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Mycorrhizal association of *Ochlandra travancorica* in Kerala, India

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Terminal feeder roots and rhizosphere soil samples of the Reed Bamboo *Ochlandra travancorica* were collected in July 2011 from different localities in Kollam and Thiruvananthapuram districts of Kerala State. Composite soil samples (Soil pH 3.9–5.6.) revealed the presence of nine AM fungal taxa, namely, *Claroideoglomus etunicatum*, *Glomus aggregatum*, *G. boreale*, *G. macrocarpum*, *G. multicaule*, *G. tortuosum*, *Sclerocystis clavispora*, *S. rubiformis* and *S. taiwanensis.* Root colonization was 42–72 % and extensive hyphal and vesicular stages were observed. The extra-radical hyphae were hyaline and dichotomously branched on root epidermis showed appressoria. The hyphal coils were observed in the cortex cells.

The Reed Bamboo is one of the most important sources of raw material for the paper and pulp industry, traditional cottage industries and is widely distributed in the Kerala State in Western Ghats. The genus *Ochlandra* represents seven species and a variety (Image 1a). *Ochlandra travancorica* Benth. ex Gamble is a big size reed occurring widely as an undergrowth in the low level evergreen and semi-evergreen forests. Pure patches on the banks of rivers and streams form impenetrable thickets where other tree species cannot grow but also occurs in abandoned cultivated land. The species grows profusely in southern Kerala, especially in the forests of Thiruvananthapuram, Thenmala and Ranni. It prefers diffused light, requires annual rainfall of more than 1500mm and good drainage for its luxuriant growth. The perfect growth and regeneration of these valuable plants in its restricted habit and habitat is presumed to be because of certain microbes, especially mycorrhizal fungi.

Materials and Methods: The feeder roots and rhizosphere soil samples of *Ochlandra travancorica* were collected from four sites (Bonacaud, Kottoor, Kulathupuzha and Palode) of Kollam and Thiruvananthapuram districts in Kerala in July 2011. Four soil samples were taken from each site up to the depth of 20cm and prepared into one composite sample (ca. 500gm). The soil was screened to isolate Arbuscular Mycorrhizal (AM) fungal spores by wet-sieving and decanting technique and the count expressed as spores per 100g of soil (Gerdemann & Nicolson 1963).

Terminal feeder root samples collected from a different area was processed separately; washed in running tap water, cut into small pieces about 1cm, boiled in 10% KOH (w/v) for one hour, cooled to room temperature, washed thoroughly in distilled water, stained in lactophenol cotton blue (Philips & Hayman 1970). They were observed under a binocular microscope to locate vesicles and arbuscules to evaluate the percentage of mycorrhizal colonization.

$$\text{Percentage of mycorrhizal colonization} = \frac{\text{No. of mycorrhizal root segments}}{\text{Total no. of root segment observed}} \times 100$$

The frequency of occurrence of AM fungi was calculated by using the formula:

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$$\text{The frequency (\%)} = \frac{\text{Sample unit in which fungal species occurred}}{\text{Total number sample units examined}} \times 100$$

Numerical species richness was used in the present study. Index of general diversity (H') was calculated by Shannon & Weaver (1949) cited in Odum (1971).

$$H' = \Sigma (n_i / N \log_e n_i / N)$$

where, n_i is the importance value of each species and N is the total importance value.

Index of dominance (C) (Simpson 1949).

$$C = \Sigma (ni/ N)$$

where n_i is the number of individuals of each species and N is the total number of individuals in that location.

The fungal spores were identified with the help Schenk & Perez (1990).

The soil from different sampling sites was analyzed for soil fertility such as pH (Eutech instruments pH tutor), electric conductivity (Eutech instruments), organic carbon (Walkley & Black's rapid titration method 1934), total nitrogen micro-Kjeldahl method (Jackson 1973), available phosphorous (Bray & Kurtz 1945) and available potassium was estimated by ammonium acetate method (Hanway & Heidel 1952) using flame photometer (Systronic 3292).

Result

Hyphal structure: The root samples exhibited the presence of extensive hyphal and vesicular stages of AM fungal colonization and the colonization varied from 42–72 %. The extra-radical hyphae were hyaline and dichotomously branched on root epidermis to form an appressoria. The hyphae on the root were hyaline to yellowish-brown, thick-walled, aseptate, growing inter and intra cellularly through the cortex and had penetrated the inner cortex. The hyphal coils were observed in root cortex and globose to subglobose terminal vesicles (25– 37 × 25–40 µm; Image 1b) were observed in the cortex cells.

Spore count: Arbuscular mycorrhizal fungi were found to be present in all the sampling sites in this study and spore count was 272–348 spores per 100g soil (Fig. 1). A total of nine species, namely, *Claroideoglomus etunicatum, Glomus aggregatum, G. boreale, G. macrocarpum, G. multicaule, G. tortuosum, Sclerocystis clavispora, S. rubiformis, S. taiwanensis* were isolated from the different sampling areas. Among these, *G. aggregatum, S. clavispora* and *S. rubiformis* were common to all the sites.

The frequency of occurrence of mycorrhizal species associated with *O. travancorica* varied from one locality

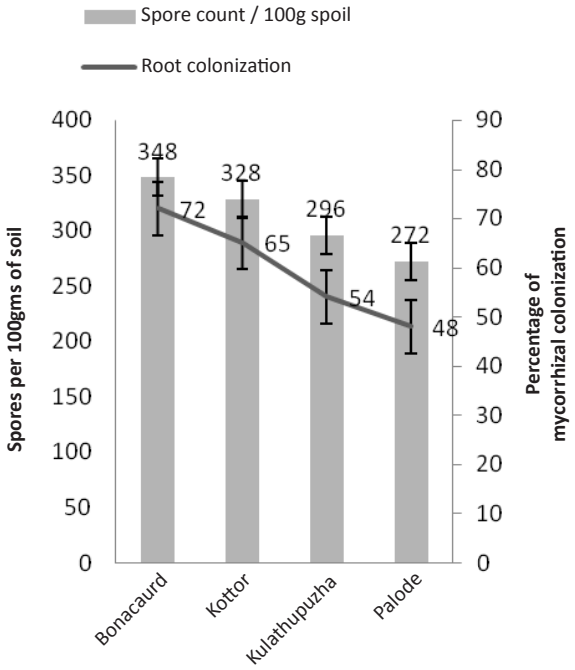

Figure 1. Arbuscular mycorrhizal fungal root colonization and spore count per 100g soil of different habitats of *Ochlandra travancorica*

to another (Table 1). *G. aggregatum, S. clavispora* and *S. rubiformis* were found in all the locations of the study; whereas, incidence of *G. macrocarpum* was 75% and *G. boreale, G. tortuosum* and *S. taiwanensis* was 50%, *Claroideoglomus etunicatum* and *G. multicaule* showed 25% of frequency. Species richness also varied. Kulathupuzha harboured more number of species than the other study localities. Simpson's diversity index (Ds) and Shannon's diversity index (Hs) was in the range of 0.6–0.8 and 2.8–4.4, respectively.

Physiochemical characters of soil: The pH of *O. travancorica* rhizosphere soils collected from the study sites was 3.9–5.6. The level of phosphorous was very low. The physiochemical characters of soil samples are in the Table 2.

Description of the species

Claroideoglomus etunicatum (Image 1c)

(W.N. Becker & Gerd.) C. Walker & A. Schüßler Gloucester, p. 22, 2010.

Glomus etunicatum W.N. Becker & Gerd., Mycotaxon 6(1): 29, 1977.

Material examined: 25.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1020).

Chlamydospores formed singly in soil or in dead roots, adherent to debris, globose to subglobose, 70–135 µm in diam., wall smooth to roughened. Spore wall 4–10

Table 1. Pattern of species richness and relative frequency of AM fungi in different habitats of *Ochlandra travancorica*

AM fungal Species	Bonacaurd	Kottor	Kulathupuzha	Palode	Relative frequency %
Claroideoglomus etunicatum	-	-	+	-	25
Glomus aggregatum	+	+	+	+	100
Glomus boreale	-		+	+	50
Glomus macrocarpum	+	+	+	-	75
Glomus multicaule	-	-	+	-	25
Glomus tortuosum	-	-	+	+	50
Sclerocystis clavispora	+	+	+	+	100
Sclerocystis rubiformis	+	+	+	+	100
Sclerocystis taiwanensis	+	+	-	-	50
Spices richness	5	5	8	5	

+ = presence; - = absence

Table 2. Soil chemical properties of *Ochlandra travancorica* habitats studied

Sampling area	PH	Electrical conductivity (ds/m)	Organic carbon (kg/ha)	Available nitrogen (kg/ha)	Available phosphorus (kg/ha)	Available potassium (kg/ha)
Bonacaud	4.4	0.3	2.96	326.14	0.12	449.63
Kottor	4.4	0.4	2.93	346.55	0.18	528.32
Kulathupuzha	5.6	0.5	0.78	250.88	0.15	589.93
Palode	3.9	0.2	0.88	275.97	0.56	102.14

µm thick, outer wall hyaline, up to 5µm thick, inner wall persistent, yellow to brown, laminated 2–8 µm thick. Intact outer wall rarely present in matured spores. Hyphal attachments to spores are one; outer wall extending down up to attached hypha for a short distance. Attached hypha thickened by extension of inner spore wall for up to 30µm, spore contents separated from attached hypha by a thin curved septum at maturity, opening occluded by inner wall thickening.

Glomus aggregatum (Image 1d)

Schenck & Smith *emend*. Koske, Mycologia 74(1): 80, 1982.

Materials examined: 25.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1041); 25.vii.2011, Palode (TBGT slide no. 1077); 20.vii.2011, Bonacaud, (TBGT slide no. 1088); 22.vii.2011, Kottur, TBGT slide no. 1090.

Chlamydospores formed in loose clusters, without peridium, hyaline to yellow, globose, subglobose, obovate, cylindrical to irregular, 40–90 x 45–110 µm; wall yellow to yellowish-brown, up to 2µm thick, outer walls slightly thicker and lighter in colour than the inner wall; walls separable with slight pressure. Subtending hyphae at the point of attachment was 4–10 µm wide, straight to sharply curved at the spore base. Pore usually open, 2–5 µm wide, often closed by a thin curved septum, cytoplasmic plug or spore wall thickening but not by hyphal wall thickening.

Glomus boreale (Image 1e)

(Thaxt.) Trappe & Gerd., in Gerd. & Trappe, Mycol. Mem. 5: 58, 1974 (*borealis*).

Endogone borealis Thaxt., Proc. Amer. Acad. Arts & Sci. 57: 318, 1922.

Material examined: 25.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1033); 27.vii.2011, Palode, coll. B. Gopakumar (TBGT slide no. 1051).

Chlamydospores mass irregular, spongy, dark to chocolate brown, 6–8 mm in diameter. Gleba with loosely woven hyphae, 10–18 µm broad, contains foreign matter and many abortive spores. Spores borne on slender hyphae and subtended by the septum, reddish-brown, broadly and symmetrically elliptical, 128–142 x 98–106 µm in diam., wall reddish-brown, up to 8µm thick.

Glomus macrocarpum (Image 1f)

Tul. & C. Tul., G. Bot. Ital. 1(7–8): 63, 1845 (*macrocarpus*).

Endogone macrocarpa (Tul. & C. Tul.) Tul. & C. Tul., Fungi Hypog.: 182, 1851.

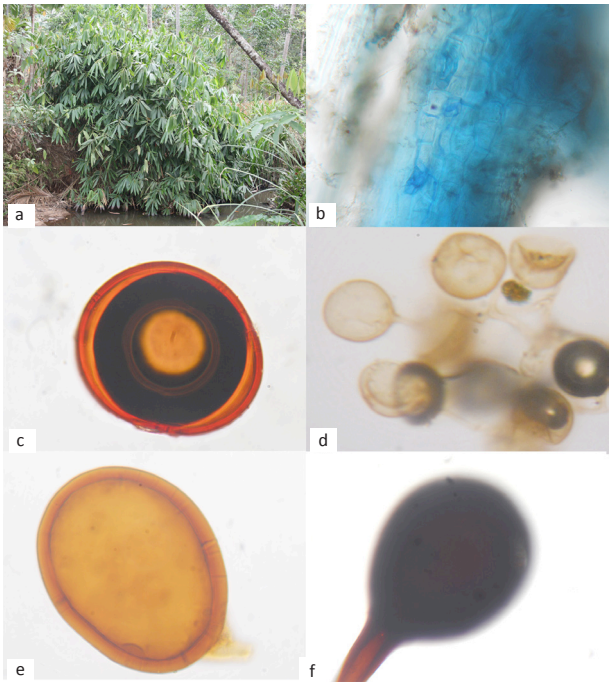

Image 1. a - Habitat of *Ochlandra travancorica* Benth. ex Gamble; b - Vesicles inside cortical cells; c - *Claroideoglomus etunicatum* (W.N. Becker & Gerd.); d - Walker & A. Schüßler; e - *Glomus aggregatum* Schenck & Smith emend. Koskee; f - *Glomus boreale* (Thaxt.) Trappe & Gerd.

Material examined: 25.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1023); 20.vii.2011, Bonacaud, coll. P.P. Rajesh Kumar (TBGT slide no. 10 99); 25.vii.2011, 22.vii.2011, Kottur, coll. P.P. Rajesh Kumar TBGT slide no. 1102).

Chlamydospores slightly longer than wide, globose, subglobose to irregular, 98–130 x 98–130µm. Spore wall composed of two layers, outer layers thin up to µm thick; inner wall layer yellow, 6–10 µm thick. Spores taper to the point of attachment, hypha single, persistent, 12µm broad at this point of attachment, inner wall occlude the pore of the attached hyphae, and the wall thickening continuous in to the subtending hyphae for up to 75µm from the spore. Pore closed by a septum. Spores characteristically bear straight, long subtending hyphae which may extend up to 100µm before branching.

Glomus multicaule (Image 2a)

Gerd. &Bakshi, Trans. Brit. Mycol. Soc. 66(2): 340, 1976 (*multicaulis*).

Material examined: 25.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1018).

Sporocarps unknown; chlamydospores dark brown, 142–240 x 125–150 µm in diam., ellipsoidal, broadly ellipsoidal, subglobose to occasionally triangular, with 1–4 hyphal attachments, attachments generally occurring at opposite ends of the spore. Spore wall 8–22 µm thick, thickest at the point of hyphal attachments, rounded projections 1–3 µm long, evenly distributed over the wall surface.

Glomus tortuosum (Image 2b)

N.C. Schenck & G.S. Sm., Mycologia 74(1): 83, 1982.

Parapseudoglomus tortuosum (N.C. Schenck & G.S. Sm.) S.P. Gautam & U.S. Patel, The Mycorrhizae: Diversity, Ecology and Applications, p. 11, 2007.

Sporocarps unknown, chlamydospores formed singly or in pairs in soil, immature spores subhyaline, without hyphal mantle. Matured spores yellow to dull greyish-brown with a mantle of sinuses hyphae closely apprised to the spore and flattened, 4–10 µm wide, forming a layer of hyphae on the spore surface, up to 20µm thick, occasionally mantle extended down to the hyphal attachment. Mantle hyphae hyaline when young, acquiring a brownish pigment with age and originating from the swelling on the hyphal attachment, 10–20 µm below the spore or arising from the other hyphae adjacent to the spore. Mantle adhered with debris and soil particles. Chlamydospores globose to subglobose, 120–210 µm (excluding mantle); spores with single laminate thin wall less than 1µm. The width of hyphal attachment at the spore base is 8–20 µm, hyaline to light yellow.

Material examined: 25.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1062); 27.vii.2011, Palode, coll. P.P. Rajesh Kumar (TBGT slide no. 1097).

Sclerocystis clavispora (Image 2c)

Trappe, Mycotaxon 6(2): 359, 1977.

Material examined: 27.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica*, Palode, coll. P.P. Rajesh Kumar (TBGT slide no. 1068); 25.vii.2011, Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1072); 20.vii.2011, Bonacaud, P.P. Rajesh Kumar (TBGT slide no. 1073); 22.vii.2011, Kottur, coll. P.P. Rajesh Kumar (TBGT slide no. 1074).

Sporocarps globose to subglobose, 400–710 x 500–710 µm, brownish-black to black, minutely verrucose, spores formed radially in a single, tightly packed layer around a central plexus of hyphae; peridium lacking. Chlamydospores brown, 142–180 x 20–38 µm, clavate to subcylindrical, tapering towards hyphal attachment, hyphal attachment 7–10 µm broad. Spore wall 1–3 µm thick at the sides, 17–22 µm thick at the apex, 5–8 µm

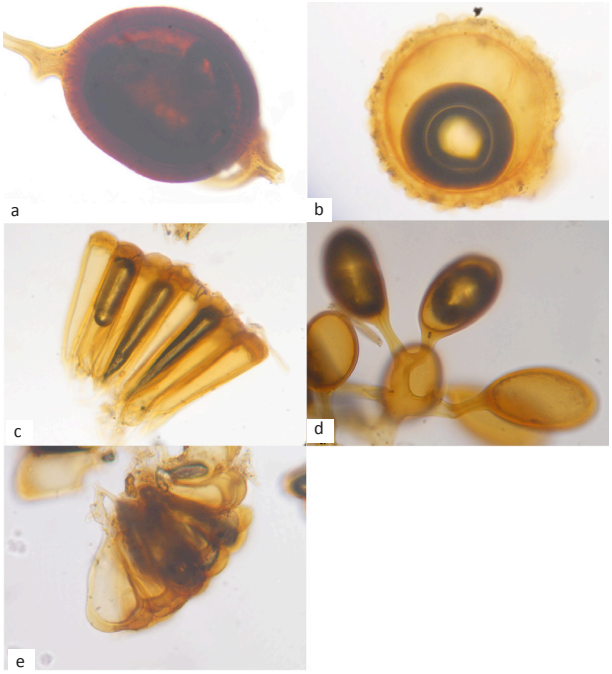

Image 2. a - *Glomus multicaule* Gerd. & Bakshib.; b - *Glomus tortuosum* N.C. Schenck & G.S.Sm.; c - *Sclerocystis clavispora* Trapped; d - *Sclerocystis rubiformis* Gerd. & Trappee; e - *Sclerocystis taiwanensis* C.G. Wu & Z.C. Chen.

thick at the base. Central plexus 145–440 µm in diam., composed of tightly interwoven, pale brown, thin walled hyphae of 3-8 µm broad.

Sclerocystis rubiformis (Image 2d)

Gerd. & Trappe, Mycol. Mem. 5: 60, 1974.

Glomus rubiforme (Gerd. & Trappe) R.T. Almeida & N.C. Schenck, Mycologia 82(6): 709, 1990.

Sclerocystis indica Bhattacharjee & Mukerji in Bhattacharjee, Mukerji & Misra, Acta Bot. Indica 8(1): 99, 1980.

Sclerocystis pachycaulis C.G. Wu & Z.C. Chen, Taiwania 31: 74, 1986.

Material examined: 25.vii.2011, Isolated from rhizosphere soil of *Ochlandra travancorica,* Kulathupuzha, coll. P.P. Rajesh Kumar (TBGT slide no. 1030); 20.vii.2011, Bonacaud, coll. P.P. Rajesh Kumar (TBGT slide no. 1032); 27.vii.2011, Palode, coll. P.P. Rajesh Kumar (TBGT slide no. 1046); 22.vii.2011, Kottur, coll. P.P. Rajesh Kumar (TBGT slide no. 1057).

Sporocarps dark brown, subglobose to ellipsoid, 180–370 x 180–370 µm, consisting of a single layer of chlamydospores surrounding a central plexus of hyphae, resembling a miniature blackberry. Peridium nearly absent, individual spores at times partially enclosed in a thin network of tightly appressed hyphae. Chlamydospores dark brown, obovoid, ellipsoid to subglobose, 32–110 x 24–80 µm with a small pore opening into the thick walled subtending hypha. Spore wall laminate, 3–8 µm thick, up to 13µm thick at spore base, often perforated projections on the inner surface. A variable stalk-like projection produced near the base of some spores.

Sclerocystis taiwanensis (Image 2e)

C.G. Wu & Z.C. Chen, Trans. Mycol. Soc. Rep. China 2(2): 78, 1987; Shaji, Rajeshkumar & Hosag., Indian J. Bot. Res. 5 (1&2): 125–126.

Glomus taiwanense (C.G. Wu & Z.C. Chen) R.T. Almeida & N.C. Schenck, Mycologia 82(6): 711, 1990.

Glomus taiwanense (C.G. Wu & Z.C. Chen) R.T. Almeida & N.C. Schenck ex Y.J. Yao, in Yao, Pegler & Young, Bull. 50(2): 306, 1995.

Material examined: 20.vii.2011, isolated from rhizosphere soil of *Ochlandra travancorica,* Bonacaud, coll. P.P. Rajesh Kumar (TBGT slide no. 1079); 22.vii.2011, Kottur, coll. P.P. Rajesh Kumar (TBGT slide no. 1080).

Sporocarps globose, brown to dark brown, 190–250 x 190–250 µm in diameter. Chlamydospores formed radially in a single, tightly packed layer around the central plexus of hyphae, clavate to cylindrical, cinnamon brown, 65–80 µm long, 28–32 µm broad at the upper portion, 9–18 µm broad at the lower portion, with or wit out septum at spore base. Wall two-layered, external one thin and hyaline, inner layer brown, apical portion of the wall deep golden brown, 9–13 µm thick, 2–3 µm thick laterally. Central portion pale yellow and typically distinct from the wall. Stalk pale brown, continuous, 9–22 x 2–4 µm, central plexus up to 70µm in diameter.

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