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Are there arbuscular mycorrhizal associations in carnivorous plants *Drosera burmanii* and *D. indica*?

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ABSTRACT: Arbuscular mycorrhizal (AM) associations have not been described within the roots of carnivorous plants *Drosera indica* and *D. burmanii* which form a part of the natural vegetation of south India. Fungal structures characteristic of an *Arum*-type AM colonization were present in both species despite their low level of colonization (<50%). Root colonization by AM fungi and spore density in the rhizosphere differed with plant species which were significantly more in *D. burmanii*. Edaphic factors such as soil pH and organic carbon content positively influenced the fungal development whilst soil moisture and phosphorus content had a negative effect. Five taxa of AM fungi belonging to the genera *Acaulospora*, *Funneliformis*, *Glomus* and *Racocetra* were extracted from the rhizosphere of *D. indica* as against three taxa belonging to the genera *Funneliformis* and *Glomus* from *D. burmanii*.

Key words: AM association, carnivorous plant, *Drosera burmanii*, *Drosera indica*

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INTRODUCTION

Arbuscular mycorrhizal (AM) fungi belonging to the phylum Glomeromycota (Schüßler *et al*. 2001) ubiquitously associate with the large majority of plant families in different ecosystems across the world (Janos 1980; Gai *et al*. 2006). This symbiosis has important consequences for the cycling of nutrients in the soil, since AM fungi provide plants with essential nutrients, such as phosphate when they are scarce or have low mobility in the soil solution. In turn, photosynthetic carbon is transported into the soil via the transfer of sugar from the roots to their endosymbiotic fungi which later translocate the carbon in the form of lipids and sugar into the external mycelium spreading in the soil (Bago *et al*. 2003).

For over a century, the roots of carnivorous plants have been classified as non-mycorrhizal (Macdougal 1899; Juniper *et al*. 1989; Brundrett 2009). Although carnivorous plants may occasionally be colonized by facultative mycorrhiza (Chambers *et al*. 2008; Fuchs & Haselwandter 2004), it is a general notion that they do not develop a mycorrhizal association owing to their unique mode of nutrient acquisition, e.g. the absorption of animal-derived minerals through their specialized leaf structures, which makes the role of the fungal partner redundant. However, entering into a mycorrhizal relationship bestows benefits to the host plant which often exceed the facilitation of nutrient acquisition e.g. abiotic and biotic stress tolerance (Augé 2001; Pozo & Azcon-Aguilar 2007), and therefore may provide the host plant with a competitive advantage particularly in a high-stress environment.

Drosera burmanii Vahl. and *D. indica* L. are two carnivorous plant species inhabiting flooded acidic south Indian soils low in nutrients (Jayaram & Prasad 2006). Despite the fact that the captured insects contribute to the plant nutrition (Perica & Berljak 1996), vigorous growth of these plants under P-limiting natural conditions led me to examine possible symbiosis between their roots and AM fungi. It is hypothesized that despite their leaf adaptations which allow them to acquire extra sources of nutrients, carnivorous plants do host AM fungi in roots.

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To test this hypothesis, I have carried out a qualitative and quantitative evaluation of AM fungi associated with two species of *Drosera* in relation to some edaphic factors.

MATERIALS AND METHODS

The study was carried out in acidic sandy (entisol) soils of Sanatana Dharma College campus situated at Alappuzha (9^0 55'N, 76^0 46'E, altitude 1 m a.s.l), Kerala in the south west coast of India. The campus had a natural vegetation of *Drosera* species viz. *D. burmanii* and *D. indica* which showed its predominance during the period from June to August of every year.

Sampling. Sampling was done from five locations (ca. 50-100 m apart) of the campus during the month of July 2011. Ten root and rhizosphere soil samples from each species were randomly taken from each location. Root samples were taken by pulling out the entire plant with utmost care to get the roots intact. The plants were gently tapped to remove the soil particles and adherent debris and rinsed in tap water. Soil samples of ca. 100 g were collected from each plant species to a depth of 10 cm. Root and soil samples from the same species were pooled to get composite samples from each location. The samples were collected separately in polythene bags, labeled and stored at 4^0C.

Soil analyses. The moisture content of the soil was determined within 2-3 h of sample collection by drying in a hot air oven at 80^0C for 24 h. pH was measured using a digital pH meter (soil:water ratio 1:2). Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley & Black 1934) and phosphorus (P) by the ascorbic acid method (Watanabe & Olsen 1965).

Assessment of AM colonization in roots. The root samples were cleared in 10% (w/v) KOH (15 min, 90^0C) and stained with 0.05% trypan blue (Phillips & Hayman 1970). Fifteen root fragments (ca. 1 cm long) were mounted on slides in a polyvinyl alcohol-lactic acid-glycerol (PVLG) mixture (Koske & Tessier 1983) and examined with a compound microscope to quantify AM colonization. The percentage of root length colonized by AM fungi was determined using the magnified line-intersect method (McGonigle *et al.* 1990).

Spore extraction and analysis. Spores were isolated from the air-dried sample by wet-sieving and decanting method of Gerdemann & Nicolson (1963). Spore density was expressed per 50 g dry soil. Spores of each morphotype were mounted on PVLG and PVLG mixed with Melzer's reagent (1:1, v:v). Taxonomic identification of spores to the species level was based on spore size, colour, ornamentation and wall characteristics (Schenck & Pérez 1990) and by comparing the original descriptions available at http://invam.caf.wvu.edu.

Statistical analysis. Results were analyzed statistically by one-way ANOVA. Means were compared using Tukey's honestly significant difference (HSD) test at the 0.05 level of probability. Pearson's correlation coefficient was used to explain the relationship between soil and mycorrhizal characteristics. Statistical analyses were performed with the Systat (version 9) statistical programme.

RESULTS AND DISCUSSION

AM colonization was evident in the root samples of both species of *Drosera* collected from different sites. The fungal structures such as intra and extra radical hyphae, vesicles and arbuscules were found in the cortex of both species in a manner typical of *Arum*-type colonization (Fig. 1a-b). Vesicles were found to aggregate at certain points in the cortex in *D. burmanii* (Fig. 1c) while they were sporadically distributed throughout the entire length of the colonized roots in *D. indica*. Vesicles were produced at the tip of intercellular hyphae (Fig. 1d). In both species, arbuscules were produced from a truncate hypha entering the host cell which showed coarse branching (Fig. 1e).

The proportion of root length colonized by AM fungi and spore density in the rhizosphere soil of *Drosera* species is given in Table 1. In general, both the plant species had a low level (<50%) of colonization by AM fungi. Root colonization and spore density were significantly more in *D burmanii* as compared to *D. indica*.

Soil collected from the rhizosphere of *Drosera* species recorded a moisture content varying from 7.2% (*D. burmanii*) to 10.6% (*D. indica*). The pH was low, ranging from 4.7 (*D. indica*) to 5.8 (*D. burmanii*). OC in the soil ranged from 0.37% (*D. indica*) to 0.48% (*D. burmanii*). The available P level was significantly (P<0.001) more in the rhizosphere soil of *D. indica* (20.4 kg h^{-1}) than that of *D. burmanii* (15.2 kg h^{-1}). Correlation analysis revealed that root colonization and spore density in the rhizosphere of *Drosera* plants were positively correlated with soil properties such as pH and OC content but negatively correlated with soil moisture and P (Table 2)

Five taxa of AM fungi belonging to the genera *Acaulospora*, *Funneliformis*, *Glomus* and *Racocetra* were isolated from the rhizosphere of *Drosera indica* (Fig. 2) but only three taxa were obtained from the rhizosphere of *D. burmanii* (Table 3).

The incidence of AM fungi with fully-developed fungal structures in *Drosera* species is consistent with the reports

Table 1. AM colonization in roots and rhizosphere spore density of two species of *Drosera*. Means in a column with different letters are significantly different ($P<0.05$) by Tukey's HSD.

Species	Root colonization (%)	Spore density
	(mean±SD)	in 50 g soil
D. burmanii	38.00[a]±5	78[a]±8
D. indica	20.00[b]±6	45[b]±3

Table 2. Pearson's correlations between soil characteristics, AM colonization and spore density in the rhizosphere of *Drosera* ($n=10$, $^{**}P<0.01$, $^{***}P<0.001$).

Soil characteristics	Root colonization	Spore density in rhizosphere soil
Moisture	-0.912***	-0.934***
pH	0.844**	0.976***
OC	0.789**	0.893***
P	-0.916***	-0.940***

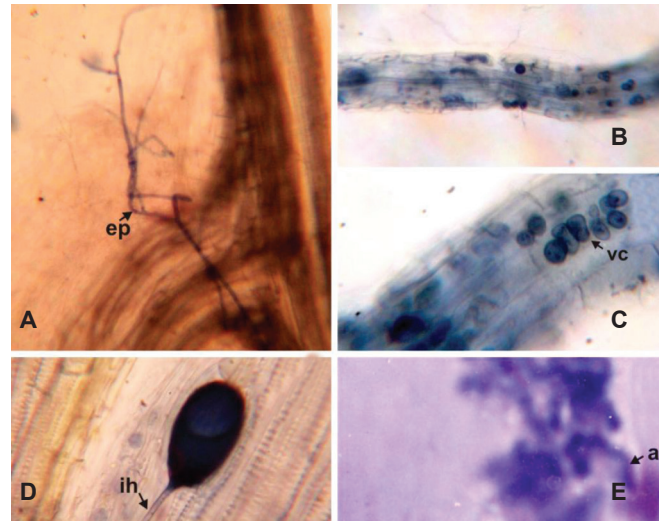

Fig. 1. Colonization of AM fungi in *Drosera* roots. **A**- Intra and extra radical hyphae with entry point (*ep*) in *D. indica*; **B**- Root colonization in *D. burmanii* with a dense vesicular cluster (*vc*); **C**, **D**- An intercellular hypha (*ih*) ending in a vacuole in the root cortex of *D. indica*; **E**- A degenerating arbuscule in the cortical cell of *D. burmanii* with arbuscular trunk (*at*) intact; *bars* **A**- 4µm; **B**- 15µm; **C**, **D**- 4 µm; **E**- 2 µm.

of Fuchs & Haselwandter (2004) and Weishampel & Bedford (2006) who observed colonization attributable to AM fungi in the roots of *D. intermedia* and *D. rotundifolia*. The fungal structures particularly, presence of arbuscules, is a *sine qua nome* for identification for AM colonization in roots (Bonfante-Fasolo 1984), since these structures are formed by all AM fungi whilst vesicles are not (Gerdemann & Trappe 1974). In the present study, intercellular aseptate hyphae, vesicles and arbuscules were the most frequent structures seen in both species of *Drosera* providing unequivocal evidence of AM colonization in their roots.

The AM in *Drosera* corresponded to the *Arum*-type characterized by intracellular hyphal growth with intracellular arbuscule formation in contrast to the *Paris*-type with extensive intracellular hyphal coils seen in plants of the natural condition. Morphological types of AM colonization in a host are influenced mainly by two factors. Firstly, the anatomical features of the host such as variations in longitudinal extent of the air space could influence mycorrhiza morphology (Brundrett & Kendrick 1990). Secondly, the identity of an AM fungus can also affect whether an *Arum*- or *Paris*-type morphology develops in a given host (Cavagnaro *et al.* 2001). However, the most likely factor that determines AM morphology in *Drosera* is to be identified through more detailed studies.

Percentage root length colonized by AM fungi was very low in root samples of *Drosera*, probably due to narrow root length and density in these plants. This is in agreement with the finding of Muthukumar *et al.* (1999) that the extent of root colonized by AM fungi in sedges is proportional to the length and density of root hairs. Furthermore, in most root samples, the fungal structures such as hyphal coils and appresoria were rarely found. The potential nutrient benefit of mycorrhizal association in *Drosera* therefore has yet to be ascertained. The present study also witnessed a positive relationship ($r=0.856$, $P<0.01$, $n=10$) between mycorrhizal root length and spore density in the rhizosphere soil which contrasts with the earlier reports of Al-Raddad (1991) that the extent of AM colonization in a host plant need not necessarily be correlated with spore density in the rhizosphere.

Variability in natural colonization by AM fungi in plants belonging to identical genera is not uncommon in the plant kingdom (Graham *et al.* 1991). Though *D. burmanii* and *D. indica* were growing in the same ecological conditions, the percentage root colonization and spore density in the rhizosphere differed between the species. This could be ascribed to the genetic variability of the host (Menge *et al.* 1978, Graham *et al.* 1991).

Other factors likely to influence root colonization and spore density are soil properties. AM colonization in roots and spore density in the rhizosphere of *Drosera*

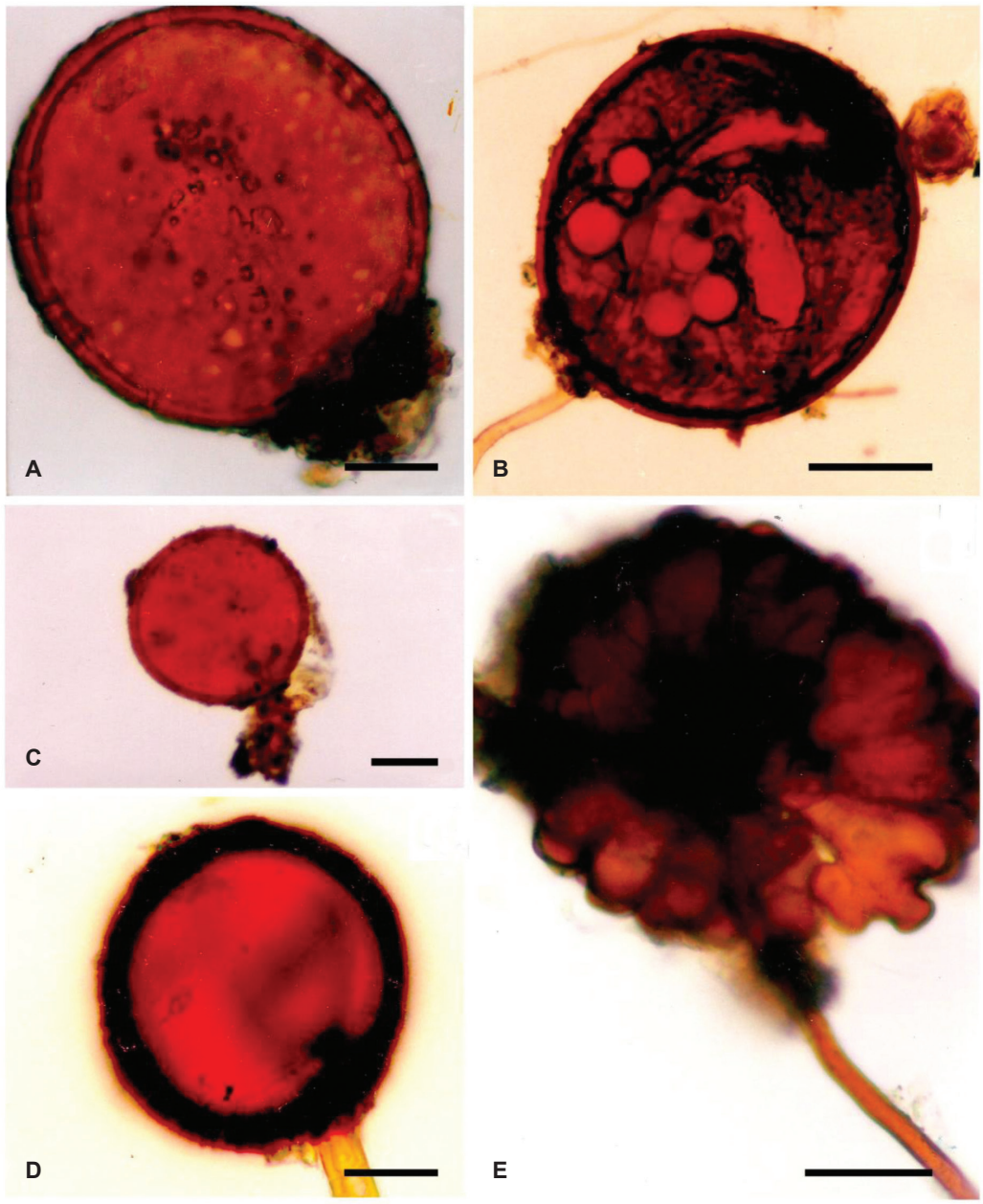

Fig. 2. AM species identified from the rhizosphere of *Drosera*. **A**- *Acaulospora lacunosa*; **B**- *Funneliformis geosporus*; **C**- *Funneliformis dimorphicus*; **D**- *Glomus invermaium*; **E**- *Racocetra verrucosa*; *bars* **A**- 10 µm; **B**- 15 µm; **C**- 30 µm; **D**- 10 µm; **E**- 15 µm.

decreased with an increase in soil moisture. Al-Agely & Reeves (1995) also noted the poor spore density and root colonization in *Oryzopsis hymenoides* due to the high soil moisture. Rhizosphere soils of *Drosera* showed their pH to be acidic. A positive correlation between soil acidity and AM colonization observed in the present study contrasts with the finding of Khaliel (1988) who concluded that no correlation exists between AM colonization and soil pH in three Indian forest trees. A correlation study between spore density and pH (Rajasree 2009) also supports the negative relationship. Soil inhabited by *Drosera* had a low level of OC which was found to have a stimulatory role on root colonization as well as spore density. Gryndler *et al*. (2002) observed that some of the organic materials in the soil could stimulate AM fungal development. The negative relationship between fungal characters and P level in the present study is consistent with the observation by other workers (Mosse 1973; Abbott & Robson 1991; Kahiluoto *et al*. 2000) who found root colonization and spore density to decrease with increasing P.

Tab. 3. Distribution of AM species in rhizosphere soils of *Drosera.*

AM fungi	*D. burmanii*	*D. indica*
Acaulospora lacunosa Morton.n.sp		+
Funneliformis dimorphicus (Boyetchko &	+	+
JP Tewari) Oehl, GA Silva & Sieverd., comb.nov		
Funneliformis geosporus (TH Nicolson & Gerd.)	+	+
C Walker & A Schüssler		
Glomus invermaium IR Hall	+	+
Racocetra verrucosa (Koske & C Walker) Oehl		+
FA Souza & Sieverding		
+ = present		

AM fungal taxa belonging to Glomerales (*Glomus*, *Funneliformis*) dominated over Diversisporales (*Acaulospora*) and Gigasporales (*Racocetra*) in the rhizosphere of *Drosera*. To assign a possible reason for this is rather complex. Nevertheless, factors such as their adaptability to acid soils, high competitiveness and/or reproductive capacity (Sieverding 1991) may be contributing to this. Prevalence of *Glomus* over *Acaulospora* and *Gigaspora* was reported earlier from cropped soils of Canada (Talukdar & Germida 1993). Another notable feature in the present study was the confinement of *Acaulospora lacunosa* and *Racocetra verrucosa* only to the rhizosphere of *D. indica.* This is probably because of the specificity of these taxa to a particular host. Previous studies also suggest the existence of some degree of fungal-host preference in AM associations (Dhillion 1992; Husband *et al*. 2002; Bever 2002).

The present results suggest that a natural AM system influenced by the host as well as edaphic factors are operating in *Drosera* species. However much research is required to unravel the exact role of the symbiosis and the stage/s at which carnivorous plants like *Drosera* are greatly dependent on it.

REFERENCES

Abbott LK & Robson AD. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture Ecosystem and Environment* **35**: 121-150.

Al-Agely AK & Reeves FB. 1995. Inland sand dune mycorrhizae: effects of soil depth, moisture and pH on colonization of *Oryzopsis hymenoides*. *Mycologia* **87**: 54-60.

Al-Raddad AM. 1991. Response of bean, broad bean and chickpea plants to inoculation with *Glomus* species. *Scientia Horticulturae* **146**: 195-200.

Augé RM. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3-42.

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ & Shachar-Hill Y. 2003. Carbon transport from arbuscular mycorrhizal roots involves the translocation of carbohydrates as well as lipids. *Plant Physiology* **131**: 1496-1507.

Bever JD. 2002. Host specific AM fungal population rates can generate feedback on plant growth. *Plant and Soil* **244**: 281-290.

Bonfante-Fasolo P. 1984. Anatomy and morphology of VA mycorrhizae. In: Powell CL & Bagyaraj DJ (eds.), VA mycorrhizas, 5-33. CRC, Boca Raton, Florida.

Brundrett MC. 2009. Mycorrhizal associations and other means of nutrition of vascular plants; understanding of the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant and Soil* **320**: 37-77.

Brundrett MC & Kendrick B. 1990. The roots and mycorrhizas of herbaceous woodland plants. II. Structural aspects of morphology. *New Phytologist* **114**: 469-479.

Cavagnaro TR, Gao L-L, Smith FA & Smith FE. 2001. Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytologist* **151**: 469-475.

Chambers SM, Curlevski NJA & Cairney JWG. 2008. Ericoid mycorrhizal fungi are common root inhabitants of non-Ericaceae plants in a south-eastern Australian sclerophyll forest. *FEMS Microbiology Ecology* **65**: 263-270.

Dhillion SS. 1992. Evidence of host-mycorrhizal preference in native grassland species. *Mycological Research* **94**: 359-362.

Fuchs B & Haselwandter K. 2004. Red list plants; colonization by arbuscular mycorrhizal fungi and dark septate endophytes. *Mycorrhiza* **14**: 277-281.

Gai JP, Christie P, Feng G & Li XL. 2006. Twenty years

of research on community composition and species distribution of arbuscular mycorrhizal fungi in China: a review. *Mycorrhiza* **16**: 229-239.

Gerdemann JW & Nicolson TJ. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society* **46**: 235-244.

Gerdemann JW & Trappe JM. 1974. The endogonaceae in the Pacific North West. *Mycologia Memoir* 5.

Graham JH, Eissenstat DM & Drouillard DL. 1991. On the relationship between a plant's mycorrhizal dependency and rate of vesicular arbuscular mycorrhizal colonization. *Functional Ecology* **5**: 773-779.

Gryndler M, Vósatka M, Hršelová H, Chavatalová I & Jansa J. 2002. Interaction between arbuscular mycorrhizal fungi and cellulose in growth substrate. *Applied Soil Ecology* **19**: 279-288.

Husband R, Herre EA, Turner SL, Gallery R & Young SPW. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. *Molecular Ecology* **11**: 2669-2678.

Janos DP. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology* **61**: 151-162.

Jayaram K & Prasad MNV. 2006. *Drosera indica* L. and *D. burmanii* Vahl., medicinally important insectivorous plants in Andhra Pradesh-regional threats and conservation. *Current Science* **91**: 943-946.

Juniper BE, Robins RJ & Joel DM. 1989. Carnivorous plants. Academic, London.

Kahiluoto H, Ketoja E & Vestberg M. 2000. Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 1. Bioassays in a growth chamber. *Plant and Soil* **227**: 191-206.

Khaliel AS. 1988. Incidence of VAM on some desert plants and correlation with edaphic factors. In: Mahadevan A, Raman N & Natarajan K (eds.), Mycorrhizae for Green Asia: Proceedings 1, Asian Conference on Mycorrhiza, Madras, India, 56-59.

Koske RE & Tessier B. 1983. A convenient permanent slide mounting medium. *Mycological Society of America Newsletter* **34**: 59.

Macdougal DT. 1899. Symbiotic saprophytism. *Annals of Botany* **13**: 1-46.

McGonigle TP, Miller MH, Evans DG, Fairchild GL & Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495-501.

Menge JA, Johnson ELV & Platt RG. 1978. Mycological dependency of several citrus cultivars under three nutrient regimes. *New Phytologist* **81**: 553-559.

Mosse B. 1973. Advances on the study of vesicular arbuscular mycorrhiza. *Annual Review of Phytopathology* **11**: 171-196.

Muthukumar T, Udaiyan K, Vasantha K, Kleiner D & Manian S. 1999. Mycorrhizae in sedges as related to root characters and its ecological significance. *Pertanika Journal of Tropical Agricultural Science* **22**: 9-17.

Perica MC & Berljak J. 1996. *In vitro* growth and regeneration of *D. spatulata* Labill on various media. *Horticultural Science* **31**: 1033-1034.

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

Pozo MJ & Azcon-Aguilar C. 2007. Unraveling mycorrhiza induced resistance. *Current Opinion in Plant Biology* **10**: 393-398.

Rajasree R. 2009. Studies on endomycorrhizal synthesis in *Sesamum indicum* L. PhD Thesis, Mahatma Gandhi University, Kottayam, India.

Schenck NC & Pérez Y. 1990. Manual for the identification of VA mycorrhizal fungi, Synergistic, Gainesville, Florida.

Schüβler A, Schwarzott D & Walker C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* **105**: 1413-1421.

Sieverding E. 1991. Vesicular arbuscular mycorrhiza management in tropical agroecosystems. Deutsche Gesellscraft fur Technische Zusammenarbeit, Bremer, Germany.

Talukdar NC & Germida JJ. 1993. Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Saskatchewan, Canada. *Canadian Journal of Microbiology* **39**: 567-575.

Walkley A & Black LA. 1934. An examination of the Degtareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science* **37**: 29-38.

Watanabe FS & Olsen SR. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extract from soil. *Soil Science Society of America Journal* **29**: 677-678.

Weishampel PA & Bedford BL. 2006. Wetland dicots and monocots differ in colonization by arbuscular mycorrhizal fungi and dark septate endophytes. *Mycorrhiza* **16**: 495-502.

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REZIME

Ima li arbuskularna mikorize kod karnivornih biljaka *Drosera burmanii* i *D. indica*?

Variampally Sankar Harikumar

Arbuskularna mikoriza nije opisana u korenima karnivornih biljaka *Drosera burmanii* i *D. indica* iz južne Indije. Ipak, fungalne strukture karakteristične za *Arum*-tip arbuskularne mikorize konstatovane su kod obe vrste iako je nizak nivo kolonizacije ovim gljivama (<50%). Kolonizacija korenova mikorizalnim gljivama kao i prisustvo spora u rizosferi se razlikuje kod ovih vrsta i bitno je izraženije kod *D. burmanii*. Edafski faktori kao što su pH zemljišta i sadržaj organskog ugljenika pozitivno su korelisani sa razvojem gljiva, dok je sadržaj fosfora i vlažnost zemljišta negativno korelisan. Pet taksona arbuskularno-mikoriznih gljiva iz rodova *Acaulospora*, *Funneliformis*, *Glomus* i *Racocetra* su izolovani iz rizosfere *D. indica* dok je iz rizosfere *D. burmanii* izolovano tri taksona iz rodova *Funneliformis* i *Glomus*.

Ključne reči: arbuskularna mikoriza, karnivorne biljke, *Drosera burmanii*, *Drosera indica*