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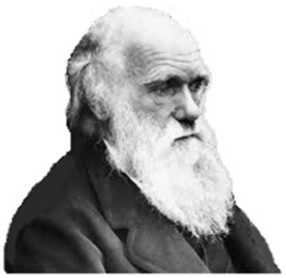

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Endomycorrhizal studies in *Curcuma aeruginosa* Roxb. of Kerala, India

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ABSTRACT

This is the first report of an ecological investigation on endomycorrhizal symbiosis in *Curcuma aeruginosa*, a medicinally and nutritionally important herb. Samples were collected from the field, where the plant grows under natural condition. AM fungal and soil fertility parameters were analysed as per standard methods. Altogether 16 different AM fungal species from seven different soil series are discovered. AM fungal species composition varied among soil series. Species richness of AM fungi was positively correlated with organic carbon content of soil. Shannon's diversity index and Simpson's index in the study area were measured. High intensity of AM fungal colonization in the roots of naturally growing *C. aeruginosa* suggests the need of proper management of the association in its cultivation practices also. Data obtained from the present investigation may help for the development of native AM fungi based biofertilizer based for a sustainable soil fertility management and cultivation of the crop.

Keywords: *Curcuma aeruginosa*, Native AM fungi, Arbuscular mycorrhiza, Ultisols

1. INTRODUCTION

Curcuma aeruginosa (*C. aeruginosa*) is an underutilized herb [1] with many medicinal and nutritional qualities [2]. *C aeruginosa* rhizomes contain 41.85% starch [3], which is used as food by many tribal and native people [4]. *C. aeruginosa* possesses antioxidant [5] and antimicrobial properties [6]. However processing and starch production from various Curcuma species in South India has declined [4] because large scale cultivation of the crop has diminished.

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Mycorrhizal status in a plant reflects the choice of specific environment by the mycorrhiza and their useful role in ecosystem [7]. The beneficial effects of AM fungi on the nutrition of agricultural plants depends on both abundance and type of fungi present in soil [8] and mycorrhization to plant is highly dependent on soil conditions [9]. Therefore, field surveys are necessary, not only to understand the abundance and type of indigenous AM fungi present in the rhizosphere of crop plants [10], but also to understand the ecology and local adaptations [11] of AM fungal species present in soils where the plant grows. Even though arbuscular mycorrhizal (AM) associations of certain Curcuma species are known [12], no reports are available about the naturally associated AM fungi of *C. aeruginosa* in its field conditions.

The objectives of the study were: (1) identification native AM fungi associated with the roots of *C. aeruginosa* from its natural growing conditions, (2) analysis of various AM fungal parameters such as spore density and root colonization in relation to soil fertility parameters, and (3) categorization of *C. aeruginosa* growing soils according to Unites States Department of Agriculture (USDA) system.

2. MATERIALS AND METHODS

2. 1. Area of study

The present study was conducted in Wayanad, Kozhikode, Kollam and Thiruvananthapuram districts of Kerala. Sampling sites were selected at random. 12 samples were collected from the different localities (**Fig. 1**), which were designated as S1 to S12. Coordinates of each sampling site were also noted by using GPS.

2. 2. Sampling procedure

Soil samples from rhizosphere zone of *C. aeruginosa* plants were collected. The whole plant was uprooted from the soil for soil and root collection. About 500 g soil each was collected from three different locations of a sampling site and mixed together to make a composite sample. 1 kg of the composite sample was collected in labelled plastic bag for laboratory studies. The fine, healthy lateral roots collected from the three locations of a site were put together in a labelled bottle, fixed in 50 mL of 10% potassium hydroxide (KOH) for assessment of percentage colonization.

2. 3. Spore identification and counting

The soil samples were analyzed for AM fungal spores, following wet sieving and decanting procedure [13]. In the laboratory, 100 gram field soil was added to 500 mL of distilled water in a beaker and mixed thoroughly. It was then filtered through soil sieve. The sieve was then washed with distilled water and the filtrate was then made up to 25 mL to represent 100 g soil in 25 mL. 1 mLwas then pipetted out in parallel lines on to a 9 cm filter paper disc placed in a Petri dish. The filter paper in wet condition was immediately observed under the microscope for identification of the spore and counting of spores of each species. The spore count was multiplied upward to represent spore density per 100 g soil. Ten plates were prepared from one soil sample and the data were represented as average. Intact and crushed spores were mounted on slides in water and examined under the microscope. Spores were also mounted on slides in polyvinyl-lactoglycerol (PVLG) and PVLG mixed with Melzer's reagent and examined under microscope. Spores were identified to species level based on spore colour, size,

surface ornamentation and wall structure based on the system proposed by Hall and Fish [15] Schenk and Perez [16] and original species descriptions of Redecker *et al*. [17] and Schüßler and Walker [18], as well as new online classification available at INVAM [19].

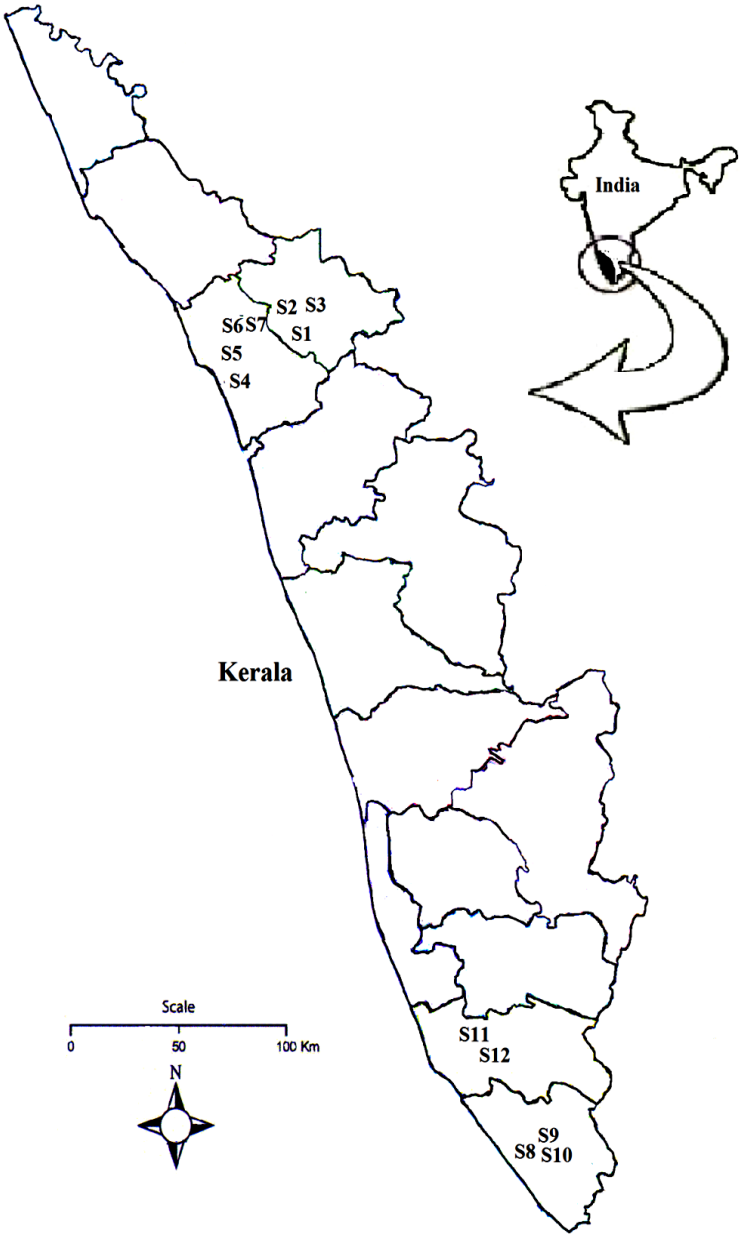

Figure 1. Study area showing sampling sites (S1 to S12)

2. 4. Study of Percent colonization

Estimation of mycorrhizal colonization of the root system was done after clearing and staining the roots [20]. The roots from each site were cut into pieces of 1cm length and boiled in 10% KOH and washed with distilled water. After washing, the root segments were

neutralized with 10% hydrochloric acid (HCl). The cleared root segments were stained with 0.05% trypan blue in lactophenol and left overnight. The root segments were then destained using lactophenol and examined for AM fungal colonization. 100 root segments were examined for mycorrhizal infection. Presences of mycelium, arbuscule or vesicle were taken as the indication of colonization. The results were expressed as percent colonization.

2. 5. Diversity indices

Various Ecological parameters of diversity of diversity calculated in the study are as follows. Isolation frequency, the percentage of soil samples, where a species or a genus occurred. Relative abundance is the percentage of the spore number of a species or a genus. Importance value is calculated as (IF + RA)/2. Shannon diversity index (H') = $-\Sigma$ *Pi* ln *Pi,* where Pi = ni/N, 'ni' is the spore numbers of a species and N is the total number of identified spore samples. Evenness (E) = H'/H'max, where H'max = lnS; S is the total number of identified species. Simpson's diversity index, DS = Σ [ni(ni – 1)/N(N – 1)], where ni is the spore numbers of a species, and N is the total number of identified spore samples. Gini-Simpson index of diversity (DG_S), where $DG_S = 1 - D_S$. Isolation frequency (IF), relative abundance (RA), **i**mportance value (IV), spore density, species richness, Shannon–Wiener index (H'), evenness (E) and Simpson's index (D_S) were carried out, as per Chen *et al*. [21] and Gini-Simpson index (1–D_S) was measured, as per Stiling [22].

2. 6. Soil analyses

Soil pH was measured (PCSTestr 35 Multi-parameter) in water (1:2.5). Micro-diffusion method [23] was employed for quantifying soil available nitrogen (SAN). Estimation of soil available phosphorus (SAP) was done as per Bray and Kurtz Method [24] using Spectrophotometer Hitachi U5100. Soil available potassium (SAK) was estimated by Flame Photometer (Systronics 128) method, as per Jackson [25]. Total organic carbon (TOC) of the soil was estimated as per Walkley and Black [26]. Taxonomy of soils was identified as per USDA system (based on the GPS coordinates measured at the time of sample collection) from already existing relevant documents on such soils [27].

2. 7. Statistics

Pearsons correlations was carried out to check the inter-relations between various AM fungal and soil fertility parameters. All the analysis including descriptive statistics was carried out using IBM SPSS software version 19.

3. RESULTS

3. 1. AM species composition and diversity

AM fungal associates of *C. aeruginosa* in the study area belonged to 8 genera (Acaulospora, Claroideoglomus, Funneliformis, Glomus, Sclerocystis, Dentiscutata, Ambispora and Gigaspora,) and 16 species (*A. capsicula, A. scrobiculata A. bireticulata, Acaulospora* sp, *Claroideoglomus etunicatum, Funneliformis mosseae, F. badium, Glomus australe, Glomus* sp1, *Glomus* sp2, *Sclerocystis rubiformis, S. clavisporum, Dentiscutata heterogama, Ambispora leptoticha, Gigaspora desipens, Gigaspora rosea*).

Among the 8 genera of AM fungi, Acaulospora represented most number of species (4 species) followed by Glomus (3 species). Species richness in sampling sites varied from 3 (S12) to 8 (S4). Among the different genera identified during the study, highest relative abundance (RA) was shown by Funneliformis followed by Acaulospora and the lowest by Ambispora (**Table 1**), whereas highest RA by a species was shown by *F. mosseae* followed by *A. scrobiculata* and lowest by *A. capsicula.* The highest isolation frequency (IF) was shown by two species of Funneliformis viz. *F. badium* and *F. mosseae* and the lowest IF by five different species such as *A. capsicula, A. sp, D. heterogama, Ambispora leptoticha* and *Gigaspora rosea.* The highest and the lowest importance value (IV) were shown by *F. mosseae* and *A. capsicula*, respectively.

Table 1. Diversity measurements of AM fungal associates under the study area

AM fungi	RA	IF	IV
Acaulospora	33.20	91.67	62.44
A. capsicula	0.022	8.33	4.18
A. sp	0.133	8.33	4.23
A. bireticulata	11.01	50	30.50
A.scrobiculata	22.04	75	48.52
Claroideoglomus	3.92	33.33	18.63
Claroideoglomus etunicatum	3.92	41.67	22.80
Funneliformis	46.40	100	73.20
F. badium	12.89	75	43.95
F. mosseae	33.51	75	54.26
Glomus	1.44	41.67	21.55
Glomus australe	0.089	8.33	4.21
Glomus sp1	0.886	16.67	8.78
Glomus sp2	0.465	16.67	8.57
Sclerocystis	14.46	75	44.73
S. rubiformis	9.70	41.67	25.69
S. clavisporum	4.76	41.67	23.21
Dentiscutata	0.16	8.33	4.24

Dentiscutata heterogama	0.16	8.33	4.24
Ambispora	0.04	8.33	4.19
Ambispora leptoticha	0.04	8.33	4.19
Gigaspora	0.38	16.67	8.52
G. desipens	0.33	16.67	8.50
G. rosea	0.04	8.33	4.19

Shannon-Wiener index, Simpson index, Gini-Simpson index and Evenness observed in the study area were 1.82, 0.20, 0.79, and 0.66, respectively.

3. 2. AM fungal spore density and root colonization

Total spore density under the study area (**Table 2**) ranged from 450 (S1) to 1595 (S6) per 100 g soil. Variation in total spore density over different sampling sites were significant ($P<0.05$). The lowest individual spore density (2.5 per 100 g soil) was observed for *Acaulospora capsicula* which was observed only in one sample (S1). The highest individual spore density (787.5 per 100 g soil) was shown by *F. mosseae* (S6). AM fungal root colonization ranged from 76% (S9) to 93% (S11).

Table 2. AM fungal and soil fertility characteristics. Values are mean ± SE of mean

Sample	Spore density (100^{-1} g soil)	% root colonization	SAN (Kg ha^{-1})	SAP (Kg ha^{-1})	SAK (Kg ha^{-1})	TOC (g kg^{-1})	pH (1:2.5)
S1	450±29.81	80.00±00	395.23±12.45	8.73±0.76	123.89±2.29	11.87±0.35	5.48±0.03
S2	1505±64.96	90.00±00	235.2±18.11	10.82±1.21	210.58±10.38	5.8±0.12	5.53±0.03
S3	550±28.87	85.00±00	193.4±13.83	15.59±0.71	194.12±33.66	4.47±0.67	5.06±0.18
S4	582.5±22.99	81.00±00	250.9±39.46	10.13±0.31	169.43±3.37	17.3±0.21	4.84±0.01
S5	580±30	85.00±00	203.84±23.95	11.06±1.14	224.1±3.86	6.8±0.40	5.18±0.2
S6	1595±11.67	91.00±00	224.75±13.83	19.08±1.47	196.57±7.43	6.2±0.3	4.99±0.09
S7	1143±80.88	79.00±00	188.16±15.68	12.92±1.34	236.85±10.13	2.83±0.22	4.96±0.06
S8	932.5±107.2	82.00±00	203.84±15.68	7.4±0.26	200.95±4.18	2.5±0.17	5.24±0.11
S9	1530±148	76.00±00	203.84±9.05	11.3±0.21	151±8.31	1.8±0.1	5.87±0.1

S10	1135±131.8	89.00±00	256.11±5.23	10.48±0.38	122.74±3.79	2.57±0.15	4.89±0.05
S11	627.5±41.07	93.00±00	240.43±13.83	8.86±0.31	116.38±3.34	2.93±0.09	5.86±0.15
S12	752.5±74.2	79.00±00	282.24±23.95	9.79±0.19	140.31±3.49	1.73±0.12	5.23±0.11

3. 3. Soil fertility characteristics

Results of various soil fertility parameters analysed are given in **Table 2**. SAN ranged from 188.16 (S7) to 395.23 kg ha^{-1} (S1), SAP from 7.4 (S8) to 19.8 kg ha^{-1} (S6), SAK from 116.38 (S11) to 236.85 kg ha^{-1} (S7), TOC from 1.73 (S12) to 17.3 g kg^{-1} (S4) and pH from 4.84 (S4) to 5.87 (S9). All the soil fertility parameters (SAN, SAP, SAK, TOC and pH) were significantly different ($P<0.05$) over sampling sites.

3. 4. Interrelationship among AM fungal and soil fertility parameters

Correlation analysis done in the study is given in **Table 3**. The AM fungal species richness and the soil organic carbon showed a strong positive correlation. SAN was negatively correlated to SAP. No other significant interrelationship was observed among the studied parameters.

Table 3. Correlation studies between AM fungal and soil fertility characteristics

	SD	**COL%**	**SR**	**SAN**	**SAP**	**SAK**	**TOC**	**pH**
SD	1							
%COL	.141	1						
SR	–.084	–.035	1					
SAN	–.373	–.110	.411	1				
SAP	.412	.258	.011	–.369	1			
SAK	.251	–.050	-.021	–.638*	.400	1		
TOC	–.393	–.084	.854**	.437	–.048	–.018	1	
pH	.104	.009	–.356	.145	–.373	–.374	–.290	1

SD - spore density, COL% - % colonization, SR - species richness, SAN - soil available nitrogen, SAP - soil available phosphorus, SAK - soil available potassium, TOC - total organic carbon. *Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level.

3. 5. Diversity of *C. aeruginosa* growing soils

C. aeruginosa growing soils identified from the study area are given in **Table 4**. Altogether 7 soil series were identified; all of them belonged to the order Ultisols.

Table 4. Diversity of soil under the study area

Soil order	Soil series and Soil subgroup	Sample number and site	AM fungi
Ultisols	Meppady Typic Kandiustults	S1, S3 Wayanad	*Acaulospora capsicula, Acaulospora species, Claroideoglomus etunicatum, Funneliformis badium, Glomus australe, Glomus* sp1, *Funneliformis mosseae*
Ultisols	Mananthavady Typic Kandihumults	S2 Wayanad	*Acaulospora scrobiculata, A. bireticulata, Dentiscutata heterogama, Funneliformis badium, Sclerocystis rubiformis*
Ultisols	Nanminda Typic Plinthustults	S4, S5 Kozhikode	*Acaulospora scrobiculata, A. bireticulata, Claroideoglomus etunicatum, Funneliformis badium, Funneliformis mosseae, Glomus* sp2, *Gigaspora decipiens, G. rosea, S. clavisporum*
Ultisols	Kunnamangalam Typic Kanhaplohumults	S6, S7 Kozhikode	*Acaulospora scrobiculata, Claroideoglomus etunicatum, Funneliformis badium, Funneliformis mosseae, Glomus* sp1, *Sclerocystis rubiformis, S. clavisporum*
Ultisols	Nedumangad Ustic Kanhaplohumults	S8, S9, S10 Thiruvananthapuram	*Acaulospora scrobiculata , A. bireticulata, Claroideoglomus etunicatum, Funneliformis badium, Funneliformis mosseae, Glomus* sp1, *Glomus* sp2, *Gigaspora decipiens, Sclerocystis rubiformis, S. clavisporum*
Ultisols	Ummannoor Typic Plinthustults	S11 Kollam	*Acaulospora scrobiculata, Funneliformis badium, Sclerocystis rubiformis, S. clavisporum*
Ultisols	Karavaloor Typic Plinthohumults	S12 Kollam	*Acaulospora scrobiculata, Funneliformis mosseae, Sclerocystis rubiformis*

Spore density, AM fungal colonization and all other soil fertility characters were also analysed at the level of each soil series. It may be noted that the AM fungal spore density, soil pH, SAK, SAP and TOC were significantly different over soil series.

4. CONCLUSION

AM fungal composition in the rhizosphere soil of a plant species is an important determinant of plant growth. The frequent and dominant occurrence of *F. mosseae*, *F. badium*, *A. bireticulata* and *A. scrobiculata* observed in the present study indicates their good adaptation to the host plant and soil types. AM fungi identified in the present study may be helpful in better cultivation of *C. aeruginosa* in a sustainable way. Moreover, in-depth field investigations and experiments are needed to understand adaptations of AM fungal population in relation to various soil types.

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