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Variation in Arbuscular Mycorrhizal Fungi and Phosphatase Activity Associated with *Sida cardifolia* in Karnataka

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Abstract: An investigation was carried out to study the AM fungal association in *Sida cardifolia* from different parts of Karnataka. AM colonization in the roots, spore load and Phosphatase activity in the root zone soil collected from different parts of Karnataka were determined. AM root colonization was more in the roots of *Sida cardifolia* collected from Kolar and least in case of root samples collected from Tumkur. Soil collected from Kolar had a highest spore density and least spore density was observed in case of soils collected from Mandya. Alkaline phosphatase activity of the rhizosphere soils collected from Tumkur was more compared to other four locations. Acid phosphatase activity was high in Kolar soils. Phosphatase activity was much related to AM fungal activity. There were 64 isolates comprising 15 species of AM fungi .Important genera of AM fungi are *Glomus*, *Acaulospora* and *Scutellospora* .Genus *Glomus* was found to be more dominant in all the locations. *Acaulospora lacunosa, Glomus melanosporus, Glomus fulvum* and *Glomus fasciculatum* were the dominant species prevalent. AM fungal diversity in the rhizosphere soils of *Sida cardifolia* was highest in Hassan and least in case of Bangalore.

Key words: AM fungi · *Sida cardifolia* · Diversity · Root colonization

INTRODUCTION

Sida cardifolia an important medicinal plant, also known as country mellow, belongs to the family Malvaceae. This normally grows in waste lands of Karnataka.The presence of medicinally important alkaloids like ephedrine, pseudoephidrine, vascicinol, vasicinone and vascine present in seeds, leaf and roots gave prominence to this plant. The plant in natural habitat forms association with large number of microorganisms; one among them is AM fungi. AM fungi are an important component in the soil microbial mass which regulates several essential biological processes at the plant soil interface. The majority of higher green plants and large number of AM fungi are involved in mycorrhiza formation [1]. They regulate the abundance of plant community [2]. They help in improved uptake of diffusion limited macro and micro nutrients, increased tolerance to biotic and abiotic stresses, beneficial alterations of plant growth regulators in synergistic interactions with beneficial rhizosphere microorganisms [3]. It has been observed variation in AM fungal diversity with the change in plant species [4]. Plants of a particular family are colonized by specific type of AM fungi, a few AM fungal genera were found only in the plants of a particular family. However there may be considerable variation in AM fungal species colonizing the same plant in different geographical locations. Hence this investigation was under taken to study AM fungi in rhizosphere soils of *Sida cardifolia* in different locations of Karnataka.

MATERIALS AND METHODS

Five sampling sites were chosen in five different districts of Karnataka (Bangalore, Tumkur, Kolar, Hassan and Mandya). Five root zone soil samples were collected along with fine roots of *Sida cardifolia* from each of the five sampling sites and brought to the laboratory. pH of the soil was measured with a glass electrode (1:2.5 soil: water ratio) as per the procedure given by Jackson [5]. Available phosphorus content in the soil samples was measured by the method given by Watanable and Olson [6]. Acid and Alkaline Phosphatase activities were estimated as per the procedure given by Eivazi and Tabatabi [7].

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Staining of root segments was carried out as per the procedure proposed by Phillips and Hayman [8]. The percent mycorrhizal root colonization was determined following the gridline intersect method proposed by Giovannetti and Mosse [9]. Soil samples (50g) collected from the root zone soil was subjected to wet sieving and decantation as outlined by Gerdmann and Nicolson [10] to estimate the AM fungal spore density.

Identification of AM fungi was done by host baiting technique or trap pot culturing. The composite samples obtained from the sampling points are brought to the laboratory; the test soil samples were mixed with sterile sand and soil mix (1:1) and planted with trap plant sorghum. After 3-4 months, the potting mix was wet sieved and the spores were observed under microscope. Morphologically similar spores were picked, surface sterilized with aqueous solution containing 200ppm streptomycin sulphate and 2% chloromine T. and were mounted on a glass slide in lacto-glycerol. The spores were identified with the help of "Manual for Identification of VA mycorrhizal fungi" by Schenk and Perez [11]. The data were statistically analyzed by M stat. The diversity index was determined by following Shanon-weaver diversity index.

RESULTS

AM fungal colonization, spore density, acid and alkaline phosphatase activity in *Sida cardifolia* along with available phosphorus in rhizosphere soil and soil pH are shown in Table 1.

AM fungal colonization ranges between 26.80 to 68.00 percent. Highest percent root colonization (68) was observed in root samples collected from Kolar followed by Bangalore (52), Mandya(50.40) and Hassan(49.80). A least of 26.80 percent was observed in root samples of Tumkur. A M spore density per 50 gram of soil was significantly more in root zone soils collected from Kolar (194) followed by Bangalore (136.8), Tumkur (132.6), Hassan (121.8) and least in case of root Samples of Mandya (120). A total of 64 isolates of AM fungi were recorded from five locations of Karnataka. A total of 15 species of AM fungi were found in five locations of Karnataka (Table 2). Highest number of spores being 15 in Bangalore followed by 12 in Hassan, 11 in Mandya,13 in Kolar and 13 in Tumkur. AM fungal species abundance was measured in all the five locations (Table 3) .The maximum number of spore were of *Glomus claroideum* (46 spore 50^{-1}gsoil) and a

Table 1: A M fungal colonization, spore density, available 'P', pH, acid and alkaline phosphatase activity in rhizosphere soil of *Sida cardifolia* in different locations of Karnataka

Location	Percent rootcolonization	Spore density 50-1g soil	Acid Phosphatase Activity (µg/h/g of soil)	Alkaline Phosphatase Activity (µg/h/g of soil	Available 'P' (mg/g of soil)	pH of soil
Hassan	49.80	121.80	59.24	57.20	0.29	6.22
Tumkur	26.80	132.60	61.68	84.20	0.30	7.02
Bangalore	52.00	136.80	41.17	80.67	0.19	6.60
Kolar	68.00	194.00	62.33	58.68	0.33	5.43
Mandya	50.40	120.00	43.27	52.71	0.25	6.35
LSD(p>0.05)	13.18	51.14	25.53	25.65	0.11	0.48

Table 2: A M fungal species distribution in rhizosphere soils of *Sida cardifolia* from different places of Karnataka

	Locations				
AM fungal species	Hassan	Mandya	Kolar	Tumkur	Bangalore
Acaulospora lacunosa	+	+	+	+	+
Acaulospora mellea	+	_	+	+	+
Acaulospora nicolsonii	+	_	_	+	+
Acaulospora spinosa	+	+	+	+	+
Glomus albidus	+	+	+	_	+
Glomus aggregatum	+	+	+	_	+
Glomus borealis	+	+	+	_	+
Glomus claroideum	+	+	_	+	+
Glomus citricolum	_	+	+	+	+
Glomus fasciculatum	+	+	+	+	+
Glomus fulvum	+	+	+	+	+
Glomus geosporum	_	_	+	+	+
Glomus occultum	_	_	+	+	+
Glomus melanosporum	+	+	+	+	+
Scutellospora calospora	+	+	+	+	+
Total species	11	11	13	12	15

Table 3: Abundance of spores of different A M fungal species in the rhizosphere of Sida cardifolia

	Hassan		Mandya		Kolar		Tumkur		Bangalore	
A M fungal species	No. of spores (50-1g soil)	Percent abundance	No. of spores (50-1g soil)	Percent abundance	No. of spores (50-1g soil)	Percent abundance	No. of spores (50-1g soil)	Percent abundance	No. of spores (50-1g soil)	Percent abundanc
A. lacunosa	30	15.63	20	18.52	82	35.34	20	8.13	112	48.70
A. mellea	3	1.56	ND	_	5	2.15	4	1.63	5	2.17
A. nicolsonii	2	1.04	ND	_	ND	_	20	8.13	2	0.87
A. spinosa	11	5.73	10	9.26	3	1.30	3	1.22	6	2.61
G. aggregatum	30	15.63	13	12.04	6	2.58	ND	_	11	4.78
G. albidus	5	2.60	5	4.63	4	1.72	ND	_	2	0.87
G. borealis	5	2.60	7	6.48	8	3.45	ND	_	18	7.83
G. citricolum	ND	_	7	6.48	8	3.45	2	0.81	3	1.30
G. clareoideum	46	23.98	6	5.55	ND	_	47	19.10	15	6.52
G. fasciculatum	20	10.42	23	21.30	30	12.93	23	9.35	13	5.65
G. fulvum	10	5.21	10	9.26	60	25.86	100	40.65	20	8.70
G. geosporum	ND	_	ND	_	4	1.72	3	1.22	4	1.74
G. occultum	ND		ND		3	1.30	12	4.88	7	3.04
G. melanosprum	15	7.81	4	3.70	11	4.74	10	4.06	6	2.61
S. calospora	15	7.81	3	2.78	8	3.45	2	0.81	6	2.61

Note: A- Acaulospora, G- Glomus, S-Scutellospora, ND-not detected

Table 4: A M fungal species diversity of Sida cardifolia in different locations of Karnataka

Location	No. of AM fungalspecies	Shanon-weaverDiversity index
Hassan	12	3.19
Mandya	11	3.11
Kolar	13	2.75
Tumkur	12	2.66
Bangalore	15	2.64

least count of *Acaulospora nicolsonii* (2 spores 50^{-1} g soil) were found in Hassan. Their percent spore abundance was 23.98 and 1.04, respectively. The spores of *Glomus fasciculatum* was maximum (23 spores 50^{-1} g soil) and *Scutellospora calospora* was least (3 spores 50^{-1} g soil) in Mandya soils. Their percent spore abundance was 21.30 and 2.78 respectively. Maximum number of *Acaulospora lacunosa* spores (82 spores 50^{-1} g soil) were found in Kolar samples and a least of *Glomus occultum* (3 spores 50^{-1} g soil) seen in the same sample. Their percent spore abundance was 35.34 and 1.30 respectively. In Tumkur samples *Glomus fulvum* was more abundant (100 spores 50^{-1}g soil) and least was *Scutellospora calospora* (2 spores 50^{-1} g soils) and their percent spore abundance was 40.65 and 0.8 respectively. *Acaulospora lacunosa* spores were more abundant in Bangalore samples (112 spores 50^{-1} g soils) and *Acaulospora nicolsonii* was the least (2 spores 50^{-1} g soil) their percent abundance was 48.7 and 0.87 respectively.

Acaulospora lacunosa, *Acaulospora spinosa Glomus melanosporum*, *Glomus fulvum*, *Glomus fasciculatum*, *Scutellospora calospora* were distributed in all the locations where as, *Acaulospora mellea, Glomus citricolum Glomus albidus*, *Glomus boreale, Glomus claroideum*, *Glomus aggregatum* were distributed in four locations and others, *Acaulospora nicolsonii*, *Glomus occultum* and *Glomus geosporum* were distributed in only three locations. The diversity index for AM Fungal species in different locations is given in Table 4. Shanon-Weaver diversity index of AM fungi was more in Hassan (3.19) followed by Mandya (3.11), Kolar (2.75), Tumkur (2.66) and least in Bangalore (2.64).

DISCUSSION

The variation in AM fungal colonization pattern within the same species in different location was earlier noticed by Lakshmipathy *et al.* [12] in their study with medicinal plants in Karnataka. In the present study also there was a variation in AM root colonization in different locations of Karnataka. The variation of AM fungal root colonization in different locations could be due to the change in the habitat, environmental factors, soil fertility and acclimatization of a particular AM fungal genus/species to a particular location [13].This study also reveals that AM Fungal colonization pattern is related to soil pH and available phosphorous in the soil. AM Fungal colonization was high in slightly acidic soils compared to

the neutral and alkaline soils. The root zone soil with high available P supported good AM colonization. Koide [14] had observed a hindrance in colonization of AM fungi at very low (<3 ppm) and high (>9 ppm) phosphorus content of soil will hinder colonization of AM fungi. According to Sumana [15], the acid phosphatase activity increases with increased colonization by AM fungi. AMF colonization is known to alter the inherent phosphorous supply by increasing the Phosphatase activity in the rhizosphere [2].

In this study it was observed that there were variations in AM Fungal spore density. In support of these findings studies, conducted in different locations of natural systems have also been reported. Pincone [16] recorded more sporulation by AM fungi in pastures compared to forests. Further, spore densities in pastures were found to be more compared to secondary forests [17]. Sieverding and Leihner [18] reported that combination of graminaceous and leguminous crops generally increase mycorrhizal population. The other plants associated with *Sida cardifolia* in the natural habitats may influence the variations in spore density.

Genus *Glomus* was more abundant compared to *Acaulospora* and *Scutellospora* among these three genera *Acaulospora lacunosa*, *Glomus melanosporum*, *Glomus fulvum* and *Glomus fasciculatum* were more abundant and present in all the five locations. The variation in spore abundance of different AM fungi in different locations was observed in earlier studies. Schenk and Kinloch [19] observed the dominance of species in *Gigaspora margarita*, *Gigaspora gigantea* and *Gigaspora gregaria* in Soya bean fields while *Glomus fasciculatum* and *Glomus clarum* in bahia grass and *Acaulospora* in cotton and peanut fields. The same results were obtained by Lakshmipathy *et al*., [20] in cashew plantations where *Glomus etunicatum* was most abundant. Vijayalakshmi and Rao[21] working with mycorrhizal association in ten members of Asteraceace and seven Amaranthaceace growing in different locations having different soil types observed variation in mycorrhizal association and they found that *Glomus mosseae* and *Glomus macrocarpum* were dominant. The plant community composition is likely to affect mycorrhizal fungi [22,23]. Baylis [24] observed that Arbuscular mycorrhizal populations readily respond to mycotrophic plants in a community.

In this study variation in Shanon-Weaver diversity index of AM fungal species in rhizosphere soils of *Sida cardifolia* in different locations of Karnataka was noticed. This kind of diversity was noticed earlier. Oehl *et al.* [25] in their study recorded variation in diversity index in different land use types, a maximum diversity index being in grasslands compared to cultivated fields. However Johnson and Wedin [26] did not find any significant variation in diversity due to change in land use types. The variation in AM fungal diversity in rhizosphere soil of a particular plant in different locations may be due to the influence of adjoining plants [27,28]. However the qualitative and quantitative differences in diversity and other parameters like pH, available phosphorous and phosphatase activity with regard to AM fungal association with *Sida cardifolia* was observed.

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