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MYCORRHIZA NEWS

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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

Role of Brassinosteroids in Arbuscular Mycorrhiza Formation in Tomato Roots

*Aswathy Nair and Sujata Bhargava**

Introduction

Arbuscular mycorrhizal (AM) symbiosis involves an exchange of mineral nutrients and photoassimilates between the host plant and AM fungus (Harrison 2005). Phytohormones are known to regulate different stages of AM colonization and play a role in the establishment of successful symbiosis. Growth promoting hormones, such as auxins, cytokinins, and gibberellins, are known to alter root growth and suppress host defence responses in response to AM colonization (Walters and McRoberts 2006). On the other hand, stress hormones, such as abscisic acid, jasmonate (JA), ethylene, and salicylic acid (SA) are involved in activation of plant defence responses against the AM fungus. For example, a transient increase in SA levels during early stages of AM colonization was reported in rice plants during colonization by *Glomus mosseae*, which led to the activation of plant defence responses (Blilou *et al.* 2000). JA levels were seen to increase in the roots of *G. intraradices* colonized barley plants and remained high even after establishment of symbiosis (Hause and Schaarschmidt 2009). Crosstalk between phytohormones is important not only in initiation and propagation of AM colonization but also in limiting the extent of colonization such that the association remains symbiotic and does not become pathogenic (Gutjahr and Paszkowski 2009).

The role of brassinosteroids (BRs) in AM colonization is controversial. Some reports indicate that BR levels do not differ in non-colonized and AM colonized plants, and the *lkb* mutant in pea, which is defective in BR synthesis and showed no difference from wild type plants in the extent of AM colonization (Foo *et al.* 2013). However, the BR biosynthesis deficient tomato mutant *dumpy* showed reduction in the extent of AM colonization as compared to wild type plants, indicating that BR does play a role in regulating AM colonization (Bitterlich *et al.* 2014). BR is known to play a role in regulating plant defence responses by interacting with other hormones (Divi *et al.* 2010). For example, BR downregulated the expression of the JA biosynthesis pathway gene, allene oxide synthase, in rice plants infested with root knot nematodes (Nahar *et al.* 2013). On the other hand, JA signalling was shown to inhibit a BR biosynthesis pathway gene Dwarf4 (DWF4) (Ren *et al.* 2009). BR and JA were also shown to act synergistically in *Nicotiana attenuata* plants, where antisense inhibition of the BR signalling genes, *BRI1* and *BAK1*, led to a decrease in JA and JA-Ile levels (Yang *et al.* 2013).

BR is also known to play a role in carbohydrate partitioning to sink tissues during the growth and development of plants (Goetz *et al.* 2000). About 20% of total photoassimilates are thought to be provided to the AM fungus by the host plant (Doidy *et al.* 2012), and BR possibly plays a role in regulating this photoassimilate allocation to the AM fungus. The expression of a cell wall invertase gene (*Lin6*) was seen to increase during AM colonization (Schaarschmidt *et al.* 2006) as well as in response to BR (Goetz *et al.* 2000). BR is also known to positively regulate the expression of sucrose synthase 1 (Suc1) (Yu *et al.* 2011) as well as that of genes involved in sucrose transport (Bitterlich *et al.* 2014).

In this paper, attempts have been made to study the role of BR in AM symbiosis using tomato mutants defective in either BR synthesis (*dumpy*) or signalling (*curly*). Expression of genes involved in sucrose metabolism and JA responses in the mutants and their

Department of Botany, University of Pune, Pune 411 007, India
*Corresponding author, Email: sujata@unipune.ac.in

wild types indicated that BR interacts with the JA pathway to regulate AM colonization.

Materials and Methods

Plant material and AM fungal inoculation

Seedlings of tomato variety *Solanum pimpinellifolium* and its BR signalling mutant (*curly*) and *S. lycopersicum* and its BR synthesis mutant (*dumpy*) were obtained from Tomato Genetics Resource Centre, UC Davis, California. These were raised in seedling trays and transplanted after 15 days to bags containing about 1 kg of autoclaved soil. Inoculation with *G. fasciculatum* was done using a soil-based inoculum (supplied by CNBRCD, Bangalore, India) at the time of transplantation. Controls consisted of plants to which no mycorrhizal inocula were applied. All plants were maintained in a greenhouse under diffused daylight and temperature ranging from 25–28°C.

Measurement of shoot and root biomass

Root fresh weights were determined after washing the soil from the roots, blotting them dry, and weighing the root biomass. Shoots were detached from the roots and weighed separately. Three independent plants were used for biomass measurements.

Measurement of the extent of AM colonization

Colonization measurements were done using roots from three independent plants, 52 days post-sowing, by clearing root pieces in 10% hot KOH solution followed by staining in 0.5% trypan blue. Extent of colonization was measured from 30 root segments of 2 cm length per plant using the computer programme 'Mycocalc' (Trouvelot *et al.* 1986).

Gene expression

RNA isolation from root tissue was carried out with Trizol reagent (Sigma Aldrich, USA) and cDNA was prepared using RNA (1 µg) as a template for reverse transcription with ImProm II reverse transcriptase (Promega, USA). Primers were designed for amplification of 12-oxophytodienoate reductase 3 (OPR3, NM001246944), coronatine-insensitive 1 (COI1, AY423550.1), sucrose synthase (*SuSy*, AJ011319.1), Lin6, cell wall invertase (INVCW, AB004558.1), and vacuolar invertase (INVA, Z12027.1). The primers were first used for amplifying the cDNA using semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR) and the products sequenced to confirm the transcripts being amplified. Real-time RT-PCR was carried out in a thermocycler (RealPlex 2, Eppendorf, Germany) using the same cDNA. The assay was carried out using QuantiFast SYBR Green PCR kit (Qiagen, USA), following the manufacturers protocol. The 10 µL reaction mix consisted of 1X SYBR Green (PCR mix), 5–7.5 pmol of primers, and 1 µL of cDNA as template. Amplification was carried out at 95°C for 5 min, followed by 40 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Elongation factor 1α (EF1α) was used as an internal control for normalizing the expression levels of gene of interest. Gene expression levels were determined from leaves of three independent plants, each with two technical replicates. The relative mRNA expression was calculated by the $2^{-\Delta\Delta C}{}_{t}$ method (Livak and Schmittgen 2001).

Statistics

Colonization between the wild types and their respective mutants was compared using Student's *t*-test. Gene expression studies were carried out using root tissue from three independent plants. ANOVA and Tukey's two-way comparisons were made for multiple comparisons between AM colonized and non-colonized wild and mutant plants for gene expression levels (SPSS 16.0 Statistical Software).

Results

Measurement of the extent of AM colonization

The extent of colonization was reduced in both, the

Table 1 Measurement of the extent of *G. fasciculatum* colonization in roots of *S. lycopersicum* (wild type) and its brassinosteroid synthesis mutant *dumpy* and *S. pimpinellifolium* (wild type) and its brassinosteroid signalling deficient mutant *curly* at 52 dpi. Values represent means ($n = 3$) and their standard deviations

Colonization parameter	Mean values (in %)			
	S. lycopersicum	*dumpy*	*S. pimpinellifolium*	*curly*
Frequency of mycorrhiza in root system	92 ± 2.1	64 ± 2.3*	92 ± 2.1	65 ± 6.6*
Intensity of mycorrhizal colonization in root fragment	33 ± 2.7	23 ± 0.5*	35 ± 3.2	26 ± 4.7*
Intensity of mycorrhizal colonization in root system	27 ± 2.3	19 ± 0.7*	28 ± 3.7	19 ± 5.1*
Arbuscule abundance in mycorrhizal part of root fragment	37 ± 2.4	13 ± 1.4*	35 ± 3.5	13 ± 2.1*
Arbuscule abundance in root system	22 ± 1.9	8 ± 0.9*	20 ± 2.9	8 ± 0.6*

*Means differ significantly between the respective wild types and mutants ($P < 0.05$)

BR synthesis (*dumpy*) and signalling (*curly*) mutants, as compared to their respective wild types *S. lycopersicum* and *S. pimpinellifolium* (Table 1). The frequency as well as intensity of colonization in the mutants was lower than that in the respective wild types. The most severely affected parameter was arbuscule abundance, which, in proportion to the intensity of colonization, was about 50% lower in the mutants as compared to the respective wild types.

Root and shoot biomass

The AM colonized plants showed a significantly higher growth in terms of fresh weight of the shoots and roots as compared to the non-colonized plants of *S. pimpinellifolium* and *S. lycopersicum*. However, the mutant plants did not show any significant differences in root or shoot biomass between the controls and AM colonized plants (Table 2).

Gene expression

Expression of three genes involved in sugar metabolism, namely sucrose synthase (*SuSy*), vacuolar acid invertase (*INVA*), and cell wall invertase (*INVCW*), and two genes involved in JA synthesis (OPR3) and signalling (COI1) showed differential expression in the roots of AM colonized plants and non-colonized plants. The AM colonized roots of the wild type plants, *S. lycopersicum* (Figure 1A) and *S. pimpinellifolium* (Figure 1B) showed an increased expression of the sucrose metabolism genes (*SuSy* and *INVA*) and also of the JA biosynthesis (*OPR3*) and signalling (*COI1*) genes as compared to the non-colonized plants. The cell wall invertase (*INVCW*) did not show differential expression in response to colonization. The JA biosynthesis and signalling pathway genes and the sucrose metabolism genes were not induced in the AM colonized plants of the BR mutants *dumpy* and *curly*.

Table 2 Shoot and root biomass in wild type *Solanum spp.* and their respective brassinosteroid biosynthesis (*dumpy*) and brassinosteroid signalling (*curly*) mutants, in response to AM colonization at 52 dpi. Values represent means ($n = 3$) and their standard deviations

Plant name	Shoot biomass (g)		Root biomass (g)	
	Non-colonized	AM colonized	Non-colonized	AM colonized
S. lycopersicum	8.6 ± 1.31	14.1 ± 0.12*	1.3 ± 0.04	1.6 ± 0.040*
Dumpy	0.31 ± 0.028	0.33 ± 0.050	0.18 ± 0.016	0.19 ± 0.006
S.pimpinellifolium	8.5 ± 1141	12.8 ± 0.18*	1.4 ± 0.037	2.3 ± 0.036*
Curly	0.31 ± 0.021	0.38 ± 0.041	0.17 ± 0.028	0.20 ± 0.007

*Means differ significantly between AM colonized and non-colonized plants ($P < 0.05$) of the wild types

Discussion

The BR mutants showed dwarf phenotypes with severe reduction in shoot and root growth. However they did show AM colonization, albeit with a significant decrease in all the colonization parameters, particularly arbuscular abundance, as compared to the respective wild types. Arbuscules, being the main sites of exchange of nutrients between the fungus and root cells, play an important role in bringing about mycorrhization effects in terms of an increase in root and shoot biomass. Reduction in the number of arbuscules in the BR mutants probably abolished the mycorrhization effects, as was also observed in the BR deficient tomato DWARF mutant (Bitterlich *et al.* 2014).

Functional colonization involves two main aspects from the point of view of AM fungus, namely the ability of the fungal partner to escape defence responses of the host plant and the ability of the fungus to obtain carbon nutrition from the host plant. The host plant on the other hand has to turn-off the defence responses to establish a symbiotic relationship with the AM fungus and at the same time has to ensure that the AM fungal colonization does not go beyond a certain threshold, which would upset the cost–benefit ratios of the two interacting partners. Using the BR mutants in tomato, we studied the role of BR in regulating JA-mediated defence responses and sugar metabolism in the host plants to bring about a successful symbiotic interaction with the AM fungus *G. fasciculatum*.

Our results showed that while expression of the JA biosynthesis (*OPR3*) and signalling (*COI1*) genes increased in the AM colonized wild type plants, there was no induction of these genes in the BR mutants. AM colonization is known to be accompanied by an increase in the endogenous JA levels of plants, which could be correlated to higher expression of the JA response genes observed in the wild type AM

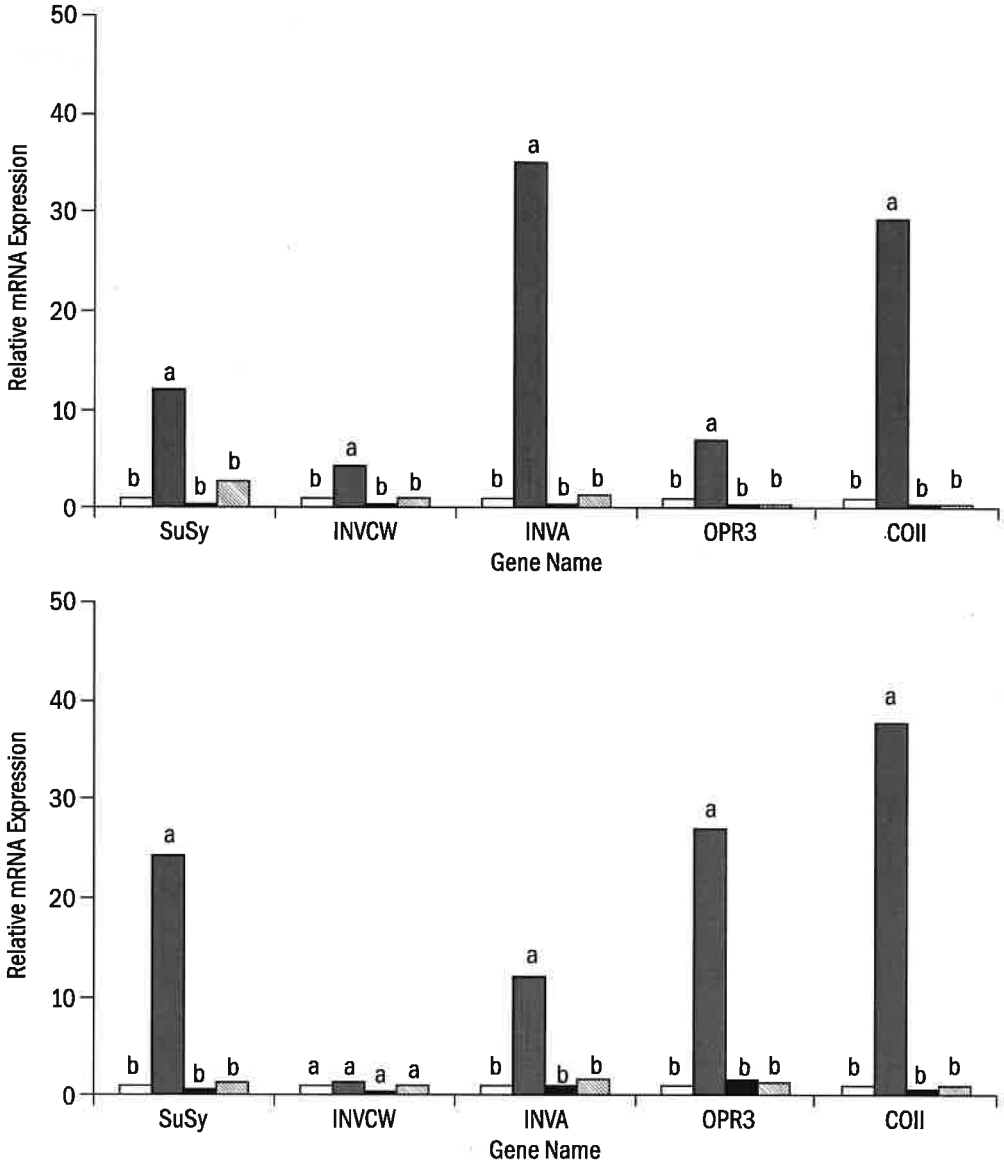

Figure 1 Quantitative RT-PCR analysis of the levels of expression of sucrose synthase (SuSy), cell wall invertase (INVCW), vacuolar invertase (INVA), JA biosynthesis (OPR3), and JA response (COI1) genes in AM colonized and non-colonized plants of (A) wild type (*S. lycopersicum*) and BR synthesis mutant *dumpy* and (B) wild type (*S. pimpinellifolium*) and BR signalling mutant *curly*. Relative mRNA levels of these genes were calculated after normalization with constitutively expressed *EF 1α*. Three independent plants per treatment were used for the analysis. Bars represent means ($n = 3$) of the relative mRNA expression in wild type (white bars), AM-colonized wild type (grey bars), mutants (black bars), and AM-colonized mutants (striped bars) with their standard deviations

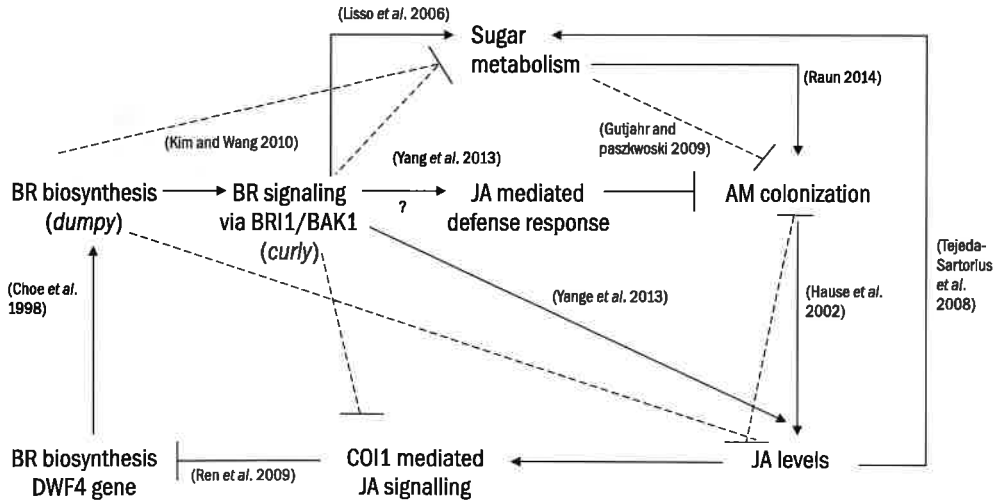

Figure 2 Model showing the possible role of BR in fine tuning JA responses, which regulate AM colonization. Arrows headed indicate activation and lines with a bar indicate inhibition. '?' indicates that there are controversial reports on BR signalling leading to enhanced JA levels or JA-mediated defence responses.

colonized plants (Nair *et al.* 2015). The JA-mediated defence responses have been reported to play a role in limiting the extent of AM colonization, such that the association remains mutualistic and does not become pathogenic (Gutjahr and Paszkowski 2009). Lack of induction of the JA pathway genes in the BR mutants was suggestive of a positive involvement of BR in regulation of JA biosynthesis and signalling genes. The involvement of BR signalling in JA responses during interactions between *N. attenuata* and the herbivore *Madhuca sextanta* was studied by silencing the genes coding for BR receptor (BRI1) and its associated receptor kinase (BAK1). The silencing of these genes showed low JA and JA-Ile levels, respectively, and an increased susceptibility to this insect (Yang *et al.* 2013). Hence, BR signalling appears to play a role in the positive regulation of JA-induced defence responses. However, there are reports suggesting antagonistic interactions between BR and JA signalling pathways. For example, the BR deficient mutant lines (*d2-1* and *d2-2*) of rice showed enhanced levels of JA biosynthesis and signalling genes and expressed resistance against the root knot nematode *Meloidogyne graminicola* (Nahar *et al.* 2013). The role of JA in regulation of BR biosynthesis has also been reported. It was shown that JA signalling through the COI1 pathway led to inhibition of the *DWF4* gene that plays a role in BR biosynthesis (Ren *et al.* 2009).

Besides the role of BR in regulating JA-mediated defence responses against AM colonization, this hormone is also known to play an important role in regulating sugar metabolism and transport in plants, particularly to sink tissues (Blee and Anderson 2000; Bitterlich *et al.* 2014). In our experiments, two genes involved in sucrose metabolism, namely, sucrose synthase (*SuSy*) and vacuolar acidic invertase (*INVA*), showed an increased expression in the AM colonized plants of the wild type tomato plants and also to some extent, but not significantly, in the BR mutants. These enzymes play an important role in providing the AM fungus with hexoses that are taken up by the fungal hexose transporters (Helber *et al.* 2011). A DWARF1 mutant of tomato that was defective in BR synthesis showed reduced AM colonization as well as reduced expression of a sucrose transporter gene (*SUT2*) (Bitterlich *et al.* 2014). Protein–protein interaction between the BR receptor kinase (BAK1) and *SUT2* has been postulated to regulate sucrose flux to the AM fungus (Kuhn 2016). The lower expression of the sucrose metabolism genes in BR mutants as compared to the wild type plants could be correlated to the lower number of arbuscules in these mutants, as well as to the inhibition of BR-induced JA signalling pathway. JA is known to play a role in inducing the expression of genes involved in sucrose metabolism and transport, and JA-deficient mutant spr2 in tomato showed lower expression of these genes as well as the extent of AM colonization (Tejeda-Sartorius *et al.* 2008). Hence, BR may play a role in regulating the extent of AM colonization and sugar metabolism through its interaction with the JA pathway. The use of BR mutants in our studies provided evidence for this role of BR during AM symbiosis. Based on the reports on the role of BR in JAsignalling and regulation of sugar metabolism, an autoregulatory model has been proposed, wherein BR is shown to fine tune expression of genes involved in JA-mediated defence responses as well as in sugar metabolism. Hence, BR may act through the JA pathway in regulating AM colonization to levels that would support a mutualistic association (Figure 2).

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References

Bitterlich M, Kugel U, Boldt-Burisch K, Franken P, and Kuhn C. 2014. The sucrose transporter *SlSUT2* from tomato interacts with brassinosteroid functioning and affects arbuscularmycorrhiza formation. *The Plant Journal* **78**: 877–89.

Blee KA and Anderson AJ. 2000. Defense responses in plants to arbuscular mycorrhizal fungi. In: Podila GK, and Douds DD(Eds),*Current Advances in Mycorrhizae Research.* Minnesota, USA:The American Phytopathological Society, USA, pp. 27–44.

Blilou I, Ocampo JA, and Garcia-Garrido JM. 2000. Induction of Ltp (Lipid transfer protein) and Pal (phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomusmosseae. The Journal of Experimental Botany* **51**: 1969–77.

Divi UK, Rahman T, and Krishna P. 2010. Brassinosteroid-mediated stress tolerance in *Arabidopsis* shows interactions with abscisic acid, ethylene and salicylic acid pathways. *BMC Plant Biology* **10**: 151.**doi**10.1186/1471-2229-10-151

Doidy J, Grace E, Kuhn C, Simon-Plas F, Casieri L, and Wipf D. 2012. Sugar transporters in plants and in their interactions with fungi. *Trends in Plant Science* **17**: 413–22.

Foo E, Ross JJ, Jones WT, and Reid JB. 2013. Plant hormones in arbuscularmycorrhizal symbioses: An emerging role for gibberellins. *Annals of Botany* **111**: 769–79.

Goetz M, Godt DE, and Roitsch T. 2000. Tissue-specific induction of the mRNA for an extracellular

invertaseisoenzyme of tomato by brassinosteroids suggests a role for steroid hormones in assimilate partitioning. *The Plant Journal* **22**: 515–22.

Gutjahr C and Paszkowski U. 2009. Weights in the balance: Jasmonic acid and salicylic acid signalling in root-biotroph interactions. *Molecular Plant-Microbe Interactions* **22**: 763–72.

Harrison M. 2005. Signalling in the mycorrhizal symbiosis. *Annual Review of Microbiology* **59**: 19–42.

Hause B and Schaarschmidt S. 2009. The role of jasmonates in mutualistic symbioses between plants and soil-born microorganisms. *Phytochemistry* **70**: 1589–99.

Helber N, Wippel K, Sauer N, Schaarschmidt S, Hause B, and Requena N. 2011. A versatile monosaccharide transporter that operates in the arbuscularmycorrhizal fungus *Glomussp* is crucial for the symbiotic relationship with plants. *The Plant Cell* **23**: 3812–23.

Kuhn C. 2016. Review: Post-translational cross-talk between brassinosteroid and sucrose signalling. *Plant Science* 248: 75–81.

Livak KJ and Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **25**: 402–08.

Nahar K, Kyndt T, Hause B, Hofte M, and Gheysen G. 2013. Brassinosteroids suppress rice defense against root-knot nematodes through antagonism with the jasmonate pathway. *Molecular Plant-Microbe Interactions* **26**: 106–15.

Nair A, Kolet SP, Thulasiram HV, and Bhargava S. 2015. Role of methyl jasmonate in the expression of mycorrhizal induced resistance against *Fusarium oxysporum in tomato plants. Physiological and Molecular Plant Pathology* **92**: 139–45.

Ren C, Han C, Peng W, et al. 2009. A leaky mutation in DWARF4 reveals an antagonistic role of brassinosteroid in the inhibition of root growth by jasmonate in *Arabidopsis. Plant Physiology* **151**: 1412–20.

Schaarschmidt S, Roitsch T, and Hause B. 2006. Arbuscularmycorrhiza induces gene expression of the apoplasticinvertase*LIN6* in tomato *(Lycopersiconesculentum)* roots. *The Journal of Experimental Botany* **57**: 4015–23.

Tejeda-Sartorius M, Martinez de la Vega O, and Delano-Frier JP. 2008. Jasmonic acid influences mycorrhizal colonization in tomato plants by modifying the expression of genes involved in carbohydrate partitioning. *Physiol Plantarum* **133**: 339–53.

Trouvelot A, Kough JL, and Gianinazzi-Pearson V. 1986. Mesure du taux de mycorhization VA d'un system radiculaire. Recherche de methods d'estimation ayant une signification fonctionnele. In: Gianinazzi PV and Gianinazzi S (Eds), *Mycorrhizae: Physiology and Genetics.* Paris: INRA, pp. 217–722.

Walters DR and McRoberts N. 2006. Plants and biotrophs: A pivotal role for cytokinins? *Trends in Plant Science* **11**: 581–86.

Yang DH, Baldwin IT, and Wu J. 2013. Silencing brassinosteroid receptor BRI1 impairs herbivory-elicited accumulation of jasmonic acid-isoleucine and diterpene glycosides, but not jasmonic acid and trypsin proteinase inhibitors in *Nicotianaattenuata. Journal of Integrative Plant Biology* **55**: 514–26.

Yu X, Li L, Zola J,*et al.* 2011. A brassinosteroid transcriptional network revealed by genome-wide identification of *BESI* target genes in *Arabidopsis thaliana. The Plant Journal* **65**: 634–46.

Constraints in AM Inoculum Production with Reference to Oilseed Production

*C Manoharachary[a] and Uttara Shankar[b]**

Introduction

In India, oilseed crops are grown in phosphorus (P) and moisture-deficient soils. Therefore, the need to improve oilseed production has acquired importance. AM fungi plays a significant role in the uptake of minerals and water, besides improving plant growth, yield, and productivity. The main handicap in AM studies has been inoculum production technology as they can only be multiplied on the suitable host plant. Few attempts have been made in this direction, including the culturing of endophytes. The logical approach in this regard is to study the formation, development, and functioning of AM. The mycorrhizal potential has to be re-established by inoculating the appropriate AM fungi, selecting effective AM fungi, and investigating their competitive abilities. AM fungi associated with sesame cultivars have been collected, multiplied, and tested for cultivar response. Selection of suitable host and the most responsive AM fungi were identified. Their formation, development, and establishment in the most suitable host have also been studied. Pot culturing and field studies in relation to plant growth and yield in Gowri cultivar have also been made.

AM fungi characterized by worldwide distribution are found on numerous plants (Gerdemann 1968). Recent research has shown that AM symbiosis in crop plants plays a major role in nutrient acquisition from soils, besides supporting plant growth with improved water uptake (Hayman 1982). Substantial information about AM symbiosis and plant growth for groundnut and soybean is available while there is no such account for *Sesamum indicum*. An important oil source, sesame is widely grown in semi-arid tropics. The role of AM fungi in increasing plant growth is well documented in pot experimentation. There is a need for the application of AM fungi as biofertilizer in increasing oilseed production. However, AM fungi being obligate symbionts could not be multiplied on artificial media. Therefore, the available literature reveals their multiplication with the most susceptible and responsive host plant root through pot culture technique (Wood 1984).

In the agricultural system of tropical countries, the dependence of cultivated plants on AM is greater as the soils are low in phosphorus and other nutrients. Field inoculation requires glass house selection of the most efficient fungal species in relation to specific host plant growing in a specific soil, assuming that the same growth responses are reproduced in the field (Abbot and Robson 1982; Bagyaraj 1984, 1986; Chambers *et al.* 1980; Daft and El-Giahmi 1975). Researchers have attempted in multiplying the inoculum and transplanting such inoculum under field conditions (Ferguson 1981; Mosse 1981; Sreenivasa and Bagyaraj 1988). Commercial production of AM inoculum is crux of the problem in utilizing AM fungi in practical agriculture. If such a bottleneck is broken down, mycorrhizal fungi can be used as a bio-fertilizer, in the fields, by the farmers. This article presents data pertaining to the association of AM fungi, their formation, colonization and establishment, role in plant growth, and yield in *Sesamum indicum* besides discussing the constraints involved in inoculum production for oilseed production.

Materials and Methods

Rhizosphere soil samples were collected from local fields and studies were conducted selecting six sesame cultivars, that is, Gowri, JE Xpulatil, E-8, TMV-1, TMV-2, and T-4. Spore types of AM fungi and their quantification in rhizosphere soil samples were recorded after extraction by the wet-sieving and decanting technique (Gerdemann and Nicolson 1963).

Soil samples were analysed for pH, soil type, moisture, organic carbon, available phosphorus, nitrogen, and potassium (Jackson 1958). Root pieces collected from naturally-grown sesame plants were stained (Phillips and Hayman 1970) and studied for percentage root colonization (Toth and Toth 1982). AM fungi collected were identified up to species level (Schenck and Perez 1987).

Mycorrhizal cultures were raised using sterile alfisol soil having soil and sand in the ratio 1:1. *Cenchrus ciliaris* has been used for multiplying AM inoculum. Pot culture experiments under glass house conditions were conducted using Gowri cultivar. Cultivar seeds were surface sterilized with 0.01% $HgCl_2$ and washed several times with distilled water. Nutrient solution (Hoagland and Arnon 1950)

[a]NASI Senior Scientist, Department of Botany, Osmania University, Hyderabad, India
[b]Project Associate, The Energy and Resources Institute (TERI), India Habitat Centre, Lodhi Road, New Delhi 110 003, India
*Corresponding author, Email: uttara.shankar@teri.res.in

was supplied to the plants twice a month to all the treatments, including the control plants. Pots were maintained with requisite moisture percentage and water-holding capacity. In addition, plant height, weight, and oil contents were also estimated.

The alfisol soil used contains 6.4.mg of P per kg. Autoclaved soil: sand mixture was used along with rock phosphate (containing 30% P_2O_5) to analyse the efficiency of rock phosphate in enhancing the AM activity in relation to plant growth.

Field inoculation was conducted under natural conditions and laid out in a randomized block. The total area for this experiment was 100 sq. m and each treatment was in four replicate rows. The control and AM inoculated rows consist of 25 plants in each row. The treatments administered were: Gowri cultivar grown in field soil; Gowri cultivar in field soil + *Glomus fasciculatum*; Gowri cultivar in field soil + *Gigaspora margarita*. Mycorrhizal inoculum consisting of soil + sand mixture with 10 spores/g (surface sterilized with 0.5% sodium hypochlorate) and infected mycorrhizae root bits were used. A layer of mycorrhizal inoculum was first laid out in furrows followed by a thin layer of soil and subsequent sowing of surface sterilized Gowri seeds. No fertilizer was applied to the soil. All the plants were removed and studied for plant growth and yield at the end of 90 days growth. However, the percentage AM root colonization was estimated at the end of 30th, 60th, and 90th day of crop growth, respectively.

Results and Discussion

The field soil samples were collected and subjected to analysis of physico-chemical factors. Soils were slightly alkaline and sandy loam with limited nutrients (Table 1).

Table 1 Physico-chemical factors of the soil

Cultivar	pH	Sand	Silt	Clay	Moisture (%)	Water holding capacity (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)
Gowri	7.3	53	26	21	20	60	870	8.0	520
JE Xpulatil	7.2	49	28	23	19	56	840	8.3	510
E-8	7.1	51	27	22	20	58	860	9.2	520
TMV-1	7.4	50	27	23	20	60	850	9.0	530
TMV-2	7.4	50	28	22	19	60	850	9.2	530
T-4	7.2	50	27	23	19	58	840	9.2	

Altogether eleven AM fungi were collected but only six AM fungi were identified and these were associated with rhizosphere soil samples of six sesame cultivars (Table 2). Of the six cultivars surveyed, Gowri cultivar has been colonized by maximum AM propagules and AM fungal species. Kharif crop has supported more propagules than Rabi crop (Table 3). Percentage AM root colonization has revealed the formation of

Table 2 Occurrence of AM fungi

AM Species	Gowri	JEX P	E-8	TMV-1	TMV-2	T-4
Acaulospora morrowae Spain and Schenck	+	+	+	-	-	+
Glomus fasciculatum (Thaxter sensu Gerd) Gerd and Trappe	+	+	+	+	+	+
Glomus epigaeum Daniels and Trappe	+	-	-	-	-	-
Glomus caledonium (Nicol and Gerd) Trappe and Gerd	+	-	-	-	-	-
Glomus monosporum Gerd and Trappe	+	-	+	-	-	-
Gigaspora maragarita Becker and Hall	+	+	+	-	-	+

Table 3 AM propagules number per 100 g Rhizosphere soil

AM Fungus	Gowri		JEXP		E-8		TMV-1		TMV-2		T4	
	1	2	1	2	1	2	1	2	1	2	1	2
Acaulospora morrowae	15	18	5	5	9	8	-	-	-	-	10	8
Glomus fasciculatum	20	30	6	14	9	16	8	9	7	16	8	18
Gigaspora margarita	12	26	5	5	7	6	-	5	5	10	6	10

vesicles, arbuscules, and spores sporocarps by the 20th day. Colonization percentage reaches maximum by 90th day of crop growth (Table 4). AM fungi was established and played a significant role in plant growth from 30th day of the crop. Gowri cultivar was found colonized heavily by the AM fungi. Of the six AM fungi, *Acaulospora morrowae*, *Glomus fasciculatum*, and *Gigaspora margarita* were found to benefit the Gowri cultivar to the maximum extent. Addition of rock phosphate has enhanced the mycorrhizal effectiveness in increasing crop growth and yield. There has been positive correlation between the increased percentage AM root colonization and plant height in pot experiments, rock phosphate amended soil, and field experimental soil (Table 6). The yield in terms of number of capsules per plant, grain weight, and percentage of oil content has increased with AM inoculation in pot experiments. The addition of rock phosphate enhanced the growth and yield under mycorrhizal inoculum. Further increase in plant growth and yield were seen when the Gowri cultivar got inoculated with AM under field conditions. Of the three AM fungi chosen, *Gigaspora margarita* and *Glomus fasciculatum* were the most effective. Various amendments introduced, such as rock phosphate, cow dung, and green manure have increased the number of AM propagules and percentage root colonization by AM (Tables 5–7). It is pertinent to mention here

Table 4 Percentage of AM root colonization in different cultivars

AM Fungus	Gowri			JE Xpulatil			E-8			T4		
	D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3
Control	-	-	-	-	-	-	-	-	-	-	-	-
Acaulospora morrowae	25.6	55.7	70.6	10.1	25.6	30.8	15.4	25.5	50.7	15.6	25.6	45.4
Glomus fasciculatum	30.8	66.1	90.6	10.2	20.7	35.4	15.5	34.6	60.6	10.2	20.9	40.6
Gigaspora margarita	35.6	71.7	95.8	5.0	25.3	30.9	10.0	20.3	40.6	15.4	25.7	50.8

D_1 = 30 days
D_2 = 60 days
D_3 = 90 days

Table 5 Number of AM propagules and percentage of AM root colonization in Gowri cultivar (pot experiments) in relation to amendments

	1		2		3		4		5		6		7	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Glomus fasciculatum	28	90	36	92	32	90	34	92	38	96	41	95	46	96
Gigaspora margarita	30	95	42	96	38	96	46	98	46	98	49	96	54	98

1= Gowri cultivar
2= Rock phosphate (RP)
3= Cow dung (CD)
4= Green manure (GM)
5= Gowri + RP + CD
6= Gowri + RP + GM
7= Gowri + RP + CD + GM

A= Control
B= Treatment

Table 6 Percentage of AM root colonization and plant height

Treatment	Pot experiment		Rock phosphate		Field experiment	
	1	2	1	2	1	2
Control	-	37.1	-	36.8	8.4	40.0
Acaulospora morrowae	70.6	63.0	60.8	48.6	48.0	78.0
Glomus fasciculatum	90.6	79.7	75.6	57.3	55.9	91.3
Gigaspora margarita	95.8	105.9	80.7	76.5	70.8	100.0

Table 7 Yield in relation to AM colonization

Treatment	No. of capsules/ Plant			Grain weight/g/plant			Oil content (10 g seed) (%)		
	1	2	3	1	2	3	1	2	3
Control	4.0	12.0	22.0	1.3	7.0	8.8	24.3	26.0	30.0
Acaulospora morrowae	4.7	20.0	32.0	2.1	8.0	12.0	32.0	30.0	38.0
Glomus fasciculatum	5.0	26.0	43.3	2.8	13.0	15.0	36.4	38.0	45.5
Gigaspora margarita	7.7	40.0	58.0	3.3	16.0	20.7	39.8	45.0	54.0

1= Pot experiments
2= Rock phosphate (Under pot experiment)
3= Field inoculation

that the most commonly used methods, such as axenic culturing, solution cultures, and NFT system may not prove beneficial under field conditions. Thus, it is imperative that it is essential to use indigenous strains of AM fungi and attempts should be made in multiplying them using rock phosphate, cow dung, and green manure. It is a well-accepted fact that mass cultivation of AM fungi is an uphill task for the mycorrhizologists. Inspite of difficulties in achieving a suitable technology for AM commercial exploitation, a great deal has been achieved in understanding their role in crop productivity.

Earlier, researchers have obtained similar results while working on different crops other than the oilseed crops.

It is essential to understand the germination of AM spores, inhibitors of spore germination, survival of inoculum, suitable site of infection, increasing the levels of mycorrhizal effectiveness, and discovering suitable technology for mass production.

REFERENCES

Abbott LK and Robson AD. 1982. Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soils. *Australian Journal of Agricultural Research* **33**: 1049–59.

Bagyaraj DJ. 1984. Biological interactions with VA mycorrhizal fungi. In: Powell CL and Bagyaraj DJ (Eds.) *VA Mycorrhiza*, pp. 131–53. Boca Raton, USA: CRC Press.

Bagyaraj DJ. 1986. Mycorrhizal association in crop plants and their utilization in agriculture. In: Nair MC and Balakrishnan S (Eds.) *Beneficial Fungi and their Utilization*, pp. 59–72. Jodhpur, India: Scientific Publ.

Chambers CA, Smith SE, and Smith FA. 1980. Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of *Trifolium subterraneum*. *New Phytologist* **85**: 47–62.

Daft MJ and El-Giahmi AA. 1975. Effects of Glomus infections on three legumes. In: Sanders FE, Mosse B and Tinker PB (Eds.) *Endomycorrhizas*, pp. 581–92. London: Academic Press.

Ferguson JJ. 1981. Inoculum production and field application of vesicular-arbuscular mycorrhizal fungi. PhD Dissertation, University of California, Riverside, 117 pp.

Gerdemann JW. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *Annual Review of Phytopathology* **6**: 397–418.

Gerdemann JW and Nicolson TH. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**: 235–44

Hayman DS. 1982. Practical aspects of VAM. In: Rao Subba NS (Ed.) *Advances in Agricultural Microbiology*, pp. 325–37. New Delhi, Oxford, IBA.

Hoagland DR, and Arnon DI. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station*. 347 p.

Jackson ML.1958. *Soil Chemical Analysis*. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Mosse B.1981. Vesicular arbuscular mycorrhiza research for tropical agriculture. *Research Bulletin* **194**: 5–81. Hawaii Institute of Tropical Agriculture and Human Resources.

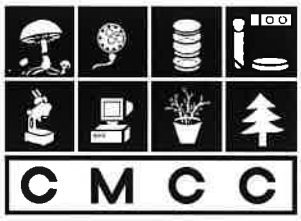
Phillips JM, and Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* **55**: 158–161.

Schenck NC and Perez Y. 1987. *Manual for Identification of VA Mycorrhizal Fungi*. Gainesville, FL.

Sreenivasa MN and Bagyaraj DJ. 1987. Selection of a suitable substrate for mass multiplication of *Glomus fasciculatum*. In: Verma AK, Oka AK, Mukerji KG, and Tilak KVBR (Eds.) *Mycorrhiza Round Table: Proceedings of a Workshop*, pp. 592–99. New Delhi, India: Janak Raj [JNU-IDRC, Canada Sponsored National Workshop on Mycorrhizae, New Delhi, India, March 13–15, 1987].

Toth R and Toth D. 1982. Quantifying vesicular-arbuscular mycorrhizae using a morphometric technique. *Mycologia* **74**: 182–87.

Wood T. 1984. A mycorrhizae and reclamation of arid and semi-arid lands. *Proceedings of the National Conference on Mycorrhiza Oregon, USA*, 84pp.



Morpho Taxonomy of *Glomus claroideum/Claroideoglomus claroideum* (Accession CMCC/AM-2701)

*Aditi Pandit and Alok Adholeya**

One of the most unique groups of microorganism is arbuscular mycorrhiza fungi (AMF), which display distinct morphological characters across different families and genera. AMF are considered as one of the most important part of the soil micro-flora as well as other microorganisms, which are present in the soil rhizosphere and hence can directly influence the soil microbial community. These are well-known to be cosmopolitan in nature and are distributed among various ecological and geographical regions. AMF have been recovered from a variety of habitats as they are found in the soil, in the form of chlamydospores. As we know, India is rich in ecological regions; hence, AMF population is diverse and unique in its different agricultural lands and forests. Mostly, mycorrhiza research is conducted on taxonomy, phylogeny, ecology, functional symbiosis, and genetics. But out of all these, morphological characterization is the preliminary step for the identification of any given isolate. Identification of morphological features of an AMF species includes spore morphology (structure, shape, and size), wall layers, surface ornamentations, hyphal attachment, and reaction of wall layers towards different reagents. In continuation to the previous series of articles regarding morphotaxonomic studies of our successfully isolated and established AMF species, the study of an extremely well–known genus that has been successfully maintained in Centre for Mycorrhizal Culture Collection (CMCC) of TERI with the accession number CMCC/AM-2701 has been taken up in this article. The researchers have enlisted a detailed morphological description of this accession that was critically observed to reach a level of morphotaxonomic identification.

Monosporal Establishment

This culture of accession CMCC/AM-2701 was isolated and raised from the soil of Rajasthan, Jaisalmer (India). For proliferation of indigenous mycorrhiza trap cultures were raised in pot conditions with a suitable host for a period of 3 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). Firstly, the soil was suspended in water and was allowed to pass through series of sieves of 60,100, and 300 British Standard Size (BSS) and then were analysed for AMF diversity and density. The sieving was critically analysed under stereo microscope and morphologically looking similar and healthy spores were categorized and were used to obtain pure single species of AMF. The morphotaxonomic characterization was done after preparing voucher specimens. For voucher slide preparation, two mounting agents, polyvinyl lacto glycerol (PVLG) and polyvinyl lacto glycerol: Melzer's reagents (1:1) were used. After a successful growth period of 3 to 6 months, the host roots were evaluated for root colonization. Cultures showing colonized roots and spores were considered as successful cultures. Cultures are considered to be pure when the spores isolated from them were morphotaxonomically similar to the voucher specimen prepared from the mother cultures that were used during the initiation of the monosporals (Figure 1).

Spore Morphology and Shape

Spores isolated from the monosporals were borne singly without sporocarp in the soil and each of them were suspended with a single prominent subtending hyphae. Most of the spores were light yellow to dark brown in colour. The shape of the spores varied from globose to subglobose. Scanning Electronic Micrograph (SEM) of the spores showed that the outer surface of the spores is slightly rough. The outer wall of the spores is mucilaginous and appears rough

Centre for Mycorrhizal Research, Biotechnology and Management of Bioresources, The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi 110 003, India
* Corresponding author, Email: aloka@teri.res.in

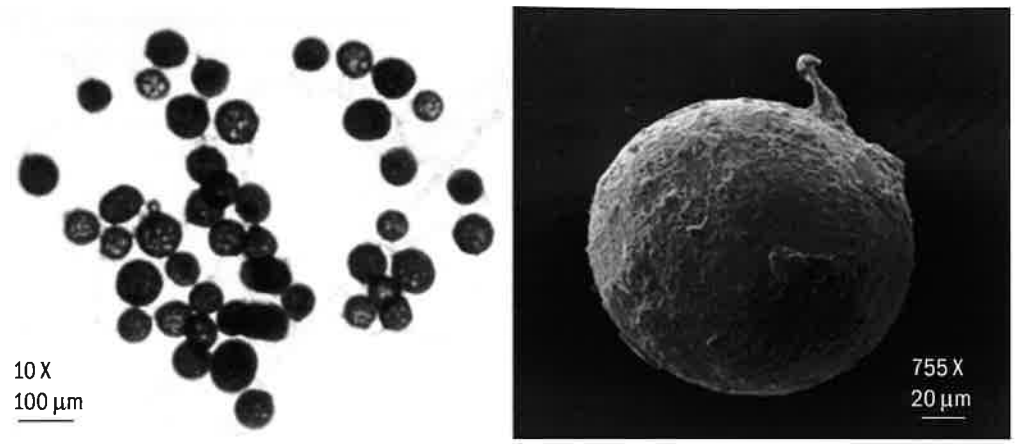


Figure 1 Compound microscopic images (10 x) and scanning electron micrographs (SEM) of CMCC/AM-2701 showing spore with a small subtending hyphae

with organic debris adhered to it (Figure 2). The average diameters of the spores were found to be in the range of 50–(86)–125 µm (Figure 3).

Subcellular Structure of the Spore

Spores of this accession show no or very less reaction with PVLG and polyvinyl lacto glycerol: Melzer's reagents. However, mature spores are composed of the following subcellular structures and wall layers (Figure 4):

- **Spore Wall Layer 1 (L1):** The first and the outermost hyaline mucilaginous layer of the spore designated as L1. The average thickness of this outer wall layer is (0.5)–(0.75)–(0.95) µm. This layer is usually evanescent, deteriorates and gets sloughed off with the ageing of the spore, and thus, is rarely present in the spore wall of the

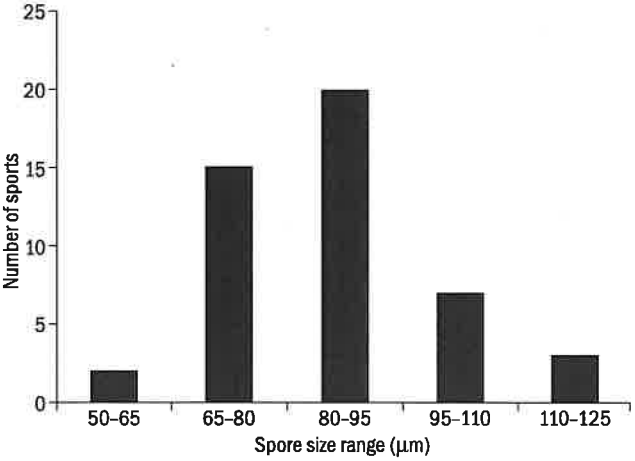


Figure 3 Analysis of spore diameter of 50 healthy and cleaned spores obtained from 1-year-old monosporal culture of accession CMCC/AM-2701

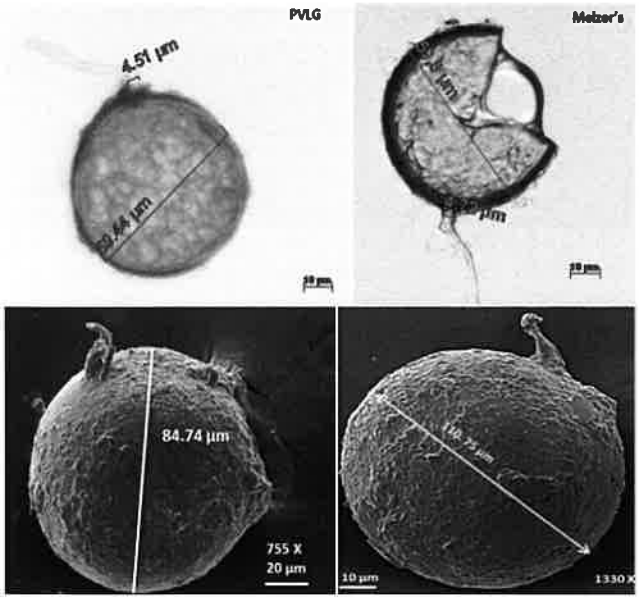


Figure 2 Compound microscopic images (10x) and SEM (755 x) of mature spores of accession CMCC/AM-2701 showing globose - to subglobose -shaped spores with a single subtending hyphae. SEM images reveal rough outer layer of the spore

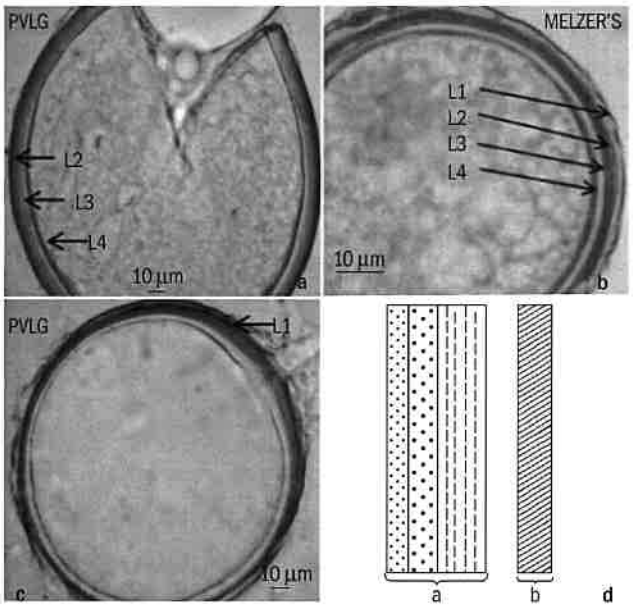


Figure 4 Compound microscopic images of spore wall layers of CMCC/AM-2701 after mounting in PVLG: PVLG (a and c), and Melzer'sreagent (b). Murograph (Walker 1983) of the mature spore showing three distinct wall layers (d)

mature specimen. This layer is mucilaginous but reactive in Melzer's reagent.

- **Spore Wall Layer 2 (L2):** This layer is hyaline in juvenile spores with L1. The average thickness of this spore wall layer is 0.6–2.0 µm thick. This is degrading concomitant with L1 and shows no reaction with Melzer's reagent.
- **Spore Wall Layer 3 (L3):** This layer is thin and consists of tightly adherent pale yellow sublayers (or laminated). The average thickness of this layer is 2.8–6.2 µm.
- **Spore Wall Layer 4 (L4):** The innermost wall layer of the spore wall; appear quite thin because it is concolorous with L3. Its appearance resembles that of a flexible inner germinal wall, but it extends into the subtending hypha as part of the hyphal wall. Since it originates as part of the subtending hypha, it is considered as the innermost layer of the spore wall.

Subtending Hyphae

All the spores show intact, cylindrical to slightly flared subtending hyphae. The width of the subtending hyphae at the point of attachment with the spore base ranges 4.18–(5.15)–7.5 µm. The hyphal wall of the subtending hyphae has three wall layers (L1, L2, and L3) continuous with the outer layer of the outer wall (Figure 5).

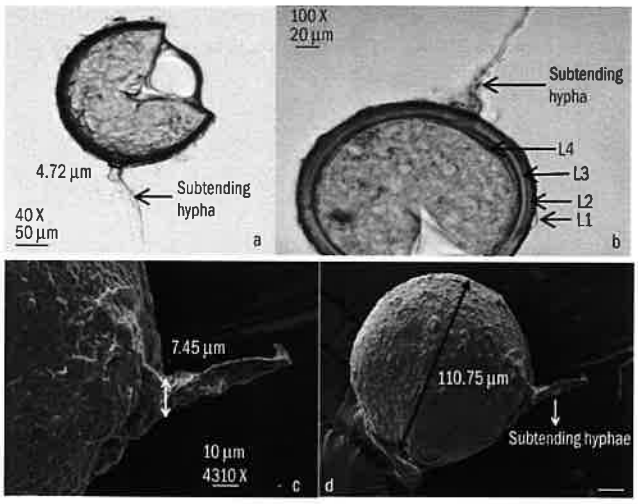


Figure 5 Compound microscopic images of spore of CMCC/AM-2701 showing cylindrical subtending hyphae after mounting it with PVLG: Melzer's reagent (a and b). SEM images of subtending hyphae (c and d)

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. The septum is continuous with the innermost sub-layers of the spore either with L3 and L4 or L4 alone.

Mycorrhiza

Mycorrhiza structures, such as arbuscules, extra and intraradical hypha, were observed in *Sorghum bicolor* roots when stained in ink vinegar. Root colonization assays showed the presence of both extraradical and intraradical hyphae. However, the extraradical hyphae are more abundant in specimen observed in pot cultures. Arbuscules are abundant and are dispersed evenly through the root cortex. Intraradical vesicles are present. Extraradical hyphae were abundant and were usually seen bearing spores bearing or in a group of two to three (Figure 6).

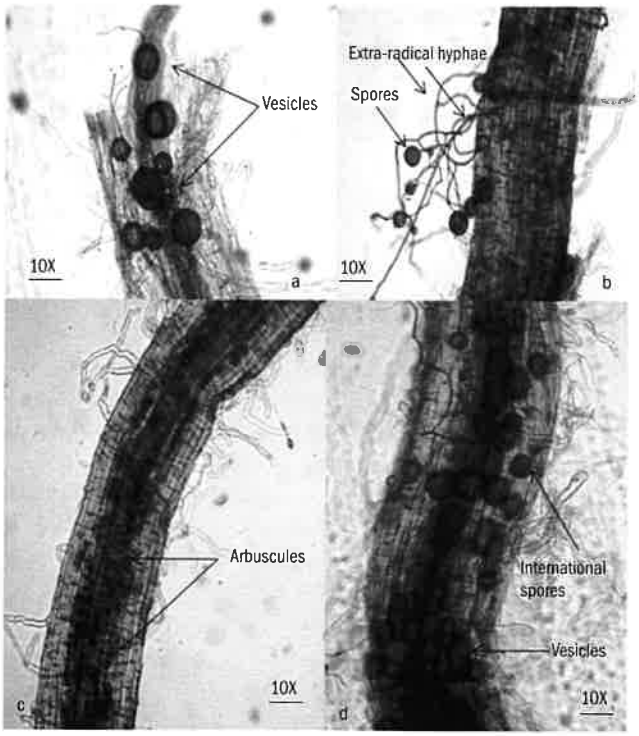


Figure 6 Compound microscopic images of roots of *Sorghum bicolor* stained in ink vinegar and observed for root colonization by CMCC-AM-2701 showing extra radical hypha (a) and arbuscules (c) and abundant vesicles in the cortical cells (a and d). Spores in the extraradical hyphae are also seen in small clusters (b)

Conclusion and Classification Level

On the basis of above morphotaxonomic analysis of the accession CMCC/AM-2701, 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 with four with layered spore walls.
- Spore wall layer is composed of outer hyaline mucilaginous layer followed by one inner concomitant layer followed by a thin and tightly adherent third layer and innermost layer that is continuous with the subtending hyphal wall.
- Spores are of varying shapes and sizes ranging from globose to sub-globose.
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intra cellular arbuscules.
- All these features suggest that the culture CMCC/AM-2701 belongs to the family Claroideoglomeraceae (Walker and Schüßler 2004).

Following are some of the unique morphotaxonomic features of the accession:

- Light yellow to dark brown, globose, asexual spores produced singly with layered spore walls; spores are of varying shapes and sizes ranging from globose to sub-globose. Size ranges 50–(86)–125 µm.
- Spore wall layer is composed of outer mucilaginous layer, inner concomitant layer, a third thin and tightly adherent and innermost layer that is continuous with the subtending hyphal wall; L2 is the thickest and most prominent layer.
- Presence of intact, cylindrical, and slightly flared subtending hyphae. The width of the subtending hyphae at the point of attachment at the spore base ranges 4.18–(5.15)–7.5 µm.
- Formation of both intraradical and extraradical hyphae and abundant vesicles and intracellular arbuscules.

The taxonomic feature of the accession CMCC/AM-2701 matches the characters of *Glomus claroideum* (Schenck and Smith 1982).

Systematic Classification

Glomeromycotina
Glomeromycetes
Glomerales
Glomeraceae
Claroideoglomeraceae
Claroideoglomus

Most of the work on AMF focusses on phylogeny, ecology, genetics, taxonomy, and functional properties. Identification of morphological features of an organism has been used as a conventional method for identifying an organism and comparing it with existing reference or type species. Morphological features about AMF spore morphology, wall layers, hyphal attachment, reaction with reagents, and so on help to reveal information about genera and species to which the AMF belongs. But sometimes this approach can create a problem in identification of the organism at species level. Therefore, using alternative methods have more significant advantages over traditional taxonomic approaches. Characterization techniques, such as biochemical characterization through fatty acid methyl ester profiles and sequencing of ITS region of 18s rDNA (molecular characterization) has become more popular. Analyses of rDNA regions have often confirmed the morphologically defined species. It is therefore advised to our distinguished readers to kindly correlate their morphotaxonomic studies with molecular phylogenetic results.

Acknowledgements

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References

Gerdemann JW and Nicolson TH. 1963. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**: 235–44.

Hall IR and Fish BJ. 1979. A key to the Endogonaceae. *Transactions of the British Mycological Society* **73**: 261–70.

Morton JB. 1990. Species and clones of arbuscular mycorrhizal fungi (Glomales, Zygomycetes): their role in macro- and microevolutionary processes. *Mycotaxon* **37**: 493–515.

Schenck NC and Smith GS. 1982. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. *Mycologia* **74**: 77–92.

Schüßler A and Walker C. 2010. The Glomeromycota: A species list with new families and new genera. In: Schüßler A, Walker C, and Gloucester (Eds.), *libraries at The Royal Botanic Garden Edinburgh*, The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University. Last accessed from: www.amf-phylogeny.com.

Souza T. 2015. *Handbook of Arbuscular Mycorrhizal Fungi.* Switzerland: Springer.

Walker C. 1983. Taxonomic concepts in the Endogonaceae: Spore wall characteristics in species descriptions. *Mycotaxon* **18**: 443–55

Walker C, and Schüßler A. 2004. Nomenclatural clarifications and new taxa in the Glomeromycota. *Mycological Research* **108**: 981–82.

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Mirshad PP, Puthur JT*. 2017	**Drought tolerance of bioenergy grass *Saccharum spontaneum* L. enhanced by arbuscular mycorrhizae** *Rhizosphere* **3**(1): 1–8 [*Plant Physiology and Biochemistry Division, Department of Botany, University of Calicut, C.U. Campus P.O., Kerala 673635, India]
John L*, Huriel P–S, Ignacio D–A, Vilma C–M, Lorena C–M, Javier V–M. 2017	**Interactions between microbial plant growth promoters and their effects on maize growth performance in different mineral and organic fertilization scenarios** *Rhizosphere* **3**(1): 75–81 [*Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro 8701, Col. Ex Hacienda de San José de la Huerta, 58190 Morelia, Michoacán, México]
Erdem Y*, Mehmet S. 2017	**The role of organic/bio–fertilizer amendment on aggregate stability and organic carbon content in different aggregate scales** *Soil and Tillage Research* **168**: 118-24 [*Department of Soil Science and Plant Nutrition, Faculty of Agriculture, University of Akdeniz, 07030, Antalya, Turkey]

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 asterisk)
Magdalena MW*, Evan SK, Lynette PR, Erik A L. 2017	**Interactive plant functional group and water table effects on decomposition and extracellular enzyme activity in Sphagnum peatlands** *Soil Biology and Biochemistry* **108**: 1–8 [*School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931, USA]
Sibylle F, Stefanie H, Christopher N, André S, Meike O, Joachim R, Daniel G, Rainer GJ*. 2017	**Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization** *Applied Soil Ecology* **114**: 82–89 [*Soil Biology and Plant Nutrition, University of Kassel, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany]
Jaseetha AS*, Mohammed AAH, Nilanjana D. 2017	**Microbial-enhanced lindane removal by sugarcane (*Saccharum officinarum*) in doped soil-applications in phytoremediation and bioaugmentation** *Journal of Environmental Management* **193**: 394–99 [*Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin 682016, India]
Andrea B*, Sonia D, Erica L, Nobuo K, Valentina S, Valeria B. 2017	**Wild *Camellia japonica* specimens in the Shimane prefecture (Japan) host previously undescribed AMF diversity** *Applied Soil Ecology* **115**: 10–18 [*Institute for Sustainable Plant Protection, UOS Torino – National Research Council, Torino, Italy]
Jerusa S*, Jochen B, Wesley de Melo R, Luiz Roberto Guimarães G. 2017	**Potential of different AM fungi (native from As-contaminated and uncontaminated soils) for supporting *Leucaena leucocephala* growth in As-contaminated soil** *Environmental Pollution* **224**: 125–35 [*School of Civil Engineering, Architecture and Urban Design – FEC, Sanitation and Environment Dept., State University of Campinas – UNICAMP, PO Box 6021, 13083-889, Campinas, SP, Brazil]
Marta P*, Szymon R, Krzysztof S, Jacek K, Aleksandra S, Eugeniusz M, Dorota C, Sebastian W, Jon Paul M, Michal M, Hazem MK. 2017	**Relationships between soil parameters and physiological status of Miscanthus x giganteus cultivated on soil contaminated with trace elements under NPK fertilisation vs. microbial inoculation** *Environmental Pollution* **225**: 163–74 [*Institute for Ecology of Industrial Areas, 6 Kossutha Street, 40-844 Katowice, Poland]
Guadalupe Z–H, Miguel Bernardo BN–R, del-Val E, Alejandro A, Trevor J, John L*. 2017	**Multitrophic interactions between maize mycorrhizas, the root feeding insect *Phyllophaga vetula* and the entomopathogenic fungus *Beauveria bassiana*** *Applied Soil Ecology* **115**: 38–43 [*Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México (UNAM), Antigua Carretera a Pátzcuaro 8701 Ex-hacienda San José de la Huerta, 58190 Morelia, Michoacán, Mexico]
Waed T, Claudia R*, Anna T, De Cillis. F, De Mastro G. 2017	**Influence of arbuscular mycorrhizae on plant growth, essential oil production and phosphorus uptake of *Salvia officinalis* L.** *Industrial Crops and Products* **102**: 144–53 [*Department of Agricultural and Environmental Science, University of Bari "A. Moro", Bari- via Amendola, 165/A – 70126, Italy]

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 asterisk)
Brandon KW*, Belinda EH, Richard AF. 2017	**Arbuscular mycorrhizal fungi in Australian stormwater biofilters** *Ecological Engineering* **102**: 483–89 [*Department of Environmental Health Sciences, University of California Los Angeles, Box 951772, Room 46-078 CHS, Los Angeles, CA 90095-1772, USA]
Heather R*, Alan CG. 2017	**Microbial inoculants as a soil remediation tool for extensive green roofs** *Ecological Engineering* **102**: 188–98 [*School of Biological Sciences, Royal Holloway, University of London, Egham Hill, Egham, Surrey, United Kingdom, TW20 0EX]
Catherine B, Pierre-Louis A, Vincent C, Stéphanie H, Stéphane D*, Sylvie C. 2017	**Tracing native and inoculated *Rhizophagus irregularis* in three potato cultivars (Charlotte, Nicola and Bintje) grown under field conditions** *Applied Soil Ecology* **115**: 1–9 [*Université Catholique de Louvain, Earth and Life Institute, Applied Microbiology, Mycology, Croix du Sud 2, Box L7.05.06, 1348 Louvain-la-Neuve, Belgium]

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FORTHCOMING EVENTS CONFERENCES, CONGRESSES, SEMINARS, SYMPOSIUMS, AND WORKSHOPS

Niagara Falls, Ontario, **Canada**
June 4–8, 2017

IOBC-WPRS Working Group "Integrated Control in Protected Crops, Temperate climate"

Email: questions@iobccanada2017.ca
Website: http://iobccanada2017.ca

Bangkok, **Thailand**
July 3–5, 2017

7th World Summit on Plant Genomics Theme: Frontiers in Plant Genomics: From Discovery to Applications

Tel.: 1-650-889-4686
Email: plantgenomics@geneticconferences.com
Website: http://plantgenomics.conferenceseries.com/scientific-program

Valencia, **Spain**
July 9–13, 2017

The 7th Congress of European Microbiologists (FEMS 2017)
Rue François-Versonnex 7, 1207 Geneva, Switzerland

Tel.: + 41 315 280432 ext. 60
Fax: + 41 22 906 9140
Email: reg_fems17@kenes.com , fems@kenes.com
Website: http://fems-microbiology2017.kenes.com/congress-information/general-information

Melbourne, **Australia**
July 24–25, 2017

5th International Conference and Exhibition on Pharmacognosy, Phytochemistry & Natural Products
Theme: New Trends & Effective Strategies for Medicinal & Natural Products

Tel.: 91-40-71279012
Email: pharmacognosy@conferenceseries.net
Website: http://pharmacognosy-phytochemistry-natural-products.pharmaceuticalconferences.com/registration.php

Walnut Creek, CA, **USA**
July 24–26, 2017

2017 Microbial and Plant Systems Modulated by Secondary Metabolites Meeting
DOE Joint Genome Institute, 2800 Mitchell Drive, Walnut Creek, CA 94598

Tel.: 925-296-5670, 925-927-2541
Email: mlballon@lbl.gov
Website: http://jgi.doe.gov/events/2017-microbial-plant-systems-modulated-secondary-metabolites-meeting/

San Francisco, CA, **USA**
July 29–August 4, 2017

2017 International Congress on Membranes and Membrane Processes (ICOM 2017)
University of Toledo, MS 305, 2801 West Bancroft Street, Toledo, OH USA

Tel.: +1.419530-8088
Email: chairs@icom2017.org
Website: http://www.icom2017.org/registration.html

Prague, **Czech Republic**
July 30–August 4, 2017

ICOM 9: 9th International Conference on Mycorrhiza
Conference Secretariat, GUARANT International, Na Pankráci 17, 140 21 Prague 4, Czech Republic

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Email: icom9@guarant.cz
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