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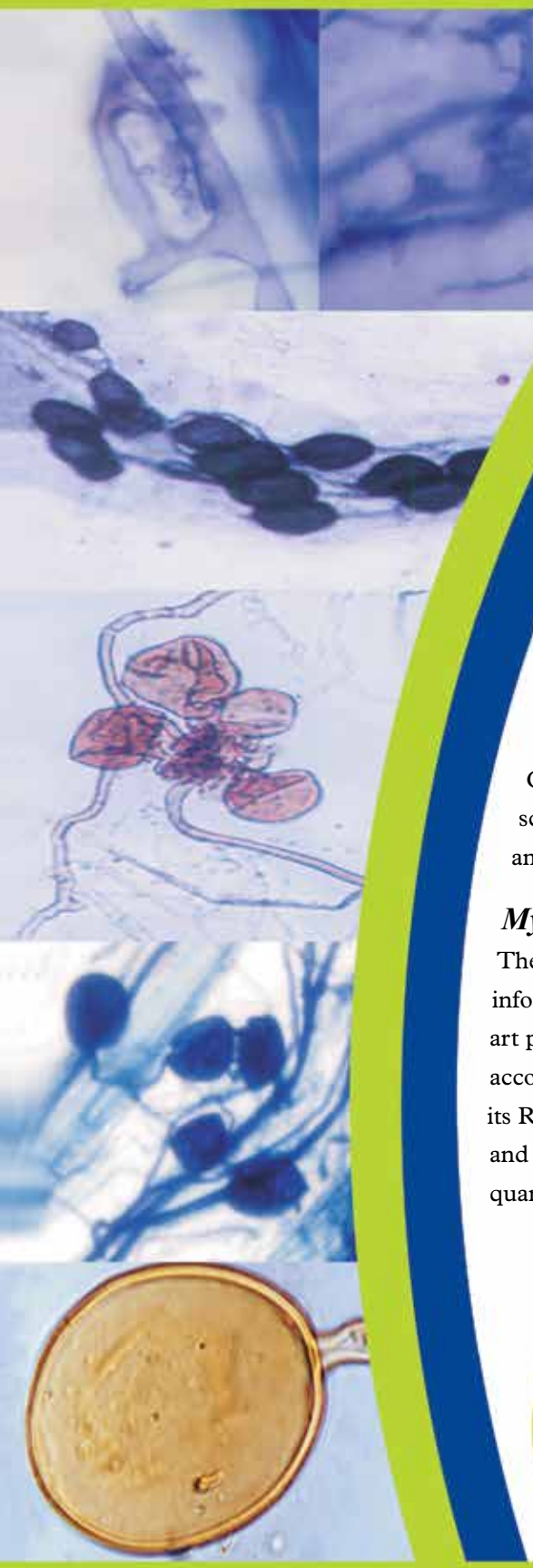

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Mycorrhiza News

The *Mycorrhiza News* provides a forum for the dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

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Research finding papers

Decomposed Coconut coir pith and Arbuscular Mycorrhizal fungal inoculum - A good Nursery Media mix for *Citrus limon* (L.) Burm. f.

H.C. Lakshmana, M.N. Srineevasab and Jyoti Puttaraddera*

Introduction

Arbuscular mycorrhizal fungi (AMF) are important soil inhabiting fungi in mobilizing phosphorus (P) nutrient in many soils through hyphal transport to the plant (Anjana and Lakshman 2004; Sylvia 1988). Nutrient change between two symbionts is at the core of the arbuscular mycorrhiza (AM) association and reciprocal transfer is a requirement for a functioning symbiosis (Fitter 2006; Dudhane et al. 2010). Nearly, 90 per cent of terrestrial plants are infected with AMF as a microsymbiont (Lakshman 1992, 1996). With rising environmental concern about heavy fertilization polluting the soil and water, it is very important to produce mycorrhizal inoculation in order to reduce the amount of chemical fertilizer. Very recently there has been a great demand for organic based agriculture. Most horticultural plants are colonized by AMF. Their presence can enhance the growth of the host plant (Ortas and Varma 2007). Significance of AMF on plant growth and nutrients uptake by many plant species was reported to be have AM fungal association (Mosse 1973; Nalini et al. 1986; Smith and Read 1997). Healthy and vigorous seedlings will stand better in plantations and hence a nursery mix that promotes better germination and seedling vigour is a must to sustain the demand for seedlings.

Citrus limon (L.) Burm. f. is the most important medicinal plant, belonging to family Rutaceae. It grows as shrub bushes reach up to 1.25 meter in height. Its fruits are rich in sugars, pectin, cellulose and vitamin C. Most of the citrus plants are temperamental about soils, often suffering from trace element deficiency. In general, they can be grown in fertile, light loamy soils. Plants grow at very slow rate at nursery stage. They normally raised in a medium containing red earth, farm yard manure and fine sand in equal volumes. Hence, the present investigation was undertaken to standardize a media mix suitable for *Citrus limon* (L.) Burm.f. using Mycorrhizal fungus (*G. leptonicum*) with Coconut Coir pith, which is available in plenty in most of the southern states of India, that is, Karnataka, Tamil Nadu, and Kerala.

Materials and Methods

Preparation of AM Fungal Inoculum

Chlorios gayana Munch. (Rhode grass) is a potential host plant, this grass was used for mass culturing of mycorrhiza (*G. leptonium*). Therefore, the grass was grown in separate earthen potsmeasuring (20×25 cm) maintained 1:1 sterile garden soil: pure sand to culture *G. leptonium*. Mixed mycorrhiza inoculum of the pot cultured of 15 g. was placed 4cm below the soil of each experimental pot with 15 g. decomposed coconut coir pith (DCCP) before sowing the seeds of *Citrus limon* (L.) Burm.f.

Preparation of Decomposed Coconut Coir Pith

The raw Coconut Coir pith (RCCP) was collected from Sirsi area of north Canara district. It was

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decomposed using edible oyster mushroom fungus, *Pleurotus platypus* following the method of Nagarajan et al. (1985) and modified by Theradimani and Marimuthu (1992) and Kumar and Marimuthu (1997) for laboratory conditions. Well decomposed Coconut Coirpith available after 30 days of inoculation was shade dried for 120 days and then thoroughly mixed with normal nursery mix (NNM) in varying proportions (V/V) to get the following treatments.

- NNM
- Non mycorrhizal (NM)
- DCCP
- RCCP
- M + DCCP 25 per cent
- M + DCCP 50 per cent
- M +DCCP 75 per cent

The experiment was conducted in randomized block design replicated four times. Seeds were collected from four –year- old plant of *Citrus limon* (L.) Burm.f., where the plants were maintained at Karnatak University Botanical Garden, Dharwad. The media mixes were separately filled in plastic trays of size 26 × 26 × 100cm. Well-filled seeds were handpicked with the aid of hand lens (5×) from the seed lot and were sown in line on the media mixes contained in earthen pots measuring (15×20 cm length × breadth), care has to be taken that to prevent any weed or contamination to experimental pots. The experimental pots are watered on alternate day, 15 ml minus P Hoagland nutrient solution given to each pot once in 15 days. The different growth parameters were observed and seedling vigour index (SVIN) in nursery was calculated based on formula suggested by Abdul-Bakri and Anderson (1973), which was modified to calculate SVIN.

SVIN = Survival percentage of seedlings on 30 days after sowing × root length + shoot length. Similarly growth parameters like shoot length, root length and dry matter of seedlings were calculated after 30 day till 90 days. In the present study, we have given the data for 90 days only.

Table 1: Effect of AM fungus (G. *leptonicum*) with Coconut coir mix on the vigour of *Citrus limon* (L.) Burm.f. shoots length, root length, dry matter, and leaves for 90 days

AMF + Media mix	Seedlings vigour index in nursery	Shoot length (cm)	Root length (cm)	No of leaves per plant	Dry weight per plant (mg / plant)
NM + NNM	18.24	2.1	1.1 1.1	2.1	21.4
M + NNM	123.12	5.6	2.4 2.4	4.8	58.2
M + RCCP	148.4	3.9	1.8 1.8	4.0	26.1
M + DCCP 25%	479.6	14.7	2.6 2.6	5.4	57.4
M + DCCP 50%	915.3	19.8	4.8 4.8	8.7	73.4
M + DCCP 75%	728.2	15.1	3.4 3.4	5.2	63.1
(CD at 0.05%)	105.2	0.8	0.4 0.4	1.3	6.2

M,= Mycorrhizal; NM, = Non mycorrhizal; NNM,- Normal Nursery Mix.

Table 2: Effect of AMF (G. leptonicum) with coconut coir mix on the Citrus limon (L.) Burm.f. percent AMF colonization, spore number, and nutrient content in dry weight of shoots for 90 days

AMF + Media mix	Percentage of AM Colonization	No. of spores/ 50 g. soil	Percentage N	Percentage P	Percentage K	Percentage Mg	percentage Cu
NM + NNM	–	–	0.26	0.05	0.31	0.18	0.14
M + NNM	47.4	102	0,21	0.15	0.34	0.16	0.18
M + RCCP	36.2	198	0.27	0.046	0.29	0.11	0.14
M + DCCP 25%	52.6	196	0.31	0.17	0.37	0.13	0.15
M + DCCP 50%	61.3	142	0.38	0.21	0.34	0.15	0.18
M + DCCP 75%	59.2	102	0.32	0.19	0.39	0.14	0.17
(CD at 0.05%)	19.0		0.11	0.004	0.12	0.12	0.09

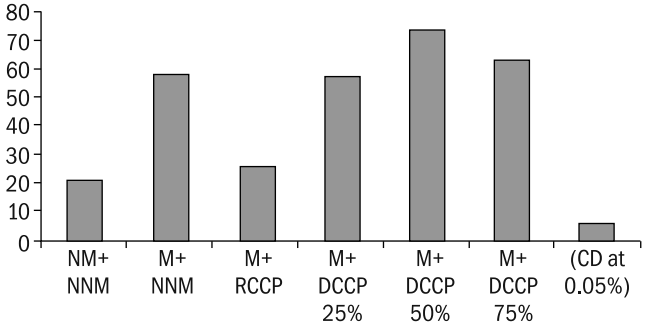

Fig 1: Effect of AM fungus (*G. leptonicum*) with coconut coir mix on the vigour of *Citrus limon* (L.) Burm.f. on dry matter for 90 days

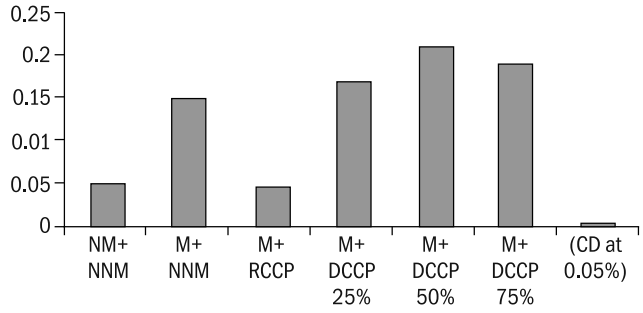

Fig 2: Effect of AM fungus (*G. leptonicum*) with coconut coir mix on the vigour of *Citrus limon* (L.) Burm.f. on dry matter for 90 days

Results and Discussion

The SVIN shoot and root length, number of leaves and dry weight of *Citrus limon* (L.) Burm.f. are presented in (Table 1and Figure 1). The SVIN of 123.12, 148.4, 479.6, 915.3, 726.2 was recorded compared to NNM, it was testing 18.24 percentage RCCP, DCCP. The maximum SVIN was recorded with the inoculation of mycorrhiza (*G.leptonicum*) + DCC P50 per cent which is followed by M + DCCP 75 per cent and M + DCC P25 per cent over the (control),that is NM or non-mix of coconut coir pith either in RCCP or DCCP without mycorrhizal inoculation. However, the M+DCCO 75 per cent and M+DCCP 25 per cent did not differ significantly from each other with respect to the production of leaves. In case of shoot length and dry matter, production was significantly higher with M+DCCP 25 per cent, 57.4 mg; M+DCCP 50 per cent, 73.4 mg; and M+DCCP 75 per cent, 63.1 mg/ plant was recorded over the control plants. Therefore M + DCCP 50 per cent followed by M + DCCP 75 per cent and M + DCCP 25% per cent favoured better shoot length and dry matter than NM+ DCCP or RCCP alone.

Percentage of AM fungal colonization in roots and spore number was higher in such plants that were treated with M + DCCP 50 per cent, M + DCCP 75 per cent and M + DCCP 25 per cent respectively. Similarly, there was a significantly higher percentage of N, P, K concentration in the shoots of *Citrus limon* (L.) Burm. f. when the plants were inoculated with M + DCCP 50 per cent, M + DCC 75 per cent and M + DCCP 25 per cent than NM + NNM (Control) plants as shown in (Table 2 and Figure 2). In contrast to this, Mg concentration did not increase in plant treated with M + DCCP 50 per cent or M + DCCP 75 per cent or M + DCCP 25 per cent of Coconut Coir pith with mycorrhizal inoculation.

In the present investigation the M+DCCP with 50 per cent and 75 per cent (V/V) to NNM successful boosted the vigour of the *Citrus limon* (L.) Burm.f. seedling. The M+DCCP 50 per cent is being recommended as a soil amendment to reclaim problem soils (Ramaswami and Kothadaraman 1985; Nagarajan et al. 1986). This organic manure might help in increasing the vigour of seedlings through (a) supply of plant nutrients and (b) improvement in physical, chemical and biological properties of soil. Dry matter production and number of leaves per seedling were also more in M+DCCP 50 per cent with mycorrhiza amended media mix than in others. It is also possible to raise seedlings in water deficient area using M+DCCP 50 per cent with mycorrhiza improves the water holding capacity of soils in small earthen pots or Polythene bags. The re-establishment of mycorrhizal relationship in denuded areas, mined spoils, and coastal zones has come to be recognized as an important biofertilizer component and an overall reclamation strategy (Peterson and Fargular 1994; Jasper et al. 1987; Smith and Read 1997; Lakshman 2002; Clarson 1986).

Rapid reestablishment of *Citrus limon* (L.) Burm.f. plants in earthen pots contained M+DCCP 50 per cent with mycorrhiza is to be a major concern for reclamation strategy and growth of plants. There is tremendous potential for practical research on this aspect of AM fungi and ectomycorrhiza *(Pleurotus platypus)* association (Felker 1999); this study suggests that plants with dual symbiotic association or successful as primary colonization of pioneer habitats such as temperature area or dry lands (Sen 1992). Mycorrhizal colonization level differed in different levels of M+DCCP treatment. Mycorrhiza can improve the performance of seedlings quality by stimulating nutrient uptake (Ortas et al. 2004). Mycorrhizal colonization level differed in different levels of M+DCCP treatment. Mycorrhiza can improve the performance of seedling quality by stimulating nutrient uptake (Ortas et al. 2004). Manipulation of symbiotic associations for re-vegetation purposes has great potential in reclamation of drastically disturbed lands. In conclusion, there will, however, be a need to select promising AM fungal species or strains suitable to reach category of stree land. Possibilities of using a combination of symbiotic/

non-symbiotic nitrogen fixers/ phosphate solubilizers with DCCP (50 per cent) that may give maximum benefit to the plants growing under stress conditions will have to be explored.

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Application of Native Arbuscular Mycorrhizal Fungi for Cultivation of Aromatic Plant *Cymbopogon flexuosus* in Alkaline Soil

*Harbans Kaur Kehri**,Varun Khare, Ovaid Akhtar, and Pragya Srivastava*

Introduction

Economic significance of aromatic plants can be traced by the fact that aroma chemicals contained in them play a vital role in our day to day living as spices; condiments and food flavouring agents; perfumes, '*ittars*', and deodorants; drugs and ointments; gum exudates and oleoresins; antibacterial, germicidal, and insecticidal agents; etc. Because of such vast and varied applications, essential oils are in consistent demand all over the world and constitute an important sector of trading in international market. Owing to the considerable diversity of edaphic and climatic conditions, ranging from tropical to temperate, an array of aromatic flora occurs in wild state in India. However, one of the major constraints in the large scale production of aromatic plants for commercial purposes is the availability of land, as because of ever increasing population in developing countries like India, there is a considerable pressure on land on account of the competing land uses. The arable lands are not used for the cultivation of non-conventional crops like aromatic plants. Therefore, for large-scale production of aromatic plants, focus should be turned towards the marginal lands with less fertile and problem soils, which are lying fallow at present. One of the important categories of such soil is alkaline soil. In India, about 8.6 mha land is salt affected, out of which about 3.0 mha is alkaline in nature that occupies an extensive area in the Indo-Gangetic plains of northern India (Singh 1992).

Poor physical property, high pH, high exchangeable sodium percentage (ESP), and accumulation of CO_3^{--} and HCO_3^{-} ions of the soil render it inhospitable for normal crop production. Application of organic/chemical amendments with moderately salt-tolerant plants is a common practice to bring such soil under cultivation. However, application of some native biological input such as arbuscular mycorrhizal (AM) fungi is relatively a new approach and seems to be a promising option. Plants exhibit considerable dependence on mycorrhizal association for an adequate supply of nutrients and water, enabling them to thrive under stress conditions. In a number of recent reports, it has been well established that the AM fungi enhance the ability of plants to cope with environmental stresses, generally prevalent in the degraded ecosystems, by providing a number of nutritional and physiological benefits (Ruiz-Lozano 2003; Giri and Mukerji 2004; Al-Karaki 2006; Khare et al. 2008). Although little work has been done for the cultivation of aromatic plants in alkaline soil but no work is reported regarding application of AM fungi in this reference.

The present study has been planned to explore the potentiality of indigenous AM fungi dominant in alkaline soil in improving the establishment, survival and performance of aromatic plant, *Cymbopogon flexuosus* in alkaline soil.

Materials and Methods

Soil Collection

To set the experiment, alkaline soil was collected from the fallow fields of Handia site (250°23′N, 820°11′E), located in the Gangetic plains of India near district Allahabad, Uttar Pradesh. The pH of the soil was 10.2 with 0.20 per cent organic carbon, 2.25 per cent nitrogen, 32 kg/ha phosphorus, and 125 kg/ha potassium.

Experimental Plant Material

The slips of the experimental plant material, that is. *C. flexuosus* (Steud) Wats *var.* Krishna, were procured from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. *Cymbopogon flexuosus*, commonly known as lemon grass, is known to grow successfully in the alkaline soil having pH up to 9.8 and ESP up to 55 per cent as it has an ability of Na^+ exclusion.

Mycorrhizal Inoculum Production

A consortium of native AM fungi, dominant in the alkaline soil of the selected site was applied as mycorrhizal inoculum in the experiment. Alkaline soil collected was mixed with sterilized sand in 1:1 ratio (v/v) and the mycorrhizal inoculum was produced in trap cultures using *Sorghum bicolor* as host plant under greenhouse conditions for about four months. Mycorrhizal inoculum consisted of soil having spores (35 AM spores/10 g soil), mycelia, and infected root fragments (~80 per cent root length colonization).

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Acaulospora bireticulata, A. longula, Glomus claroideum, G. constrictum, G. fasciculatum, G. invermaium, G. mosseae, Glomus sp.UVKGL1, *Glomus* sp.UVKGL2 and *Glomus* sp.UVKGL3 were the dominant AM fungi in the mycorrhizal inoculum.

Experimental Design

To evaluate the impact of native AM fungi on the performance of *C. flexuosus* raised in alkaline soil, a pot experiment was conducted with complete randomized design under greenhouse conditions. Four different series were maintained in the experiment. Among the three non-mycorrhizal series, a control was maintained where no amendments were added in the alkaline soil. The soil was supplemented with gypsum in the second series and gypsum along with organic matter (2 per cent compost w/w) in the third series, because addition of gypsum and organic matter is a common practice at farmer's level to bring alkaline soil under cultivation. In the fourth series, plants were made mycorrhizal by adding mycorrhizal inoculum in alkaline soil supplemented with gypsum and organic matter. Single slip of aromatic plant was planted in each pot. In mycorrhizal series, 200 g soil inoculum (35 AM spores/10 g soil and root fragments infected up to 80 per cent) was added in each pot by layering method (Menge et al. 1977) before plantation of the slips.

Measurement and Analysis

Four plants per treatment were harvested at the end of 12 months to collect the data on growth, essential oil yield, total protein content, and physiological and stress parameters. Data on mycorrhization were collected three times in the year at four-month intervals and expressed as average. All data were expressed as the mean of three replicates. For determination of mycorrhization, root bits were stained in 0.05 per cent trypan blue (Philips and Hayman 1970) and percentage of root bits infection and percentage of root length colonization was determined by slide method (Giovannetti and Mosse 1980). Fifty grams air dried soil was taken for spore extraction by wet sieving and decanting method (Gerdemann and Nicolson 1963) and spore population was counted by using stereobinocular (Gaur and Adholeya 1994). Freshly collected sample of the aromatic plant *C. flexuosus* was chopped and soaked in water for about half an hour and oil was extracted by steam distillation method with an essential oil extraction apparatus (Clevenger type). Essential oil content and the yield were determined as follows:

$$\text{Essential Oil Content} = \frac{(\text{Oil produced (ml)})}{(\text{Fresh weight of sample (g)})}$$

$$\text{Essential Oil Yield (\%)} = \frac{\text{Weight of oil (g)}}{\text{Fresh weight of sample (g)}} \times 100$$

Photosynthetic pigments were extracted in 80 per cent acetone and concentration was determined by method given by Lichtenthaler and Welburn (1983). Total protein content was determined by method given by Lowry et al. (1951). Relative permeability was measured in terms of percentage of electrolyte leakage as described by Gong et al. (1998). Proline content was estimated according to method given by Bates et al. (1973). Activity of enzymatic anti-oxidative catalase was determined by monitoring the consumption of H_2O_2 by Aebi (1984) method. Non-enzymatic anti-oxidant ascorbate was determined by method given by Oser (1979).

Statistical Analysis

Data on each parameter studied were subjected to one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple range test at $P \leq 0.05$. Statistical software use was SPSS 16.0 for Windows 8.1.

Results

Mortality

Effect of consortium of native AM fungi on the rate of mortality of *C. flexuosus* has been presented in Figure 1. Rate of mortality ranged from 8.3 to 25 per cent. Plants raised in alkaline soil showed the maximum mortality while minimum mortality was observed in mycorrhizal series and about 66.8 per cent reduction over control was recorded.

Mycorrhization

Effect of consortium of native AM fungi on the mycorrhizal intensity in terms of root colonization and spore population in rhizospheric soil of *C. flexuosus* has been presented in Table 1. Average percentage of root bit infection ranged from 31 to 55 per cent and average percentage of root length colonization ranged from 15.6 to 38.6 per cent (Table 1). Average percentage of root bit infection increased 77.4 per cent and average percentage of root length colonization increased 146.4 per cent over control in mycorrhizal series. Average spore population in the rhizospheric soil ranged from 32.6 to 107 spores/50 g air dried soil. In comparison to control, all other series showed a significant increase in AM spore population. However, maximum spore population was recorded in mycorrhizal series. An increase of 227.2 per cent over control was recorded in mycorrhizal series.

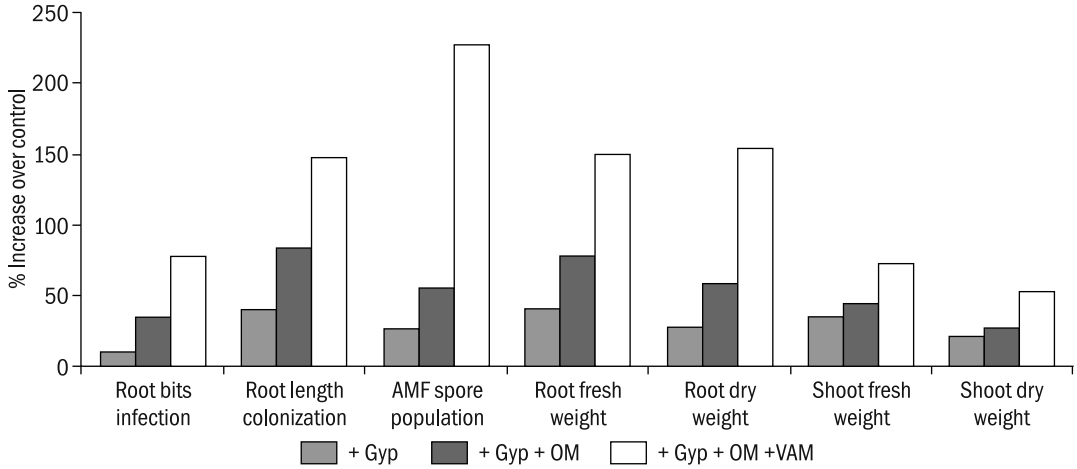

Figure 1: Percentage increase in mycorrhization and growth of *C. flexuosus var.* Krishna plants raised in alkaline/sodic soil, amended with gypsum and organic matter under pot conditions

Table 1: Effect of AM inoculation on mycorrhization and growth of *C. flexuosus var.* Krishna plants raised in alkaline/sodic soil, amended with gypsum and organic matter under pot conditions

	Mycorrhization			Growth			
				Root		Shoot	
Treatments/Samplings	Percentage of root bits infection	Percentage of root length colonization	AM spore population (per 50g air dried soil)	Fresh weight (g/plant)	Dry weight (g/plant)	Fresh weight (g/plant)	Dry weight (g/plant)
Alkaline/Sodic Soil	31.0[a]	15.7[a]	32.7[a]	60.2[a]	22.2[a]	65.2[a]	30.2[a]
+ Gyp	34.0[a]	22.0[b]	41.3[ab]	84.5[b]	28.6[b]	88.5[b]	36.6[b]
+ Gyp + OM	42.0[b]	28.7[c]	50.7[b]	107.1[c]	35.2[c]	94.1[b]	38.2[b]
+ Gyp + OM + AM	55.0[c]	38.7[d]	107.0[c]	150.3[d]	56.3[d]	112.3[c]	46.3[c]

Gyp, Gypsum; OM, Organic matter (compost); AM, Arbuscular mycorrhiza

All data were subjected to one-way ANOVA; the mean values were compared by Duncan's multiple range test ($P \leq 0.05$), and the mean with same superscript are not significantly different.

Growth

Root/shoot fresh weight of *C. flexuosus* ranged from 60.2 to 150.3 g/plant and 65.2 to 112.3 g/plant, respectively. Likewise root/shoot dry weight ranged from 22.2 to 56.3 g/plant and 30.2 to 46.3 g/plant, respectively, at harvest (Table 1). Minimum root/shoot growth was recorded in control series. In comparison to control, all other series showed a significant increase in root/shoot growth. Maximum root/shoot growth was recorded in mycorrhizal series. An increase of 149.7 per cent over control in root fresh weight and 153.6 per cent increase over control in root dry weight and 72.2 per cent increase over control in shoot fresh weight, and 53.3 per cent increase over control in shoot dry weight was recorded in mycorrhizal plants (Figure 1).

Essential Oil Production

Essential oil content ranged from 0.72 to 1.05 ml/100 g while essential oil yield ranged from 0.62 to 0.97 per

cent at harvest (Table 2). Minimum oil content and oil yield was obtained from control series, that is, alkaline/sodic soils without any amendment. In comparison to control, all other series showed a significant increase in the oil content as well as in oil yield. However, maximum oil content and oil yield was obtained from the AM inoculation series (Figure 2).

Photosynthetic Pigments

Total chlorophyll content and carotenoid content were affected significantly by alkaline/sodic soil. Total chlorophyll content ranged from 0.69 to 0.96 mg/g fresh matter and carotenoid content ranged from 0.38 to 0.50 mg/g fresh matter at harvest (Table 2). Maximum chlorophyll (39.1 per cent increase over control) and carotenoid (31.6 per cent increase over control) contents were recorded in the AM inoculation series (Figure 2).

The results showed that the Chl a pigment was more sensitive to the stress conditions in comparison to Chl b (Table 2). Chl a content ranged from 0.50 to 0.71 mg/g fresh matter at harvest, whereas Chl b content ranged from 0.19 to 0.25 mg/g fresh matter. Minimum Chl a and b contents were recorded in control series and maximum in series inoculated with AM fungi. A progressive increase was recorded in Chl

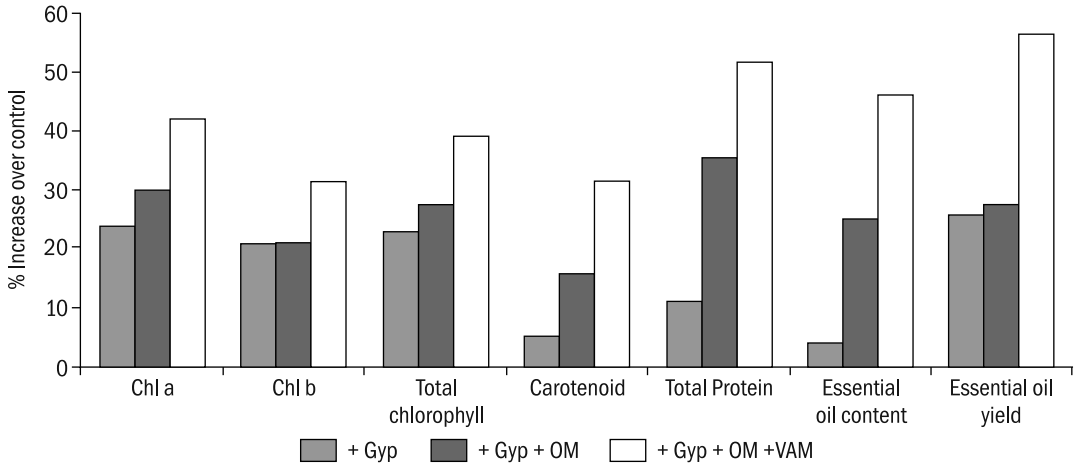

Figure 2: Percentage increase in physiological parameters and essential oil production of *C. flexuosus var.* Krishna plants raised in alkaline/sodic soil, amended with gypsum and organic matter under pot conditions

Table 2: Effect of AM inoculation on physiological parameters and essential oil production of *C. flexuosus var.* Krishna plants raised in alkaline/sodic soil, amended with gypsum and organic matter under pot conditions

	Photosynthetic pigment					Essential oil production	
Treatments/Samplings	Chl a (µg/ml)	Chl b (µg/ml)	Total chlorophyll (µg/ml)	Carotenoid content (µg/ml)	Total protein content (mg/g FM)	Essential oil content (ml/100 g fresh weight)	Essential oil yield (%)
Alkaline/Sodic Soil	0.50[a]	0.19[a]	0.69[a]	0.38[a]	99[a]	0.72[a]	0.62[a]
+ Gyp	0.62[b]	0.23[b]	0.85[b]	0.40[a]	110[b]	0.75[a]	0.78[b]
+ Gyp + OM	0.65[b]	0.23[b]	0.88[c]	0.44[b]	134[c]	0.90[b]	0.79[b]
+ Gyp + OM + AM	0.71[c]	0.25[b]	0.96[d]	0.50[c]	150[d]	1.05[c]	0.97[c]

Gyp, Gypsum; OM, Organic matter (compost); AM, Arbuscular mycorrhiza

All data were subjected to one-way ANOVA; the mean values were compared by Duncan's multiple range test ($P \leq 0.05$), and the mean with same superscript are not significantly different.

a/b ratio with the decline in stress conditions. The ratio of Chl a/b was gradually increased from 2.63 in control up to 2.84 in mycorrhizal series (Table 2).

Total Protein Content

Total protein content ranged from 99 to 150 mg/g fresh matter at harvest (Table 2). Minimum protein content was recorded in control series. In comparison to control, all the other series showed a significant increase in the protein content. However, maximum protein content was recorded in mycorrhizal series. An increase of 51.5 per cent over control was recorded in mycorrhizal series (Figure 2).

Relative Permeability

Electrolyte leakage ranged from 60 to 84 per cent at harvest (Table 3). Alkaline soils increased the electrolyte leakage while AM inoculation reduced it significantly. A reduction of 28.6 per cent over control was recorder in mycorrhizal series (Figure 3).

Proline Content

AM inoculation series also reduced the proline content. Proline content ranged from 48 to 82 µg/g fresh matter at harvest (Table 3). Maximum proline content was recorded in control series. In comparison to control, all other series showed a significant reduction in proline content. However, minimum proline content was recorded in the AM inoculation series. A reduction of 41.5 per cent over control was recorded in mycorrhizal series (Figure 3).

Ascorbic Acid Content and Catalase Activity

Ascorbic acid content ranged from 1,010 to 1,340 µg/g fresh matter while catalase activity ranged from 26 to 50 mg H_2O_2 broken/mg protein/hour at harvest (Table 3). Highest activity of catalase and ascorbate were recorded in the control plants grown in alkaline/sodic soil, while the activity of catalase and ascorbate decreased when the soil was amended with gypsum

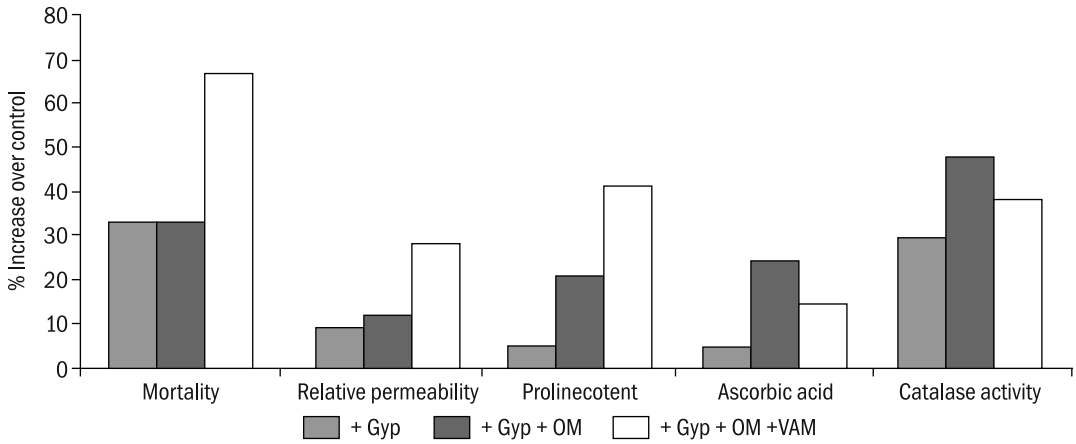

Figure 3: Percentage decrease in mortality, permeability, and stress parameters of *C. flexuosus var.* Krishna plants raised in alkaline/sodic soil, amended with gypsum and organic matter under pot conditions

Table 3: Effect of AM inoculation on mortality, permeability, and stress parameters of *C. flexuosus var.* Krishna plants raised in alkaline/sodic soil, amended with gypsum and organic matter under pot conditions

Treatments/Samplings	Mortality (%)	Relative permeability (%)	Proline content (µg/g FM)	Ascorbic acid content (µg/g FM)	Catalase activity (mg H_2O_2 broken/mg/protien/h)
Alkaline/Sodic Soil	25.0[a]	84[a]	82[a]	1340[a]	50[a]
+ Gyp	16.7[b]	76[b]	78[b]	1270[b]	35[b]
+ Gyp + OM	16.7[b]	74[b]	65[c]	1010[d]	26[c]
+ Gyp + OM + AM	8.3[c]	60[c]	48[d]	1140[c]	31[b]

Gyp, Gypsum; OM, Organic matter (compost); AM, Arbuscular Mycorrhiza

All data were subjected to one-way ANOVA; the mean values were compared by Duncan's multiple range test ($P \leq 0.05$), and the mean with same superscript are not significantly different.

and organic matter. However, under similar soil conditions, mycorrhization further increased the activity of catalase and ascorbate in the plants (Figure 3).

Discussion

Reduced mortality, increased biomass production, and oil yield in mycorrhizal plants under stressed conditions, compared to non-mycorrhizal plants, show that mycorrhizal symbiosis with consortium of AM fungi native to the stressed soil can alleviate the deleterious effects of alkaline/sodic stress.

The ameliorative effect of the AM fungi can be attributed to the fact that mycorrhizal association improves rooting and root hair production, increases the absorptive surface manifold for the better uptake of nutrients and water, thereby helps in better growth performance of the host plants (Sirohi and Singh 1993; Al-Karaki 2000; Cantrell and Linderman 2001). Improvement in plant nutrient uptake, particularly P, due to AM colonization is one of the most important mechanisms of stress tolerance in mycorrhizal plants (Hirrel and Gerdemann 1980; Al-Karaki 2000).

However, the advantages of AM fungi for plant growth and development under stress conditions are not always related to nutrient status improvement. Poss et al. (1985), Ruiz-Lozano et al. (1996), and Feng et al. (2000) have shown that mycorrhizal plants grow better than non-mycorrhizal plants under stress conditions even when both have similar P status. This indicates that the contribution of the mycorrhizal symbiosis to plant stress tolerance results from a combination of physical, nutritional, biochemical, physiological, and cellular effects. This includes enhanced osmotic adjustment, reduced oxidative damage caused by reactive oxygen species (ROS), improved physiological processes such as increased photosynthesis rate, CO_2 exchange rate, stomatal conductance, and transpiration and water use efficiency.

In the present study, reduced plant growth and biomass in the plants exposed to stress might be due to the depressed photosynthetic process resulting from substantial decrease in the contents of photosynthetic pigments and direct effects on primary photosynthetic reactions. It has been demonstrated by many workers that AM colonization increases the rate of photosynthesis by stimulating the synthesis of photosynthetic pigments (Mathur and Vyas 2000; Feng et al. 2002; Beltrano and Ronco 2008). The results of the present study also indicate the positive influence of the mycorrhizal symbiosis on photosynthetic pigments chl a, chl b, and carotenoid. Mycorrhizal association with consortium of native AM fungi increased the chlorophyll and carotenoid content in the plants significantly (Figure 3).

Reduction in proline concentration, lower permeability of the root plasma membrane, and increased protein content in the mycorrhizal plants, compared to non-mycorrhizal plants, in the present study also indicates that the mycorrhizal *C. flexuosus* plants were less affected by the alkaline/sodic stress.

Plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes in order to defend themselves against ROS causing oxidative damage. In the present study, activity of enzymatic antioxidant catalase and non-enzymatic antioxidant ascorbate was measured. The results of the present study exhibit a strong correlation between the antioxidant defence system and alkaline/sodic stress tolerance in the plants (Figure 3). Increased catalase and ascorbate activity in mycorrhizal plants compared to non-mycorrhizal plants supports the view that increased antioxidant activity could be involved in the beneficial effects of mycorrhizal colonization on the performance of the plants grown under alkaline/sodic stress conditions. Similar findings have also been reported by Alguacil et al. (2003) and Ghorbanli et al. (2004).

Conclusion

Present study shows the efficiency of native AM fungi in reducing stress imposed by alkaline/sodic soil to the aromatic plant *C. flexuosus*. The package of AM fungal consortium along with gypsum and compost could be a beneficial and cost-effective approach for reclamation and management of alkaline/sodic soils.

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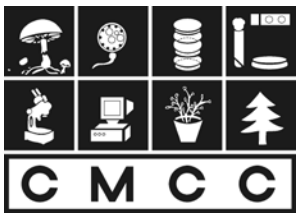

Centre for Mycorrhizal Culture Collection

Morpho Taxonomy of *Glomus hoi* (accession CMCC/AM-1301)

*Kiran Sunara, Priya Ahujaa, and Alok Adholeyab**

Arbuscular Mycorrhiza Fungi (AMF) is a unique group of microorganisms that show distinct morphological characters across families and genera. The taxonomy of AMF belonging to Glomales is based principally on the structure of their spores or sporocarps. Most of the characters are not genetically governed and are more or less influenced by the environment. However, the key characters like spore wall, hyphal wall, nature of attachment, and branching and sporulation pattern remains the same (Morton 1990). So far, new technologies and advancement in microscopy and staining procedure have greatly aided towards identifying specific characters and developing reliable taxonomic keys for species identification. Such characters were used to construct a dichotomous key (Hall and Fish 1979) and later a synoptic key (Trappe 1982). One of the most important characters that have been given weightage and considered to be a key factor in determining genera and species is the wall character and has been extensively used to develop taxonomic keys of almost all the Glomales (Walker 1983). Considering the importance of taxonomic characterization in nomenclature of AMF, we have been presenting detail description of AMF isolates that are being maintained in our Centre for Mycorrhiza Culture Collection (CMCC) germplasm bank. In this article, we present the morphotaxonomic analysis of one of a unique AMF bearing accession number CMCC/AM-1301, collected from the forests of Panchmari, Madhya Pradesh, India.

Monosporal Establishment

The isolate designated as CMCC/AM-1301 was isolated from the trap raised from the soil collected from Pachmarhi district of Madhya Pradesh. Trap cultures were raised for proliferation of indigenous mycorrhiza in pot conditions with a suitable host for a period of three months. After adequate growth cycle of the host plant, spores propagated in trap culture were checked by wet sieving and decanting method (Gerdemann and Nicolson 1963) and were categorized on the basis of their morphology. In the next step, all healthy spores were grouped according to their size, structure, and colour. Morphologically, similar types of spores were grouped or isolated for obtaining pure single species culture of AMFs. Voucher specimen of all the potential monosporal was prepared and morphotaxonomic analysis of the spore and its wall layers, hyphal attachment, etc. were carried out under compound microscope (10×, 40×, and 100×) after mounting in Polyvinyl Lacto glycerol and Polyvinyl Lacto glycerol: Melzer's reagents (1:1). Selected healthy single AMF spores were used to raise monospecific cultures that were inoculated to pre-germinated seed of a suitable host. After a successful growth period of three to six months, the host roots were evaluated for colonization and sporulation. Cultures showing colonized roots and spores were considered as successful cultures for raising monosporals and were considered to be pure when the spores isolated from them were morophotaxonomically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals (Figure 1). In case of CMCC/AM-1301, full sporulation state was attained after three cycles of the host plant.

A detailed morphotaxonomic description of this accession has been presented as adopted by various workers for identification.

Spore Morphology and Shape

Spores isolated from the monosporal cultures were borne singly or in small clusters of three or four in the extraradical region; they were devoid of sporocarps and each of them were suspended with a single subtending hyphae. Most of the spores were gloobose

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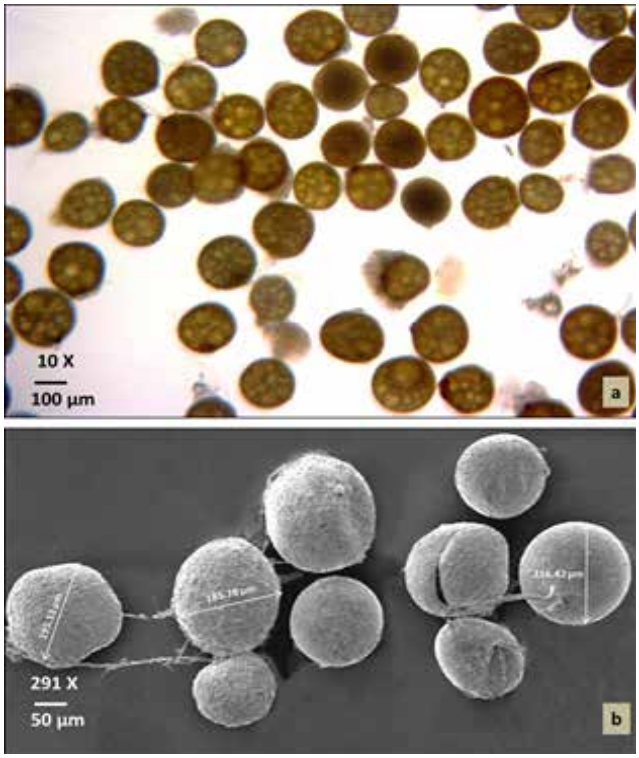

Figure 1: Compound microscopic (10×) and scanning electron microscopic (291×) images of identical spores obtained from monosporal culture of isolate CMCC/AM-1301

to sub-globose in shape with only a few of them as ellipsoidal and irregular. Mature spores were pale yellow to orange-yellow in colour whereas young spores appeared light yellow in colour. Scanning electron micrograph (SEM) of the spore surface revealed an outer slightly warty wall surface in mature spores with no any prominent pits. The outer most surface of the spore is mucilaginous and appears rough with the organic debris adhered to it (Figure 2). The average diameter of the spore was found to be in a range of 145.19–(192.76)–239.19 µm (Figure 3).

Subcellular Structure of Spore

Mature spores are composed of the following sub-cellular structures and wall layers:

Spore Wall Layer 1 (SWL-1): The first layer of the spore designated as (SWL-1) is mucilaginous, hyaline, and roughened in mature spores. The average thickness of this outer wall layer is (1.02)–(1.45)–(1.7) µm thick. This layer is somewhat roughened and deteriorates and gets sloughed off with the ageing of the spore and thus is rarely present in the spore wall of the mature specimen. This layer is highly mucilaginous and stains pinkish-red in Malzer's reagent (Figure 4c).

Spore Wall Layer 2 (SWL-2): The inner wall after the first layer is a laminated, smooth, and yellow to pale yellow in colour. This layer remains prominent

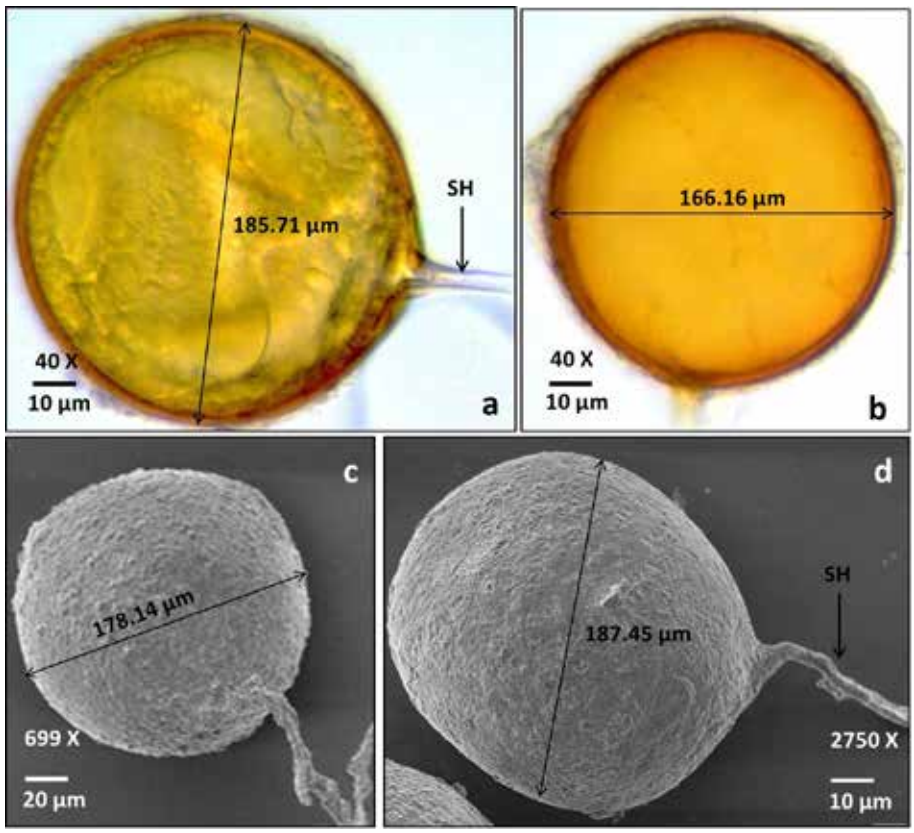

Figure 2: Compound and SEM of spores of CMCC/AM-1301 showing globose spore with single subtending hyphae. Spore mounted in PVLG (a) and PVLG: Melzer's reagents (b); SEM images of the spores (c and d)

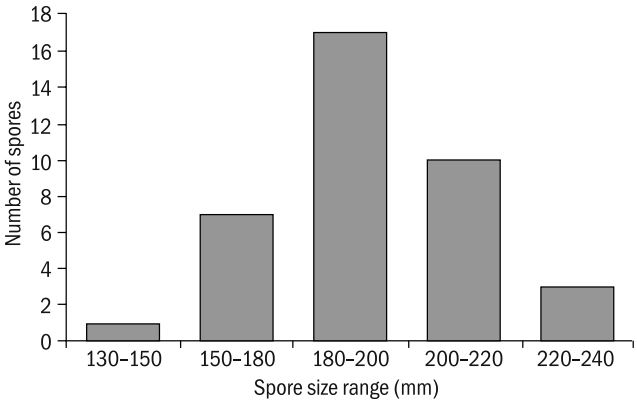

Figure 3: Analysis of spore diameters of 50 healthy spores obtained from one-year-old monosporal culture of CMCC/AM-1301

when the outer layer sloughs off in the mature spores. The wall thickness ranges from (1.3–) 1.97(2.5) µm. This wall layer is a firm wall made of thin sub layers that tightly adhere to each other (Figure 4).

Spore Wall Layer 3 (SWL-3): The innermost wall layer of the spore wall, this layer is flexible to semi-flexible in nature, yellow-brown in colour, and remains tightly adhered to the lower surface of the second layer. This layer is continuous with the subtending hyphae until the formation of an occlusion. Both the layers 2 and 3 remain inseparable at the base of the spore. The third layer is the layer that reacts strongly with the Melzer's reagent and appears dark brown in colour (Figure 4).

Subtending Hyphae

All the spores show intact, straight, or recurved subtending hyphae. The width of the subtending hyphae at the point of attachment at the spore base ranges from 9.78–(12.15)–12.9 µm. The hyphal wall of the subtending hyphae is brownish-orange in colour and is incessant with the second and the third layer of the spore that are continuous with the wall layers of the subtending hyphae. Layer two (HWL-2) is colourless and is generally the only layer that remains adhered to the mature spore. The average width of HWL-1 is around 4.5 µm and that of HWL-2 is 3.5 µm (Figures 5 and 6).

Occlusion

Most of the spores in low resolution appear to have an open pore, but higher magnification of the spore base reveals a thin curved septum, which is on an average 10–12 µm away from the point of attachment. The septum is continuous with the innermost sub-layers of the laminated SWL-3 (Figure 6).

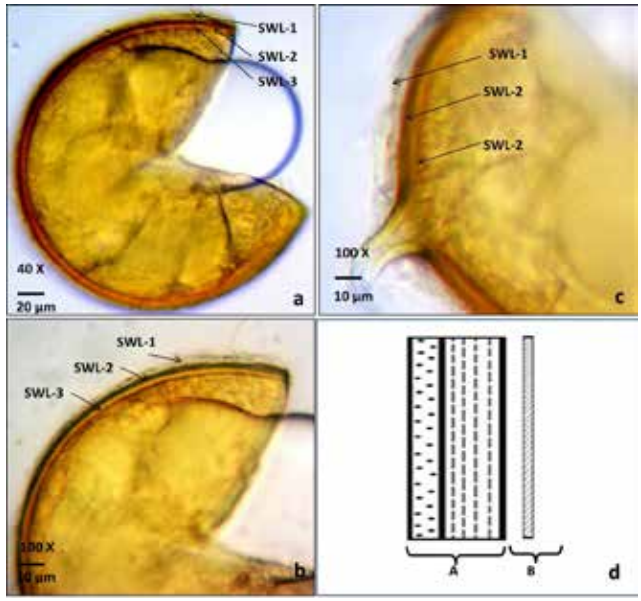

Figure 4: Compound microscopic images of spore wall layers of CMCC/AM-1301 after mounting in PVLG: PVLG (a) and Melzer's reagent (b and c). The outer layer SWL-1 is mucilaginous while the inner wall layers SWL-2 and SWL-4 are laminated. Murograph (Walker 1983) of the mature spore showing three distinct wall layers (d)

Mycorrhiza

Mycorrhiza structures such as arbuscules (AR), extra and intraradical hypha observed in *Sorghum bicolor* roots are deeply stained in ink vinegar. Root

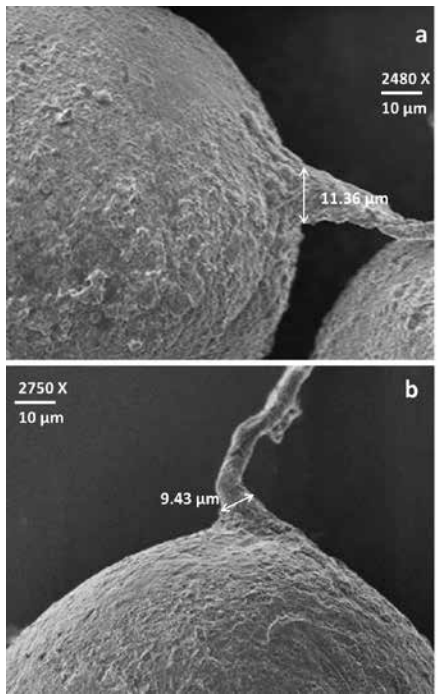

Figure 5: SEM of spores of CMCC/AM-1301 showing slightly flared subtending hyphae attached to a globose spore

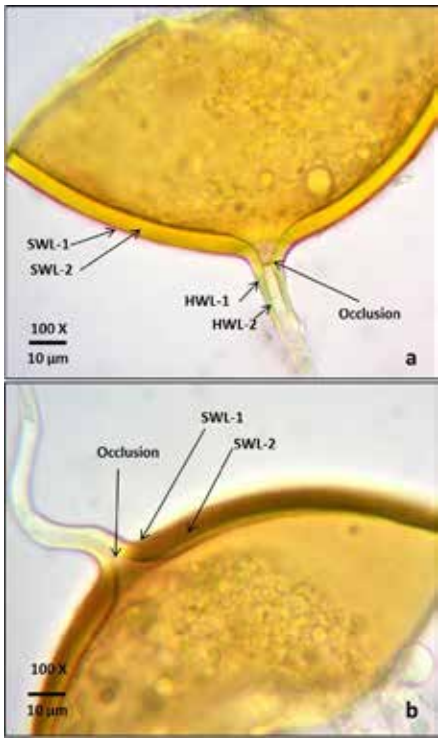

Figure 6: Compound microscopic images of spore of CMCC/AM-1301 showing flared subtending hyphae after mounting it with PVLG: Melzer's reagent. The recurved septum is continuous with the innermost layer of the laminated wall layer of the spore (a). Hyphal layers HWL-1 and HWL-2 are in continuum with the spore wall layers SWL-2 and SWL-3, respectively (a & b)

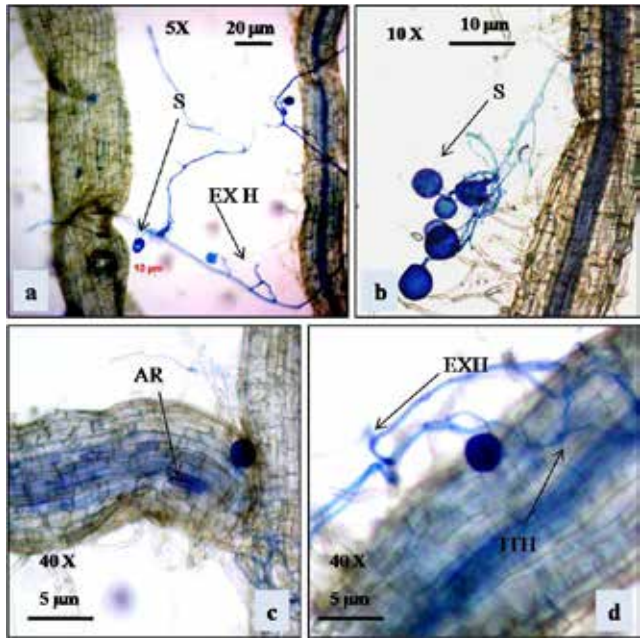

Figure 7: Compound microscopic images of roots of *Sorghum bicolor* stained in ink vinegar observed for root colonization by CMCC/AM-1301 showing extraradical (EXH) and intraradical (ITH) hypha (a & b) and AR in the cortical cells (c & d). Spores (S) in the extraradical hyphae is also seen in small clusters (b)

colonization assays showed the presence of both extraradical and intraradical hyphae. However, the extraradical hyphae are more abundant in specimen observed in pot cultures. AR are not abundant and are dispersed unevenly through the root cortex. Intercalary vesicles are absent but are present in the extraradicular region along the root surface. Extraradical hyphae were abundant and were usually seen bearing spores bearing or in a group of two to three (Figure 7).

Conclusion and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-1301, many distinguishing features regarding the family, genera, and the species could be derived. The following features were taken into consideration for characterization and identification:

- Globose, asexual spores produced singly or in loose aggregates of three or four with layered spore walls.
- Spore wall layer is composed of outer mucilaginous layer followed by two smooth inner laminated layers that are continuous with the subtending hyphal wall.
- Spores are of varying shapes and sizes ranging from globose to sub-globose and sometimes irregular.
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intra cellular AR.

All these features suggest that the culture CMCC/AM-1301 belongs to the family **Glomeraceae** (Walker and Schüßler 2004).

Some of the unique morphotaxonomic features of the accession are as under:

- Pale yellow to orange-yellow, globose, asexual spores produced singly with layered spore walls; spores are of varying shapes and sizes ranging from globose to sub-globose and sometimes irregular. Size ranging from 145.19–(192.76)–239.19 µm.
- Spore wall layer is composed of outer mucilaginous layer and two inner laminated layers that are continuous with the subtending hyphal wall.

- Presence of intact and slightly flared subtending hyphae. The width of the subtending hyphae at the point of attachment at the spore base ranges from 9.78–(12.15)–12.9 µm.
- Presence of a thin curved septum, which is on an average 10–12 µm away from the point of attachment.
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intra cellular arbuscules.

The taxonomic feature of the accession CMCC/AM-1301 matches the characters of *Glomus hoi* Berch and Trappe**,** a species originally described by S M Berch and J M Trappe in the year 1985, from Pacific Northwest, the species named in honour of a dedicated researcher of mycorrhiza Dr Iwan Ho, belonging to family Glomeraceae. The holotype of *G. hoi* has been selected from spores extracted from a pot culture with *Zea mays* L. as the host plant inoculated with roots of *Fragaria chiloensis* (L.) Duch. collected from Oregon, Linn Co., Tombstone Pass. on September 14, 1969 (Berch and Trappe 1985). This holotype shows similar characters to our described species.

Systematic Classification

Glomeromycota
Glomeromycetes
Glomerales
Glomeraceae
Glomus hoi

Conventional methods for identifying a species are usually based on taxonomic identification of organisms, assembling key characters, and comparing it with existing reference or type species. In practice, this approach is useful for taxa that are well known and when individuals can be readily identified, but it can be a serious problem when species cannot be identified based on existing references. Therefore, traditional taxonomic approaches to characterize an individual may not have significant advantages over alternative methods. Identities of individual organisms that are closely related to each other are made easier and accurate by sequencing the conserved sequences. Analyses of rDNA regions have often confirmed the morphologically defined species, and the molecular data have characterized new genera and families in AM taxonomy. It is therefore advised to our distinguished readers to kindly correlate their morphotaxonomic studies with molecular phylogenetic results.

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Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals:

- *Applied Soil Ecology*
- *Fungal Ecology*
- *Italian Journal of Agronomy*
- *Journal of Degraded and Mining Lands Management*
- *Journal of Science and Technology of Greenhouse Culture*
- *Mycorrhiza*
- *Pedosphere*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*
- *Symbiosis*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author marked with an *)
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