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Incidence of arbuscular mycorrhizal (AM) fungi in some angiosperms with underground storage organs from western ghat region of Goa

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Arbuscular mycorrhizal (AM) fungi are the most common types of all mycorrhizae and their occurrence as root symbionts has been reported from exceptionally wide range of plants. However, Taber & Trappe (1982) reported for the first time, the presence of AM fungi in the vascular system of rhizomatous tissue and the scale like leaves of *Zingiber officinale* L. Later Nazim (1990) reviewed the presence of AM fungi associated with the portions other than roots in twenty one angiosperms and some non-angiosperm species. Incidence of AM fungal colonization has been reported in scale leaves and leaf bases of *Curcuma longa* L. (Sampath & Sullia 1992), corms of *Amorphophallus commutatus* Engler (Rodrigues 1995) and tubers of *Pueraria tuberosa* (Willd.) DC (Rodrigues 1996). Arbuscular mycorrhizal fungi have been documented in tubers of *Colocasia esculenta* (L.) Schott (Bhatt & Kavreiappa 1997), garlic bulbs (Kunwar *et al*. 1999) and tubers of *Gloriosa superba* L. (Khade & Rodrigues 2003). No information is available on the arbuscular mycorrhizal fungal association in angiosperms with underground storage organs from western ghat region of Goa which is a hot spot of biodiversity. Therefore, studies were carried out on arbuscular mycorrhizal status of twelve angiosperms with underground storage organs in this paper.

Root samples and rhizosphere soil samples of twelve plant species having underground storage organs (tubers, rhizomes, corms and bulbs) belonging to five families (Liliaceae, Dioscoreaceae, Amaryllidaceae, Zingiberaceae and Araceae) were randomly collected during June-July 2001 from four localities (Valpoi, Collem, Honda, Neturli) of western ghat region of Goa. Five plant species *viz., Curcuma longa*, *Amorphophallus campanulatus*, *Colocasia esculanta*, *Colocasia* sp. and *Alocasia macrorrhiza* are edible and medicinal while remaining are wild species. Five plants per species were dug out, placed in polythene bags and transported to the laboratory. Root samples were freshly processed whereas the rhizosphere soil samples were stored at 4^0C until they were processed.

For AM colonization assessment, root samples were cleared with 10% KOH, acidified with 1N HCl and stained in lactoglycerol trypan blue (0.05%). Quantification of AM fungal colonization was carried out according to grid intersection method and expressed as percentage root length colonized (Mc Gonigle *et al*. 1990). The stained roots were mounted on microscopic slides with 18 mm coverslips. At least 150 intersections were examined by light microscopy at x 200 magnification and root length colonized by hyphae, arbuscules, vesicles and total root colonization of AM fungi were quantified.

Spores of arbuscular mycorrhizal fungi were

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extracted from 100 g rhizosphere soil using wet sieving and decantation method. For this, 100 g of soil was dispersed in 1000 ml of water and the supernatant was decanted through stacked sieves (500 µm - 35 µm). Debris on the sieves was collected in beaker. Spores were recovered on filter paper and quantification of spores was carried under stereo microscope. Diagnostic slides with spores/sporocarps were prepared using polyvinyl alchol lactoglycerol (PVLG) as mountant. Both broken and unbroken spores were examined under compound microscope. Spores of AM fungi were identified according to spore morphology and wall characters (Almedia & Schenck 1990; Morten & Benny1990; Schenck & Perez 1990; Wu 1993). Frequency of occurrence (%) for each AM fungal species was calculated as a fraction of number of soil samples possessing spores of the species.

The data on arbuscular mycorrhizal colonization were arcsine transformed, whereas the data on spore density of AM fungi were log transformed prior to statistical analysis. Pearson's correlation was used to assess the relationship between mycorrhizal variables. Data on root colonization and spore density of AM fungi were subjected to analysis of variance (ANOVA) to investigate their variation within the different plant species. Data was statistically analyzed using MSTAC package.

All the plant species studied, exhibited AM fungal association (Table 1). Arbuscular mycorrhizal colonization was restricted to the epidermal and parenchymatous cells near the root bases of the underground storage organs and in the root region. Our study reported the presence of hyphae, arbuscules and vesicles in all plants except *Crinum vivipara* var. *viviparum* which lacked arbuscules indicating the active growth of AM fungi within the host roots (Table 1). Presence of extramatrical hyphae was observed in four species (*Dioscoria deltoidea*, *Crinum vivipara*, *Curcuma deciepiens* and *Colocasia* sp). The average root length colonized by hyphae was 16.1%. The highest and the lowest root length colonized by hyphae was recorded in *Crinum vivipara* var. *viviparum* (27.8%) and *Curcuma longa* (7.2%) respectively (Table 1). Further, the root length colonized by arbuscules ranged from nil in *Crinum vivipara* to 28.6% in *Colocasia esculenta*. The average root length colonized by arbuscules was 9.3% (Table 1). Also, the average root length colonized by vesicles was 12.3% with colonization varying from as low as 3.4% (*Asparagus* sp.) to as high as 34% (*Crinum vivipara*) (Table 1). However,

Table 1. Arbuscular mycorrhizal status of plant species having underground storage organs from western ghats region of Goa; values are mean ± 1 SE.

Plant species	Root length colonized (%)			*Mean total root colonization(%)	*Mean spore density 100 g soil^{-1}	Species richness
	Hyphae	Arbuscules	Vesicles			
Asparagus sp.	20 ± 4.7	19.4 ± 0.8	3.4 ± 0.7	42.8 ± 1.1	300.4 ± 10.1	7
Smilax sp.	16 ± 3.4	8 ± 1.39	5.4 ± 1.0	29.4 ± 1.8	432.6 ± 11.7	5
Dioscoria deltoidea L.	25 ± 5.8	7.36 ± 1.3	19.4 ± 1.5	52.0 ± 2.9	528.2 ± 14.9	6
Dioscoria pentaphylla L.	17.6 ± 3.1	7.4 ± 1.3	5.6 ± 0.8	30.6 ± 1.8	284.6 ± 28.6	4
Crinum vivipara var. *viviparum* Ansari & Nair	27.8 ± 5.4	-	34 ± 1.4	61.8 ± 3.4	12.6 ± 0.8	5
Curcuma decipiens Dalz.	16.8 ± 3.1	4.8 ± 0.62	5 ± 0.5	26.6 ± 0.7	510.8 ± 9.0	4
Curcuma longa L.	7.2 ± 1.1	2.8 ± 0.58	2.3 ± 1.8	19.4 ± 0.8	16.2 ± 0.8	2
Amorphophallus commutatus Englee	13.2 ± 2.4	7.6 ± 1.0	25.2 ± 1.7	46.0 ± 2.4	280.8 ± 5.9	4
Amorphophallus campanulatus Blume	12.6 ± 3.1	2.8 ± 1	7.2 ± 1	24.2 ± 2.4	36.6 ± 1.8	3
Colocasia esculanta L.	16.6 ± 3.3	28.6 ± 2	17.8 ± 0.6	63.0 ± 2.1	128.0 ± 2.6	3
Colocasia sp.	22.5 ± 4.9	10.8 ± 1	22.5 ± 1.3	55.0 ± 2	79.8 ± 3.7	4
Alocasia macrorrhiza Schott	9.4 ± 2.4	12.4 ± 1.1	9.4 ± 0.9	31.2 ± 1.7	249.6 ± 8.34	4
Average	17.5	9.3	13.6	40.2	283.3	-
C. D. at 0.05%	5.7	NS	9.4	10.6	0.6	-

* F test significant at 0.05 level of probability. NS- F test not significant at 0.05 level of probability.

the average total root colonization was 40.2%. The highest and the lowest total root colonization was recorded in *Colocasia esculanta* (63%) and *Curcuma longa* (19.4%) respectively (Table 1). Our study differs from the earlier investigations in quantification of AM fungal structures within the roots, whereas the earlier reports on AM fungal association in plants with underground storage organs reported either the incidence of colonization or estimated total root colonization of AM fungi (Bhat & Kaveriappa 1997; Rodrigues 1995; Rodrigues 1996; Sampath & Sullia 1992). Spore density in the present study ranged from 122 to 528 spores 100 g^{-1} rhizosphere soil (Table 1). The average spore density of AM fungi was 283 spores 100 g^{-1} rhizosphere soil. Thus, presence of high spore density is in agreement with the work of Kunwar *et al*. (1999). Also, higher number of spores in the soil and relatively low levels of root colonization of AM fungi in the present study coincided with the early growing stage of the plants where they develop adequate root system, with the AM fungal spores beginning to germinate to actively colonize the host roots. Similar results were reported from studies carried out on tubers of *Gloriosa superba* L. in Goa (Khade & Rodrigues 2003).

Further, spore density showed no correlation with total root colonization indicating that AM fungal sporulation, germination and root colonization is dependant on a wide range of host fungal and environmental factors. However, a significant correlation was observed between root length colonized by arbuscules and total root colonization ($r = 0.75$; $P > 0.05$) thus providing information on mycorrhizal functionality (Siqera & Saggin-Junior 2001). Root colonized by hyphae and vesicles and total root colonization varied significantly within the plant species indicating the variability of AM susceptibility among the plants. However, root colonized by arbuscules did not show significant variations (Table 1). Spore density of arbuscular mycorrhizal fungi also varied significantly within the plant species.

Nineteen species of arbuscular mycorrhizal fungi belonging to four genera (*Acaulospora, Glomus, Gigaspora* and *Scutellospora*) were recovered from the rhizosphere soil of tuberous, rhizomatous, bulbous and cormatous plants (Table 2). Arbuscular mycorrhizal fungi belonging to genus *Glomus* (11) were dominant followed by *Acaulospora* (4), *Scutellospora* (3) and *Gigaspora* (1) with species number given in parenthesis (Table 2). Similarly, *Acaulospora, Glomus* and *Gigaspora* species were recorded from tubers of *Gloriosa superba* L. from Goa (Khade & Rodrigues 2003). The presence of so many AM fungal species in the rhizosphere soil of plants with underground storage organs from ecologically important region is interesting. While Bhat & Kaveriappa (1997) reported the presence of *Acaulospora, Glomus* and *Gigaspora* in the rhizosphere of *Colocasia esculenta,* our study recorded *Glomus, Gigaspora* and *Scutellospora* in same species. In the present study, *Glomus fasiculatum* (58%) showed the highest frequency of occurrence followed by *Gigaspora margarita* (50%). Species richness of AM fungi ranged from 2 species per plant in *Curcuma longa* to 7 species per plant in *Asparagus* species respectively (Table 1). This confirms our contention that plants with underground storage organs from western ghat region of Goa are colonized by arbuscular mycorrhizal fungi.

Table 2. Frequency of occurrence of AM fungi in plants with underground storage organs.

Arbuscular mycorrhizal fungi	Frequency (%)
A. elegans Trappe & Gerdemann	8.3
A. myriocarpa Sieverding et Schenck	8.3
A. scrobiculata Trappe	8.3
A. undulata Sieverding	16.7
G. dimorphicum Bovetchro & Tewari	8.3
G. fasiculatum (Thaxt. & Gerd.) Gerdemann & Trappe	58.3
G. formosanum Wu & Chen	16.7
G. geosporum (Nicol. & Gerd.) Walker	16.7
G. heterosporum Smith & Schenck	25.0
G. macrocarpum Tul. & Tul	8.3
G. maculosum Miller *et* Walker	50.0
G. magnicaule Hall	16.7
G. rubiformis (Wu & Chen) Almeida & Schenck	16.7
G. sinuosa (Gerd. & Bakshi) Almeida & Schenck	16.7
G. taiwainensis (Wu & Chen) Almeida & Schenck	33.3
G. margarita Hall & Abbott	50.0
S. gregaria Schenck & Nicolson	16.7
S. pellucida (Nicol. & Schenck) Walker & Sanders	8.3
S. weresubiae Koske *et* Walker	16.7

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