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Isolation, Identification and Evaluation of AMF Isolated From Rhizosphere Soils of Cassava Growing Tracts

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ABSTRACT

A study on diversity and density of various AMF strains of cassava, crop in Pathanamthitta district of Kerala was conducted. Rhizhosphere soil samples from 20 locations coming under three types of soil (laterite, forest and alluvial) were analysed. The dominating genera in the all soil type were Glomus which is present in cassava rhizhosphere associated worldwide. Analysis of morphological characters of the spores revealed that isolates are coming under three genera namely Glomus, Aculospora and Gigaspora. On evaluation of soil AMF isolates for the potentiality and effectiveness G. *mossae* from alluvial soils of Pullupara, *G. fasciculatum* from laterite soils of Vallikode and *G. fasciculatum* from laterite soils of Pullad had influenced the growth attributes and nutrient concentration of sorghum.

Key Words: Soil, Fungi, Inoculation, Spore density.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) form symbiotic association with most of the cultivated crop plants and they help plants in phosphorus nutrition and protecting them against biotic and abiotic stresses (Nanjundappa *et al*,2019) The AMF found in the rhizosphere of several vascular plants has got important role in sustainable agriculture and agroecosystem management. The beneficial effect of indigenous AMF in nutrition of crop plants depends on, both the abundance and type of fungi present in the soil (Abbot and Robson,1991). AM fungi are considered as one of the predominant components of soil microflora, form symbiotic association and are known for enhancing plant growth (Harikumar and Potty, 2002). The plant growth is generally improved when mycorrhizal fungi colonize the root system because of more efficient nutrient uptake. The plant-acquired carbon is traded for various mycorrhizal benefits in the host plant (Rai, 2006). AM fungal hyphae exclusively colonize the root cortex and form highly branched structures inside the cells called arbuscules, which are considered as the functional site of nutrient exchange (Balestrini *et al,* 2015). The extent to which crop relies on AMF association to achieve maximum growth or yield, depends on crop species, varieties, levels of available soil nutrients particularly, P and Zn.

Cassava is known to be highly mycotrophic and its response to phosphate fertiliser application seems to depend upon the mycorrhizal association. The beneficial effect of mycorrhizae is of special importance for those plants having coarse and poorly branched root system, since mycorrhizae hyphae can extend up to 8cm away from the root region (Potty, 1990). Olugbemi (2016) reported that optimum cassava tuber yield obtained significantly higher with mycorrhizal inoculation compared to other treatments without AMF. The AM fungi diversity in cassava rhizhosphere of the area is not yet explored hence a study was required to understand the abundance and type of indigenous AMF present in the rhizosphere of the cassava and comparison on the efficacy ,growth attributes and nutrient concentration in jowar was conducted.

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Table 1. List of Soil AMF status of cassava rhizosphere soil of PTA.

Location No	Soil Type	Spore load/ g of soil	Place	GPS coordinates	
1	Forest soil	112	Kottanad	9^0 40’74.2” N	76^0.73’24.4” E
2	Forest soil	85	Perupatty	9^0 39’96.4” N	76^0.73’87.1” E
3	Alluvial soil	106	Mallapally	9^0 44’84.8” N	76^0.66’72.4” E
4	Laterite soil	Nil	Kunnamthanam	9^0 43’50.0” N	76^0.60’06.1” E
5	Laterite soil	64	Kaviyoor	9^0 40’02.4” N	76^0.61’26.0”E
6	Laterite soil	83	Kallopara	9^0 41’11.4” N	76^0.62’64.5” E
7	Laterite soil	71	Eraviperoor	9^0 37’57.1” N	76^0.61’49.1” E
8	Laterite soil	89	Pullad	9^0 36’74.7” N	76^0.67’42.8” E
9	Laterite soil	107	Puramattom	9^0 38’41.5” N	76^0.65’51.3” E
10	Laterite soil	126	Nellimala	$9^0$36’68.4” N	76^0.64’32.3” E
11	Laterite soil	61	Kuravankuzhy	9^0 36’96.9” N	76^0.67’58.5” E
12	Laterite soil	123	Valakuzhy	9^0 40’48.1” N	76^0.68’67.8” E
13	Laterite soil	52	Theadical	9^0 38’87.2” N	76^0.73’50.4” E
14	Alluvial soil	53	Pullupara	9^0 38’03.0” N	76^0.76’51.5” E
15	Alluvial soil	66	Pazhavagady	9^0 38’80.4” N	76^0.80’61.0” E
16	Laterite soil	83	Elanthoor	9^0 28’75.1” N	76^0.73’33.6” E
17	Laterite soil	107	Chennirkara	9^0 23’98.5” N	76^0.72’85.1” E
18	Alluvial soil	81	Vallikodu	9^0 22’49.6” N	76^0 .76’78.5” E
19	Laterite soil	52	Kulanada	9^0 25’56.1” N	76^0.63’09.2” E
20	Laterite soil	113	Mezhuvely	9^0 20’52.3” N	76^0.56’72.7” E

MATERIALS AND METHODS

Twenty soil samples were collected from the cassava growing tracts of Pathanamthitta (PTA) district, Kerala in three different soil types such as forest, laterite, and alluvial soil. Rhizosphere soil and root samples were collected up to a depth of 20cm with a quantity of 500g of soil from each location by pooling the soil collected from different sites of a single location. The spore density was determined by wet sieving and decanting method (Gerdemann and Nicholson, 1963) and spore density was expressed in number per gram of soil. The spore type was observed under stereo and trinocular research microscope to identify the spore types on the basis of morphological features like size, shape, surface characteristics and nature of hyphal attachment following the identification key of Schenck and Peres (1990).The isolated AMF were compared with six standard mycorrhizal cultures received from Kerala Agriculture University (KAU) for their growth enhancing potential and effectiveness. A pot culture experiment was undertaken in Completely Randomised Design (CRD) with 26 treatments and two replication using sorghum as the trap crop. Sorghum was grown for a period of 30 d and there after it has been harvested to record the biometric observations and plant analysis for nutrient concentration. The observations were recorded at 30 days after planting (DAP) for height of plant, fresh and dry weight of plant,fresh and dry weight of roots and nutrient concentration of plants.

The leaf, stem and root/tuber obtained were analysed for N by modified Microkjeldhal method

Table 2. Evaluation and ranking of soil AMF isolates based on their influence on growth attributes of sorghum.

Soil AMF isolate No	Plant height at harvest (cm)	Ranking	Fresh weight of plant (g)	Ranking	Fresh weight of root (g)	Ranking	Dry weight of plant (g)	Ranking	Dry weight of root (g)	Ranking
1	18.25	23	47.30	18	127.85	24	13.75	18	17.60	21
2	21.25	19	50.00	15	168.48	10	16.60	10	21.81	5
3	28.00	7	60.30	5	169.85	8	18.80	3	20.20	9
4	27.95	8	59.10	7	180.65	4	17.80	6	22.90	4
5	30.55	4	58.60	8	134.50	19	17.00	9	19.00	15
6	22.70	16	46.45	19	112.50	26	12.83	23	15.10	26
7	23.55	14	56.90	11	140.50	18	16.45	11	18.65	19
8	32.00	1	75.90	1	195.70	3	19.65	1	25.20	2
9	29.25	5	61.55	4	168.30	11	16.25	13	18.70	18
10	24.30	13	51.70	12	161.70	13	13.35	21	18.91	16
11	25.25	12	48.20	17	145.80	16	16.30	12	18.80	17
12	18.95	22	42.60	23	168.90	9	13.35	22	19.25	13
13	16.90	26	39.20	25	150.40	15	11.35	26	18.35	20
14	31.25	2	65.30	3	210.20	1	18.70	4	24.60	3
15	18.10	25	40.45	24	119.30	25	12..6	24	16.80	24
16	18.25	24	37.90	26	129.50	21	12.30	25	17.40	22
17	21.15	20	46.25	20	164.50	12	13.50	20	19.40	12
18	30.75	3	73.95	2	198.90	2	19.30	2	27.40	1
19	23.05	15	45.60	21	128.70	23	14.50	17	16.40	25
20	19.75	21	44.20	22	129.20	22	13.70	19	17.35	23
21	25.90	11	50.70	14	179.10	5	15.20	16	21.05	6
22	27.50	9	60.10	6	169.90	7	17.25	8	20.70	8
23	22.60	17	51.55	13	158.30	14	17.90	5	19.50	11
24	26.40	10	49.50	16	130.40	20	15.40	15	19.05	14
25	28.95	6	58.00	9	178.60	6	15.90	14	20.90	7
26	22.20	18	58.00	10	142.90	17	17.60	7	19.80	10
CD (0.05)	4.10		13.18		17.81		2.95		2.11	

Table 3. Evaluation and Ranking of Soil AMF isolates based on their influence on nutrient concentration of sorghum .

Soil AMF isolate No	N (%)	Ranking	P (%)	Ranking	K (%)	Ranking	Zn (ppm)	Ranking	Cu (ppm)	Ranking	Fe (ppm)	Ranking
1	0.81	20	0.11	25	0.50	25	68.95	15	46.27	16	4766.50	8
2	0.80	21	0.16	18	0.80	21	50.15	26	35.70	24	1345.00	26
3	0.77	23	0.14	20	0.77	13	83.20	8	35.70	25	1390.50	24
4	0.83	17	0.06	26	0.83	20	71.65	13	63.40	7	2416.00	21
5	0.93	9	0.17	13	0.93	3	52.85	24	45.95	18	7555.00	4
6	1.08	6	0.48	4	1.08	8	58.45	21	50.45	14	4514.00	10
7	0.88	12	0.13	22	0.88	14	72.05	12	76.10	2	4594.00	9
8	2.14	1	1.02	1	2.14	2	89.20	5	66.40	6	6662.00	5
9	1.17	5	0.14	21	1.17	1	81.75	10	34.10	26	1807.50	23
10	0.83	16	0.13	23	1.92	6	89.60	3	46.10	26	1358.00	25
11	0.78	22	0.16	15	1.77	9	52.30	25	41.05	21	3915.50	14
12	0.81	19	0.37	6	1.35	16	61.50	19	54.45	13	3715.00	15
13	1.05	7	0.18	12	2.18	5	86.80	6	59.55	10	6333.00	6
14	1.45	4	0.40	5	1.62	11	67.50	16	49.65	15	3453.00	16
15	0.82	18	0.16	16	0.59	24	57.55	22	45.30	19	2241.50	22
16	0.68	26	0.16	17	1.22	18	82.60	9	75.55	3	7850.00	3
17	0.89	10	0.29	7	0.68	23	73.85	11	55.35	12	8440.00	1
18	1.45	3	0.90	2	2.28	4	91.55	2	71.45	4	4200.00	12
19	0.87	13	0.28	8	0.39	26	89.40	4	78.50	1	2625.00	19
20	0.74	25	0.20	11	0.80	21	70.55	14	62.85	9	2784.00	17
21	1.02	8	0.15	19	1.02	8	61.60	18	39.70	23	2720.00	18
22	0.85	14	0.21	9	0.85	14	58.85	20	43.40	20	3990.50	13
23	0.76	24	0.17	14	0.76	24	98.05	1	63.25	8	2506.00	20
24	0.88	11	0.21	10	0.88	11	85.05	7	57.35	11	8283.00	2
25	0.84	15	0.12	24	0.84	15	54.50	23	40.70	22	5603.50	7
26	1.60	2	0.61	3	0.84	15	64.40	17	66.55	5	4283.50	11
CD (0.05)	0.57		0.29		0.89		19.34		24.49		4101419.10	

(Jackson, 1973), total P by Vanodomolydo phosphoric yellow colour method (Jackson, 1973) and total K and Ca by Flame photometric method (Jackson, 1973).

RESULTS AND DISCUSSION

The analysis of rhizosphere soil sample to find out diversity of AMF showed that isolates were belonging to 3 genera. Among the genus Glomus was the dominant one. Two species were identified and among the identified *G. fasciculatum* showed dominance. The highest spore density was observed in laterite soil (50 to 123nos/g) followed by Alluvial soil (53-113nos/g) and forest soil (85-112 nos/g). The nutrient absorption efficiency of the AMF isolates was evaluated in comparison with standard AMF isolates. *G fasciculatum* (No8), *G fasciculatum* (No18) and *G mossase* (No14),showed relatively higher impact on growth attributes of Sorghum at 30DAP.Such AMF isolates had increased the plant height, fresh dry weight of roots (Table 2). The cassava plants surveyed in different soil types and sampling sites showed in variation in spore density and root infestation between soil types might be due to this edaphic factor like soil pH, moisture content, soil organic content and available phosphorus (Udaiyan *et al*,1996, Weber and Claus, 2000 ; Johnson *et al,* 1991). The variability of spore density between soil isolates again depends on physio-chemical and biological properties of soil, season and age of plants. (Gemma *et al*, 1989)

The diversity and adaptation of AMF is governed by the AMF physiology and genetics and the response to host plants and surroundings (Allen *et al*, 1995). The diversity of species distribution observed in the present study is due to the ability of cassava to associate under different species of different species of AMF dominatingly in various soil types like forest, laterite and alluvial soils. These results were in concurrence with the findings of Harikumar (1997) and Serry *et al* (2016) in tuber crops. The results had brought to the notice that most of the AM fungal spores associated with rhizosphere soils of cassava belong to the genus Glomus in the root zone indicates either the influences of soil or host plants (Nasim and Rajwa,2005).It has also been reported that continues cultivation of same crop for a longer period in the same soil might have reduced the incidence of other genera ,encouraging the occurrence of Glomus alone (Siverding, 1991). The results were in agreement with the findings of Iqbal and Nair (1991) The dominance of Glomus in the rhizosphere may be due to the preferential association of AMF species to host plants (Hartnett and Wilson,1999: Bever, 2002: Bouamri, *et al*,2006).

On evaluation of 29 soil AMF isolates for its potential, the soil AMF isolate No14 coming under *G. mossae* isolated from the alluvial soils of Pullupara (location No14), soil AMF isolate NO18 coming under *G. fasciculatum* isolated from laterite soils of Vallikode (location No 18) and soil AMF isolate NO8 coming under *,* isolated from laterite soils of Pullad (location No 8) had preferably influenced the growth attributes of sorghum and increased the nutrient concentration of N,P,K ,Fe, Zn and Cu showing the efficiency and effectiveness of mycorrhizal colonisation. Hence, these three cultures were selected for further inoculation studies in cassava for growth parameters ,tuber production and qualitative characters at different levels of P nutrition.

CONCLUSION

It can be concluded from the study that genus Glomus was dominating in all types of soil coming under the cassava growing tracts of Pathanamthitta. Among the species of Glomus, *G. mossae* from alluvial soils of Pullupara (AMF culture No1), *G. fasciculatum* from laterite soils of Vallikode (AMF culture No2) and *G. fasciculatum* from laterite soils of Pullad (AMF culture No3) were found to be effective and potential. Hence it can be selected and evaluated for inoculation studies in cassava for growth, tuber yield.

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