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## About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI (The Energy and Resources Institute, formerly known as the Tata Energy Research Institute) was established in 1974. TERI's focus is on research in the fields of energy, environment, and sustainable development, and on documentation and information dissemination. The genesis of these activities lie in TERI's firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

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Focusing on ecological, environmental, and food security issues, the activities of the Biotechnology and Management of Bioresources Division include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grassroots level, with village communities.

## Mycorrhiza Network

The Biotechnology and Management of Bioresources Division's Mycorrhiza Network works through its three wings—the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhizal Culture Collection), and *Mycorrhiza News*.

The MIC is primarily responsible for establishing databases on Asian mycorrhizologists and on mycorrhizal literature (RIZA), which allows information retrieval and supplies documents on request. The CMCC has the main objectives of procuring strains of both ecto and vesicular arbuscular mycorrhizal fungi from India and abroad; multiplying and maintaining these fungi in pure cultures; screening, isolating, identifying, multiplying, and maintaining native mycorrhizal fungi; developing a database on cultures maintained; and providing starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction or handling.

## Mycorrhiza News

*Mycorrhiza News* – a quarterly newsletter publishing articles compiled from RIZA – invites short papers from budding and senior mycorrhizologists, giving methodologies being used in mycorrhizal research, providing information on forthcoming events on mycorrhiza and related subjects, listing important research references published during the quarter, and highlighting the activities of the CMCC.

# Contents



# Taxonomic findings [Series 1]

# *Acaulospora* from Goa


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## 1 ***Acaulospora delicata*** Walker, Pfeiffer, and Bloss

Spores were formed singly in soil, laterally borne on the neck of a sporiferous saccule. The saccule is broad, with a cross sectional area 60 × 80 µm to 100 × 120 µm, hyaline, and consists of the swollen tip of a thin-walled coenocytic hypha. It is 10–12 µm in diameter, with a single wall of 122 µm thick. The spore is hyaline to pale yellowish-cream, globose to sub-globose, and is 80–105 µm broad (Figure1). The spore has four walls in two groups (A and B). Wall group A consists of a thin hyaline, outer evanescent wall (wall 1), which is 1 µm thick. Closely attached to wall 1 is wall 2, which is a relatively thick (2.5–3.5 µm) laminated wall with up to six sub-equal laminations. Wall group B consists of two thin, hyaline membranous walls (wall 3 and 4, which are 0.5 µm and 0.75–1 µm thick, respectively). Wall 3 is covered by minute granular excrescences.

***Distinguishing feature:*** Presence of granular excrescences on wall 3 of Group B of the spore.
***Distribution*:** Recorded from old Goa with 37% frequency of occurrence.
***Association*:** Found in association with *Carica papaya* L. plants from coastal area of Goa, India.

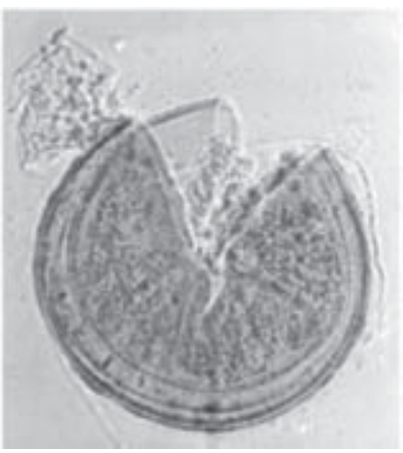

**Figure 1** A crushed spore of *Acaulospora delicate* showing its spore contents (× 400)

## 2 ***Acaulospora foveata*** Trappe and Janos

Spores were formed singly in soil, borne laterally on the neck of a sporiferous saccule. The saccule is globose and 180–200 mm in diameter. It consists of the swollen tip of a thin-walled funnel–shaped hypha, which is about 75 mm in diameter at the apex and widens at the point of attachment. The saccule empties and collapses with spore maturity.


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The spore is yellowish brown to reddish brown, globose to subglobose, 210–237 × 350–400 µm in diameter**.** The spore surface is uniformly pitted with round to oblong or occasionally irregular depressions (6–10 × 4–16 µm in diameter) with rounded bottoms, separated by ridges 1–12 µm broad. The outer spore wall is yellowish or reddish brown, 11–15 µm thick, with an adherent but separable, hyaline inner layer that is 3 mm thick. The spore contains small hyaline guttules with fine, tapering hyphae subtending below the point of attachment.

***Distinguishing feature*:** Spore surface is uniformly pitted with round to oblong or occasionally irregular depressions.
***Distribution*:** Recorded from Collem (37% frequency of occurrence), Quepem and Valpoi (33% and 50% frequency of occurrence, respectively).
***Association*:** Found in association with *C. papaya* L. plants from the Western Ghats, plateaus of South Goa, and the Western Ghats of North Goa, India.

## 3 *Acaulospora mellea* Spain and Schenck

Spores were formed singly in soil, borne laterally on the neck of a sporiferous saccule. The saccule is globose, 90–100 µm in diameter, and consists of the swollen tip of thin-walled hyphae. The saccule is white, emptying its contents during spore formation, which results in a transparent to hyaline receptacle attached to the spore. The spores are honey-coloured to yellow-brown, globose to subglobose (Figure 2), 96–130 × 78–92 µm in diameter, ellipsoidal, 96–130 × 78–92 µm in diameter. The spore wall 6–8 µm thick, consisting of three separable walls. The outermost wall (wall 1) is yellowish brown to dark brown, 2–6 µm thick, laminate, inseparable from the middle wall (wall 2), which is 0.5 µm thick. Wall 3 is hyaline to light yellow and 0.5–1 µm thick. Walls 3, 4, and 5 are membranous.

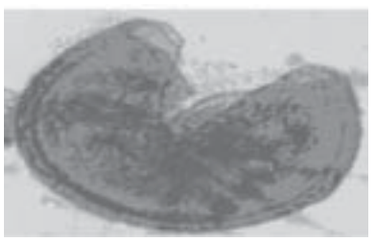

**Figure 2** A crushed spore of *Acaulospora mellea* (× 200).

***Distinguishing feature*:** Outer spore wall laminated.
***Distribution*:** Recorded from Colva with 16.66%, Valpoi with 25%, Kodar with 12.5%, and Old Goa with 25% frequency of occurrence, respectively.
***Association*:** Found in association with *C. papaya* L. plants from Western Ghats and plateaus and coastal areas of Goa, India.

## 4 *Acaulospora myriocarpa* Spain, Sieverding, and Schenck

Spores were formed singly in soil, borne on a short pedicel 4–8 µm long and 3–6 µm wide, terminating in globose terminus of 47–65 µm in diameter. The spores are hyaline to yellow, globose to subglobose, 104–114 × 80–96 µm in diameter**,** with reticulate spore content (Figure 3). The spore wall structure is of three walls in one group. Wall 1 is rigid (0.75–2 µm thick)**,** wall 2 is rigid (0.3–1.5 µm thick), and wall 3 is membranous (<0.3 µm thick) and is closely appressed to wall 2.

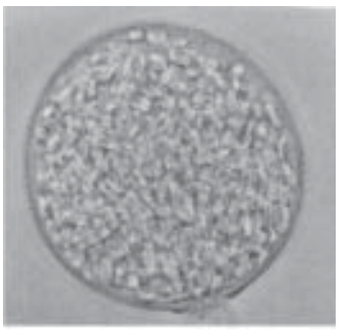

**Figure 3** A globose hyaline spore of *Acaulospora myriocarpa* with reticulate content (× 1000)

***Distinguishing feature:*** Spore hyaline with single wall group and reticulate content.
***Distribution:*** Recorded from Old Goa with 100% and Valpoi with 37.5% frequency of occurrence, respectively.
***Association*:** Found in association with *C. papaya* L. plants from Western Ghats and coastal areas of Goa, India.

## 5 *Acaulospora nicolsonii* Walker, Reed, and Sanders

Spores were formed singly in soil, laterally on the neck of the sporiferous saccule that collapses after the spores mature. The saccule is hyaline to white, globose, and 160–200 µm in diameter. The spores are hyaline to pale yellowish brown, globose to subglobose (Figure 4), 80–100 × 100–120 µm in diameter, attached to the saccule by a slightly raised collar (2–4 µm wide). The outer, brittle spore wall group (Group A) (walls 1–3) encloses the inner membranous wall (Group B) (wall 4). Wall group A has an outer thin, hyaline evanescent wall (wall 1), (0.5–1 µm thick), tightly adherent to a thick, brittle, hyaline to pale yellowish brown laminated wall (wall 2). This wall is 3–10 µm thick with up to 13 sub-equal laminae, enclosing a loosely adherent, pale yellow, brittle, unit wall, and 0.5–1.5 µm thick (wall-3). Wall 1 is smooth or roughened as it breaks up and sloughs, leaving

granular fragments attached to wall 2 which may crack in an irregular network. The inner wall (Group B, wall 4) is very thin, hyaline, and membranous (<0.5 µm). The spore contents appear vacuolated due to the presence of many oil droplets, but later becoming reticulate as the droplets apparently coalesce.

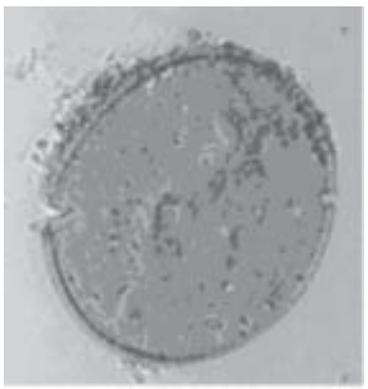

**Figure 4** A spore of *Acaulospora nicolsonii* (× 400).

***Distinguishing feature:*** Spore wall brittle and laminated.
***Distribution*** Recorded from Colva with 16.66%, Valpoi with 12.50%, Kodar with 37.5%, and Old Goa with 12.5% frequency of occurrence, respectively.
***Association*:** Found in association with *C. papaya* L. plants from Western Ghats, plateaus, and coastal areas of Goa, India.

## 6 *Acaulospora scrobiculata* Trappe

Spores were formed singly in soil, and are sessile, borne laterally on a wide, thin-walled, hyaline hypha that terminates nearby in a thin-walled globose sporiferous saccule of 100–160 µm in diameter (Figure 5A). The saccule becomes empty and collapses with spore maturity. The spore is sub-hyaline in the early stages and turns light olive to light brown at maturity (Figure 5A). It is globose to broadly ellipsoid, 100–240 × 100–220 µm in area. The spore surface is evenly pitted with depressions that are 1–1.5 × 1–3 µm in size (Figure 5C), separated by ridges 2–4 µm thick. The mouths of the depressions are circular to elliptical or occasionally linear to Y–shaped (Figure 5D).

The spore wall is 4–8.5 µm thick and consists of four layers: a rigid, pitted, outer layer, 3–6 µm thick and three inner smooth layers (<1 µm thick) (Figure 5B). The spore contains small, relatively uniform guttules.

***Distinguishing feature:*** The spore surface is evenly pitted with depressions.
***Distribution*:** Recorded from Collem with 100% frequency of occurrence and from Valpoi, Kodar, Old Goa, and Colva with 62.5% frequency of occurrence.

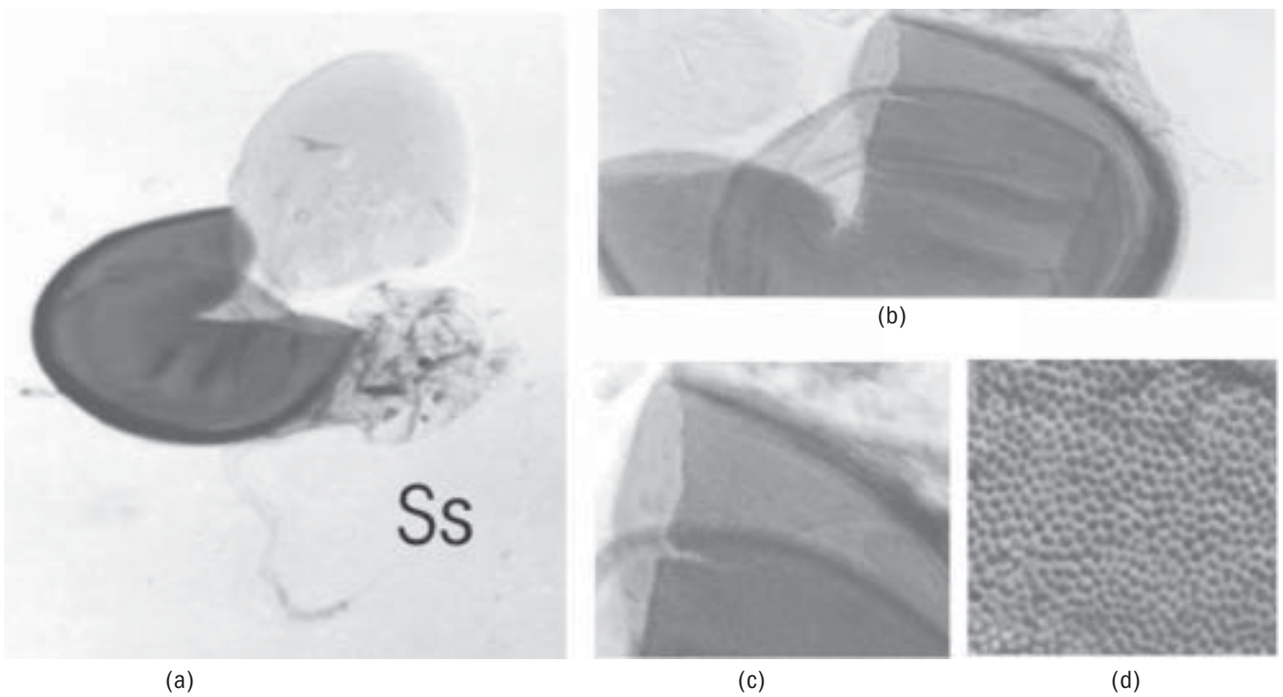


**Figure 5** (a) A crushed spore of *Acaulospora scrobiculata* attached to sporiferous saccule (Ss) showing its contents (×100) (b) A portion of spore of *Acaulospora scrobiculata* showing the wall layers (× 200) (c) A portion of spore surface evenly pitted with depressions (×200) (d) Spore surface evenly pitted with polygonal shaped depressions separated by ridges (× 1000).

***Association*:** Found in association with *C. papaya* L. plants from Western Ghats, plateaus and coastal area of Goa, India.

## 7 *Acaulospora spinosa* Walker and Trappe

Spores were formed singly in soil, laterally borne on the neck of a sporiferous saccule. The saccule is globose, approximately the same size as the spore. The spore consists of the swollen tip of a thin-walled, funnel-shaped to cylindrical hypha. The spore is attached by a collar, 8–15 µm broad, to the side of the hypha. The saccule empties and collapses by spore maturity. The spores are hyaline, dull yellowish brown (Figure 6) to dark reddish brown, globose to subglobose, and 100–150 × 100–180 µm in diameter. The spore surface is crowded with numerous blunt spines that are 1–4 µm high and 1 µm in diameter at the polygonal base, tapering to 0.5 µm at the tip. The spines are separated by ± 0.2 µm, sometimes adhering in lines to form an irregular, partial reticulum on parts of the spore surface. The spore wall is three layered. The outer layer is yellowish brown to reddish brown, 4–10 µm thick and contains spines. It encloses two membranous hyaline walls, each 0.2–1 µm thick.

***Distinguishing feature:*** The spore surface is ornamented with crowded with numerous blunt spines with polygonal bases.
***Distribution*:** Recorded from Collem with 83.33%, Quepem and Colva with 50%, Kodar with 37.5%, and Old Goa with 87.58%–100% frequency of occurrence.

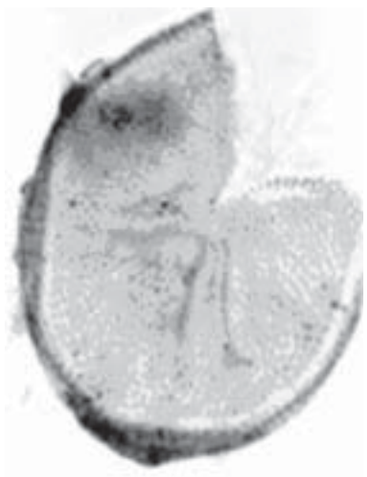

**Figure 6** Crushed spore of *Acaulospora spinosa* with spore surface crowded with blunt spines (× 400)

***Association*:** Found in association with *C. papaya* L. plants from Western Ghats, plateaus and coastal area of Goa, India.

## 8 *Acaulospora splendida* Sieverding, Chaverri, and Rojas

The spores were formed singly in soil, laterally borne on the neck of sporiferous saccule. The saccule is broadly ellipsoidal or irregular in shape, 160–300 × 150–200 µm in diameter. It collapses but usually remains attached to the spore after its formation. The spores are hyaline, light yellow to brown, and 150–250 µm in diameter. The five spore walls are divided into two groups (A and B), which are 2.5–5 µm thick. Wall 3 of group B is finely wrinkled, allowing the flow of oily spore content (Figure 7).

***Distinguishing feature:*** The spore consists of five walls in two groups (A and B) with wall 3 of group B finely wrinkled allowing the flow of oily spore content.
***Distribution*:** Recorded from Collem, rare in occurrence (12.5%).
***Association*:** Found in association with *C. papaya* L. plants from Western Ghats of Goa, India.

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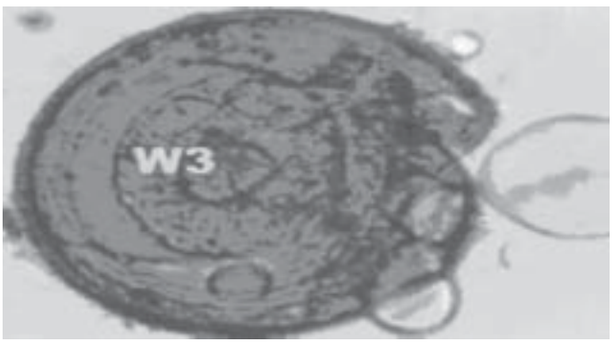


**Figure 7** Crushed spore of *Acaulospora splendida* with finely wrinkled wall 3 of Group B allowing the flow of oily spore content (× 1000)

# Research finding papers

## Interactions between *Acaulospora* and *Azospirillum* and their synergistic effect on rice growth at different sources and regimes of soil phosphorus

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### Introduction

Plants and AM (arbuscular mycorrhizal) associations are common in natural and agro-ecosystems. AMF (arbuscular mycorrhizal fungi) are considered to be essential for increasing the sustainability of agricultural systems (Gianinazzi and Schüepp 1994). In agricultural soils, they are probably the most abundant fungi, accounting for ≅ 50% of the soil microbial biomass (Olsson, Thingstrup, Jakobsen, *et al.* 1999). They represent the most efficient nutrient uptake facilitators, particularly in nutrient-deficient soils of tropical regions. The ability of AMF to enhance host-plant uptake of relatively immobile nutrients, in particular phosphorus and zinc, (Khaliq and Sanders 2000) is well documented. In addition, they affect and improve the disease fighting ability of plants (Graham 2001). These fungi have a wide range of applications in sustainable agricultural systems (Schreiner and Bethlenfalvay 1995).

The rhizosphere colonizing PGPR bacteria like *Azotobactor, Azospirillum*, and others produce substances that stimulate plant growth. Bacterial phytohormone production and nitrogen fixation are recognized as processes involved in plant growth promotion by *azospirilli*, leading to better root development and enhanced water and mineral uptake (Steenhoudt and Vanderleyden 2000; Dobbelaere, Croonenborghs, Thys, *et al.* 2001). *Azospirillum* inoculation increased the yield of cereals (Kapulnik, Sarig, Nur, *et al.* 1981). Inoculation of host by AMF and *Azospirillum* increased plant growth (Barea, Bonis, and Olivares 1983; Subba Rao, Tilak, and Singh 1985) in nitrogen- and phosphorus-deficient soils.

Phosphorus is a costly primary plant nutrient, since the manufacture of phosphorus-fertilizers requires phosphorus-rich rock, sulphur, and energy (Sharma and Prasad 2003). It is also a major macronutrient for all organisms and serves multiple functions. The use of microbial inoculants such as AMF and *Azospirillum* would enable us to reduce the application of fertilizers, thereby justifying sustainable environmental requirements with a possible cost benefit. The AMF inoculants might also reduce the inorganic fertilizer inputs and sustain plant productivity in agriculture (Mc Gonigle 1988). Mohammad, Mitra, and Khan (2004) reported that mycorrhiza-inoculated plants grew better and yielded more at lower levels of phosphorus fertilizer.

In Assam, rice is the principal grain crop, which occupies 96% of the total area under cereal cultivation (Anonymous 2001). There have been several investigations on the beneficial effects of AMF on rice (Gupta and Ali 1993; Secilia and Bagyaraj 1994) but only a few have been carried out on the interaction between *Azospirillum*, VAM (vesicular arbuscular mycorrhiza), and rice (Subba Rao, Tilak, Lakshmikumari, *et al.* 1979). Relatively little is known about the effect of *Azospirillum* on AM fungal colonization and consequently crop growth and yield. This paper investigates the role of the tripartite association between *Acaulospora*, *Azospirillum*, and rice in phosphorus nutrition, and growth and yield of rice at different phosphorus levels.

### Materials and methods

#### *Collection and treatment of soil*

The test soil was collected from the Botanical Garden, Department of Botany, Gauhati University, Guwahati, India. The soil, which had a pH of 5.77, phosphorus (total) 0.12%, nitrogen (total) 0.106%, and potassium 1.3 ppm, was sieved (4 mm) and mixed with building sand in 2:1 ratio. This sand–soil mixture was steam sterilized at 1 kg/cm$^2$ for 1 h. Sterilization was repeated twice with an interval of 24 h in between. Earthen pots, 20 cm in diameter, with a drainage hole were filled with this soil-sand mixture.

The soil was amended with single super-phosphate and tricalcium phosphate (mg per pot) and mixed thoroughly. Applications of phosphorus were equivalent to 0 mg, 25 mg, 50 mg, and 75 mg (per pot).

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### *Host plant and inoculation with symbionts*

Rice plant (*Oryza sativa* L. var. Lachit) was used as the test plant and seeds were collected from the Department of Agriculture, Government of Assam. The seeds were surface sterilized with 1%–2% sodium hypochlorite for 1 min followed by 70% alcohol for 30 s and washed several times with double distilled sterilized water. The surface sterilized seeds were soaked in sterile water and then germinated on a cotton cloth. The germinated seeds were then sown on sterilized sand in trays. The mycorrhizal inocula consisted of spores, soil, and infected root fragments. Each mycorrhizal pot received 10 g inoculum at 2 cm below the soil surface. The non-mycorrhizal pots received the same quantity of autoclaved inoculum.

*Azospirillum* isolated on nitrogen-free Nfb media were enriched on nutrient media before it was used. After 24 h, the cells were collected by centrifugation at 2000 rpm for 20 min and then suspended in ¼-strength Ringer's solution so as to get $5 \times 10^5$ cells/ml. Each *Azospirillum* treated pot obtained 3 ml of this suspension.

### *Growth conditions*

The pot experiment was conducted in a net house at Gauhati University, Guwahati, India, under natural conditions. Plants were watered, up to saturation level, daily. Throughout the whole experiment the plants received basal nutrient solution twice. The experiment was carried out from August to November 2005.

### *Colonization of plants and their growth performance*

Rice plants with intact root systems were carefully excavated from each pot at the end of the experiment. The percentage of root length colonized by VAM fungi was estimated by examining the stained root samples (Koske and Gemma 1989) microscopically (Brundrett, Piche, and Peterson 1984). Gerdemann and Nicolson's (1963) method was followed for counting VAM spores (50 g/ads) in the soil. At each harvest, the shoot length was measured. The rice plants were dried at 60 °C for 48 h, cooled in a dessicator, and their shoot and root dry weight was determined.

### *Determination of plant nutrient concentration*

The shoot phosphorus (%) and nitrogen content (%) was determined by using ascorbic acid procedure (Okalebo, Gathua, and Woomer 1993) and indophenol blue method (Allen 1974) respectively.

### *Statistical analysis*

Results were analysed by two-way and three-way analysis of variance and the significance was determined according to the least significant difference test (Gomez and Gomez 1984). The experiment consisted of two phosphorus sources at four soil phosphorus concentrations with (1) *Acaulospora*, (2) *Azospirillum*, (3) *Acaulospora* and *Azospirillum*, and (4) without both *Acaulospora* and *Azospirillum*. The 32 soil treatments were replicated three times for a total of 96 pots. The experiment comprised of a fully crossed 4×4×2 factorial split plot randomized block design.

## Results and discussion

Shoot dry biomass and height of plants strongly depended on phosphorus application and mycorrhizal colonization (Tables 1 and 2). The mycorrhizal plants grew better at low soil phosphorus addition, their non-mycorrhizal counterparts, however, required more phosphorus to attain the same growth. Highly significant F value ($F_{9, 24}$=209.177, phosphorus<0.01) obtained for the interaction effect between phosphorus and microbial applications show that the responses of plant dry biomass to microbial inoculations were not same at different rates of phosphorus and, similarly, that phosphorus responses differed between inoculated and non-inoculated plants. The highly significant variation in dry biomass between inoculated and uninoculated plants (Table 2) revealed that the presence or absence of both the microorganisms affected the dry biomass irrespective of phosphorus doses. The results indicate the influence of microbial inoculants in reducing the inorganic fertilizer demand of control by 50%. Biomass also differed significantly between the same or different treatments at the same level between the SSP and TCP fertilization (Table 2). The present results together with previous reports (Rubio, Borie, Schalchli, *et al.* 2003) clearly indicated that inoculation of plants with AMF and PGPR-enhanced dry matter (Table 2). Non-inoculated plants always had significantly lower dry weights than plants colonized by AMF indicating that rice crop was positively responsive to colonization by AMF. This was also supported by the higher dry weights obtained for the plants colonized by AMF and *Azospirillum* than for non-inoculated plants (Table 2). The results also indicate that plants grew better on phosphorus fertilized than non-fertilized soils.

Mycorrhizal inoculation responded more on TCP treated soils as compared to the soluble SSP added soils. This is in agreement with the earlier works (Rubio, Borie, Schalchli, *et al.* 2003). Dual inoculation of VAM and fluorescent *Pseudomonas* sp. at the pre-transplant stage of rice significantly increased their biomass over corresponding singly inoculated ones (Dhillion 1992). The positive influence of dual inoculation may be due to the phytohormones produced by the symbionts for additional growth in colonized plants (Barea, Bonis, and Olivares 1983; Tien, Gaskins, and Hubbell 1979), and stimulation of mycorrhizal roots by the bacteria (Balota, Lopes, Hungria, *et al.* 1995) which otherwise could be achieved by the use of fertilizers on non-mycorrhizal plants (Maria and Justo 1988). The non-mycorrhizal plants responded more to SSP-fertilization than the mycorrhizal ones (Jha, Sharma, and Mishra 1993).

**Table 1** Effect of *Acaulospora* and *Azospirillum* on plant height (cm) at two different sources and levels of phosphorus fertilization

| | *SSP* (mg per pot) | | | | *TCP* (mg per pot) | | |
|---|---|---|---|---|---|---|---|
| Treatment | 00.00 | 25.00 | 50.00 | 75.00 | 25.00 | 50.00 | 75.00 |
| Control | 62.00 | 62.00 | 63.00 | 64.00 | 71.00 | 70.00 | 68.00 |
| *Acaulospora* | 67.00 | 66.00 | 68.00 | 69.00 | 72.00 | 70.00 | 67.00 |
| *Azospirillum* | 67.00 | 70.00 | 72.00 | 74.00 | 71.00 | 69.00 | 67.00 |
| *Acaulospora* + *Azospirillum* | 65.00 | 70.00 | 68.00 | 69.00 | 66.00 | 65.00 | 64.00 |

Data average of three replications
$LSD_{0.05}$ = 1.406; $LSD_{0.01}$ = 1.906 for comparison between two treatment means averaged over all phosphorus sources and levels
$LSD_{0.05}$ = 2.813; $LSD_{0.01}$ = 3.813 for comparison between two treatment means averaged over all phosphorus sources at the same or different phosphorus levels
$LSD_{0.05}$ = 3.239; $LSD_{0.01}$ = 4.350 for comparison between two phosphorus sources at the same combination of phosphorus level and treatment

**Table 2** Effect of *Acaulospora* and *Azospirillum* on shoot biomass at two different sources and levels of phosphorus fertilization

| | *SSP* (mg per pot) | | | | *TCP* (mg per pot) | | |
|---|---|---|---|---|---|---|---|
| Treatment | 00.00 | 25.00 | 50.00 | 75.00 | 25.00 | 50.00 | 75.00 |
| Control | 5.30 | 6.04 | 7.29 | 6.20 | 5.60 | 5.65 | 6.00 |
| *Acaulospora* | 5.95 | 7.26 | 6.12 | 5.86 | 6.07 | 7.35 | 6.40 |
| *Azospirillum* | 5.45 | 6.30 | 6.90 | 8.20 | 6.09 | 7.40 | 7.26 |
| *Acaulospora* + *Azospirillum* | 6.08 | 7.30 | 7.00 | 7.20 | 7.23 | 7.42 | 7.30 |

Data average of three replications
$LSD_{0.05}$ = 0.103; $LSD_{0.01}$ = 0.139 for comparison between two treatment means averaged over all phosphorus sources and levels
$LSD_{0.05}$ = 0.205; $LSD_{0.01}$ = 0.278 for comparison between two treatment means averaged over all phosphorus sources at the same or different phosphorus levels
$LSD_{0.05}$ = 0.281; $LSD_{0.01}$ = 0.377 for comparison between two phosphorus sources at the same combination of phosphorus level and treatment

Plants had significantly highest proportion (F=491.76, phosphorus<0.01) of root length colonized by *Acaulospora* sp. than colonized by both *Acaulospora* sp. and *Azospirillum* sp. at all the regimes of insoluble phosphorus (Figure 2). However, this proportion of root length colonized by *Acaulospora* sp. in soil supplemented with SSP was significantly lower than those colonized by both the inoculants (Figure 1). The percentage of total root infected by *Acaulospora* sp. decreased with increase in phosphorus concentration. The pots given phosphate had lower intensity of infection than their respective control pots not supplied with phosphorus of SSP source. Mycorrhizal root infection in plants also decreased with the increase in phosphorus level in both the phosphorus sources. This is in accordance with the earlier works that found that phosphorus addition to plant by soil (Jha, Sharma, and Mishra 1993; Thingstrup, Rubæk, Sibbesen *et al.* 1998) or by foliar application decreased VAM colonization. The number of mycorrhizal spores decreased significantly with increasing soluble phosphorus-application (Figure 1) as reported by Thompson, Robson, and Abbot (1992); Rubio, Borie, Schalchli, *et al.* (2003); and Mohammed, Mitra, and Khan (2004).

There was significant difference in plant height amongst the treatments at both the sources and all levels of phosphorus fertilization (Table 1). At both the sources of phosphorus, with regard to plant height, the non-mycorrhizal and non-PGPR plants responded more to phosphorus fertilization. The growth of mycorrhizal and *Azospirillum* inoculated plants was greater at low phosphorus application than the non-mycorrhizal and without *Azospirillum* inoculated plants that required more phosphorus to attain approximately the same height. At both the sources of phosphorus applications, plants inoculated with *Acaulospora* sp. attained more height than plants inoculated either with *Azospirillum* or both *Acaulospora* sp. and *Azospirillum*.

Dually inoculated seedlings possessed higher shoot phosphorus concentration (Table 3) than those with only *Azospirillum* or non-mycorrhizal only when no phosphorus was added to soil. The ANOVA of shoot phosphorus concentration revealed significant effect of phosphorus level ($F_{3, 6}$=12.40, phosphorus<0.01), treatments ($F_{3, 24}$=33.22, phosphorus<0.01) and phosphorus source ($F_{1, 32}$=10.78, phosphorus<0.01) on shoot phosphorus concentration.

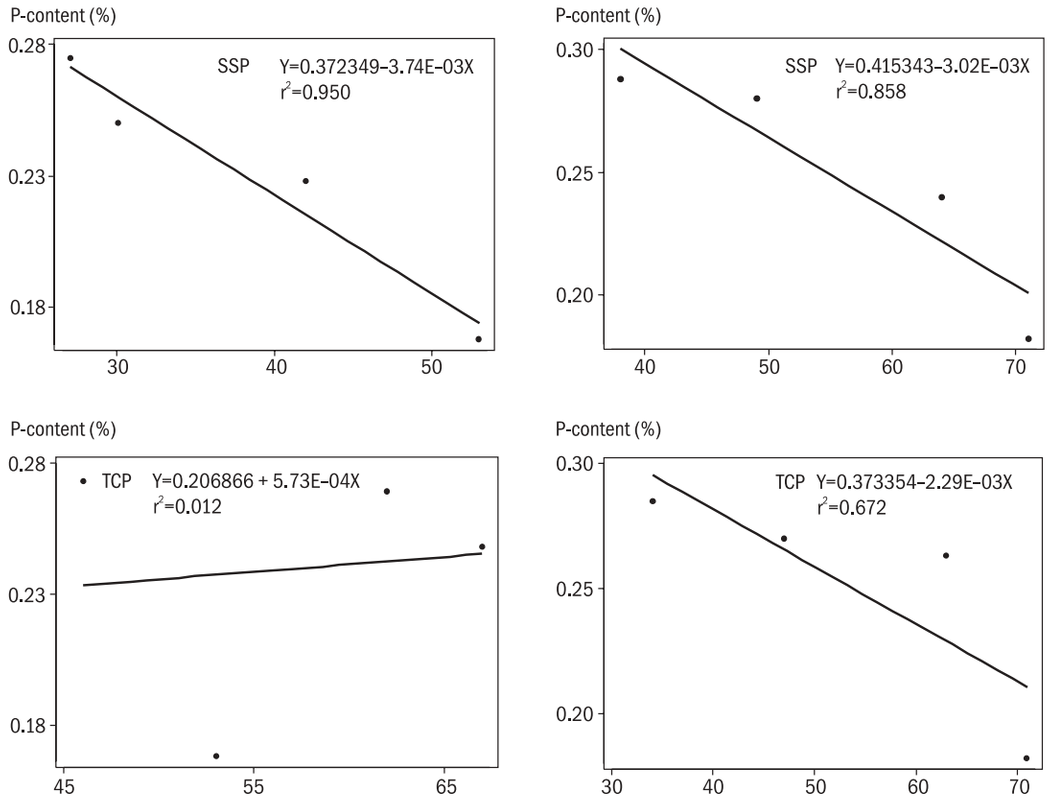


**Figure 1** Fitted line plot (regression) of plant phosphorus concentration (mg per g) vs root colonization (%) of *Acaulospora* and dual inoculated plants grown on different sources and concentration of soil phosphorus

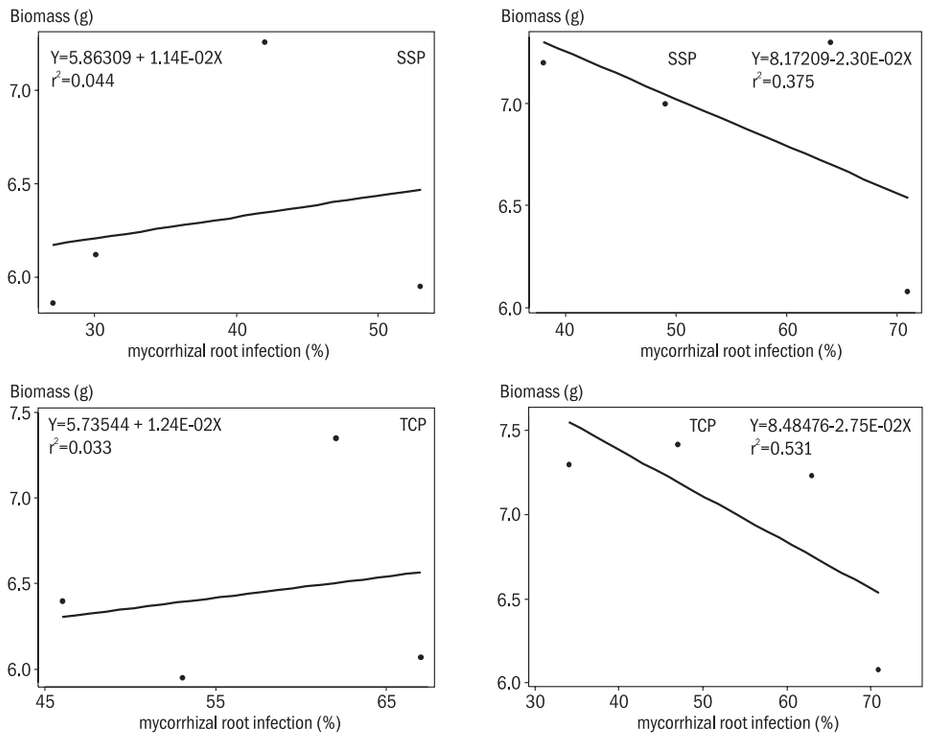


**Figure 2** Fitted line plot (regression) of biomass vs root colonization (%) of *Acaulospora* and dual inoculated plants at different sources and concentration soil phosphorus

**Table 3** Mycorrhizal spore population (50 g/ads) in rice rhizosphere as affected by *Acaulospora*, *Azospirillum,* and different sources and levels of soil phosphorus

| | *SSP* (mg per pot) | | | | *TCP* (mg per pot) | | |
|---|---|---|---|---|---|---|---|
| Treatments | 00.00 | 25.00 | 50.00 | 75.00 | 25.00 | 50.00 | 75.00 |
| Control | 000 | 000 | 000 | 000 | 000 | 000 | 000 |
| *Acaulospora* | 106 | 170 | 138 | 111 | 143 | 164 | 183 |
| *Azospirillum* | 000 | 000 | 000 | 000 | 000 | 000 | 000 |
| *Acaulospora* +*Azospirillum* | 121 | 230 | 185 | 117 | 170 | 209 | 240 |

Data average of three replications
$LSD_{0.05}$ = 0.737; $LSD_{0.01}$ = 0.999 for comparison between two treatment means averaged over all phosphorus sources and levels
$LSD_{0.05}$ = 1.474; $LSD_{0.01}$ = 1.998 for comparison between two treatment means averaged over all phosphorus sources at the same or different phosphorus levels
$LSD_{0.05}$ = 2.337; $LSD_{0.01}$ = 3.139 for comparison between two phosphorus sources at the same combination of phosphorus level and treatment

**Table 4** Mycorrhizal dependency (%) of *Oryza sativa* as affected by *Acaulospora*, *Azospirillum,* and different sources and levels of soil phosphorus

| | *SSP* | | | | *TCP* | | |
|---|---|---|---|---|---|---|---|
| Treatments | 00.00 | 25.00 | 50.00 | 75.00 | 25.00 | 50.00 | 75.00 |
| Control | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *Acaulospora* | 53 | 42 | 30 | 27 | 67 | 62 | 46 |
| *Azospirillum* | 00 | 00 | 00 | 00 | 00 | 00 | 00 |
| *Acaulospora* + *Azospirillum* | 71 | 64 | 49 | 38 | 63 | 47 | 34 |

Data average of three replications
$LSD_{0.05}$ = 0.793; $LSD_{0.01}$ = 1.074 for comparison between two treatment means averaged over all phosphorus sources and levels
$LSD_{0.05}$ = 1.586; $LSD_{0.01}$ = 2.149 for comparison between two treatment means averaged over all phosphorus sources at the same or different phosphorus levels
$LSD_{0.05}$ = 3.031; $LSD_{0.01}$ = 4.072 for comparison between two phosphorus sources at the same combination of phosphorus level and treatment

Levels of phosphorus applications (F = 19.97, phosphorus<0.01) (Table 4) and presence or absence of microbial inoculants significantly influenced the total number of spores produced in the rice rhizosphere 50 per g soil. Inoculation of plants with *Azospirillum* stimulated sporulation of mycorrhizal fungi.

A positive but insignificant correlation [r = 0.209 (SSP) and r = 0.183 (TCP)] between percentage root colonization and biomass was obtained for plants inoculated with *Acaulospora* sp. and the correlation was negative [r = –0.613 (SSP) and r = –0.728 (TCP)] for plants inoculated with both *Acaulospora* sp. and *Azospirillum* growing on soils amended with SSP. A negative correlation was

**Table 5** Effect of *Acaulospora* and *Azospirillum* on shoot phosphorus concentration (mg/g) at two different sources and levels of phosphorus fertilization

| | *SSP* | | | | *TCP* | | |
|---|---|---|---|---|---|---|---|
| Treatments | 00.00 | 25.00 | 50.00 | 75.00 | 25.00 | 50.00 | 75.00 |
| Control | 0.153 | 0.197 | 0.226 | 0.23 | 0.175 | 0.183 | 0.157 |
| *Acaulospora* | 0.168 | 0.228 | 0.25 | 0.275 | 0.248 | 0.269 | 0.273 |
| *Azospirillum* | 0.162 | 0.216 | 0.26 | 0.28 | 0.185 | 0.248 | 0.251 |
| *Acaulospora* + *Azospirillum* | 0.182 | 0.24 | 0.28 | 0.288 | 0.263 | 0.27 | 0.285 |

Data average of three replications
$LSD_{0.05}$ = 0.002; $LSD_{0.01}$ = 0.003 for comparison between two treatment means averaged over all phosphorus sources and levels
$LSD_{0.05}$ = 0.004; $LSD_{0.01}$ = 0.005 for comparison between two treatment means averaged over all phosphorus sources at the same or different phosphorus levels
$LSD_{0.05}$ = 0.006; $LSD_{0.01}$ = 0.008 for comparison between two phosphorus sources at the same combination of phosphorus level and treatment

obtained between percentage root colonization and plant phosphorus-content when plants were growing in soil amended with SSP (r = -0.975 for *Acaulospora* sp. and –0.926 for *Acaulospora* sp. and *Azospirillum)*. However, in TCP fertilized plots, *Acaulospora* sp. inoculated plants correlated positively but insignificantly (r = 0.109). These results suggest that growth performance of rice can be reasonably improved by mycorrhizal colonization. It is also clear from the results that the combined microbial inoculations and their synergistic response can be exploited for increasing the crop productivity with minimum input of inorganic fertilizers. This, however, would require assessment of selected microorganisms with specific soil plant combinations to ascertain their growth stimulation ability.

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# Synergistic effects of AMF and bioformulations on softwood grafting in jamun (*Syzygium cuminii* Skeels)

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## Introduction

Jamun, also known as Indian black berry, is an indigenous under-exploited fruit of high commercial value. It has recently attained utmost importance as an arid-zone horticultural crop because of its hardy nature, high yielding potential, quality fruits, and nutritive and medicinal properties. The increasing concern about the environment and socio-economic impact of chemical agriculture has led many farmers and consumers to seek organic cultivation. This shift necessitates raising the seedlings/rootstock organically to ensure higher graft-take and subsequent growth for production of organic nursery plants of jamun.

## Material and methods

An experiment was conducted at the nursery of the Department of Pomology, Kittur Rani Channamma College of Horticulture, Karnataka, during 2004-06, with three replications in factorially randomized design.

Factor-I: AMF (arbuscular mycorrhizal fungi) (*Sclerocystis dussii*, *Glomus monosporum*, *G. intraradices*, *G. fasciculatum*, and Control-1, that is, non-mycorrhizal)

Factor-II: Control-2, that is, without bioformulations, *amrit pani*, microbial consortium and *panchagavya*

The AMF were inoculated to rootstocks at the time of sowing. Mature scions were cured two weeks prior to the grafting day by defoliation in order to activate terminal buds. The AM (arbuscular mycorrhizal) fungal inoculated rootstocks were subjected to softwood grafting four months after sowing. The polybags were watered at weekly intervals for six months after the first week after grafting.

To each polybag, 10 ml of bioformulations (3% concentrated) was applied as soil drenching, immediately after irrigation. Parameters of the grafts, such as graft-take after three months of grafting, graft survival at the sixth month, sprout height, number of leaves, and stionic ratio were recorded.

$$\text{Stionic ratio} = \frac{\text{Diameter of scion above joint (mm)}}{\text{Diameter of rootstock below joint (mm)}}$$

## Results and discussion

Data recorded on graft-take and survival as influenced by AMF and bioformulations are tabulated in Table 1. Among the AMF, *G. fasciculatum* registered significantly highest graft-take (73.33%) and survival (98.80%) followed by *G. intraradices* (graft-take – 65.83% and survival – 98.49%), *G. monosporum* (graft-take – 54.19% and survival – 96.76%), and *S. dussii* (graft-take – 53.75% and survival – 93.45%). Non-mycorrhizal grafts show significantly lower graft-take (44.17%) and survival (87.21%).

Among bioformulations, microbial consortium recorded significantly higher graft-take (63.33%) and survival (98.67%), followed by *panchagavya* (graft-take – 62.67% and survival – 95.84%) and *amrit pani* (graft-take – 54.67% and survival – 94.64%). Grafts that did not receive bioformulations recorded significantly lower graft-take (52.33%) and survival (90.62%). For graft-take, interaction effects were found to be significant (Table 1). Significantly highest graft-take (83.33%) was noticed in the grafts inoculated with *G. fasciculatum* and supplemented with microbial consortium, which was statistically on par with the *G. fasciculatum* rootstock along with application of *panchagavya* (80.00%). Significantly least graft-take of 26.67% was recorded where neither AMF inoculation was done nor bioformulations were applied (Table 1).

There were significant differences among AMF and bioformulations on sprout length at all stages of growth, that is, DAG (days after grafting). Among the AMF, grafts inoculated with *G. fasciculatum* recorded significantly highest sprout length (8.75, 9.50, 11.63,

**Table 1** Effect of AMF and bioformulations on per cent graft-take and survival in jamun

| Treatment | *Per cent graft success* | | *Per cent graft survival* | |
|---|---|---|---|---|
| *Sclerocystis dussii* + Control-2 | 55.00 (47.89) | | 93.94 (81.63) | |
| *S. dussii* + *amrit pani* | 53.33 (46.93) | | 87.27 (72.77) | |
| *S. dussii* + microbial consortium | 63.33 (52.77) | | 100.00 (90.05) | |
| *S. dussii* + *panchagavya* | 43.33 (41.18) | | 92.59 (80.67) | |
| Mean for *S. dussii* | 53.75 (47.19) | | 93.45 (81.28) | |
| *Glomus monosporum* + Control-2 | 55.67 (48.86) | | 94.44 (82.01) | |
| *G. monosporum* + *amrit pani* | 43.33 (41.18) | | 92.59 (77.06) | |
| *G. monosporum* + microbial consortium | 50.00 (45.02) | | 100.00 (90.05) | |
| *G. monosporum* + *panchagavya* | 66.67 (54.78) | | 100.00 (90.05) | |
| Mean for *G. monosporum* | 54.17 (47.46) | | 96.76 (84.79) | |
| *Glomus intraradices* + Control-2 | 53.33 (46.93) | | 93.94 (78.34) | |
| *G. intraradices* + *amrit pani* | 66.67 (54.78) | | 100.00 (90.05) | |
| *G. intraradices* + microbial consortium | 70.00 (56.87) | | 100.00 (90.05) | |
| *G. intraradices* + *panchagavya* | 73.33 (58.96) | | 100.00 (90.05) | |
| Mean for *G. intraradices* | 65.83 (54.39) | | 98.49 (87.12) | |
| *Glomus fasciculatum* + Control-2 | 70.00 (56.87) | | 95.21 (79.69) | |
| *G. fasciculatum* + *amrit pani* | 60.00 (50.81) | | 100.00 (90.05) | |
| *G. fasciculatum* + microbial consortium | 83.33 (65.99) | | 100.00 (90.05) | |
| *G. fasciculatum* + *panchagavya* | 80.00 (63.47) | | 100.00 (90.05) | |
| Mean for *G. fasciculatum* | 73.33 (59.29) | | 98.80 (87.46) | |
| Control-1 + Control-2 | 26.67 (31.09) | | 75.56 (60.57) | |
| Control-1 + *amrit pani* | 50.00 (45.02) | | 93.33 (81.19) | |
| Control-1 + microbial consortium | 50.00 (45.02) | | 93.33 (81.19) | |
| Control-1 + *panchagavya* | 50.00 (45.02) | | 86.50 (68.84) | |
| Mean for Control-1 | 44.17 (41.54) | | 87.21 (72.95) | |
| Mean for Control-2 | 52.33 (46.33) | | 90.62 (76.45) | |
| Mean for *amrit pani* | 54.67 (47.75) | | 94.64 (82.2) | |
| Mean for microbial consortium | 63.33 (53.13) | | 98.67 (88.28) | |
| Mean for *panchagavya* | 62.67 (52.68) | | 95.84 (83.93) | |
| For comparing the means of | S Em± | CD at 5% | S Em± | CD at 5% |
| AMF | 0.842 | 2.390 | 1.750 | 4.967 |
| Bioformulation | 0.752 | 2.136 | 1.563 | 4.439 |
| AMF x bioformulation | 1.682 | 4.780 | 3.495 | NS |

[Figures in parenthesis pertain to the angular transformation of data]

Control-1 – non-mycorrhizal; Control-2 – without bioformulations; NS – non-significant; AMF – arbuscular mycorrhizal fungi

and 15.51 cm at 90, 120, 150, and 180 DAG, respectively). Significantly least sprout length was recorded in Control-1 (7.24, 7.87, 10.02, and 13.05 cm at 90, 120, 150, and 180 DAG, respectively). Among the bioformulations, microbial consortium applied grafts recorded significantly maximum sprout length of 8.34, 9.19, 12.15, and 15.86 cm at 90, 120, 150 and 180 DAG, respectively. As interaction effects, significantly highest sprout length was recorded in *G. fasciculatum* inoculated and supplemented with microbial consortium throughout the growth phases (Table 2). Significantly least sprout length was observed in absolute control (Control-1 + Control-2).

The influence of AMF and bioformulations on the number of leaves in grafts was highly significant (Table 3). Among AMF, grafts inoculated with *G. fasciculatum* registered significantly maximum number of leaves. Among bioformulations, significantly highest value was recorded in microbial consortium during 90 and 150 DAG, while *panchagavya* had significantly higher value during 120 and 180 DAG. However, the values of these two treatments were statistically similar at all stages of growth. Grafts inoculated with *G. fasciculatum* and applied with microbial consortium registered significantly maximum number of leaves (8.00, 10.03, 15.63, and 21.23 at 90, 120, 150, and 180 DAG, respectively). Exclusion of AMF and bioformulations either individually or in combination resulted in significantly least number of leaves (Table 3).

**Table 2** Effect of AMF and bioformulations on sprout length of jamun grafts

| | *Sprout length* (cm) | | | |
|---|---|---|---|---|
| Treatment | 90 DAG | 120 DAG | 150 DAG | 180 DAG |
| *Sclerocystis dussii* + Control-2 | 7.05 | 7.89 | 9.56 | 12.60 |
| *S. dussii* + *amrit pani* | 7.84 | 8.29 | 10.44 | 13.55 |
| *S. dussii* + microbial consortium | 8.66 | 9.41 | 13.65 | 16.48 |
| *S. dussii* + *panchagavya* | 8.02 | 9.00 | 10.34 | 14.92 |
| Mean for *S. dussii* | 7.89 | 8.65 | 11.00 | 14.39 |
| *Glomus monosporum* + Control-2 | 7.34 | 7.96 | 9.62 | 12.61 |
| *G. monosporum* + *amrit pani* | 8.00 | 8.91 | 10.53 | 13.56 |
| *G. monosporum* + microbial consortium | 8.32 | 9.14 | 12.46 | 15.93 |
| *G. monosporum* + *panchagavya* | 8.21 | 9.23 | 11.59 | 15.37 |
| Mean for *G. monosporum* | 7.97 | 8.81 | 11.05 | 14.37 |
| *Glomus intraradices* + Control-2 | 7.15 | 7.92 | 9.53 | 12.42 |
| *G. intraradices* + *amrit pani* | 7.95 | 8.40 | 10.59 | 13.76 |
| *G. intraradices* + microbial consortium | 8.19 | 9.40 | 1.39 | 15.18 |
| *G. intraradices* + *panchagavya* | 8.11 | 9.16 | 11.64 | 13.60 |
| Mean for *G. intraradices* | 7.85 | 8.72 | 10.79 | 13.74 |
| *Glomus fasciculatum* + Control-2 | 7.93 | 8.59 | 10.30 | 13.30 |
| *G. fasciculatum* + *amrit pani* | 8.58 | 9.34 | 11.87 | 13.95 |
| *G. fasciculatum* + microbial consortium | 9.41 | 10.16 | 12.80 | 18.49 |
| *G. fasciculatum* + *panchagavya* | 9.07 | 9.91 | 11.56 | 16.28 |
| Mean for *G. fasciculatum* | 8.75 | 9.50 | 11.63 | 15.51 |
| Control-1 + Control-2 | 6.94 | 7.46 | 9.05 | 12.04 |
| Control-1 + *amrit pani* | 6.86 | 7.77 | 10.02 | 13.30 |
| Control-1 + microbial consortium | 7.14 | 7.84 | 10.46 | 13.22 |
| Control-1 + *panchagavya* | 8.01 | 8.41 | 10.56 | 13.66 |
| Mean for Control-1 | 7.24 | 7.87 | 10.02 | 13.05 |
| Mean for Control-2 | 7.28 | 7.97 | 9.61 | 12.59 |
| Mean for *amrit pani* | 7.85 | 8.54 | 10.69 | 13.62 |
| Mean for microbial consortium | 8.34 | 9.19 | 12.15 | 15.86 |
| Mean for *panchagavya* | 8.28 | 9.14 | 11.14 | 14.77 |

| For comparing the means of | S Em± | CD at 5% | S Em± | CD at 5% | S Em± | CD at 5% | S Em± | CD at 5% |
|---|---|---|---|---|---|---|---|---|
| AMF | 0.034 | 0.097 | 0.065 | 0.183 | 0.076 | 0.217 | 0.134 | 0.379 |
| Bioformulation | 0.030 | 0.087 | 0.058 | 0.164 | 0.068 | 0.194 | 0.119 | 0.339 |
| AMF x bioformulation | 0.068 | 0.197 | 0.129 | 0.367 | 0.153 | 0.434 | 0.267 | 0.759 |

Control-1 – non-mycorrhizal; Control-2 – without bioformulations; AMF – arbuscular mycorrhizal fungi; DAG – days after grafting

**Table 3** Effect of AMF and bioformulations on number of leaves on jamun grafts

| | *Number of leaves* | | | |
|---|---|---|---|---|
| *Treatment* | *90 DAG* | *120 DAG* | *150 DAG* | *180 DAG* |
| *Sclerocystis dussii* + Control-2 | 6.37 | 8.63 | 12.60 | 17.47 |
| *S. dussii* + *amrit pani* | 7.07 | 9.07 | 12.83 | 17.47 |
| *S. dussii* + microbial consortium | 7.53 | 9.60 | 14.73 | 19.47 |
| *S. dussii* + panchagavya | 7.70 | 9.60 | 14.83 | 19.47 |
| Mean for *S. dussii* | 7.17 | 9.23 | 13.75 | 18.47 |
| *Glomus monosporum* + Control-2 | 6.33 | 8.57 | 13.07 | 18.53 |
| *G. monosporum* + *amrit pani* | 7.04 | 9.08 | 14.73 | 19.40 |
| *G. monosporum* + microbial consortium | 7.75 | 9.00 | 14.50 | 19.36 |
| *G. monosporum* + *panchagavya* | 7.77 | 9.95 | 14.80 | 19.59 |
| Mean for *G. monosporum* | 7.22 | 9.30 | 14.28 | 19.22 |
| *Glomus intraradices* + Control-2 | 6.90 | 9.04 | 14.63 | 19.80 |
| *G. intraradices* + *amrit pani* | 7.02 | 9.21 | 14.20 | 18.33 |
| *G. intraradices* + microbial consortium | 7.08 | 9.73 | 14.37 | 18.65 |
| *G. intraradices* + *panchagavya* | 7.82 | 9.67 | 14.43 | 19.63 |
| Mean for G. *intraradices* | 7.39 | 9.41 | 14.41 | 19.11 |
| *Glomus fasciculatum* + Control-2 | 6.93 | 8.63 | 12.70 | 17.55 |
| *G. fasciculatum* + *amrit pani* | 7.10 | 9.86 | 14.90 | 19.69 |
| *G. fasciculatum* + microbial consortium | 8.00 | 10.03 | 15.63 | 21.23 |
| *G. fasciculatum* + panchagavya | 7.90 | 9.94 | 14.87 | 20.63 |
| Mean for *G. fasciculatum* | 7.48 | 9.62 | 14.53 | 19.78 |
| Control-1 + Control-2 | 5.67 | 8.10 | 11.77 | 16.93 |
| Control-1 + *amrit pani* | 6.03 | 8.96 | 12.63 | 18.43 |
| Control-1 + microbial consortium | 6.10 | 8.61 | 12.60 | 18.07 |
| Control-1 + *panchagavya* | 5.95 | 8.72 | 12.63 | 18.07 |
| Mean for Control-1 | 5.94 | 8.60 | 12.41 | 17.88 |
| Mean for Control-2 | 6.44 | 8.60 | 12.95 | 18.06 |
| Mean for *amrit pani* | 6.85 | 9.24 | 13.86 | 18.66 |
| Mean for microbial consortium | 7.44 | 9.51 | 14.37 | 19.36 |
| Mean for *panchagavya* | 7.43 | 9.58 | 14.31 | 19.48 |

| For comparing the means of | S Em± | CD at 5% | S Em± | CD at 5% | S Em± | CD at 5% | S Em± | CD at 5% |
|---|---|---|---|---|---|---|---|---|
| AMF | 0.054 | 0.153 | 0.060 | 0.170 | 0.065 | 0.185 | 0.088 | 0.250 |
| Bioformulation | 0.048 | 0.137 | 0.054 | 0.152 | 0.058 | 0.166 | 0.079 | 0.224 |
| AMF x bioformulation | 0.011 | 0.307 | 0.120 | 0.340 | 0.130 | 0.370 | 0.176 | 0.500 |

AMF – arbuscular mycorrhizal fungi; DAG – days after grafting

The values of the diameter below and above the graft joint and their ratio are presented in Table 4. The AMF had significant influence on graft stem diameter. The diameter below graft union was maximum in *G. intraradices* (8.43 mm). However, the diameter values in *G. fasciculatum*, *S. dussii*, and *G. monosporum* were statistically similar with each other.

The diameter above graft union was maximum in grafts inoculated with *G. fasciculatum* (7.86 mm), which was statistically on par with other AMF. Significantly least diameter below and above graft joint (7.40 and 6.80 mm, respectively) was recorded in uninoculated control when compared to inoculated grafts.

Shivashankar and Iyer (1988) reported that the higher root infection and colonization by AMF might exert a direct effect on germination, and root and shoot growth in the subsequent stages of plant growth. AMF are known to secrete plant growth regulators like gibberellins (Allen, Moore, and Christensen 1980), auxins, and cytokinins (Edriss, Davis, and Burger 1984). The growth promoting substances in the rhizospheres were taken up by the inoculated seedlings/rootstocks leading to enhanced growth and vigour. Enhancement of plant growth by AM fungal inoculation was also reported by earlier workers in mango (Santosh 2004 and Bassanagowda 2005), citrus (Venkat 2004), papaya (Duragannavar 2005, Kareddy and Rangarajan, 2001), khirni (Sreeramulu, Gowda, and Bagyaraj 1998), and grape (Usha, Mathew, and Singh 2004).

The work carried out on microbiological parameters of *amrit pani*, microbial consortium, and *panchagavya* revealed that they contain a lot of saprophytic bacteria, actinomycetes, fungi, yeasts, nitrogen fixers, phosphorus-solubilizers, growth promoting PGPRs, and biocontrol agents (Santosh 2004). For instance, *panchagavya* works on with cosmic energy and with a production of certain plant growth stimulants such as hormone and enzymes with enormous increase in beneficial microorganisms (Natarajan 2002). In the present study, it was observed that there were synergistic interaction between AMF and bioformulations as evident from the amplified responses due to combined treatments (Tables 1, 2, and 3).

The AM fungal inoculation and bioformulation application did not alter the graft compatibility as evident from the stionic ratio (Table 4). Choudhari, Patil, and Mali (1977) categorized the graft compatibility based on the stionic ratio as the ratio ranging between 0.57 and 0.76 indicating typical bottleneck appearance; 0.77 and 0.89 over growth of stock; 0.90 and 0.99 smooth graft union, and above 1.00 slight bulging at union, while 1.01 and above have been classed as over growth of scion. Thus, the grafts resulting from AM inoculation and bioformulation application fall under smooth graft union category (Table 4) and promotion of organic nursery on fruit crops like jamun and others can be done with

**Table 4** Effect of AMF and bioformulations on diameter of graft stem and stionic ratio

| | *Diameter of stem* (mm) | | |
|---|---|---|---|
| Treatment | *Stionic ratio* | *Above union* | *Below union* |
| *Sclerocystis dussii* + Control-2 | 7.750 | 8.296 | 0.937 |
| *S. dussii* + *amrit pani* | 7.747 | 8.405 | 0.923 |
| *S. dussii* + microbial consortium | 7.460 | 8.397 | 0.887 |
| *S. dussii* + *panchagavya* | 6.813 | 8.089 | 0.860 |
| Mean for *S. dussii* | 7.442 | 8.297 | 0.902 |
| *Glomus monosporum* + Control-2 | 7.677 | 8.137 | 0.943 |
| *G. monosporum* + *amrit pani* | 7.488 | 8.069 | 0.930 |
| *G. monosporum* + microbial consortium | 7.860 | 8.202 | 0.960 |
| *G. monosporum* + *panchagavya* | 7.765 | 8.041 | 0.967 |
| Mean for *G. monosporum* | 7.698 | 8.112 | 0.950 |
| *Glomus intraradices* + Control-2 | 7.599 | 8.274 | 0.923 |
| *G. intraradices* + *amrit pani* | 7.916 | 8.298 | 0.953 |
| *G. intraradices* + microbial consortium | 7.329 | 8.672 | 0.847 |
| *G. intraradices* + *panchagavya* | 7.624 | 8.489 | 0.900 |
| Mean for *G. intraradices* | 7.617 | 8.433 | 0.06 |
| *Glomus fasciculatum* + Control-2 | 7.766 | 8.279 | 0.940 |
| *G. fasciculatum* + *amrit pani* | 7.673 | 8.199 | 0.933 |
| *G. fasciculatum* + microbial consortium | 8.263 | 8.770 | 0.940 |
| *G. fasciculatum* + *panchagavya* | 7.756 | 8.247 | 0.940 |
| Mean for *G. fasciculatum* | 7.865 | 8.374 | 0.938 |
| Control-1 + Control-2 | 6.538 | 7.172 | 0.913 |
| Control-1 + *amrit pani* | 7.113 | 7.445 | 0.953 |
| Control-1 + microbial consortium | 6.634 | 7.514 | 0.883 |
| Control-1 + *panchagavya* | 6.894 | 7.478 | 0.923 |
| Mean for Control-1 | 6.795 | 7.402 | 0.918 |
| Mean for Control-2 | 7.466 | 8.032 | 0.931 |
| Mean for *amrit pani* | 7.588 | 8.083 | 0.939 |
| Mean for microbial consortium | 7.509 | 8.311 | 0.903 |
| Mean for *panchagavya* | 7.370 | 7.478 | 0.918 |

| For comparing the means of | S Em± | CD at 5% | S Em± | CD at 5% | S Em± | CD at 5% |
|---|---|---|---|---|---|---|
| AMF | 0.138 | 0.391 | 0.176 | 0.498 | 0.013 | NS |
| Bioformulation | 0.349 | NS | 0.157 | NS | 0.012 | NS |
| AMF x bioformulation | 0.275 | NS | 0.350 | NS | 0.026 | NS |

NS – non-significant; AMF – arbuscular mycorrhizal fungi

AMF and application of bioformulations like microbial consortia, *panchagavya* or *amrit pani*.

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# Efficacy of AM fungi in improving the growth performance of *Cyamopsis tetragonoloba* under saline soil conditions


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## Introduction

Nearly 7% of the world's land is saline due to the presence of excess soluble salts in the soil (Dudal and Purnell 1986). In India, the area with potential salinity is about 20 Mha (million hectares), out of which 7 Mha is severely affected. The presence of an excess amount of salt in soil inhibits the growth of new roots, which is essential for the healthy growth of plants. Because of this, the reclamation programmes of such soils leads to a high mortality of plants and are not economical for sustainable agriculture (Cantrell and Linderman 2001).

There are conclusive evidences that show the importance of AMF (arbuscular mycorrhizal fungi) in reclamation of such soils. AM (arbuscular mycorrhizal) association protects plants from salt stress by regulating ionic exchange and enhancing nutrient uptake (Hartmond, Schaesberg, Graham, *et al.* 1987; Dixon, Garg, and Rao 1993). The present study was undertaken to evaluate the effect of AMF on the growth and establishment of *Cyamopsis*


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*tetragonoloba* (L.) Taub. under varying concentrations of saline soils —25%, 50%, and 75%.

## Materials and methods

A soil sample was collected from the site located at Handia, Allahabad, where high salt concentration (pH = 10.2, EC = 4.06 dS/m) has rendered it into an unproductive saline fallow land. This soil was mixed with sterilized sand in a 1:1 ratio and trap culture was maintained on *Cenchrus ciliaris* for four months to raise mycorrhizal inoculum. After four months *Cenchrus* roots were assessed for AM colonization by a method proposed by Phillips and Hayman (1970). The percentage AM infection in roots was estimated by the root slide technique (Nicolson 1960). AM fungal spore population in rhizospheric soil was also estimated by the wet sieving and decanting method (Gerdemann and Nicolson 1963). The soil inoculum contained about 35 AM spores/10 g of soil and AM infection level in the *Cenchrus* root bits was upto 80%.

The experiment was designed under greenhouse conditions and in the following eight series.

1. Control (agricultural soil)
2. Control (agricultural soil) + AMF
3. Agricultural soil + saline soil (3:1)
4. Agricultural soil + saline soil (3:1) + AMF
5. Agricultural soil + saline soil (1:1)
6. Agricultural soil + saline soil (1:1) + AMF
7. Agricultural soil + saline soil (1:3)
8. Agricultural soil + saline soil (1:3) + AMF

Five pots were maintained under each series and five seeds of *C. tetragonoloba* were sown in each pot. A 300 g soil inoculum was added by the layering method (Menge, Lembright, and Johnson 1977) in conditions 2, 4, 6, and 8 (+ AMF series).

Pre-emergence and post-emergence mortality was recorded for each series. Plants were uprooted at vegetative, flowering, and fruiting stages of growth. The root and shoot biomass was determined after oven drying the samples at 70 °C for 72 h. The yield, in terms of dry weight of grains per plant and number of pods per plant, was determined after the harvest.

## Results and discussion

In saline soils, AMF successfully colonized the roots of *C. tetragonoloba* (Table 1). Although the level of colonization varied in each series, it was significantly higher in inoculated ones. Earlier reports have also shown an increased AM fungal sporulation and

**Table 1** Mycorrhizal intensity in roots and spore population in the rhizosphere of *Cyamopsis tetragonoloba*

| Treatment | *AMF spore population* (per 10 g soil) | | | | *AMF association* (% root bits infected) | | | |
|---|---|---|---|---|---|---|---|---|
| | V | Fl | Fr | Av | V | Fl | Fr | Av |
| Control | 34 | 52 | 58 | 48 | 58 | 72 | 74 | 68 |
| Control + AMF | 76 | 89 | 99 | 88 | 80 | 89 | 89 | 86 |
| 25% Saline | 28 | 40 | 43 | 37 | 38 | 51 | 55 | 48 |
| 25% Saline + AMF | 58 | 79 | 85 | 74 | 63 | 73 | 80 | 72 |
| 50% Saline | 18 | 23 | 28 | 23 | 30 | 46 | 44 | 40 |
| 50% Saline + AMF | 52 | 79 | 79 | 70 | 57 | 68 | 73 | 66 |
| 75% Saline | 6 | 10 | 14 | 10 | 18 | 25 | 63 | 32 |
| 75% Saline + AMF | 48 | 67 | 77 | 64 | 29 | 62 | 62 | 51 |
| CD | 4.53 | 4.29 | 5.60 | – | 4.33 | 4.31 | 4.99 | – |

V – vegetative; Fl – flowering; Fr – fruiting stages of growth; Av – average; CD – minimum difference required for significance at 5% level

**Table 2** Effect of salinity and arbuscular mycorrhizal fungi on mortality, length, dry weight, and yield of *Cyamopsis tetragonoloba*

| | *Mortality* (%) | | *Length* (cm) | | *Dry weight* (g/plant) | | *Yield* | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Pre-emergence | Post-emergence | Root | Shoot | Root | Shoot | Po | GDW |
| Control | 0.0 | 0.0 | 8.1 | 23.8 | 0.25 | 2.67 | 60 | 4.1 |
| Control + AMF | 0.0 | 0.0 | 9.5 | 40.3 | 0.43 | 4.84 | 80 | 5.0 |
| 25% Saline | 11.1 | 0.0 | 8.1 | 22.3 | 0.21 | 1.48 | 50 | 3.5 |
| 25% Saline + AMF | 5.6 | 0.0 | 6.6 | 33.1 | 0.26 | 2.86 | 60 | 3.9 |
| 50% Saline | 11.1 | 5.5 | 5.9 | 15.8 | 0.14 | 1.34 | 30 | 1.4 |
| 50% Saline + AMF | 0.0 | 0.0 | 6.0 | 18.3 | 0.25 | 1.66 | 60 | 2.1 |
| 75% Saline | 27.8 | 8.3 | 4.9 | 13.5 | 0.08 | 0.58 | 30 | 1.4 |
| 75% Saline + AMF | 11.1 | 0.0 | 5.3 | 16.8 | 0.20 | 1.66 | 50 | 2.3 |
| CD | 1.01 | 0.60 | 0.91 | 2.21 | 0.06 | 0.49 | 6.62 | 0.56 |

Po – number of pods/10 plant; GDW – dry weight of grains (g/10 plant); CD– minimum difference required for significance at 5% level

colonization under saline conditions (Aliasgharzadeh, Rastin, Towfighi, *et al.* 2001).

Significant reduction was observed in the mortality rate of mycorrhizal plants in comparison to non-mycorrhizal plants (Table 2). There was also considerable reduction in root and shoot biomass under salt stress conditions. However, AMF inoculation successfully recovered more than 60% suppression (Table 2). The results supported previous findings that mycorrhizal plants grow better than non-mycorrhizal plants under salinity stress (Cantrell and Linderman 2001).

Similar observations were recorded in terms of yield. Significant increase was observed in a number of pods and dry weight of grains per plant (Table 2). Furthermore, the pods obtained from the mycorrhizal plants were found to be healthy whereas those obtained from the non-mycorrhizal plants were smaller, sickle shaped, and without grains.

The data clearly shows that AM fungal inoculation promotes the growth of the plants in saline soil. Improvement in growth may also be regulated by improved nutrient supply (Ojala, Jarell, Menge *et al.* 1983). Absorption of phosphorus and its supply to the root system of the mycorrhizal plant under salt stress condition is a major contribution of AMF (Pfeiffer and Bloss 1988). Mycorrhizal inoculation also enhances magnesium uptake and reduces sodium concentration in plants. This in turn helps in increasing the chlorophyll content and improves the overall growth performance of mycorrhizal plants (Rozema, Asp, Van Diggeln, M-Van Esbrock, Brokeman, and Punte *et al.* 1986; Giri, Kapoor, and Mukerji 2002).

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# Spatial variations of AM fungi in *Terminalia crenulata* Roth. from the Western Ghat region of Goa, India

*Sharda W Khade* and B F Rodrigues*

## Introduction

AMF (arbuscular mycorrhizal fungi) is important in influencing the diversity and distribution of plant communities. AMF colonize roots and subsequently provide plants with an increased ability to take up scarce nutrients resulting in increased growth, drought tolerance, and protection against diseases. In return, AMF are dependent on plants for their carbohydrate requirements. Pot experiments show that different species of AMF clearly invoke different growth responses of individuals within a plant species (Mangan, Eom, Adler, *et al.* 2004).

The state of Goa lies in the heart of the Western Ghats, which is one of the hotspots of biodiversity (Khade and Rodrigues 2002). The major portion of the slopes of the Western Ghats belt falls in this region, encompassing luxuriant forests with good diversity (Rao 1985). AM (arbuscular mycorrhizal) association in pteridophytes, medicinal plants, and forest trees from Western Ghat region of Goa have been documented (Khade and Rodrigues 2002). However, studies investigating the ecological importance of beneficial fungi such as AMF are in rudimentary stage (Mangan, Eom, Adler, *et al.* 2004). Therefore, in present paper studies on spatial variations of AMF associated with *Terminalia crenulata* Roth., a timber yielding tree commomly occurring in forest area of Western Ghat region of Goa, was undertaken.

## Material and methods

*T. crenulata* Roth., which belongs to the family Combrataceae and commonly occurs in four forest areas from the Western Ghat region of Goa, was considered for the study (Table 1).

| Site no. | *Site Name* |
|---|---|
| 1 | Mollem |
| 2 | Collem |
| 3 | Dharbandoda |
| 4 | Anmod |

Root and rhizosphere soil samples of five trees/site were collected during March 2001. Care was taken to trace back the roots of the selected trees while sampling. Rhizosphere samples were collected upto a depth of 0–25 cm. Samples were packed in polyethylene bags and transported to the laboratory. Root samples were freshly processed, whereas the soil samples were stored at 4 °C until analysed. The roots were cleared and stained in 0.05% trypan in lactoglycerol (Phillips and Hayman 1970) and the degree of colonization was estimated by slide method (Giovannetti and Mosse 1980). Spores of AMF were isolated by wet sieving and decanting method (Gerdemann and Nicolson 1963) and quantification of spore density was carried out (Gaur and Adholeya 1994). AMF were identified to species level using bibliographies provided by Schenck and Perez (1990) and Walker and Vestberg (1998). Taxonomic identification of spores was matched with the descriptions provided by the *International Collection of Vesicular Arbuscular Mycorrhizal Fungi*.[1] Standard deviation was calculated for mean root colonization and mean spore density of AMF.

### *Diversity indices*

Species richness per site is the mean number of AM fungal species associated with each site (Beena, Raviraja, Arun, *et al.* 2000).

### *Frequency of occurrence*

Frequency of occurrence of AMF was calculated using the following formula (Beena, Raviraja, Arun, *et al.* 2000).

$$\text{Frequency (\%)} = \frac{\text{Number of soil samples that possess spores of particular species}}{\text{Total number of soil samples screened}} \times 100$$

## Results and discussion

Data on AM status of *T. crenulata* Roth. is presented in Table 2. AM colonization was

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[1] Details available at <http://invam.caf.wvu.edu>

**Table 2** Arbuscular mycorrhizal status of *Terminalia crenulata* Roth. from the Western Ghat region of Goa

| | *Sites* | | | |
|---|---|---|---|---|
| AM fungal parameters | 1 | 2 | 3 | 4 |
| Type of colonization | HV | HV | HV | HV |
| Total root colonization (%) | 50 ± 4.82 | 33 ± 6.40 | 37 ± 2.21 | 30 ± 1.78 |
| Spore density/100g soil | 24 ± 4.8 | 116 ± 15.42 | 512 ± 0.76 | 80 ± 2.4 |

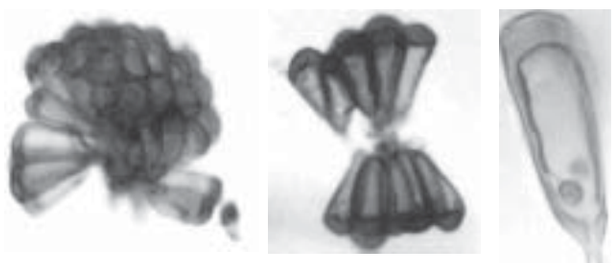
AM – arbuscular mycorrhizal; H – Hyphal colonization; V – Vesicular colonization

characterized by the presence of hyphae and vesicles. This is in accordance with the findings of Khade and Rodrigues (2003) who reported hyphal and vesicular colonization in *T. crenulata* Roth. collected from Site 1. The lowest and highest root colonization was recorded from Site 4 (30%) and Site 1 (50%) and the minimum and maximum spore density was recorded from Site 1 (20 spore/ 100 g soil) and Site 3 (512 spore/100 g soil). Similarly**,** Khade and Rodrigues (2003) reported 84% root colonization and 340 spore/100 g soil in *T. crenulata* Roth. from Site 1.

The diversity of AMF associated with *T. crenulata* Roth. is presented in Table 3. A total of six species belonging to three genera – *Acaulospora*, *Glomus*, and *Scutellospora* – were recorded in the present study. Similarly, Khade and Rodrigues (2002) reported 16 AM fungal species belonging to four genera – *Acaulospora*, *Glomus*, *Sclerocystis*, and *Scutellospora* – in medicinal plants from the Western Ghats and adjoining region. Further, Khade and Rodrigues (2002) reported 17 AM fungal species belonging to five genera – *Acaulospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora* – in commonly occurring pteridophytes from Chorlem and Site 1.

In the present study, the genus *Glomus* was dominant and recovered from all the study sites. The highest frequency of occurrence (100%) was recorded in *Glomus taiwanensis* (Wu and Chen), followed by *G. fasiculatum* (Thaxt.) Gerd. (Almeida and Schenck**),** and Trappe emend. Walker and Koske (75%) at Site 4. However, AMF belonging to two other genera recovered from Site 4 – *Acaulospora* and *Scutellospora* – recorded a frequency of occurrence of 25% each. In the present study, the minimum species richness (1 species/tree) was recorded at Site 1, while maximun species richness (4 species/tree) was recorded at Site 3 and Site 4 respectively.

Consequently, the present study brings out spatial variability in root colonization, spore population, and species diversity of AMF in *T. crenulata* Roth. from the Western Ghats of Goa.



a b c

**Figure 1** (a) A portion of sporocarp of *Glomus taiwanensis* (×100)
(b) Cluster of spores of *Glomus taiwanensis* (× 100)
(c) A single spore of *Glomus taiwanensis* (× 1000)
[**Note** The thickened spore wall at the apex of the spore]

## Acknowledgement

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**Table 3** Diversity of AM fungi associated with *Terminalia crenulata* Roth. from the Western Ghat region of Goa

| | *Sites* | | | | |
|---|---|---|---|---|---|
| AM fungal parameters | 1 | 2 | 3 | 4 | *Frequency of occurrence* (%) |
| *Acaulospora scrobiculata* Trappe | - | - | - | + | *25* |
| *Glomus fasiculatum* (Thaxt.) Gerd. and Trappe emend. Walker and Koske | - | + | + | + | *75* |
| *Glomus clariodeum* Schenck and Smith emend. Walker and Vestber | - | - | - | + | *25* |
| *Glomus multicaule* Gerd. and Bakshi | - | - | + | - | *25* |
| *Glomus taiwanensis* (Wu and Chen) Almeida and Schenck | + | + | + | + | *100* |
| *Scutellospora fulgida* Koske and Walker | - | - | + | - | *25* |
| Species richness | *1* | *2* | *4* | *4* | - |

AM – arbuscular mycorrhizal; + – presence of AMF; - – absence of AMF

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## Centre for Mycorrhizal Culture Collection

# Disposal of black liquor wastewater through high rate transpiration system using selected species of plants and mycorrhiza

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## Introduction

In recent years, the effluent discharged from pulp and paper mills into land and water bodies has become a problem of immense importance. Pulp and paper mills are categorized as one (out of seventeen) of the most polluting industries by the CPCB (Central Pollution Control Board) (CPCB 1999). Increasing public concern regarding proper disposal of potential toxicants and an anticipated future, filled with stringent regulatory measures, led to the development of several abatement processes in the industry. Unfortunately, none of the existing wastewater treatment processes offer a perfect solution.

The effluents generated by pulp and paper mills, utilizing lignocellulosics such as straw and bagasse, constitute an issue of significant environmental concern. In India, small paper mills, whose the two main raw materials are straw and bagasse, account for about half of the total installed capacity. The combined pollution load of these mills, in terms of lignin, is about four times higher than that of larger paper mills. The wastewater discharged by this industry is highly

heterogeneous. It contains compounds from wood and/or other raw materials, processed chemicals, and other compounds formed during processing.

The Indian paper industry is more than a hundred years old. Most mills, except for a few new and modernized ones, are built based on obsolete process technologies as far as effluent handling is concerned. There are, at present, 9000 large and medium industries in India, approximately 300 of which are paper mills. Among these, 40 are large, producing more than 35 tonnes of paper per day, and the rest are medium or small. Besides being pollution intensive, the paper making process is energy, chemical, and water intensive. (Nemade, Kumar, and Alappat 2003). It ranks fifth among the major industries contributing to water pollution (Springner 1993). Effluent discharged from these mills has a mixture of chemicals used in the digestion of raw wood chips, cellulose fibres, dissolved lignin, and wood preservatives (Patel and Gadhiya 2000).

Taking into consideration the above facts, there is a real need for the development of technology related to low-cost materials that could be applied to industrial effluent in order to reduce their pollution load on land and water bodies. The pulp and paper industry is likely to face stringent regulations on the quality of effluent entering water bodies. The problems faced by the industry relate to residual COD (chemical oxygen demand), toxicity, and colour.

For small-scale paper mills using agricultural residues as their raw material, the installation of CRP (chemical recovery plant) for soda recovery has been suggested. However, the high cost of establishing a conventional CRP is a major deterrent for most of the small paper mills. There is another option of soda ash recovery, which is slightly cheaper to establish and is presently being exercised by some paper mills like Shreyans papers, Ahmedgarh, Punjab. The apprehension among paper mills is that, if all of them go for soda ash recovery, it would not be economically feasible to get a good market for it.

The HRTS (high rate transpiration system) is a low-cost alternative to conventional wastewater-treatment systems for industries that use large areas of cultivable land for disposing their effluents. This is a promising technology, where selected plant species along with mycorrhiza are used for reclamation, as well as providing an option of loading huge volumes of wastewaters in a small area of land with no obvious side effects.

## Materials and methods

The present study was conducted at the Madhya Bharat Paper Mills Ltd, Champa, Chhattisgarh, India. In order to implement the HRTS technology, seven raised field-beds, 112–119 m long and 4.5 m wide at the base, were prepared to a height of 1 m. The field-beds were made using soil dug from the region contaminated with the effluent as well as good earth brought from outside. The base of the trenches was layered with poly-sheet to control any percolation into the groundwater and soil. Thereafter, fly ash was spread over the raised beds (Figure 1). A single drip irrigation tube, with 0.5 m spacing, was placed in the centre of each raised bed surface.

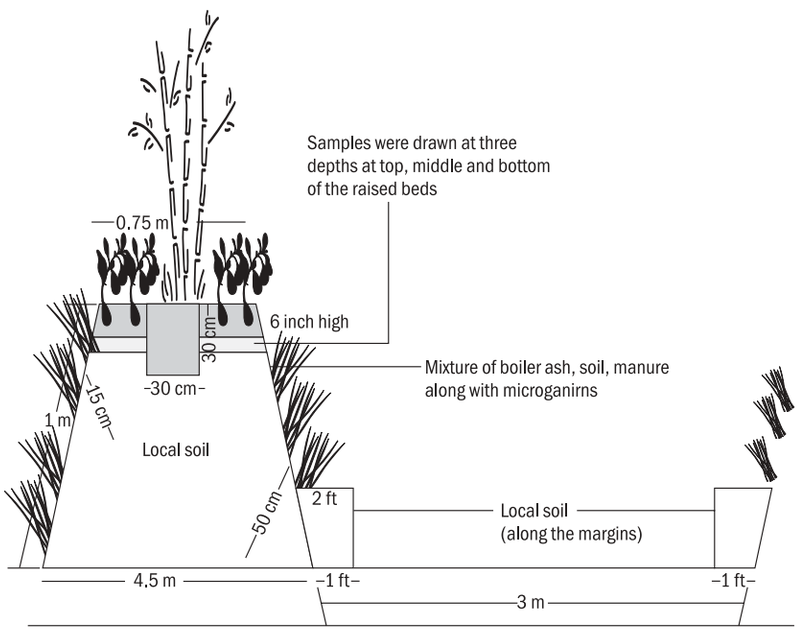


**Figure 1** Design of raised beds and sampling depths at the Madhya Bharat Paper Mills Ltd site

At first, green manuring was carried out by growing Dhaincha (*Sesbania aculeate*). Other selected plants such as *jatropha*, Paras Peepal (*Ficus* sp), Marigold (*Tagetus*), *Casurina*, Peelu (*Salvadora*), Akkuwa (*Calotropis*), and grasses like *Phragmites*, *Vetiver*, and *Salicornia* (Necklace weed) were then planted, with an inter-plant spacing of 1 m. These plants were pre-inoculated with specific mycorrhizal species, making the plants more efficient towards drawing nutrients from available and non-available parts.

Physico-chemical characterization of this effluent was carried out using standard methods (APHA 1995). Effluent loss from the trenches was monitored daily and a monthly average was calculated.

## Results and discussion

The physico-chemical composition of fresh black liquor and black liquor filled in the trenches, along with permissible limits defined by CPCB, is presented in Table 1. After one year of initiating the project, a green cover enveloped the raised beds. Black liquor consumption in different months is given in Table 2. The maximum per day consumption was observed in May (105 $m^3$), while highest rate per acre was recorded in February (78 $m^3$). Higher transpiration rate per unit area was achieved, resulting in higher rate of removal of effluent within a smaller area. Also, a much higher rate of effluent loss was observed in HRTS region as compared to the open lagoon region. Plants and microorganisms are able to thrive and take resources from the effluent in the form of nutrients and moisture for their survival and growth. The technology confirms no percolation to neighbouring soils and groundwater due to poly-lining at the base.

The HRTS technology, as implemented at the Madhya Bharat Paper Mills Ltd has, so far, been effective in managing the black liquor released by the industry. This is done using selected species of plants and microbes in a relatively smaller area, with provisions of preventing contamination of groundwater and neighbouring soils through leaching, percolation, and surface run-off.

**Table 2** Black liquor consumption using HRTS technology at Madhya Bharat Paper Mills Ltd

| Month of loading | *Rate per day* ($m^3$) | *Rate per acre* ($m^3$) |
|---|---|---|
| December 2005 (zero time) | 92.88 | 57.33 |
| January 2005 | 79.09 | 48.82 |
| February 2005 | 93.96 | 78.03 |
| March 2005 | 78.48 | 48.44 |
| April 2005 | 89.97 | 39.02 |
| May 2005 | 105.45 | 65.01 |

HRTS – high rate transpiration system

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**Table 1** Physico-chemical profile of fresh black liquor and trench black liquor along with permissible limits of CPCB

| Parameter | *Fresh liquor* | *Trench black liquor 1* | *Trench black liquor 2* | *Permissible limits (CPCB)* |
|---|---|---|---|---|
| pH at 25 °C | 8.9 | 8.7 | 8.8 | 5.5 to 9.0 |
| Conductivity (mS/cm) | 12.5 | 15.6 | 16.3 | Not specified |
| Total dissolved solids (g/litre) | 34 | 46 | 48 | Not specified |
| Total suspended solids (g/litre) | 12 | 15 | 16 | 0.1 (g/litre) |
| Total silica % (w/v as $SiO_2$) | 1.38 | 1.15 | 1.22 | Not specified |
| Total sodium % (w/v) | 18.7 | 12.98 | 12.65 | Not specified |
| COD (g/litre) | 6.5 | 7.5 | 7.65 | Not specified |
| BOD (g/litre) | 0.85 | 1.25 | 1.32 | 0.1 (g/litre) |
| Total alkalinity ( as $CaCO_3$) | 1325 | 1565 | 1585 | Not specified |

CPCB – Central Pollution Control Board; mS/cm – milli Siemens per centimeter; w/v – weight per volume

# Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information.

The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge. This list consists of papers from the following journals.

- *Applied Soil Ecology*
- *Arab Gulf Journal of Scientific Research*
- *Biology And Fertility of Soils*
- *Bioresource Technology*
- *Brazilian Journal of Microbiology*
- *Compost Science and Utilization*
- *Ecological Applications*
- *European Journal of Soil Science*
- *Forest Ecology and Management*
- *Fungal Genetics and Biology*
- *International Journal of Phytoremediation*
- *Journal of Applied Botany and Food Quality-Angewandte Botanik*
- *Journal of Ecology*
- *Journal Of Experimental Botany*
- *Journal of Plant Diseases and Protection*
- *Journal of Plant Nutrition*
- *Microbe Interactions*
- *Mycorrhiza*
- *New Phytologist*
- *Pesquisa Agropecuaria Brasileira*
- *Physiologia Plantarum*
- *Plant and Soil*
- *Plant Biosystems*
- *Plant Growth Regulation*
- *Revista Fitotecnia Mexicana*
- *Scientia Horticulturae*

Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

| 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) |
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# Forthcoming events

## Conferences, congresses, seminars, symposia, and workshops

24–29 August 2008
Torino, **Italy**

**ICPP 2008: 9th International Congress of Plant Pathology**
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10–14 September 2008
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**International Life Science Students Conference**
The University of Warsaw and The Warsaw University of Life Sciences

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