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Arbuscular Mycorrhizal Fungi of the 'Khazan Land' Agro-Ecosystem

B.F. Rodrigues[1]* and Anuradha N. Naik[2]
[1]Department of Botany, Goa University, Goa 403 206, India
[2]National Institute of Oceanography, Dona Paula, Goa 403 004, India
*(*E-mail: felinov2001@yahoo.co.uk)*

Introduction

'Khazan Lands' are partially modified mangrove and coastal wetland ecosystems of the west coast of India. Water with variable salinity is the dynamic element of this managed agro-ecosystem. As Khazan Lands are the sites of extreme conditions (e.g., salinity, flooding, physiological drought, siltation), it is interesting to study the interactions between plants and microbes, particularly arbuscular mycorrhizal (AM) fungi associated with pioneering vegetation. The presence of AM fungi and their restricted association with plant species indicates their tolerance and survival under stress conditions of Khazan Lands. The present chapter deals with the occurrence and diversity of AM fungi in association 28 plant taxa belonging to 16 genera that grow in Khazan Lands with a view to understand their ecology, role in rehabilitation and the reclamation of Khazan Lands and similar biomes of coastal region.

Khazans

The term 'Khazan' is a vernacular name in Konkani language of Goa for coastal saline soils. In Maharashtra, similar soil habitats are called 'Khar' or 'Kharvat', in Karnataka as 'Gazzaani', and in Kerala as 'Pokkal' and 'Chemeen Kattu'. Khazans are the integrated agro-aqua ecosystems that are managed in Goa and its territory. These lands have been reclaimed over centuries from marshy mangrove swamps and developed by the local people using their traditional knowledge of climate, tidal cycles, geomorphology, precipitation, runoff, sediment dynamics, soil properties and drainage characteristics of estuarine lands (Sonak, 2005). These

land productive for cultivation as well as aquaculture. Besides paddy cultivation, Khazans are also used in some areas as saltpans and for the production of lime from shells.

Estuaries, mangroves, outer and inner embankments, backwaters, creeks, sluice gates and drainage canals are important components of the Khazan system. The Khazans of Goa protect agricultural fields and villages from tidal ingress through a system of bunds (dykes) (Fig. 1). The outer embankments built by locally available laterite stones, mud and clay are protective against tidal activities. Mangrove species are usually planted near the outer embankment, which act as wave breakers. The inner embankments are made up of indigenous mud, straw and poles, which prevent soil erosion as well as protect fields from nutrient leaching. A wooden sluice gate plays an important role to regulate the water level in fields of Khazan. The adjoining fields are irrigated with water during the monsoon and when the water is least saline, sluice gates regulate water movement by the simple arrangement of shutters. The opening or closing of the wooden shutters protects the tidal flow and its impact on agricultural fields.

Study Site

Goa is situated along the central west coast (15°40′00″ to 14°43′54″ N; 74°21′13″ to 73°40′33″ E). The study site selected was Nivel Cantor, which is a typical Khazan area encompassing 13 hectares situated in Madkai village in Ponda with a sluice gate. It is a one-crop pattern Khazan, wherein the area is under paddy cultivation from June to October and rest of the months it remains dry. Apart from paddy, the vegetation in the surrounding area includes mangroves, mangrove associates, herbaceous annuals, and hydrophytes.

Arbuscular mycorrhizae

Mycorrhizas are widespread under natural conditions and occur in almost all soils, including mine soils (Jasper *et al.*, 1989) to agricultural soils (Abbott and Robson, 1982) and their symbiotic association with plants have been well documented (Godeas *et al.*, 1999; Setua *et al.*, 1999). The AM fungi are known to benefit the host plant species in a variety of ways (e.g., enhancement of growth, stimulation of root nodulation in legumes, exploitation of phosphorus in deficient soils, nutrient cycling, stress management). Literature search revealed inadequate information on AM fungi of mangroves including Khazan Lands (see Chapter 10 for AM fungi and salinity stress in crop plants).

Roots

Roots of 28 plant taxa belonging to 16 families (Mathew, 1991; Mohanan and Henry, 1994; Naithani *et al.*, 1997; Rao, 1985, 1986) along with rhizosphere soil was sampled from different locations of the Khazan land. Roots were washed gently and fixed in formalin-acetic-alcohol (FAA) and transported to the laboratory for processing. Fixed roots were rinsed to free FAA and cut into 1 cm pieces,

cleared in 2.5% KOH, acidified with 5N HCl and stained in trypan blue (0.5% in lactophenol) (Koske and Gemma, 1989). Those roots remained dark after clearing and were bleached in alkaline H_2O_2 prior to acidification. The stained roots with trypan blue were examined with a compound microscope (× 200-400) for AM fungal structures (arbuscules, vesicles and hyphae) and root colonization percentage was estimated according to magnified intersection method (Mc Gonigle *et al.*, 1990).

Rhizosphere soil

The rhizosphere soil samples collected from individual plant species were packed in polyethylene bags and stored at 4°C for a short duration until processing. The soil pH was determined after 1:1 dilution with distilled water soon after sampling. The same solution was used to assess the electrical conductivity (EC) (Bower and Wilcox, 1965). The total nitrogen and available phosphorus were assessed according to Jackson (1971) and exchangeable potassium was evaluated after extraction with ammonium acetate (Jackson, 1971). Soil organic matter was detected employing Walkley and Black's rapid titration method (Jackson, 1971).

AM fungal spores

The soil samples were analyzed for AM fungal spores. For quantification of spore density, 100g air-dried composite soil sample was assayed using wet-sieving and decanting method (Gaur and Adholeya, 1994; Gerdemann and Nicolson, 1963). Each composite sample was processed in triplicate and spores were observed under a stereomicroscope to assess their structure and morphology. Baiting of native AM fungi was also carried out in open pot cultures (Gilmore, 1968) using finger millet, *Eleusine coracana* (L.) Gartner as host. Plants in five pot cultures were maintained per AM fungi in green house and watered adequately. After 45 days, roots of host species were screened for AM fungal colonization. Pots showing successful colonization were maintained for up to six months and watering was reduced at the last three weeks to maximize AM fungal spore production (Menge, 1982). At the end of six months, plants were cut at the base, and the soils along with the roots were air-dried and checked for the presence of spores. The spores isolated from pot cultures were assessed and identified. Diagnostic slides containing intact and crushed spores and sporocarps of AM fungi were prepared in polyvinyl lacticglycerol (PVLG) (Koske and Gemma, 1989). Spore morphology as well as characteristics of wall layers were considered for the identification up to species level (Almeida and Schenck, 1990; Bentivenga and Morton, 1995; Morton and Benny, 1990; Morton and Redecker, 2001; Redecker *et al.*, 2000; Schenck and Perez, 1990; Walker and Vestberg, 1998). Spore identification was carried out by matching the descriptions provided by International Collection of *Vesicular Arbuscular Mycorrhizal* Fungi (INVAM; http://invam.caf.wvu.edu). Names and epithets of AM fungi were followed according to the recommendations by Walker and Trappe (1993).

Observations and Discussion

Soil quality

Khazan rhizosphere soils were clayey in nature with pH ranging between 5.3 and 7.1 (mean, 6.2). The EC ranged from 4.81 to 32.6 m mhos/cm (mean, 17.2 m mhos/cm). Such fluctuations in the pH and EC can be attributed to the constant flushing and washing of salt water, which leads to the deposition of salt at different regions. The rhizosphere soils were rich in organic carbon (1.09%), low in available phosphorus (30.42 kg/ha) and total nitrogen (1.99%), while the available potassium was appreciably high (1729 kg/ha). Constant flushing and leaching might be responsible for decreased total nitrogen, while low available phosphorus could be owing to the fact that tropical soils are usually of phosphate fixing. High EC as well as available potassium indicates the saline nature of Khazan soils.

Table 1. *Arbuscular mycorrhizal colonization in roots and spore density in rhizosphere of plant species of Khazan Land (see Fig. 1)*

Plant species	Family	Colonization*			Colonization (%)	Spore density**
		V	A	H		
Acanthus ilicifolius L.	Acanthaceae	-	-	-	00	123
Acrosticum aureum L.	Pteridaceae	-	-	-	00	127
Ammannia multiflora Roxb.	Lythraceae	+	-	+	54	310
Avicennia officinalis L.	Verbenaceae	-	-	-	00	66
Ceratopteris thalictroides (L.) Brongn.	Parkeriaceae	+	-	+	80	370
Cyprus cyperoides (L.) O. Kuntze.	Cyperaceae	+	-	+	39	330
Cyperus rotundus L.	Cyperaceae	+	-	+	33	78
Derris uliginosa Benth.	Papilionaceae	-	-	-	00	156
Echinochloa colonum (L.) Link.	Poaceae	-	-	-	00	64
Eclipta alba (L.) Hassk.	Asteraceae	-	-	-	00	88
Eleocharis geniculata (L.) Roem & Schult.	Cyperaceae	-	-	-	00	96
Excoecaria agallocha L.	Euphorbiaceae	+	-	+	36	100
Fimbristylis aestivalis (Retz.) Vahl.	Cyperaceae	-	-	-	00	87
Fuirena ciliaris (L.) Roxb.	Cyperaceae	+	-	+	37	104
Ipomoea muricata Jacq.	Convolvulaceae	+	-	+	35	98
Isachne globosa (Thunb.) O. Kuntze.	Poaceae	+	-	+	27	210
Ludwigia perennis L.	Onagraceae	+	-	+	45	63
Nymphaea nouchali Burm.	Nymphaeaceae	-	-	-	00	61
Oryza sativa L. var. Jyoti	Poaceae	+	-	+	32	134
Oryza sativa L. var. Asgo.	Poaceae	+	+	+	57	110
Oryza sativa L. var. Korgut	Poaceae	+	-	+	42	116
Paspalum scrobiculatum L.	Poaceae	+	-	-	23	53
Phyllanthus niruri L.	Euphorbiaceae	-	-	-	00	78
Salvinia molesta Mitch.	Salviniaceae	-	-	-	00	23
Scirpus lateriflorus Gmel	Cyperaceae	+	-	+	34	60
Sesuvium portulacastrum L.	Aizoaceae	+	+	+	76	93
Sphaeranthus africanus L.	Asteraceae	+	+	+	81	266
Sphenoclea zeylanica Goert. Frud.	Companulaceae	-	-	-	00	59

*V, Vesicles; A, Arbuscules; H, Hyphae
**Spores/100 g rhizosphere soil (mean, n=3)

Colonization

The extent of root colonization of AM fungi of 28 plant taxa is presented in Table 1. Roots of sixteen taxa were colonized by AM fungi with high vesicle colonization. Root colonization ranged between 23% (*Paspalum scrobiculatum*) and 81% (*Sphaeranthus africanus*). Some reports are available on the absence of AM fungal colonization in members belonging to the family *Nymphaeaceae* (Bagyaraj *et al.*, 1979; Dharmarajan *et al.*, 1993; Khan, 1974). Our study also revealed no AM fungal colonization in the roots of *Nymphaea nouchali*. Similarly, no AM fungal colonization was seen in *Acanthus ilicifolius, Acrostichum aureum, Avicennia officinalis* and *Derris uliginosa* as reported earlier (Gupta *et al.*, 2002; Mohankumar and Mahadevan, 1986). The presence of AM fungal colonization in roots of *Sesuvium portulacastrum* confirms the earlier report by Bhaskaran and Selvaraj (1985). Roots of *Ludwigia perennis* were colonized by AM fungi, while *Fimbristylis aestivalis* were devoid of it. However, Dharmarajan *et al.* (1993) reported the absence of AM colonization in *L. perrenis* and colonization of *Fimbrystylis* sp. In our study, four out of six Cyperaceae members showed colonization. The family, *Cyperaceae* has been previously categorized as non-mycorrhizal because roots of a few species were evaluated for AM fungi (Brundrett, 1991; Newman and Reddell, 1987; Peat and Filter, 1993; Powell, 1975; Smith and Read, 1997), but later there have been several reports of mycorrhizal colonization in certain *Cyperaceae* taxa (Lovera and Cuenca, 1996; Wetzel and Van der Valk, 1995). A variation in the mycorrhizal condition in Cyperaceae may be due to the environmental and edaphic factors rather than its phylogenetic constraint per se (Read, 1984), because AM fungal colonization seems to be negatively correlated with soil moisture (Anderson *et al.*, 1984). It has been speculated that non-mycorrhizal state of some Cyperaceae members is due to their occurrence in marshy and anaerobic soils rather than their taxonomic position (Tester *et al.*, 1987).

Spores

Rhizsphere spore density of AM fungi of 28 plant taxa is given in Table 1. The spore density varied between 53 (*Salvinia molesta*) and 370 (*Ceratopteris thalictroides*) per 100 g rhizosphere soil. Muthukumar *et al.* (2001) suggested that the spore production of AM fungi was strongly influenced by environmental factors with host species and soil types. Khan (1974) also reported that the soil type influences the dissemination of AM fungi. Twelve plant taxa (*Avicennia officinalis, Phyllanthus niruri, Sphenoclea zeylanica, Nymphaea nouchali, Echinochloa colonum, Salvinia molesta, Eclipta alba, Eleocharis geniculata, Acanthus ilicifolius, Fimbristylis aestivalis, Derris uliginosa* and *Acrosticum aureum*) although possess AM fungal spores in the rhizosphere (23-126 spores/ 100 g), were devoid of root colonization. This observation indicates that the mere presence of AM spores in the rhizosphere will not prove root colonization and the possibilities of association with other microbes to overcome the stress conditions prevail in Khazan habitats.

AM fungi

A total of 17 AM fungi belonging to three genera (*Acaulospora, Glomus* and *Scutellospora*) were recovered from the pot cultures (Table 2). Among them, *Acaulospora* and *Glomus* with a total of eight species each dominated the Khazan Lands, *Glomus claroideum* was most abundant followed by *Glomus fasciculatum*

Table 2. *Arbuscular mycorrhizal fungi of rhizosphere soils of selected plants growing in Khazan Lands (see Fig. 1)*

Plant species	AM fungal species
Acanthus ilicifolius L.	*Glomus claroideum, G. geosporum, G. multicaule*
Acrosticum aureum L.	*Acaulospora delicata, A. spinosa, Glomus claroideum*
Ammannia multiflora Roxb.	*Glomus claroideum G. fasciculatum, G. formosanum,*
Avicennia officinalis L.	*Acaulospora dilatata, A. nicolsonii*
Ceratopteris thalictroides (L.) Brongn.	*Glomus aggregatum, G. claroideum, G. geosporum, G. intraradices, Scutellospora weresubiae*
Cyprus cyperoides (L.) O. Kuntze.	*Acaulospora dilatata, A. Delicata, A. mellea, A. myriocarpa, A. spinosa, Glomus aggregatum, G. claroideum, G. fasciculatum*
Cyperus rotundus L.	*Glomus claroideum, G. fasciculatum*
Derris uliginosa Benth.	*Acaulospora delicata, A. spinosa, Glomus fasciculatum*
Echinochloa colonum (L.) Link.	*Glomus claroideum, G. geosporum, G. multicaule*
Eclipta alba (L.) Hassk.	*Glomus claroideum, Glomus formosanum*
Eleocharis geniculata (L.) Roem & Schult.	*Acaulospora nicolsonii, Glomus claroideum, G. multicaule*
Excoecaria agallocha L.	*Acaulospora delicata, A. rehmii, A. spinosa,Glomus claroideum*
Fimbristylis aestivalis (Retz.) Vahl.	*Glomus claroideum, G. formosanum G. geosporum, Glomus mosseae Fuirena ciliaris* (L.) Roxb. *Glomus claroideum, G. geosporum*
Ipomoea muricata Jacq.	*Acaulopsora dilatata, Glomus geosporum*
Isachne globosa (Thunb.) O. Kuntze.	*Glomus fasciculatum, Acaulospora nicolsonii*
Ludwigia perennis L.	*Acaulospora dilatata, Glomus aggregatum, G. claroideum*
Nymphaea nouchali Burm.	*Acaulospora dilatata, Glomus formosanum, G. mosseae*
Oryza sativa L. var. Asgo	*Acaulospora delicata, A. mellea, Glomus fasciculatum*
Oryza sativa L. var. Jyoti	*Acaulospora mellea, Glomus formosanum*
Oryza sativa L.var. Korgut	*Glomus claroideum*
Paspalum scrobiculatum L.	*Acaulospora nicolsonii, Glomus fasciculatum*
Phyllanthus niruri L.	*Acaulospora laevis, Glomus claroideum, G. fasciculatum*
Salvinia molesta Mitch.	*Acaulospora mellea, A. spinosa,*
Scirpus lateriflorus Gmel	*Glomus claroideum, G. fasciculatum, G. multicaule*
Sesuvium portulacastrum L.	*Acaulospora delicata, A. dilatata, A. nicolsonii, Glomus aggregatum, Scutellospora weresubiae*
Sphaeranthus africanus L.	*Acaulospora delicata, A. laevis, A. myriocarpa, Glomus claroideum, G. mosseae*
Sphenoclea zeylanica Goert. Frud.	*Glomus formosanum, G. geosporum*

and *Acaulospora delicata*. The genus *Scutellospora* was represented by a single species, *Scutellospora weresubiae*, which was recovered from the pot cultures of *Certopteris thalictroides* and *Sesuvium portulacastrum*. The differences observed in the absence or presence of AM fungal genera might be attributed to the edaphic factors of soil and the host plant interactions at the study sites.

Conclusions and Outlook

Although Khazan Land agro-ecosystems exhibit variations in physicochemical properties, the vegetation consists of a variety of AM fungi. Seventeen AM fungi belonging to three genera in Khazan Land indicate their adaptability to stress conditions (flooding, salinity, physiological drought). It also reveals that these fungi have an important role to play in plant growth promotion and survival under adverse conditions. Abundance of *Acaulospora delicata, Glomus claroideum* and *G. fasciculatum* in Khazan Lands indicates their importance as biofertilizers. Further investigations and developments in Khazan Land may realize the importance of AM fungi and avoid disrupting this valuable plant-microbe link. This study forms a baseline for future investigations on the ecology and application of AM fungal species for rehabilitation, reclamation and to improve productivity of Khazan Land and associated biomes.

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