several (3–4) times with sterilized distilled water.

Quantification of AM fungi

Root samples and soil samples were collected at an interval of 15 days. AM colonization of root was examined after staining with 0.1% acid fuchsin [11]. Root segments, each approximately 1 cm long, were selected at random from a stained

sample and mounted on microscopic slides in group of 10. Minimum 100 root segments were examined for each pot. Slide with stained root segments were carefully observed under microscope (Leica DML 30) under suitable magnifications. Per cent root colonization was calculated using following formula:

$$\% \text{ colonization} = \frac{\text{total number of colonized root pieces}}{\text{total number of root pieces examined}} \times 100 \quad (\text{Eq. 1})$$

Effectiveness of AM fungi

Effectiveness of consortium of AM fungi was determined by taking plant height and dry weight after 90 days of growth period.

Isolation and identification of AM spores

AM spores were determined per 25 g of soil. Spore isolation was done by wet-sieving and decanting method [8]. Identification of AM fungal spores was done as per manual [20].

Data analysis

The criteria for statistical significance for per cent colonization was set at a table wide value at $P < 0.05$ using LSD [21]. Standard deviation is reported in plant height and dry weight.

Results

As evident from the results, colonization was highest (92%) in *H. vulgare* and minimum (62%) in *P. vulgaris* after 60 days of inoculation (*Table 1*).

The results envisage that in *H. vulgare* colonization of AM fungi was highest in all the growth periods i.e., from 15 days to 60 days. Further, amongst the all host species the rate of colonization in *H. vulgare* was higher during later phase of growth period. Statistical analysis of the data for colonization showed significant differences when compared with initial and higher growth intervals (LSD-14.9 at $P < 0.05$). On the whole, AM colonization in *H. vulgare* was significantly higher after 60 days than the other three host species. On the contrary minimum colonization occurred in *P. vulgaris*. Colonization by AM fungi appears to be lower in both the leguminous hosts (*P. vulgaris* and *P. mungo*) as compared to the graminaceous host. Similar trends as the rate of colonization were observed during different period of growth.

Spore population was minimum (25 spores) in *P. vulgaris* and maximum (36 spores) in *H. vulgare* after 30 days (*Fig. 1*). *H. vulgare* appeared to be a host with higher AM spore production: 74 spores per 25 g soil and 92% AM colonization after 60 days. It is interesting to note that the spore population was lower in *T. aestivum* (28 after 30 days and 57 after 60 days) though it has comparatively higher AM colonization. However, higher number spores were lower% colonization was observed in *P. mungo*. In general it appears that the AM spore population followed the trend of colonization after 30 to 60 days of growth periods. A positive correlation was observed between root colonization and spore production in these species. (*Table 1, Fig. 1*).

Table 1. Arbuscular mycorrhizal colonization (%) in four hosts plant used for mass production of AM fungi

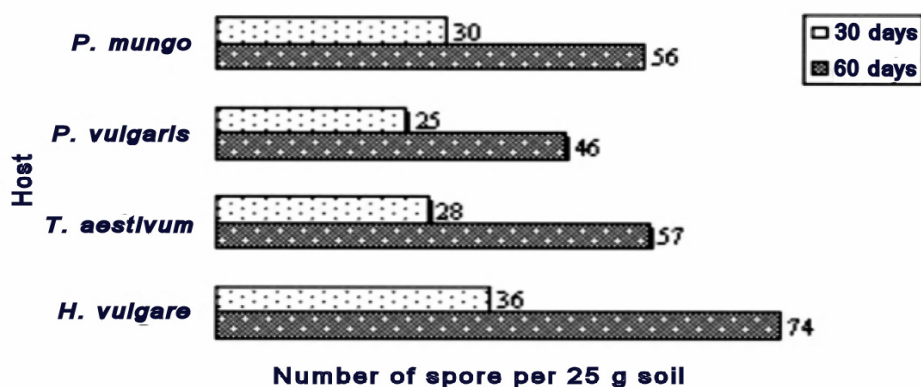
host plant	period (days)			
	15	30	45	60
<i>H. vulgare</i>	54 ^{cde}	60 ^{def}	72 ^f	92 ^g
<i>T. aestivum</i>	40 ^{abc}	49 ^{abcd}	59 ^{def}	71 ^f
<i>P. vulgaris</i>	37 ^a	44 ^{abc}	50 ^{abcd}	62 ^{def}
<i>P. mungo</i>	38 ^{ab}	41 ^{abc}	52 ^{bcd}	66 ^{ef}
				LSD – 14.97

Values with different alphabets are significantly different at $P < 0.05$

Results of growth performance of host after inoculum of consortium of AM fungi clearly indicated that AM inoculation increased the height of host plants compared to uninoculated plants. Dry weights of inoculated host plants were also found increased in all the host species (Table 2).

Dry weight of host was higher in inoculated plants of *H. vulgare* (1.44 g per plant) than in the uninoculated plants (1.10 g per plant). However it was 1.40 g per plant in inoculated and 0.93 g per plant in uninoculated plant of *T. aestivum*. Comparatively a little increase in dry weight was recorded in inoculated plant of *P. mungo* and *P. vulgaris* (8–9%).

Maximum increase in height was observed in inoculated leguminous plants, i.e. *P. vulgaris* and *P. mungo* (41%) as compared to uninoculated. However, this increase in height was comparatively of lower magnitude in graminaceous plant, i.e. *H. vulgare* (24%) and *T. aestivum* (14%). A very interesting trend was observed with dry weight of host plants, where the increase in inoculated plants was more in *H. vulgare* (30.90%) and *T. aestivum* (50.53%) as compared to *P. vulgaris* (9.18%) and *P. mungo* (8.00%) (Table 2). It appears that as far as the productivity of the host plant is concerned, the maximum effect of AM inoculation occurred in graminaceous than in the legumes.

**Figure 1.** Spore population of AM fungi per 25 g soil from four host used for mass production

After 90 days, AM spores were isolated from the pots. In all, eleven different species of AM fungi were identified from the rhizosphere soil of inoculated plants (Table 3, Fig. 2). Maximum numbers of AM species were found associated with rhizosphere soil of *H. vulgare* in comparison of other host plants. Six of these species belong to genus *Glomus* namely *Glomus aggregatum* Schenck & Smith emend. Koske, *G. ambisporum* Smith & Schenck, *G. fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker & Koske, *G. heterosporum* Smith & Schenck, *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe, *G. microaggregatum* Koske, Gemma & Olexia. Three species of AM fungi, i.e. *Glomus aggregatum*, *G. fasciculatum* and *Sclerocystis pakistanica* were found dominated in the rhizosphere soil of *H. vulgare* in comparison to rest of host species (Table 3).

Table 2: Height and dry weight of control (C) and AM inoculated (In) plant after 90 days

host plant	height (cm)		% increase over control	plant dry weight (g)		% increase over control
	C	In		C	In	
<i>H. vulgare</i>	33.00±1.94	41.00±1.78	24.24	1.10±0.30	1.44±0.36	30.90
<i>T. aestivum</i>	32.00±1.85	36.50±1.14	14.06	0.93±0.17	1.40±0.14	50.53
<i>P. vulgaris</i>	21.00±1.53	29.75±1.48	41.66	0.98±0.21	1.07±0.18	9.18
<i>P. mungo</i>	23.00±0.63	32.50±1.87	41.33	1.00±0.2	1.08±0.32	8.00

Values are mean of six replicates \pm SD

Discussion

AM fungal colonization and subsequent spore formation and production depends upon the type of host as well as the duration of infection of these symbiotic organisms. Generally, with increase in the growth period after infection, root colonization of host increase. However, this increase in colonization and period do not have greater bearing on spore production. It is the host type, which is more important for spore production. Therefore, the relationship between colonization and rate of colonization with growth period do not vary greatly in different hosts. Positive effect of AM colonization on increasing AM population were found in *H. vulgare* and *P. mungo*. Al-Raddad [3] also found highest spore population with cereals plant followed by chickpea and beans. However, this relationship does not exist in *T. aestivum* and *P. vulgaris* where with higher root colonization, the number of spores were not high. The present results are in accordance with earlier findings where no correlation could be established between spore number and root colonization [4].

Results of the experiments for mass production of AM inoculum with four selected host plants indicate a gradual increase in root colonization and spore number with the period of growth and increase in the size of plant.

This trend was more evident in *H. vulgare* in comparison to other hosts. Among the different hosts, *H. vulgare* and *T. aestivum* had a larger root system as they belong to graminaceous crop. Therefore, it seems that monocots with rapidly developing fibrous root system can be considered as ideal trap plants for producing AM spores. Higher inoculum densities were often found associated with higher root colonization in the early growing season in *H. vulgare* and *T. aestivum*. Larger plant size has been associated with

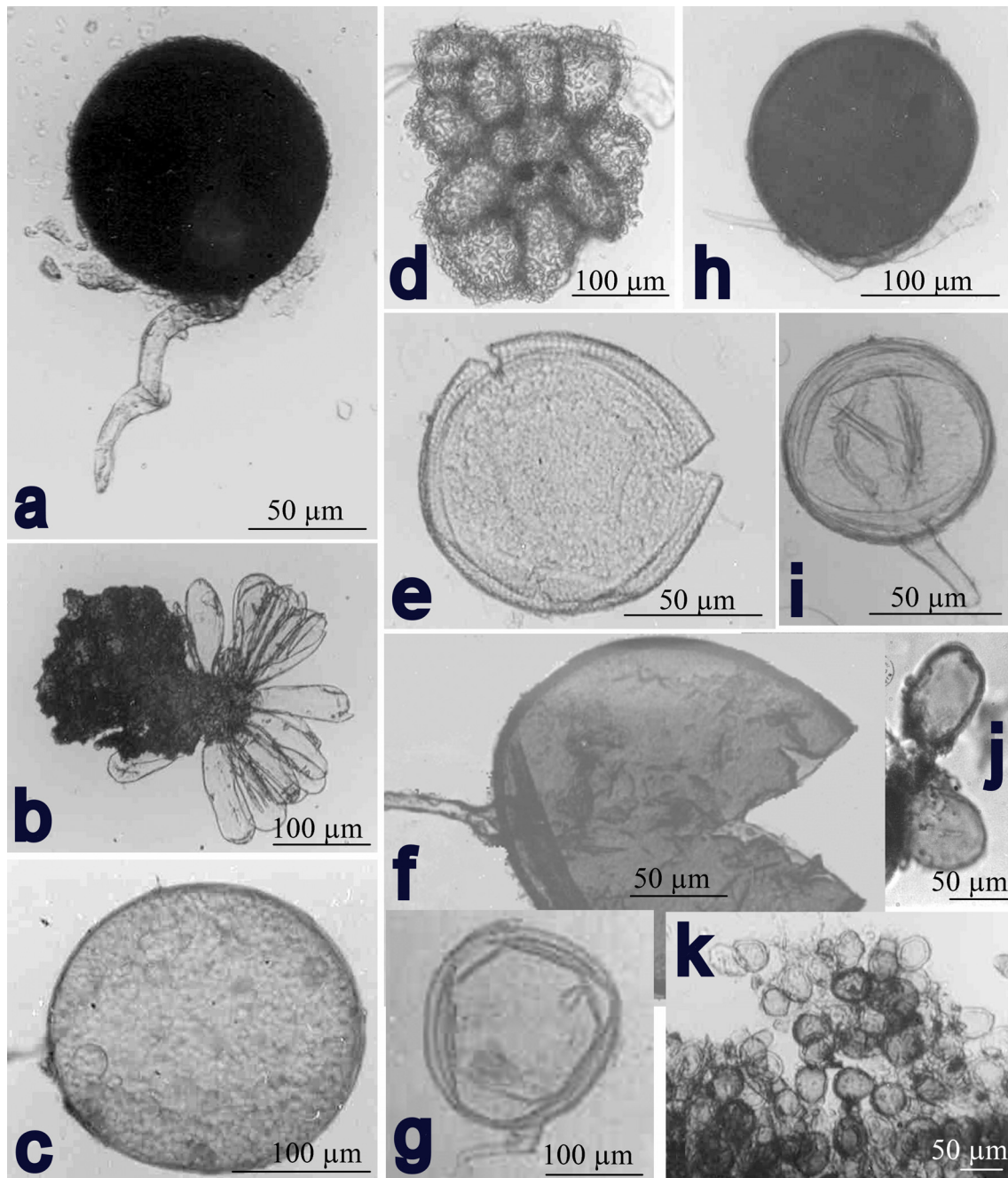


Figure 2. Identified arbuscular mycorrhizal fungal (AMF) species. (a) *Glomus ambisporum*; (b) a part of sporocarp with peridium of *Sclerocystis pakistanica*; (c) *Acaulospora* sp.; (d) sporocarp of *Sclerocystis sinuosa*; (e) *Acaulospora scrobiculata*; (f) *Gigaspora* sp.; (g) *Glomus mosseae*; (h) *Glomus heterosporum*; (i) *Glomus fasciculatum*; (j) *Glomus aggregatum*; (k) *Glomus microaggregatum*.

Table 3. Number of AM species identified from rhizosphere soil of inoculated plants

Name of AM species		<i>H. vulgare</i>	<i>T. aestivum</i>	<i>P. vulgaris</i>	<i>P. mungo</i>
1	<i>Acaulospora scrobiculata</i>	++	—	—	—
2	<i>Acaulospora</i> sp.	—	+	—	—
3	<i>Glomus aggregatum</i> Schenck & Smith emend. Koske	++	+	+	+
4	<i>Glomus ambisporum</i> Smith & Schenek	+	++	—	—
5	<i>Glomus fasciculatum</i> (Thaxter) Gerdemann & Trappe emend. Walker & Koske	+++	+	+	+
6	<i>Glomus heterosporum</i> Smith & Schenck	+	—	+	+
7	<i>Glomus mosseae</i> (Nicolson & Gerdemann) Gerdemann & Trappe	+	—	+	+
8	<i>Glomus microaggregatum</i> Koske, Gemma & Olexia	—	+	—	—
9	<i>Sclerocystis pakistanica</i> Iqbal & Bushra	+++	++	++	+
10	<i>Sclerocystis sinuosa</i> Gerdemann & Bakshi	—	+	—	+
11	<i>Gigaspora</i> sp.	—	—	+	—

+ poor; ++ moderate; +++ frequent

increased levels of sporulation, probably because larger plant often have more extensive root system than smaller plants, allowing greater mycorrhizal colonization and sporulation [18].

It is also evident from the data that AM association has further contributed to the growth of all inoculated host plant. This increase in growth on account of AM inoculation is now a well-established fact and in a number of studies from different parts of the world on different plants, such conclusions have been drawn [2].

In the present investigation the high percentage of root colonization and spore population resulted in increased height and dry weight of both graminaceous host in comparison to leguminous host. It has been observed that higher root colonization at the time of sampling may be attributed to high number of secondary roots, and its density, which favour active mycorrhizal colonization [14, 19]. Similar results were obtained by [12]. Rangeley *et al.* [16] have also reported that colonization of root is directly proportional to root growth.

The intensive root colonization of host resulted in the better plant growth in term of dry matter. These results are in line with that of Abbott and Robson [1]. It was also observed that colonization and spore population vary with time and increases with advancing growth stages of the plant [10]. As evident from the results in the pot experiments, rapid AM colonization and higher spores production was observed in *H. vulgare*. In this experiment maximum effectiveness of AMF species was also observed in *H. vulgare*. Therefore, soil of *H. vulgare* can be used as inoculum and this host can be used for mass propagation of pure cultures of AMF. Results also envisaged that either host plants favour the association of particular AM species or AM fungi may show some preference for the host.

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