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Bio-Role of *Acacia karroo* in Nitrogen Fixation at Different Locations of North-West Egypt Region

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Abstract: It is well known, that *Acacia* species can forms symbioses with both Rhizobium and bradirhizobium. Thus, the present study was carried out at two different locations, Sadat city and Bourg El Arab city (North West of Egypt) to determine the effect of pod-collection site and crown class on nodulation, nitrogen fixation and growth response of seedlings to inoculation with Rhizobium strains; and the nitrogen-fixation potential of seedlings. Two crown classes (dominant crown and intermediate crown) were selected for pod collection. Three Rhizobia strains were used for inoculation; two native strains (AKR and LER) and one introduced (NGR234). Data recorded included pod characteristics significantly differed with locations. Results obtained clearly obvious that, pods from the Bourg El-Arab site were superior to those from Sadat site and the dominant crown class produced better pods in two seasons than the intermediate crown class. Roots of *Acacia karroo* seedlings were made to nodulate by the native Rhizobium strains (AKR and LER); the foreign strain (NGR234) was not able to compete with the native ones. The AKR caused significant differences in number of nodules, fresh and dry weights of nodules, roots and the stem length of the two study seasons. It also exhibited the highest nitrogenase activity in both seasons.

Key words: *Acacia karroo* seedlings · Rhizobia strains · Nodulation · Nitrogen fixation · Nitrogenase activity

INTRODUCTION

Acacia karroo is an important multipurpose tree with a wide distribution; from Egypt and Mauritania southwards to South Africa and eastwards to India (Asia). It is an important fast-growing tree in the northwest coast of Egypt and Sinai. *Acacia karroo* and *A. nilotica* grow as part of several different agroforestry systems in the arid and semi-arid zones, pasture lands. *Acacia karroo* is a source of various products including wood fuel and charcoal, fodder, timber and tannin [1]. Multiple uses, high ability to fix nitrogen and thrive well in dry climates have given acacias high flexibility to grow in agroforestry system under dry land conditions [2,3]. In the arid and semi-arid areas where *A. karroo* seedling growth and establishment are fast, it is desirable to enhance seedling survival. In cases where tree species form effective nodules only with specific *Rhizobium* strains, inoculation of seedlings with an effective strain may improve seedling growth [4, 5].

Based on Their association with Rhizobia, African acacias are divided into three groups as slow-growing, *Bradyrhizobium* sp., viz., *Faidherbia* (*Acacia*) *albida*, fast-growing Rhizobia, viz., *Acacia nilotica* subsp. *tomentosa, A. karroo* and *A. tortilis* var *raddiana* and fast-slow-growing Rhizobia [6]. Both the fast and slow-growing native Rhizobia strains from Australian acacias were isolated from the same plant [7,8], while the effective cross inoculation amongst *Faidherbia albida* and Acacia *nilotica* isolates were studied and reported by Sutherland [9].

Acacia species is known to form symbioses with both Rhizobia and bradyrhizobia [6]. NGR234 was the only fast-growing strain among 30 isolates prepared from *Lablab purpureus* nodules in Papua New Guinea (soil pH 8.5) [10,11]. NGR234 nodulated 28 of 53 species of subfamily Mimosoideae. NGR234 strain nodulated hosts such as *Albizia* spp., *Enterolobium contortisiliquum* and *Desmanthus illinoensis*, all of which are thought to have symbiotic preferences for slow-growing bradyrhizobia

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[12]. Interestingly, almost all the species of the *Acacia* subgenus *Heterophyllum*, *A. auriculiformis, A. cyanophylla, A. mangium, A. mearnsii, A. pendula, A. retionodes* and *A. saligna* are Australian and nodulate with NGR234, although inoculation with NGR234 gave Fix + nodules with four *Acacia* spp. (*A. auriculiformis, A. pendula, A. retinodes* and *A. saligna*). *Acacia Karroo* has indeterminate nodules by *Rhizobium* NGR234 [13]. The present study was undertaken to highlight to (a) the effect of crown classes on nodulation and nitrogen fixation, (b) growth response to inoculation and the nitrogen-fixation potential of *A. karroo*.

MATERIALS AND METHODS

This study was carried out in a greenhouse of the Forestry and Wood Technology Department at the Experimental station of Faculty of Agriculture, Alexandria University, Egypt at Abies, during two successive seasons (2007-2009) using two collected pods from two different locations.

Pod Collection and Tree Crown Classes: Pods of *Acacia karroo* were collected from trees planted in two different locations, *i.e.* from Sadat City (30°22 N, 30°27 E) and from Bourg El-Arab City (30°92 N, 29°68 E) North West of Egypt. Physical and chemical characteristics of soils at two sites are given in Table 1. Based on height and diameter of trees, three trees of *Acacia karroo* were chosen for collection of pods from two crown classes, the dominant and the intermediate crown; each tree was labeled properly. The pods were collected from all the branches around the stem at breast height (BH).

Pods Characteristic: Three samples of ten pods from each tree and crown class were collected for measuring pod length, seeds per pod, seed weight and germination percentage.

Bacteriological Studies: Rhizobia were isolated from root-nodules of *Leucaena leucocephala* (LER) and *Acacia karroo* (AKR) seedlings grown in the field of the experimental station of Faculty of Agriculture at Abies using the method of vincent. Each isolate was streaked on Yeast Extract Manitol agar (YEM) for LER and AKR Rhizobia and on Trypton Yeast (TY) for NGR234 Rhizobium strain, incubated at 28°C and checked daily for colony development. The growing isolates were maintained on (YEM) or (TY) slants at 4°C and-20°C in YEM or TY broth containing 20% (v/v) glycerol and stored at-80°C for further processing. The Rhizobium strain NGR234 was obtained from Faculty of Sciences, Dept. of Plant Biology, University of Geneva, Switzerland.

Seedling Production and Incubation with Rhizobia: In order to prevent inhibitory effect of seed coat on germination, seeds of *Acacia karroo* were surface-sterilized and treated with concentrated H_2SO_4 for 30 min. and then washed with distilled water several times until all traces of acid were removed. The seeds were soaked in water for two days and germinated in mixture of sand and vermiculite 1:1(V/V) in the greenhouse maintained at day /night temperatures of approximately 25°C. Two months after germination, the seedlings were transplanted to pots of 25 cm diameter containing approximately 10000-11000g soil each. The pots were watered manually with tap water. Seedlings growing in pots were inoculcated with 20 ml of Rhizobia strains (LER, AKR and NGR234) grown in YEM and TY broth incubated for 3 days with shaking (200 rpm) at 28°C. A total of nine seedlings were used to test the infectivity and symbiotic efficiency (nodulation) and placed in greenhouse. After 8 weeks form incubation with Rhizobia strains, the seedlings were harvested and checked for root-nodule formation. The number of nodules per plant, fresh and dry weights of nodules, roots and stem and the seedling height were determined.

Nitrogen Fixation Assays: Nitrogen-fixation activity in root nodules was determined by C_2H_2 reduction assay (ARA) to measure the nitrogenase activity of root-nodules, using gas chromatogram (Carlo ERBA strumentazione). Incubation of nodulated roots was done in three replicates at 25°C in 50 ml rubber-capped vials containing C_2H_2 (10% V/V) in air [14]. The injected bottles were incubated for zero, 30 and 60 minutes.

Table 1: Physical and chemical analyses of soils

Location	Particle size distribution (%) sand	silt	clay	Soil texture	pH	EC (mmhos/cm)	Soluble cations (meq/100g soil) Na^+	K^+	Ca^{++}	Mg^{++}	Soluble anions (meq/100g soil) HCO_3	CO_3	SO_4--	Cl^-
Bourg El-Arab	45	34	25	Sandy loam clay	8.5	2.6	2.33	0.06	0.37	0.40	0.09	--	2.53	0.43
Sadat City	74	14	12	Sandy loam	8.0	3.6	5.03	0.03	0.60	1.06	1.52	--	4.73	0.80

Statistical Analysis: Analysis of variance was carried out according to factorial experiments in CRD design [15]. Mean values of pod characteristics and seedling parameters were compared using least significant difference method at 0.05 level of probability (L.S.D) [16].

RESULTS

Effect of Seed-collection Site and Tree-Crown Class on Pod Characteristics: Data obtained in two seasons and presented in Table 2 do not demonstrate any significant differences between the pod length in both seasons, while the seed number per pod, seed weight and germination percentage differed with collection site, crown class and their interaction. Pod characteristics, except seed germination, were not affected by the collection site, tree-crown class and their interaction. Pod length, seed number per pod and seed weight showed slight increase at Bourg El-Arab location as compared with Sadat location. Seed germination was significantly affected by locations, the germination percentage being higher (80%) at Bourg El-Arab than at Sadat city (66.6%). The differences were sharp between tree-crown classes in both seasons of study. Values for various pod characteristics under dominant-crown category were distinctly higher than for the intermediate-crown in the first season, but in the second season the intermediate-crown category showed slightly higher values for seed weight and seed germination. (Table 2).

Influenced of Crown Class and Rhizobia Strain on Seedling Characters: Data presented in Table 3 indicated that seedling parameters varied with the two crown classes. No significant differences were found between the number of nodules, nodules fresh and dry weight and the fresh and dry weight of root and the stem crown classes and their interaction, while the root dry weight and stem length showed highly significant differences with crown classes, Rhizobia strains and their interaction in the first season. On the other hand, the picture was changed in the second season, fresh and dry weights of nodules and stem dry weight of roots and stem and the stem length were not significantly different from crown classes and their interaction but number of nodules and fresh weight of stem were highly significant (Table 3). The dominant-crown class recorded high values for seedling parameters in the two seasons than the intermediate-crown class except for the dry weight of nodules, fresh and either dry weightsand dry weight of stem in the first season. Rhizobia strains caused highly significant differences in seedling parameters in the both seasons. Native Rhizobia strains were able to cause significant responses for nodulations and nitrogen fixation, compared with foreign strain. *Rizobium* strain AKR excelled in seedling characters than the strains LER and NGR234 (Table 3).

Effect of Crown Class and Rhizobia Strain on Nirtogenase Activity: The nitrogenase activity in the *Acacia karroo* seedling root indicated highly significant differences between crown classes and the Rhizobia strains and their interaction for all treatments except for the crown classes and the interaction of zero minute in the two seasons. Data presented in table 4 showed that the nitrogenase activity increased with the increase in the time of exposure to C_2H_2. The dominant crown class recorded higher values than the intermediate crown class in two seasons.

Table 2: Mean values on pod and seed characteristics obtained in two seasons, showing the effect of seed-collection site and tree-crown type

	First season				
	Location		Crown classes		
Pod character	Sadat City	Bourg El-Arab City	Dominant crown	Intermediate crown	L.S.D
Pod length (cm)	11.3	10.7	11.4	10.6	1.29
Seed number / pod	8.81	8.97	9.3	8.51	0.56
Seed weight (g)	26.1	25.3	26.5	24.9	2.76
Germination (%)	66.6	80.0	83.5	63.1	10.1
	Second season				
Pod length (cm)	9.94	9.98	10.7	9.26	0.49
Seed number / pod	6.97	8.82	8.17	7.62	0.80
Seed weight (g)	22.0	32.4	25.0	29.3	3.70
Germination (%)	61.8	73.1	66.7	68.2	10.9

Table 3: Effect of crown classes and incubation of different Rhizobia strains on seedlings parameters in two seasons

	First season							
	Crown classes			Rhizobia strains				
Parameters	Dominant crown	Intermediate crown	L.S.D	Control	AKR	LER	NGR234	L.S.D.
Seedling parameters								
Number of nodules	31.4	27.8	6.94	0.00	82.3	22.2	13.8	9.82
Nodules fresh weight (mg)	3.28	3.02	2.36	0.00	6.73	3.75	2.11	3.34
Nodules dry weight (mg)	0.18	0.24	0.23	0.00	0.65	0.14	0.07	0.32
Fresh root weight (gm)	10.8	12.9	3.65	7.06	18.3	11.32	10.8	5.16
Root dry weight (gm)	1.84	3.39	0.55	1.65	2.37	2.33	4.12	0.78
Fresh stem weight (gm)	17.2	17.5	4.40	14.2	30.7	10.92	13.6	6.24
Stem dry weight (gm)	4.62	4.86	1.86	3.75	6.79	4.36	4.08	2.37
Stem length (cm)	61.8	42.3	7.15	39.0	59.8	50.8	58.5	10.1
	Second season							
Seedling parameters								
Number of nodules	47.0	23.8	17.0	0.00	83.3	36.7	21.7	24.1
Fresh nodules weight (mg)	5.38	2.68	3.61	0.00	10.3	3.88	1.95	5.10
Nodules dry weight (mg)	0.38	0.18	0.24	0.00	0.84	0.20	0.07	0.35
Fresh root weight (gm)	14.6	10.1	2.34	9.84	17.0	11.7	11.2	3.31
Root dry weight (gm)	2.79	2.23	0.67	1.91	2.81	2.83	2.50	0.95
Fresh stem weight (gm)	19.3	15.6	3.69	11.7	33.4	12.4	12.4	5.22
Stem dry weight (gm)	4.92	4.23	1.15	3.49	7.27	3.82	3.72	1.62
Stem length (cm)	68.0	64.4	14.0	54.7	70.2	64.2	75.8	19.8

(AKR): *Acacia Karroo* Rhizobium; (LER): *Leucaena leucocephala* Rhizobium; (NGR234): Papua New Guinea Rhizobium.

Table 4: Mean of the tree crown classes and Rhizobia strains on nitrogenase activity in two seasons

	First season							
	Crown classes			Rhizobia strains				
parameters	Dominant crown	Intermediate crown	L.S.D	Control	AKR	LER	NGR234	L.S.D.
Nitrogenase activities (μmol/L)								
Zero minutes	1.71	1.79	0.32	0.00	4.25	1.54	1.22	0.46
30 minutes	4.07	4.41	0.33	0.00	13.12	2.08	1.76	0.46
60 minutes	5.07	5.85	0.61	0.00	15.47	3.77	2.60	0.87
	Second season							
Zero minutes	2.71	2.47	0.75	0.00	6.40	2.36	1.60	1.06
30 minutes	6.69	6.27	1.28	0.00	14.44	9.18	2.30	1.82
60 minutes	17.01	11.41	1.51	0.00	31.86	16.69	2.28	2.14

(AKR): *Acacia Karroo Rhizobium*; (LER): *Leucaena leucocephala* Rhizobium; (NGR234): Papua New Guinea Rhizobium.

In respect of nitrogenase activity, highly significant differences were recorded between Rhizobia strains. In general, Rhizobium strain AKR proved to be most effective, followed by the strain LER, the lowest values for the same treatments were recorded with strain NGR234. The interaction of crown classes with Rhizobia strains indicated that strain NGR234 gave the lowest nitrogenase activity among the strains used (Table 4).

DISCUSSION

In this work, two locations at North-West of Egypt namely, Sadat City (30°22 N, 30°27 E) and Bourg El-Arab City (30°92 N, 29°68 E) were selected for collection of pod of *Acacia karroo*, also two crown classes (dominant and intermediate crown classes) and three Rhizobia strains, two native strains (AKR and LER) and one foreign strain NGR234 were selected to study their effects on pod characteristics, nodulation and nitrogen fixation in *Acacia karroo* seedlings. Data obtained showed that, pod charactering was differed due to change in locations. This was in harmony with findings of Sorenson *et al.* [17] who found that both the altitude and longitude responsible for variation between locations. Sedgley *et al.* [18], Shetta [19] and Abou-Gazia *et al.* [20, 21] found that seed characteristics differed with season, location and attributed. Also physical and chemical properties of soils and climatic variations play important role on productivity and pod maturity as well as affected on pod characteristics between locations. Do *et al.* [22] demonstrated that ground-water availability was the major environmental variable controlling the leaf phenology of *Acacia tortilis* var *reddiana* in dry tropical regions. Peba and Tabla [23] studied the phenology and pollination of *Manilkara zapota* at two sites (forest and homegardens) and noted significant differences due to water and soil nutrients. The main factors limiting *Manilkara zapota* fruit production in the forest was climate and soil properties.

Our observations on pod characteristics of *Acacia karroo* seedlings revealed that in the first season, the Bourg El-Arab site was superior in seed number per pod and germination percentage while the pod length and seed weight were higher at the Sadat site. In the second season, effects of the collection site were the same as in the first season. Pod characteristics were improved at Bourg El-Arab site than at Sadat site. The same trend was found by Shetta [19] and Abou-Gazia *et al.* [21]. While studying the phenology of Mediterranean woody plants in northeast Spain, Milla *et al.* [24] indicated variation in the timing of fruiting among tree species. Botges *et al.* [25] showed that the dry season affected the flowering of *Caesalpinia echinata* and seed dispersal attained the maximum in the beginning of the wet season.

The present study indicates that the dominant crown class had higher number of seeds per pod than the intermediate crown class during the two seasons of study and thus substantiates the results of Abou-Gazia *et al.* [20,21]. The roots of *Acacia karroo* seedlings were nodulated by the native *Rhizobium* strain (AKR and LER). Strain NGR234 was not able to compete with the naturalized strains. Moreover, the strain AKR caused more significant differences in nodules numbers, fresh and dry weights of nodules and stem, fresh weight of roots and the stem length in the both seasons than the other Rhizobia strains. The results are comparable to those obtained with peanut, where introduction of effective strains of Rhizobia depended on the competition between introduced strain and native strains for nodules sites [26, 27]. A high competitiveness of inoculum strains in comparison with native Rhizobia strains is as important as effectiveness of symbiotic nitrogen fixation [28]. We found that strain NGR234 was unable to compete the native strains. Also the reduction of seedlings parameters noticed with strain NGR234 might be due to weak symbiosis for *A. karroo*. This was in agreement with the observations of Pueppke and Broughton [13] and Swelim *et al.* [29]. Inoculation with strain NGR234 gave fix+ nodulation in four agroforestry tree species, while with *Acacia karroo* gave indeterminate nodules. Sanginga *et al.* [30] observed that soybean plants nodulated with local Rhizobia which was highly effective depending on the genotype and field sites. The seedlings characters were improved by the activity of *Rhizobium* strain AKR. This was in harmony with the results of Molla *et al.* [31] and Bala *et al.* [32] who demonstrated that the symbiotic interaction with the native Rhizobial populations and some ecological indicators affected the nodulation rate and effectiveness of symbiosis in four agroforestry trees. Shetta [33] indicated that the *Rhizobium* strain (RKM) isolated from *Acacia saligna* enhanced the growth parameters, nodulation and nitrogen fixation of *Acacia saligna* followed by *Rhizobium* strain (Leu), while the strain NGR234 was the least effective. Wolde-meskel *et al.* [34] obtained that the indigenous Rhizobia, a nodulating native woody species in Ethiopian soil, constituted metabolically and gnomically diverse groups that are not linked to the Rhizobia nodulating the African *Acacia* trees. Denton *et al.* [35] and Donate-Correa *et al.* [36] found that the site with large Rhizobial background had greater root nodulation and the yield of legumes varied from site to site and within the site and was related to nodulation and the difference between strains and tree species.

Nitrogen fixation in *Acacia karroo* seedlings showed that the native strain AKR caused a higher nitrogenase activity than the other strains in the two seasons. This was disagreement with results of Cash and Ditterline [37] who reported that seed size affected nodulation, plant height and acetylene reduction activity and, recommended that ARA analysis should be conducted with seed size in order to increase the variability in nitrogen fixation. Shetta [33] reported that the nitrogenase activity of *Rhizobium* isolated from *Acacia saligna* increased with increase the salinity levels, while the strain NGR234 was least effective in causing nitrogenase activity.

CONCLUSION

From the above mention results, it can be concluded that, pod collected location had affected on pod characteristics, seedling parameters and nitrogen fixation. Also crown classes showed affective differences in the same traits. Although the local Rhizobia strains had the superiority effect to fix and nodulate the *Acacia karroo* than the foreign strain which was unable to compete with the native strains under field condition that may be due to adaptation of the native strains to the environmental factors and site conditions. More studies need to be undertaken on the symbiosis of Rhizobium NGR234 under arid and dry conditions.

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