British Microbiology Research Journal
12(4): 1-8, 2016, Article no.BMRJ.21766
ISSN: 2231-0886, NLM ID: 101608140

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Acacia senegal (L.) Wild. Associates with a Diversity of Beneficial Micro-symbionts in the Arid and Semi-arid Lands of Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study and selected the study sites. Authors JMK and JMM collected samples from the selected sites and coordinated all sample analysis and statistical analyses. Author JMM tabulated the analyzed data. However, the author JMK reorganized data into its current status, wrote the first draft of the manuscript and managed all literature searches. Author DWO provided advisory role oversaw final paper shape up. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/21766
Editor(s):
(1) Raul Rodriguez-Herrera, Autonomous University of Coahuila, Mexico.
Reviewers:
(1) Mohammad Arif, Kansas State University, Manhattan, USA.
(2) Dennis Miller, University of Missouri, USA.
(3) Marcela Bianchessi da Cunha-Santino, Universidade Federal de Sao Carlos, Brazil.
(4) Mohammed Suleiman, Umaru Musa Yar'adua University, Katsina, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/12979

Original Research Article

Received 2nd September 2015
Accepted 18th November 2015
Published 15th January 2016

ABSTRACT

Aims: To determine the populations and diversity of beneficial microsymbionts (rhizobia and mycorrhiza) which associates with *Acacia senegal* varieties at selected sites in semi-arid areas of Kenya.
Place and Duration of Study: Kenya Forestry Research Institute (KEFRI) Biotechnology Laboratories and selected semi-arid sites of Kenya, between 2009 and 2010.
Methodology: We estimated rhizobia populations, identified mycorhiza abundance and diversity and estimated plant growth of *A. senegal* plants grown in soils collected from the selected semi-arid sites.
Results: Rhizobia populations were generally low, below 30 cells.g^{-1} soil, in most of the sites but were relatively higher in areas with high forest cover such as Kimalel (559 cells.g^{-1} soil) and

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Ntumburi (104 cells.g^{-1} soil). Seven mycorrhizae species were identified in the selected sites and all the species were represented in all selected sites except Gigaspora spp which was totally absent in Baringo and poorly represented in all sites. *Glomus etunicata* and *Glomus intra* were the most abundant mychorrhizal species, and were most abundant in Baringo, at Kimalel (76.7% and 58.3%, respectively) and Rimoi (54.7% and 44.7%, respectively). The same species were also abundant at Daaba (26.3% and 55.7%, respectively) in Isiolo. In overall, mychorhiza were most abundant in Baringo, where Kimalel had in overall highest numbers (20.2%), followed by Isiolo where Daaba had in overall highest mychorrhizal number (13.8%) and finally Kajiado, where Kajiado sub-site had higher mycorhizal number (4.8%) compared to the Namanga sub-site (3.3%). It was established that mycorrhiza survived in harsher conditions (Daaba) than rhizobia.

Conclusions: We concluded that drylands of Kenya have low rhizobia populations, implying need for rhizobia inoculation to enhance rhizobia benefits in *A. senegal* tree species. We also concluded that the drylands have diverse and abundant mycorrhiza species which vary across sites, and which can be utilized for enhanced mycorrhizal benefits.

Keywords: Acacia senegal; forest cover; mycorrhiza; rhizobia; semi-arid areas; Kenya.

1. INTRODUCTION

Acacia senegal (L.) Wild. is a tree native to Africa and Asia and widely distributed in arid and semi-arid areas (ASALs) of Kenya. It has characteristic three prickles of up to 0.5 cm long, the centre one sharply curved, the other two more or less straight and directed forward, flowers are white and borne in spikes while Pods are papery and dehiscent [1]. In the ASALs of Kenya three varieties have been identified; variety senegal, variety kerensis and variety leiorhachis [2]. Variety senegal grows into a distinct tree form usually with a single, grey, rough stem and a flat crown. In Kenya it is found in West Pokot, Kajiado, Kitui, Baringo, Kibwezi, Isiolo and Nakuru. Unlike other varieties is found in both humid and semi-arid areas at 1200 m above sea level.

Variety Kerensis is commonly low branched with many upright twigs, the crown eventually flattened, umbrella-shaped. Bark pale brown to pale grey, smooth in young individuals, brown scaly on the older parts, slash mottled red and white, yellow rough-pealing bark, commonly growing in multiple stems from one point. It is usually found growing on rocky limestone hills, sandy plains 400-1130 m above sea level with 300-550 mm rainfall. In Kenya the variety is found at Turkana, Samburu, Isiolo, Marsabit, Wajir, Garissa, and Mandera. Variety kerensis is the major gum-arabic producing species in Kenya. Variety Leorachis commonly found growing in association with variety kerensis [1,2].

Acacia senegal has multiple uses which includes; firewood, charcoal, poles, posts, tool handles, medicine (roots, bark), fodder (pods and leaves), dyes, bee forage, soil conservation and stabilization, fiber, and gum-arabic production [1,3,4]. Despite its multiple uses *A. senegal* is slow-growing but research has shown that its growth can be enhanced by inoculation of rhizobia and mycorrhiza (microsymbionts) either singly or in combination [5-9]. However, in Kenya, there is limited information on rhizobia populations, and mycorrhiza abundance and diversity that associate with *A. senegal* tree species in the wild, and which can be utilized to maximize benefits from the tree species. Therefore this study sought to establish rhizobia populations, and diversity and abundance of mycorrhiza associated with *A. senegal* in selected ASALs sites of Kenya.

2. MATERIALS AND METHODS

2.1 Site Selection

Three major sites were selected at Isiolo, Baringo and Kajiado in the ASALs of Kenya and at each site 4, 3 and 2 sub-sites, respectively, were selected (Table 1). At Isiolo, the sub-sites selected included Ngare Ndare, Daaba, Kulamawe and Ntumburi, while at Baringo the sub-sites included Rimoi, Solit and Kimale, and Namanga and Kajiado were the sub-sites selected for the Namanga site (Table 1).

2.2 Soil Sample Collection

In each sub-site representative soil samples were collected and analyzed for nutrients, micro-symbionts (rhizobia populations and mychrhizal diversity) and growth of *A. senegal* senegal during rhizobia population assessment. All soil samples for microbial and nutrient analyses were

Table 1. Selected sites and their geographical positioning in the ASALs of Kenya

Site	Senegal variety	Latitude	Longitude	Altitude (m)
Isiolo				
Ngare Ndare	Kerensis	0 29.697 N	37 22.728 E	1038
Daaba	Kerensis	0 32.310 N	37 22.728 E	934
Kula Mawe	Leiorhachis	0 34.362 N	38 10.762 E	750
Ntumburi	Senegal	0 11 907 N	37 30.999 E	1731
Baringo				
Rimoi	Senegal	0 39.861 N	35 34.210 E	1152
Solit	Senegal	0 25.640 N	35 53.103 E	1283
Kimalel	Kerensis	0 28.271 N	35 55.109 E	1272
Kajiado				
Namanga	Senegal	2 19.803 S	36 49.772 E	1452
Kajiado	Senegal	2 53.263 S	36 45.543 E	1741

collected using the same protocol in all selected sites. During soil collection, three sampling points in a diagonal transect across the sampling plot were identified and marked. These points were at the centre and at two edges of the diagonal transect across a square plot measuring 100 m x 100 m. Three trees were selected at each soil collection point and from each tree soil was collected by auguring at 30 cm from the tree base. The soil cores were pooled, mixed and sub samples taken for chemical and microbial analysis.

2.3 Soil Analysis and Plant Growth Assessment

Soils collected from the selected sites were analyzed for pH and macro elements as described by [10]. *A. senegal* seeds were obtained from Sultan Hamud, 100 km east of Nairobi. Growth of plants grown for the most probable number (MPN) assessment was determined by measuring plant heights, shoot and root biomass of all plants grown on soils collected from selected study sites. Dry Shoot and root biomass from the MPN experiment were assessed after drying partitioned plant parts at 80℃ for 72 hours [11].

2.4 Rhizobia Enumeration

Rhizobia enumeration was estimated using most probable number (MPN) method [12]. Briefly, clean, undamaged *A. senegal* seeds were sorted by hand, nipped, sterilized in hydrogen peroxide and soaked in sterile hot water overnight. The seeds were pregerminated in water agar and pricked onto sterilized vermiculite Leonard Jar assembly. The experiment was conducted in a greenhouse for 8 weeks, after which nodule infection was assessed [11,13].

2.5 Mycorrhiza Assesment

Soils collected from the selected sites were stored in cool boxes and stored at 4℃ immediately after arriving in the laboratory. Mycorrhiza spores were isolated from 100 g of soil by wet sieving and decanting method followed by sucrose centrifugation [14]. After centrifugation the supernatant was sieved to pass through 50-µm-pore-size mesh and immediately rinsed with clean tap water. Trapped spores were counted with a Doncaster dish placed under a dissecting microscope. For species identification and abundance estimation, the spores were grouped according to their morphological characteristics and identified to genus level and species levels. Spore identification was based on colour, wall structure size, and hyphal attachment [15]. Spore relative abundance in each site were calculated as (nj/Nj)100, where nj= number of spores that belong to species j and Nj= total number of spores in the site [16]. The data obtained in the study were analysed using GenStat for windows.

3. RESULTS AND DISCUSSION

3.1 Soil Analysis and Plant Growth Assessment

Results on soil analysis revealed that most of the soils collected from Isiolo and Baringo had a pH range of between 6.2 and 7.2, thus lying between neutral and weak acid. However, soils from Kajiado site (Namanga and Kajiado) were truly acidic having a pH of between 5.6 and 5.8. Organic carbon, nitrogen, available phosphorus and potassium were generally higher at Isiolo and Kajiado compared to Baringo site (Table 2).

Table 2. Soil chemical analysis of soils collected from selected dryland sites

Site	pH ($CaCl_2$)	Carbon (%)	Nitrogen (%)	Available phosphorus (ppm)	K (ppm)
Isiolo					
Ngare Ndare	6.6	1.5	0.16	41	225
Daaba	7.2	3.3	0.28	41	977
Kula Mawe	6.7	2.0	0.42	31	599
Ntumburi	6.3	1.5	0.36	99	594
Mean	**6.7**	**2.1**	**0.31**	**53**	**599**
Baringo					
Rimoi	6.2	1.0	0.16	41	225
Solit	7.2	0.3	0.1	2	86
Kimalel	6.5	1.5	0.38	20	378
Mean	**6.6**	**0.9**	**0.21**	**21**	**230**
Kajiado					
Namanga	5.6	1.2	0.32	65	328
Kajiado	5.8	1.4	0.31	14	515
Mean	**5.7**	**1.3**	**0.32**	**40**	**422**
SEM	0.2022	0.2284	0.064	7.07	59.7

SEM= Standard error of means

Most soils in semi-arid environments have about neutral pH because of low rainfall amounts and low organic matter presence in these areas. This is most probably why most soils from the study sites had about neutral pH. However, soils from Kajiado were acidic most probably because the sites are located within the lava flow plains near Mt. Kilimanjaro hence the acidic nature of the soils most probably due to the acidic nature of parent rock material (volcanic) of the study sites. In addition the relatively higher organic carbon, nitrogen, available phosphorus and potassium at most sub-sites in Isiolo and Kajiado were most probably because these sub-sites were noted to have relatively more forest cover which most probably added organic matter to the soils and hence nutrients especially nitrogen and phosphorus (Table 2).

Results obtained on plant growth assessment revealed a relatively higher but insignificant height of plants grown on soils from Isiolo compared to those grown on soils from Baringo and Kajiado sites. Further, with respect to plant biomass, soils collected from the same Isiolo site also resulted to relatively higher shoot and root biomass compared to soils collected from the other two sites (Table 1). The observed plant growth coincided with high nutrient contents in the selected sites and hence high organic matter in the sites. Organic matter is known to enhance plant growth [17,18] through enhanced soil nutrients.

3.2 Rhizobia Enumeration

Results on rhizobia cell estimates revealed a generally low rhizobia count, below 30 cells.g^{-1} soil, in majority of the study sites (Fig. 1). Lowest rhizobia counts were recorded in Daaba (0 cells.g^{-1} soil), Kula Mawe (5 cells.g^{-1} soil) and Ngare Ndarae (8 cells.g^{-1} soil), in the Isiolo site. However, significantly ($p<0.05$) higher rhizobia counts were recorded in Kimalel (559 cells.g^{-1} soil) and Ntumburi (104 cells.g^{-1} soil) (Fig. 1).

Results on rhizobia cell estimates revealed a generally low rhizobia count, below 30 cells.g^{-1} soil, in majority of the study sites (Fig. 1). Lowest rhizobia counts were recorded in Daaba (0 cells.g^{-1} soil), Kula Mawe (5 cells.g^{-1} soil) and Ngare Ndarae (8 cells.g^{-1} soil), in the Isiolo site. During soil collection it was noted that Daaba was very hot, stony, no surface cover and had scattered trees and thus most probably rhizobia failed to survive in the harsh environmental conditions. Other studies have shown generally low rhizobia counts in semi-arid environments [11,13]. Significantly ($p<0.05$) higher rhizobia counts were recorded in Kimalel (559 cells.g^{-1} soil) and Ntumburi (104 cells.g^{-1} soil) (Fig. 1). These two sites had been observed to have relatively higher vegetation cover than the other selected sites implying that rhizobia in the sites could have benefited from tree shading and liter fall which could have contributed to soil organic matter. Organic matter is known to increase rhizobia populations [11,19] due to enhanced soil nutrients.

Table 3. Plant heights (cm), shoot and root biomass (Mg.plant^{-1}), and root to shoot ratios of plants grown for most probable number (MPN) assessment on soils collected from selected dryland sites

Site	Plant height (cm)	Shoot biomass (mg)	Root biomass (mg)	Root to shoot ratio
Isiolo				
Ngare Ndare	11	12.4	6.8	0.5
Daaba	8.7	12.4	7.2	0.9
Kula Mawe	8.1	10.1	8.6	0.9
Ntumburi	9.7	13.0	8.3	0.6
Mean	**9.4**	**12**	**7.7**	**0.7**
Baringo				
Rimoi	8.3	9.2	8.5	0.9
Solit	8.9	9.4	6.8	0.7
Kimalel	8.8	10.5	5.1	0.5
Mean	**8.7**	**9.7**	**6.8**	**0.7**
Kajiado				
Namanga	8	11.4	8.7	0.8
Kajiado	7.9	9.1	5.8	0.6
Mean	**8**	**10.3**	**7.3**	**0.7**
SED	1.4	2.1	2.5	0.2

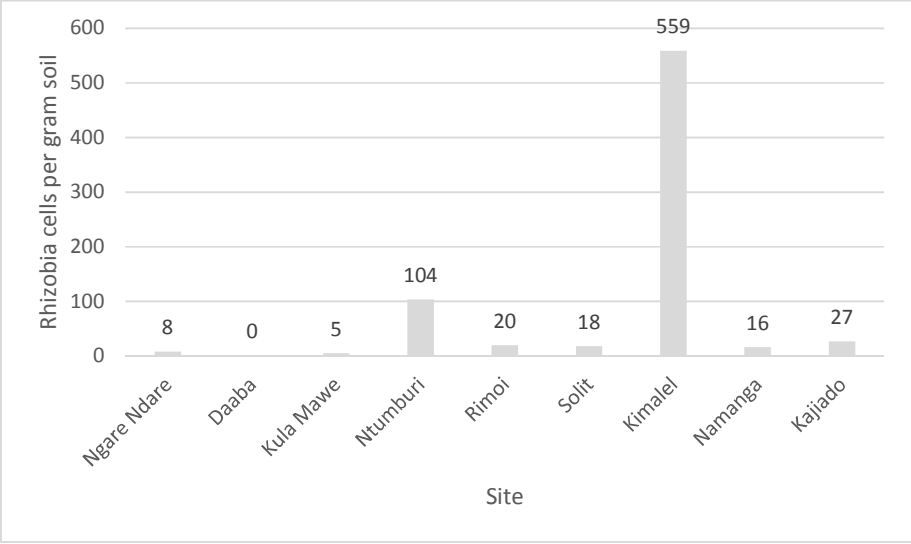

Fig. 1. Rhizobia count (cells.g^{-1} soil) in soils collected from selected dryland sites

3.3 Myocorrhiza Assessment

Results on mycorhizae species identification revealed presence of seven mycorrhizae species in the selected sites (Table 4). All the seven species were represented in all three sites except Gigaspora spp which was totally absent in Baringo and poorly represented in all sites. *Glomus etunicata* and *Glomus intra* were the most abundant mychorrhizal species, and were most abundant in Baringo, where they were most abundant in Kimalel (76.7% and 58.3%, respectively) and Rimoi (54.7% and 44.7%, respectively). The same species were also abundant at Daaba (26.3% and 55.7%, respectively) in Isiolo. In overall, mychorhiza were most abundant in Baringo, where Kimalel had in overall highest numbers (20.2%), followed by Isiolo where Daaba had in overall highest mychorrhizal number (13.8%) and finally Kajiado, where Kajiado sub-site had higher mycorhizal number (4.8%) compared to the Namanga sub-site (3.3%) (Table 4).

Table 4. Mycorrhizal diversity and abundance (%) in selected dryland sites of Kenya

Sites/AM spp	*Acaulospora*	*Glomus etunicatum*	*G. intraradices*	Gigaspora spp.	*Scutellospora nigra*	S. calospora	*S. verrucosa*	Sub-site means
ISIOLO								
Ngare Ndare	1	6	5	0	1	2	2.7	2.5
Daaba	1.3	26.3	55.7	1.3	2	2.7	7	13.8
Kulamawe	0	1.7	1.7	0	2.3	0.7	1	1.1
Ntumburi	1.3	8.3	12.7	0.3	1.7	2	3.7	4.3
Species mean	0.9	10.6	18.8	0.4	1.8	1.9	7.2	5.4
BARINGO								
Rimoi	1	54.7	44.7	0	1.7	2.3	3	15.3
Solit	1	3	3.7	0	1.7	1.7	2.3	1.9
Kimalel	1.3	76.7	58.3	0	0	2.3	2.7	20.2
Species mean	1.1	44.8	35.6	0	1.1	2.1	2.7	12.5
KAJIADO								
Namanga	1.7	5	3.3	0.3	0	1.3	1.3	1.9
Kajiado	1	17.7	7.7	0	4	1.3	1.7	4.8
Species mean	1.4	11.4	5.5	0.2	2	1.3	1.5	3.3
Grant Mean	1.1	22.1	21.4	0.2	1.6	1.8	2.8	
P value	ns	*	*	*	*	Ns	ns	
SED.	0.4	12.5	18.9	0.3	1	0.9	1.9	

These observations on mycorrhizal species distribution most probably indicated that *A. senegal* associates with a diversity of mycorrhizal species in the ASALs of Kenya and most commonly with Glomus sp, as observe in the selected study sites. Similar results were obtained by [20] in drylands of Jordan where a Glomus species was the commonest mycorrhizal species appearing in 85% of the soils sampled in the dryland sites that were studied. The results further indicated that the mycorrhizal association varied with site and increases with vegetation cover, due to presence of plants to provide roots for mycorrhizal colonization. Thus the more the plants the higher the colonization [21]. Further, it was clear that mycorrhizae were abundant at Daaba where rhizobia was absent most probably indicating that mycorrhiza are more resistant to harsh conditions than rhizobia, because Daaba site was very stony, hot and almost devoid of vegetation.

4. CONCLUSIONS

From the results obtained in this study it can be concluded that majority of the selected ASALs sites had generally low rhizobia count, below 30 cells.g^{-1} soil implying a need to inoculate the soils to boot rhizobia populations for enhanced nitrogen fixation. Further, the results indicated that the selected ASALs sites had diverse mycorrhizal species including *Acaulospora, Glomus etunicatum, G. intraradices, Gigaspora spp. Scutellospora nigra, S calospora, S. verrucosa* but their abudance varied from site to site. However, Glomus ssp were the most abundant mycorrhizal species in overall.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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