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ANALYSING ARBUSCULAR MYCORRHIZAL FUNGAL DIVERSITY IN THE RHIZOSPHERE OF *Guizotia abbysinica* (L.f.) Cass., NORTH KARNATAKA, INDIA

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ABSTRACT

Arbuscular mycorrhizal (AM) fungal species diversity patterns may vary in a plant species as influenced by soil environmental and biological factors. In the present study, *Guizotia abyssinica* (L.f.) Cass., (Niger) rhizospheric soil samples were collected from 24 niger growing places belonging to 8 districts from North Karnataka, India. Rhizosphere soil samples were analysed for spore density, relative abundance and isolation frequency of AM fungal spores at genus as well as at species level and various soil physico-chemical properties were also determined. A total of 38 AM fungal morpho-species belonging to 13 genera were recorded and Genera *Glomus* was found to be the most predominated. Spores belong to *Glomus*, *Acaulospora*, *Gigaspora* and *Sclerocystis* were most frequent and *Ambispora*, *Diversispora*, *Quatunica* and *Paraglomus* were less frequent. Maximum relative abundance and isolation frequency was found with *Rhizophagus fasciculatus* and *Glomus albidum* while, least value for relative abundance was found with *Gigaspora marginata* and isolation frequency was with many AM fungal morpho-species.

KEY WORDS : ***Rhizophagus*, *Guizotia abyssinica*, *Glomus*, North Karnataka, Relative abindance, Isolation frequency.**

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INTRODUCTION

Guizotia abyssinica (L. f.) Cass., (Niger) is an oilseed crop cultivated in Ethiopia and India. It is a dicotyledonous herb, moderately to well branched and grows up to 2 m tall. India is the major producer of niger in the Asian continent, where 90 kilotons of niger seeds were produced during 2011/2012 growing season[1]. The niger oil is used for cooking, lighting, anointing, painting and cleaning of machinery[2-4]. In addition to its oil, niger offers an important source of seed proteins, carbohydrates, vitamins and fiber that significantly contribute to the human dietary intake[5]. It is also used as a component of birdseed in USA and Europe and for cultural and medicinal purposes in Ethiopia[6]. Its refined oil has various industrial applications[7, 8]. Niger is suitable for low input agriculture as production is not dependent on high value inputs, such as fertilizers and herbicides. A niger-based agar medium can be used to distinguish *Cryptococcus neoformans* (Sant) Vaill, a fungus that causes a serious brain ailment, from other fungi[9]. There are reports that niger oil is used for birth control and for the treatment of syphilis[10]. The main components of the soil microbiota in most agro-ecosystems are the arbuscular mycorrhizal fungi (AMF) belonging to the recently raised new fungal phylum Glomeromycota[11]. These are the symbiotic fungi, which can form mutually beneficial symbiosis with 90% of vascular plants[12]. This symbiotic relationship was believed to have formed approximately 460 million years ago and would have important roles in establishment of plants on land[13]. Arbuscular mycorrhizal fungi are one of the most abundant organisms in the rhizosphere and the relationships can be found within a broad range of more than 200,000 species of host plants. AM fungi have been associated with improved plant growth for over 100 years[14]. They are generally known to increase the absorption and translocation of mineral nutrients from the soil to the host plant[15], to improve the tolerance of the host plant towards biotic[16] and abiotic stresses[17] and to build up the macro-porous structure of the soil that allows penetration of water as well as air and prevents erosion[18]. From all of these beneficial effects on plant performance and soil health, it is evident that AM fungi are crucial for the functioning of terrestrial ecosystems.The AMF diversity occurring over a broad range of agricultural land use intensity has, to our knowledge, not yet been investigated. It is evident that AM fungi are an important factor contributing to the maintenance of terrestrial ecosystem functioning. Studies have shown that the diversity of AM fungal populations in the soil can affect plant diversity and productivity and ecosystem stability[19]. Therefore, information on the species composition of the AM fungal community in roots and rhizosphere is important for an understanding of mycorrhizal function as well as for the effective management and preservation of the diversity of AM fungal populations in ecological studies. The aim of the present work was to analyse the diversity of AM fungal species in the rhizosphere of one of the most representative oil yielding species *Guizotia abbysinica* (L.f) Cass., which grows in representative agricultural ecosystems in eight districts of North Karnataka, South Western India.

MATERIALS AND METHODS

Study sites

North Karnataka is semi arid pleatue from 300 to 730 metres elevation in the Karnataka state of southwestern India. North Karnataka lies within the Deccan thorn scrub forests eco-region, which extends north into eastern Maharashtra. It includes the districts of Belagavi, vijayapur, Bagalkote, Bidar, Ballari, Kalaburagi, Yadagiri, Raichur, Gadag, Dharwad, Haveri, Koppal and Uttara Kannada Districts. *Guizotia abbysinica* (L.f.) Cass. (Niger) growing eight districts were selected for this study from North Karnataka namely, Dharwad (N15^{o}21'-E75^o.05'), Gadag (N15^o.41'-E75^o.61'), Koppal (N15^o.35'-E76^o.15'), Raichur (N16^o.2'-E77^o.37'), Bagalkote (N16^o.18'-E75^o.7'), Belagavi (N15^o.87'-E74^o.5'), Vijayapur (16^o.50'-75^o.43') and Yadagiri (16^o.46'-77^o.08').

Field sampling

Three sampling sites were chosen in an area measuring approximately 5 ha. Three

individual patches were selected at each site and three *Guizotia abbysinica* (L.f.) Cass., plants were randomly chosen within each patch. Rhizopsheric soil sample consisting of three bulked sub-samples (200 cm^3soil cores) randomly collected at 20–40 cm depths. Samples were stored in plastic bags at 4° C until processed. Soil chemical properties such as pH by water extraction method[20], organic matter by using wet oxidation method[21], available phosphorus (P) by using the Olsen method[22], extractable potassium (K) by using the molybdenum blue method and stannous chloride as the reducing agent and ammonium acetate as extractant[23, 24] and total soil nitrogen (N) content by using the Kjehldahl method[25] were measured. Root samples were examined for AM colonization[26].

Spore isolation and identification

AM fungal spores from the field collected soil were isolated by the wet sieving and decanting method[27], followed by sucrose centrifugation[28]. After centrifugation, the supernatant was poured through a 50µ mesh and quickly rinsed with tap water. Turgid spores (suggesting viability) were grouped under the dissecting microscope according to morphological characteristics. Diagnostic permanent slides were prepared for each different spore morphotype using polyvinyl-alcohol. After confirming the uniformity of the morphological groups under the optical microscope, the different morphotypes were identified to genus and, when possible, to species. Spore identification was based mainly on spore size, colour, wall structure and hyphal attachment[29-31]. Spore identification was corroborated by comparisons with the descriptions from the reference cultures in the INVAM (the International Culture Collection of vesicular Arbuscular mycorrhizal Fungi) and the BEG (International Bank for the Glomales).

Diversity index and concentration of dominance

AMF diversity was evaluated using the Shannon-Weiner diversity index which has two main components, evenness and number of species[32]. The Shannon index (H´) was calculated according to the formula $H' = -\sum(ni/N)\ \log_2(ni/N)$, where ni represents individuals of a species and N represents the total number of species. Concentration of dominance (C) was also measured by the Simpson's index[33] using the formula $C = \sum(ni/N)^2$, where ni and N are the same as for Shannon diversity index. Similarly Menhinick Index, Buzas and Gibson's Index, Equitability Index, Margalef Richness Index, and Berger-Parker index was calculated.

Statistical analysis

One-way analysis of variance (ANOVA) was carried out for root colonization and spore density. Statistical analyses were performed with the Statistical Package for Social Sciences version 16 (SPSS Inc., Wacker Drive, Chicago, IL). All factors were analyzed at P= 0.05.

RESULTS

Soil analyses

Results of the soil chemical analyses were depicted in table 1. The study revealed that, study sites were associated with slightly alkaline soil (pH 6.91 to 7.98). Electrical conductivity (EC) was ranged from 0.105 to 0.164. Levels of nutrients varied with each studied site were recorded (Table 1).

AM fungal spore community composition

Rhizosphere soil samples collected from various localities of *Guizotia abyssinica* (L.f.) Cass., growing areas revealed the presence of several species of AM fungi. A total of 38 AM fungal morpho-species belonging to 13 genera namely *Glomus, Acaulospora, Scutellospora, Gigaspora, Sclerocystis, Redeckera, Funneliformis, Claroideoglomus, Paraglomus, Rhizophagus, Diversispora, Quatunica* and *Ambispora* were found at the study site. *Glomus* was the genus with the highest number of species (14) followed by *Acaulospora* (7), *Gigaspora* (3), *Scutellopsora* (2), *Sclerocystis* (3), *Rhizophagus* (2), *Funneliformis* (1), *Diversispora* (1), *Claroideoglomus* (1), *Quatunica* (1), *Paraglomus* (1), Redekera (1) and *Ambispora* (1) (Table 3). AM fungal diversity of different locations showed significant difference based on Shannon diversity index (Table 2). However, the AM fungal communities showed different species compositions; only 12 out of 40 morpho-species were common to all the studied areas. Spores belonging to *Glomus*,

Acaulospora, *Gigaspora* and *Sclerocystis* were the most frequent in the present study, where as *Ambispora*, *Diversipsora*, *Quatunica* and *Paraglomus* were observed with lesser frequency. Maximum relative abundance and isolation frequency was found with *Rhizophagus fasciculatus* and *Paraglomus albidum* while, least value for relative abundance was found with *Gigaspora marginata* and isolation frequency was with many AM fungal morpho-species.Diversity indexes are varied for each study site. It showed that, Simpson index ranged from 0.06 to 0.62, Shannon index was ranged from 3.95 to 4.85 and Menhinick index ranged between 2.92 to 3.14. Similarly Equitability index was between 0.89 to 0.95, Margalef index was found between 5.19 to 6.93, Buzas and Gibson's index ranged from 0.77 to 0.85 and Berger-Parker Dominance index was ranged between 0.05 to 0.17 (Table 2).

DISCUSSION

There is significant evidence pointing to the importance of soil conditions in the control of mycorrhizal fungal communities[34]. With respect to edaphic controls of AM fungal communities, Johnson et al.[35] used different combinations of soil type and host species to demonstrate that the distribution of AM fungal species was dependent on soil type, some on host species and some on specific plant soil combinations. The higher concentration of soil nutrients particularly P could inhibit the root colonization. The fact was that nutrient availability had a much stronger effect on the root architecture and in low-resource environment; root system physiology adapted to maximize the nutrient uptake capacity and the role of the AM fungi was to increase the uptake capacity of the active zone[36]. Based on the morphological features of AM fungal spores, in the present study 38 AM fungal species were identified belonging to Glomeromycetes, Paraglome-romycetes and Archaeosporomycetes. Study revealed that, the presence of *Glomus* as predominated genus followed by those *Acaulospora* and *Gigaspora*. Similar reports have been observed by Souza et al.[37]. The dominance of specific genus of AMF in some environments can be attributed to various environmental factors such as physico-chemical properties of soil, morpho-physiological characteristics of plants, compatibility between the host and AMF species, dispersal fungi, among others[38]. *Glomus* presented the highest number of species in the Glomeromycota, which commonly shows the greatest number of species in different environments, such as in the semi-arid[39], and in the humid Amazon[40]. During the present investigation, greater variation was associated with various diversity indexes and these are useful to highlight the patterns of sporulation of AMF populations[41]. AMF diversity values recorded in this study are higher than those found in soils under agricultural management in Minnesota (H50.57–0.64)[42] and in soils under cocoa production in Venezuela (H50.6–0.78)[43], whereas high values of diversity were reported under agricultural management in Argentina (H.2.5)[44] and in unmanaged soils from Poland (H51.91–2.2)[45]. Ecological studies based solely on glomerospore morphology have been brought into question for not assessing the non-sporulating AMF. However, they do provide a basis for understanding the occurrence of AMF in different ecosystems. The morphological diversity of AMF may reflect the multi-functional role of these fungi in ecosystems, which was shown by the differences in the communities among the studied environments[46]. Taxonomic and ecological studies in natural and agricultural environments should be encouraged, including the use of molecular techniques, so that more complete data on the occurrence, diversity and dynamics of these symbionts can be obtained.

Table 1
Chemical properties of Niger growing rhizosphere soils collected from eight districts in North Karnataka.

Sl No	Places	pH	EC	OC (%)	$Caco_3$	Ca	Mg	Na	K	N	P
1	Bagalkote	7.94±0.08	0.14±0.03	0.52±0.1	4.38±0.57	37.96±0.95	7.38±1.02	0.32±0.04	0.58±0.08	1.24±0.16	1.16±0.13
2	Dharwad	7.92±0.03	0.156±0.00	0.8±0.08	7.20±0.11	42.16±0.71	11.24±0.30	0.64±0.05	1±0.10	1.09±0.05	1.38±0.03
3	Gadag	7.57±0.03	0.105±0.00	0.54±0.05	6.38±0.47	42.14±1.72	10.10±0.48	0.60±0.04	0.76±0.06	1.24±0.05	1.54±0.04
4	Vijayapur	7.26±0.08	0.106±0.00	0.48±0.04	3.74±0.11	22.66±4.06	13.34±0.55	1.08±0.10	0.52±0.04	1.74±0.04	1.02±0.02
5	Belagavi	7.82±0.03	0.164±0.00	0.92±0.05	6.36±0.12	40.26±0.42	10.8±0.20	0.30±0.35	0.60±0.03	0.76±0.04	1.10±0.02
6	Koppal	7.86±0.07	0.12±0.00	0.7±0.05	10.52±0.08	43.02±0.45	10.24±0.29	1.44±0.08	0.50±0.04	0.86±0.02	1.60±2.86
7	Raichur	7.98±0.23	0.118±0.01	0.98±0.11	5.18±0.38	34.88±0.99	12.92±0.36	0.86±0.02	1.22±0.11	0.84±0.04	1.76±0.02
8	Yadagiri	6.9±1.14	0.11±0.00	0.28±0.02	24.12±0.83	24.12±0.83	14.66±0.47	1.14±0.18	0.12±0.02	0.66±0.02	1.46±0.04

Table 2
Diversity of AM fungal communities at selected study sites in North Karnataka.

Biodiversity Indexes	[a]S1	S2	S3	S4	S5	S6	S7	S8
Simpson Index	0.62	0.12	0.08	0.08	0.06	0.06	0.06	0.06
Shannon Index	4.82	3.95	4.46	4.5	4.77	4.68	4.7	4.85
Menhinick Index	3.14	3.06	308	3.02	3.02	2.92	3.05	2.95
Buzas and Gibson's Index	0.83	0.77	0.81	0.78	0.83	0.83	0.84	0.85
Equitability Index	0.94	0.89	0.93	0.92	0.94	0.94	0.95	0.95
Margalef Richness Index	6.93	5.19	5.95	6.19	6.69	6.35	6.47	6.75
Berger- Parker Dominance Index	0.05	0.17	0.08	0.07	0.06	0.06	0.06	0.05

a : study sites refere table 1.

Table 3

Isolation frequency and relative abundance of different AM fungal species in the rhizosphere of Guizotia abyssinica growing areas of North Karnataka.

Sl no	↓AM fungal species study sites→	Relative abundance								Isolation frequency							
		[A]S1	S2	S3	S4	S5	S6	S7	S8	[A]S1	S2	S3	S4	S5	S6	S7	S8
1	*Acaulospora dentiscutata*	-	-	50.0	-	25.0	87.5	25	62.5	-	-	3.77	-	1.33	4.89	1.43	3.20
2	*Acaulospora dialata*	37.5	-	37.5	25.0	25.0	62.5	62.5	75	2.83	-	2.83	1.68	1.33	3.49	3.59	3.84
3	*Acaulospora foveata*	25.0	-	62.5	12.5	37.5	50.0	-	37.5	1.88	-	4.71	0.84	2	2.79	-	1.92
4	*Acaulospora laevis*	75.0	12.5	12.5	-	50.0	37.5	-	37.5	5.66	1.51	0.94	-	2.66	2.09	-	1.92
5	*Acaulospora scrobiculata*	-	25.0	50.0	-	75.0	75.0	87.5	87.5	-	3.03	3.77	-	4	4.19	5.03	4.48
6	*Acaulospora sp*	37.5	-	87.5	50.0	75.0	25.0	62.5	25	2.83	-	6.60	3.36	4	1.39	3.59	4.02
7	*Acaulospora spinosa*	-	12.5	75.0	75.0	-	-	62.5	62.5	-	1.51	5.66	5.04	-	-	3.59	3.2-
8	*Ambispora sp.*	12.5	37.5	62.5	87.5	-	87.5	50	-	0.94	4.54	4.71	5.88	-	4.89	2.87	-
9	*Clarideoglomus claroideum*	75.0	37.5	-	62.5	-	25.0	-	-	5.66	4.54	-	4.20	-	1.39	-	-
10	*Diversispora sp.*	87.5	-	87.5	-	-	12.5	50	62.5	6.60	-	6.60	-	-	0.69	2.87	3.20
11	*Funneliformis mosseae*	37.5	-	37.5	-	75.0	62.5	37.5	37.5	2.83	-	2.83	-	4	3.49	2.15	1.92
12	*Gigaspora marginata*	-	37.5	37.5	37.5	75.0	-	37.5	12.5	-	4.54	2.83	2.52	4	-	2.15	-.64
13	*Gigaspora nigra*	-	62.5	50.0	-	62.5	25.0	50	62.5	-	7.57	3.77	-	3.33	1.39	2.87	3.20
14	*Gigaspora rosea*	-	12.5	12.5	25.0	62.5	50.0	25	50	-	1.51	0.94	1.68	3.33	2.79	1.43	2.56
15	*Glomus aggregatum*	75.0	-	-	-	25.0	75.0	-	5-	5.66	-	-	-	1.33	4.19	-	2.56
16	*Glomus ambisporum*	62.5	-	-	87.5	-	50.0	-	-	4.71	-	-	5.88	-	2.79	-	-
17	*Glomus bagyarajii*	62.5	12.5	-	50.0	87.5	-	62.5	62.5	4.71	1.51	-	3.36	4.66	-	3.59	3.20
18	*Glomus citricola*	-	37.5	-	-	50.0	87.5	12.5	75	-	4.54	-	-	2.66	4.89	0.71	3.84
19	*Glomus constrictum*	-	-	37.5	87.5	75.0	-	-	25	-	-	2.83	5.88	4	-	-	1.28
20	*Glomus microaggregatum*	12.5	-	62.5	87.5	50.0	-	62.5	25	0.94	-	4.71	5.88	2.66	-	3.59	1.28
21	*Glomus monosporum*	62.5	-	62.5	37.5	25.0	37.5	62.5	87.5	4.71	-	4.71	2.52	1.33	2.09	3.59	4.48
22	*Glomus multicale*	87.5	62.5	-	12.5	12.5	50.0	37.5	-	6.60	7.57	-	0.84	0.66	2.79	2.15	-
23	*Glomus nicolsoni*	12.5	-	12.5	12.5	50.0	37.5	50	50	0.94	-	-.94	0.84	2.66	2.09	2.87	2.56
24	*Glomus reticulatum*	37.5	12.5	37.5	-	62.5	100	87.5	50	2.83	1.51	2.83	-	3.33	5.59	5.03	2.56
25	*Glomus sp*	25.0	25.0	50.0	-	112.5	62.5	37.5	87.5	1.88	3.03	3.77	-	6	3.49	2.15	4.48
26	*Glomus sp*	62.5	-	62.5	75.0	62.5	50.0	87.5	25	4.71	-	4.71	5.04	3.33	2.79	5.03	1.28
27	*Glomus sp*	50.0	-	-	37.5	-	25.0	-	25	3.77	-	-	2.52	-	1.39	-	1.28
28	*Gomus etunicatum*	-	50	50.0	12.5	87.5	87.5	50	75	-	6.06	3.77	-.84	4.66	4.89	2.87	3.84
29	*Paraglomus albidum*	-	100	50.0	75.0	12.5	50.0	50	87.5	-	12.12	3.77	5.04	0.66	2.79	2.87	4.48
30	*Quatunica erythropus*	50.0	-	-	-	25.0	-	62.5	-	3.77	-	-	-	1.33	-	3.59	-
31	*Redekera fulvum*	-	37.5	-	37.5	-	75.0	62.5	-	-	4.54	-	2.52	-	4.19	3.59	-
32	*Rhizophagus clarus*	75.0	-	12.5	75.0	87.5	-	75	50	5.66	-	0.94	5.04	4.67	-	4.31	2.564
33	*Rhizophagus fasciculatum*	62.5	100	25.0	-	50.0	75.0	25	62.5	4.71	12.12	1.88	-	2.66	4.19	1.43	3.20
34	*Sclerocystis dussii*	37.5	-	-	37.5	37.5	37.5	37.5	75	2.84	-	-	2.52	2	2.09	2.15	3.84
35	*Sclerocystis pubescence*	50.0	12.5	-	50.0	75.0	50.0	62.5	87.5	3.77	1.51	-	3.36	4	2.79	3.59	4.48
36	*Sclerocystis taiwanensis*	37.5	37.5	-	87.5	12.5	87.5	25	50	2.83	4.54	-	5.88	0.66	4.89	1.43	2.56
37	*Sctellospora nigra*	25.0	-	-	62.5	100	50.0	75	75	1.88	-	-	4.20	5.33	2.79	4.31	3.84
38	*Scutellospora calospora*	12.5	-	37.5	50.0	37.5	-	-	50	0.94	-	2.83	3.36	2	-	-	2.56

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