Potentiality of some Sudanese *Acacia* species for nodulation and nitrogen fixation

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ABSTRACT

Pot experiments were carried out to investigate the capability of seven *Acacia* species (*A. seyal*, *A. senegal*, *A. nilotica*, *A. polycantha*, *A. mellifera*, *A. nubica and A. tortilis*) for nitrogen fixation and to assess the efficiency of isolated and re-inoculated rhizobia for nodulation and N fixation. Also, the amount of N fixed by four Acacia species (*A. seyal*, *A. senegal*, *A. mellifera* and *A. nubica*) was estimated using ^{15}N isotope dilution method with *Tamarindus indica* used as a reference plant. Fertilization was carried out by adding 20 kg N/ha (5% ^{15}N atom excess) and 100 kg N/ha (1% ^{15}N atom excess) to the fixing and to the non-fixing plants, respectively. Dry biomass, number of nodules, dry weight of nodules, N% and N content were determined. Plant tissue analysis was carried out for ^{15}N and ^{14}N. Result showed significant differences between the species in the growth parameters, with *Acacia polycantha*, *A.seyal* and *A. mellifera* giving the highest values. The inoculated plants gave the highest biomass and nodulation parameters. The study revealed a strong relationship between biomass and N content. The study also showed differences in N fixation among the tested Acacias species(*A. nubica*, *A. senegal*, *A. mellifera* and *A. seyal*) both in terms of % N needed by the plant (60%, 58%, 53% and 50%) and the total N fixed (0.063, 0.104, 0.098 and 0.078 g/plant, respectively).

INTRODUCTION

Recently, there has been a growing interest in investigating the potentiality of leguminous woody plants for their role in sustainable agriculture in the arid areas. This is achieved through symbiosis with the N fixing bacteria which thereby, provide substantial nitrogen to the soil through a large biomass yield (Miettinen *et al*., 1992). The genus *Acacia*

which is considered as a member of the N fixing trees of the family *Fabaceae* (*Leguminosae*) include about 1200 species and are abundant in all tropical regions (Simmons, 1987). In the Sudan Acacias are found in various environments and form the dominant trees in some areas (El Amin, 1990). There are many uses of these trees and ways to manage them which imply a need to carefully select appropriate species from the wide variety that exist (Oba *et al*., 2001). The biological advantages of selecting indigenous species include their adaptation to the environment, their tolerance to local pests and diseases and conservation of associated fauna and flora (Brummett, 2000).

Rhizobia are soil bacteria capable of forming nodules on plants of the family *Fabaceae* (Allen and Allen, 1981). Inoculation is one way to improve the biological nitrogen fixation (BNF) in legumes (Sutherland *et al*., 2000). The success of the establishment of inoculants strain in the soil depends on the existing indigenous soil population and the suitability of the rhizobial strain for the particular crop (Montańez, 2000). Introduction of soils from an area where plants are well nodulated can work well as an inoculant of rhizobia when other methods are not practical. Inoculation of tree seedlings at the nursery stage with tested effective rhizobia may be crucial in order to exploit their N fixing capacity after transplanting in the field (Sutherland *et al*., 2000)

Knowledge of nodulation ability is very important for selection of legume tree for agroforestry (Giller and Wilson, 1991). Although several acacias form N fixing nodules (Sprent, 2001), information about their N fixing capacity is scarce due to methodological problems (Dakora and Keya, 1997). There is no simple way to measure N fixation in trees and it is especially difficult in the field. Isotope based methods provide the best approach for integrated measurements of the amount of N fixation in plants (Samba *et al*., 2002; Somado and Kuehne, 2006).

The objectives of this study were:

a) to test the capacity of some *Acacia* species for natural N fixation.

b) to assess the efficiency of isolated and re-inoculated rhizobia for nodulation and N fixation.

c) to estimate the amounts of N fixed by some *Acacia* species using ^{15}N isotope dilution method.

MATERIALS AND METHODS

Three pot experiments were conducted in the nursery of the Forestry Research Section, Gezira Research Station of the Agricultural Research Corporation at Wad-Medani, Sudan (latitude14° 24′ N, and longitude 33° 30′ E and altitude 405 m above sea level).

Experiment 1: Testing for natural nitrogen fixation

Soil samples were collected from the rhizosphere of the natural forests along the Blue Nile, Khor Abu Habil and El Gedarif. The natural forest comprised *Acacia seyal* var. seyal (Talh) , *Acacia senegal* var. senegal (Hashab), *Acacia nilotica* sub sp. nilotica (Sunt), *Acacia polycantha* sub sp. campylacantha (Kakamout), *Acacia mellifera* (Kitir), *Acacia nubica* (Laot) and *Acacia tortilis* sub sp. tortilis (Samor). Areas of survey were between 200-800 mm rainfall isohyte.

Composite samples were used by mixing the collected rhizosphere soil from the different sites for any species alone. One kilogram of the rhizosphere soil was mixed with one kilogram of the nursery soil (1 sand: 1 silt) (Table 1). The seeds were treated with conc. sulphuric acid (H_2SO_4) to enhance their germination, and then sown in transparent 20x25 cm polythene bags (4 kg capacity). Seven days after emergence, the seedlings were thinned to one plant/bag. Completely Randomized Design with 3 repl-icates was adopted. The bags were irrigated by using tap water (EC= 0.5) at seven days interval.

Number of nodules and dry biomass (whole plant) were measured. In the second season, the plant samples were analyzed to determine the total N using Kjeldahl method.

Experiment 2: *Rhizobium* inoculation of selected *Acacia* species

Isolation of *Rhizobium* spp. was carried out from the nodules of the screened species of experiment 1 according to the method employed by Vincent (1970). Soil (1 sand: 1 silt) (Table 1) was used for raising the 7 species after treating the seed with conc. H_2SO_4. The seeds were sown in 20x25 cm polythene bags (4 kg soil /pot), in a completely randomized design with 3 replicates. After four weeks, the seedlings were inoculated by dispensing 10 ml of liquid media containing the *Rhizobium* strain isolated from the same species (10^9cells / ml culture).

The following readings were recorded: Dry biomass (g), number of nodules and dry weight of nodules (g/plant). Plant samples were also analyzed to determine the total nitrogen taken up by each species using the Kjeldahl method.

Experiment 3: Estimation of the amounts of N fixed by some species of the genus *Acacia* using ^{15}N isotope dilution method

Acacia seyal, *Acacia senegal*, *Acacia mellifera* and *Acacia nubica* were tested to determine the amount of N fixed, with *Tamarindus indica* (Aradaib) used as a reference plant. The seeds were sown in April 2007, in 20x25 cm polythene bags (4 kg soil capacity) using the nursery soil (1sand: 1silt) (Table 1), after treating the seeds with conc. H_2SO_4. Completely randomized design with 3 replicates was adopted. The seedlings were inoculated by adding 10 ml suspension of the rhizobial strain isolated from the same species (10^9cells / ml culture) after two weeks from sowing.

Nitrogen fixing trees were fertilized at the rate of 20 kg N ha^{-1} (urea at 5% ^{15}N atom excess obtained by dissolving 1.848 g of the 10% atom excess labeled ^{15}N urea and 1.707 g of the ordinary urea in 2080 ml distilled water). The non-leguminous species *Tamarindus indica* (reference) were fertilized at the rate of 100 kg N ha^{-1} (urea at 1% ^{15}N atom excess obtained by dissolving 0.463 g of the 10% atom excess labeled ^{15}N urea and 3.981 g of the ordinary urea in 520 ml distilled water). Forty ml/pot of the prepared fertilizers was applied to the leguminous and the non-leguminous species. A basal dose of super phosphate was added to all experimental units at the rate of 0.16 g super phosphate/ 4 kg soil (equivalent to 50 kg P_2O_5 ha^{-1}).

After four months, the dry biomass (g) was determined and plant tissues were analyzed for $^{15}N/^{14}N$ in Siebersdorf laboratories at the International Atomic Energy Agency (IAEA), Vienna. Calculation of N_2-fixed by each species was performed by the A-value method using the following equation (Ndoye *et al*., 1995):

$$\%Ndfa_{fixing} = 100(\%Ndff_{fixing} + \%Ndfs_{fixing})$$

Where %Ndfa, %Ndff and %Ndfs refer to nitrogen derived from air, fertilizer and soil, respectively.

Analysis of variance was performed using the MSTATC package. Duncan's Multiple Range Test (DMRT) was applied to compare means.

Table 1a. Soil chemical properties.

Soil type	pH paste	E.C. dsm	Ca CO_2	N	O.C		Soluble cations			Soluble anions		Exh cations	CEC	Av. P mg kg soil
			---------% --------			Na	Ca	Mg	Cl	HCO	SO_4	Na	K	
Sand	85	0.1	1.2	0.023	0.6	10	0.0	0.1	0.5	0.0	0.52	0.04	16	1.0
Clay	79	0.4	2.4	0.054	2.1	15	0.5	2.1	2.0	0.0	1.53	0.55	35	17.6

1b. Soil physical properties.

Soil type	Mechanical analysis				Bulk density (gcm^3)		H.C. ($cm\ hr^{-1}$)
	CS	Es	Si	C	Saturation	Dry (gcm^{-3})	
Sand	96	0.1	02	01	24	1.37	57.17
Clay	02	04	69	25	55	1.32	4.15

RESULTS

Testing for natural nitrogen fixation

Dry biomass

In the first season, *A. mellifera* gave significantly ($P \leq 0.05$) the highest dry biomass over all the species while *Acacia tortilis* and *A. nubica* gave the lowest (Table 2). In the second season, *A. seyal* and *A. nilotica* gave significantly ($P \leq 0.05$) the highest, whereas *A. tortilis* gave the lowest mean and there were no significant differences between the other species (Table 2).

Number of nodules

In the first season, *A. seyal* gave significantly ($P \leq 0.05$) more number of nodules over all the other species (Table 2). In the second season, *A. polycantha* and *A. nubica* gave significantly ($P \leq 0.05$) the highest means but the latter was similar to *A. seyal* and *A. senegal*. *Acacia nilotica* gave the lowest number of nodules (Table 2).

Nitrogen content (mg/plant)

Nitrogen content was estimated in the second season only. *A. seyal, A. nilotica* and *A. senegal* gave significantly (P≤0.05) higher N-content over *A. polycantha, A. mellifera* and *A. nubica* whereas *A. tortilis* gave the lowest means (Table 2).Table 2. Dry biomass, number of nodules and N content of seven *Acacia* species using soil inoculum.

Species	Dry biomass (g/plant)	Number of nodules/plant	N-content (mg/plant)
	Season 1		
A. nubica	1.71 e	5.0 e	N.D.
A. seyal	3.56 b	14.0 a	N.D.
A. nilotica	2.79 c	8.0 c	N.D.
A. polycantha	3.51 b	9.0 b	N.D.
A. tortilis	1.51 e	5.0 e	N.D.
A. mellifera	4.85 a	10.0 b	N.D.
A. Senegal	2.10 d	6.0 d	N.D.
SE±	0.079	0.27	N.D.
		Season 2	
A. nubica	2.87 b	5.0ab	75.03 c
A. seyal	3.80 a	4.0 bc	100.10 a
A. nilotica	3.78 a	2.0 d	96.32 a
A. polycantha	3.13 b	5.0a	75.88 c
A. tortilis	1.53 c	3.0c	40.48 d
A. mellifera	3.20 b	3.0c	81.92 bc
A. Senegal	3.03 b	4.0bc	91.90 ab
SE±	0.11	0.26	4.74

Means within each column followed by the same letter(s) are not significantly different at 5 % level of significance according to Duncan's Multiple Range test.
N.D.= Not determined

***Rhizobium* inoculation of selected *Acacia* species**

Dry biomass

Generally, inoculated plants gave higher dry biomass than the non-noculated ones. Table 3 shows that in the first season, whether inoculated or not, *A. polycantha* gave significantly (P≤0.05) the highest dry weight, followed by *A senegal*, whereas, *A. tortilis* gave the lowest dry biomass. In the second season, whether inoculated or not *A. polycantha* and *A. nilotica* gave significantly (P≤0.05) the highest means (Table 3).

Number of nodules

In the first season, inoculated and non-inoculated, *A. mellifera*, *A. senegal*, *A. seyal*, as well as inoculated *A. nilotica* gave significantly (P≤0.05) the highest number of nodules (Table 3). Non-inoculated *A. tortilis* gave the lowest mean. In the second season, inoculated and non-inoculated *A. nilotica* gave significantly (P≤0.05) the highest number of nodules. Inoculated and non-inoculated, *A. mellifera* and non-inoculated *A .tortilis* gave the lowest means (Table 3).

Dry weight of nodules

In the first season, inoculated *A. senegal* and *A. polycantha* gave significantly ($P \leq 0.05$) the highest means over non-inoculated *A. polycantha* and *A. senegal*. Inoculated *A. nilotica* gave significantly higher means than inoculated *A. mellifera*, *A. seyal* and *A. nubica*. The rest of the species gave variable values but with no significant differences (Table 3). In the second season, inoculated and non-inoculated *A. polycantha* gave significantly ($P \leq 0.05$) the highest means (Table 3). Inoculated *A. senegal* and *A. nubica* and non-inoculated *A. senegal* were intermediate. The rest of the species gave significantly ($P \leq 0.05$) the lowest means.

Nitrogen content

In the first season, inoculated *A. polycantha* gave significantly ($P \leq 0.05$) the highest N content. Non-inoculated *A. tortilis* gave the lowest means, but showing no significant difference from the non-inoculated *A. seyal* and *A. nilotica* (Table 3). In the second season, inoculated *A. nilotica* and *A. polycantha* gave the highest N content but with no significant difference between them on one hand and all the other inoculated and non-inoculated species on the other hand except for the non-inoculated *A. seyal* and *A. tortilis*. Non-inoculated *A. seyal*, showed the lowest mean (Table 3).

Estimation of the amounts of N fixed by some species of the genus *Acacia* using ^{15}N isotope dilution method

Dry biomass

Acacia mellifera gave significantly ($P \leq 0.05$) the highest dry biomass over all the other species. *A. senegal* and *A. seyal* gave significantly higher means than *Tamarindus indica* and *A. nubica* which gave the lowest means (Table 4).

Nitrogen content (g/plant)

The four *Acacia* species gave significantly ($P \leq 0.05$) higher N content than the reference plant. *Acacia mellifera* and *A. senegal* significantly ($P \leq 0.05$) out-yielded the other two species. *Acacia nubica* showed the lowest mean, but higher than the reference plant (*T. indica*) (Table 4).

Table 3. Effect of inoculation on dry biomass, nodulation and N content of seven *Acacia* species.

Species	Inoculation	Dry biomass (g/plant)	Number of nodules/ plant	Nodule dry weight (g/plant)	N-content (mg/plant)
			Season 1		
A. nubica	Inoculated	5.77d	19.0bc	0.75efg	144c
A. seyal	"	4.52e	22.0ab	0.82def	118d
A. nilotica	"	5.32d	20.0abc	1.20bc	100de
A. polycantha	"	10.99a	12.0ef	1.60a	287a
A. tortilis	"	3.36fg	13.0f	0.54fgh	77fg
A. mellifera	"	7.11c	24.0a	0.94cde	196b
A. senegal	"	7.74b	23.0a	1.77a	217b
A. nubica	Non- inoculated	3.04gh	18.0cd	0.61fgh	96ef
A. seyal	"	2.65hi	22.0ab	0.33h	62gh
A. nilotica	"	3.38fg	15.0de	0.63fgh	67gh
A. polycantha	"	5.51d	11.0f	1.24b	142c
A. tortilis	"	2.28i	10.0f	0.35h	46h
A. mellifera	"	3.66f	24.0a	0.48gh	92ef
A. senegal	"	3.40fg	21.abc	1.07bcd	92ef
SE±		0.184	1.06	0.095	7.05
			Season 2		
A. nubica	Inoculated	6.1de	6.0bc	0.07b	166ab
A. seyal	"	2.11g	4.0efg	0.02ef	138abc
A. nilotica	"	8.71a	9.0a	0.03de	237a
A.polycantha	"	8.84a	6.0bc	0.09a	203a
A. tortilis	"	4.19f	5.0cde	0.03de	128abc
A. mellifera	"	5.65e	3.0gh	0.04cd	155ab
A. senegal	"	6.65cd	7.0b	0.06b	181ab
A. nubica	Non-inoculated	5.56e	5.0cde	0.03def	146abc
A. seyal	"	1.80g	3.0fgh	0.010f	45c
A. nilotica	"	7.60b	6.0bc	0.023def	197ab
A. polycantha	"	7.10bc	5.0cde	0.09a	168ab
A. tortilis	"	3.79f	2.0h	0.03de	100bc
A. mellifera	"	4.57f	2.0h	0.01f	132abc
A. senegal	"	6.09de	4.0efg	0.057bc	173ab
SE±		0.301	0.49	0.006	32.9

Means within each columns followed by the same letters are not significantly different at 5 % level of significance according to Duncan,s Multiple Range test.

^{15}N atom excess (%)

The ^{15}N enrichment in each of the four tested *Acacia* species was significantly($P \leq 0.05$)lowerthan*T. indica* (the reference plant). No significant differences were observed among the four *Acacia* species (Table 4).

Nitrogen percentage in plants (N %)

The four *Acacia* species were significantly (P≤0.05) different from the reference plant. *Acacia senegal* significantly out-yielded the other three *Acacia* species in N % (Table 4).

Nitrogen fixation (g/plant)

Senegal and *A. mellifera* showed the highest N fixation, followed by *A. seyal* with no significant differences among the three species. *Acacia nubica* showed the lowest N fixation than *A. senegal* and *A. mellifera* and with no significant difference from *A. seyal* in the Ndfa (Table 4).

Table 4. Dry biomass, percentage and amount of Ndfa of N derived from fixation of four *Acacia* species using *Tamarindus indica* as a reference tree.

Species	Dry biomass (g/plant)	N content (g/plant)	$\%^{15}N$ atom excess	N%	%Ndfa	Ndfa (g/plant)
A. mellifera	12.88a	0.18a	0.25b	1.43b	53.06b	0.10a
A. senegal	9.26b	0.17ab	0.22b	1.92a	58.14a	0.10a
A. seyal	10.31b	0.15b	0.26b	1.46b	50.05b	0.08ab
A.nubica	6.66c	0.11c	0.21b	1.60b	59.95a	0.06b
T. indica	6.84c	0.10d	0.38a	1.12c		
SE±	0.746	0.0105	0.021	0.098	1.181	0.0105

Means within each column followed by the same letter(s) are not significantly different at 5 % level of significance according to Duncan's Multiple Range test.

DISCUSSION

Capacity of some *Acacia species* for natural N fixation

In this study, seven multipurpose *Acacia* species were screened for their N fixing capacity because they are drought tolerant and are, therefore, suitable for agroforestry in the Sudan.

The results showed that the tested species were significantly different with respect to growth parameters. *Acacia polycantha*, *A.seyal* and *A. mellifera* showed significantly the highest means for most of the measured growth parameters including plant N content which implied higher N fixing capacity compared to the other species. This is in agreement with the results reported by Sprent (1995) who cited considerable variations in N fixation by different tree species. Also, Sanginga *et al*. (1990), Hardardson *et al*. (1993) and Samba *et al*. (2002) reported that there was genetic variability for N fixation among and within legume species; so it is essential to screen and use the species with higher N fixing potential.

This study showed that all the tested *Acacia* species formed nodules naturally (without seed inoculation) under nursery conditions in soil media from the rhizosphere of *Acacia* trees. *Acacia polycantha*, *A. mellifera* and *A. seyal* significantly out-yielded other species concerning number of nodule. Our findings disagreed with those of Deans *et al*. (1993) who found that only *A.seyal* seemed capable of nodulation under the traditional nursery. They suggested that formation of nodules in both *A. mellifera* and *A. senegal* appeared to require addition of peat to the growing media.

Variation in effectiveness of symbiotic association

The effectiveness of symbiotic association (determined mostly as growth performance) between native rhizobia and *Acacias species* can vary a lot (Thrall *et al*., 2000). Generally, the results of this experiment showed that the inoculated plants gave higher biomass, plant N content, number and dry weight of nodules than the non inoculated plants. These results agreed with those obtained by Sutherland *et al*. (2000) who noted that *Rhizobium* inoculation had a positive effect on seedlings growth. In contrast to our results, Deans *et al*. (1993) reported that inoculation and supplementary nutrition had no effect on nodulation and dry weight in *A. mellifera*, *A. senegal* and *A. seyal* seedlings. Also, Chan *et al*. (1999) observed that the total N content in rhizobia free seedlings was higher than that in inoculated seedlings. However, Chan *et al*. (1999) also reported that seedlings with nodules had a higher biomass than those lacking nodules, which was in conformity with our results, where *A. polycantha* seedlings gave significa-ntly the highest biomass and number of nodules. The nodulation status reflected the growth and N content of seedlings.

The total N-content of the various species was not consistent. This could be attributed to the fact that different species have different growth habits. Although *A. seyal* gave a high N %, *A. polycantha* gave significantly the highest N content (Table 2). This may be due to the fact that *A. polycantha* had the most vigorous growth that yielded the highest biomass. Hence the high leaf area increased photosynthetic efficiency and thus resulted in high nodule weight which may imply greater nitrogen fixing potential.

Estimation of the amounts of N fixed by some species of the genus *Acacia* using ^{15}N isotope dilution method

The results of this study showed that the percentage of ^{15}N atom excess was higher in the reference plant than in the *Acacia* species indicating the occurrence of N fixation in the tested *Acacia* species. Although our results showed moderate %Ndfa (*A. nubica* 60%, *A. senegal* 58%, *A. mellifera* 53%, and *A. seyal* 50%). They gave 0.063, 0.104, 0.098 and 0.078 g/plant respectively, suggesting a need for future research for more efficient rhizobia to raise this amount of N fixed from the atmosphere (%Ndfa). There are reports indicating that Acacias are poor N fixers (Sanginga *et al*., 1990). However, this evidence has been mainly based on examination of the N fixing potential of *Faidherbia albida* (synonym *A. albida*).

The results of this study showed differences in N fixation among the tested *Acacia* species both in the %Ndfa and total N fixed. This is in line with the findings of Danso *et al*. (1995). Ndoye *et al*. (1995) using ^{15}N isotope dilution method (with 5-month old plants) found that the %Ndfa was higher for *A. seyal* (63%) and *A. raddiana* (62%), giving almost twice the %Ndfa in *A. senegal* (34.4%) and *F.albida* (37.3%). They proved that *A. seyal* clearly ranked higher in the total N fixed (1.62 g N plant^{-1}) compared with an average of 0.48 g N Plant^{-1} for other *Acacia* species. Raddad *et al*. (2005) reported rates of %Ndfa between 24-61% for different *A. senegal* provenances 4 years after planting using the natural abundance method (δ^{15}N).

Our results are in agreement with the findings of Ovalle *et al*. (1996) and Ndoye *et al*. (1995) and proved that African *Acacia*s are potentially good N fixers. Also, it showed that *A. nubica* had the highest %Ndfa (although this species is given little care and is potentially active and vigorously invading marginal areas). Moreover, this species gave the lowest amount of Ndfa as a result of its lower biomass. *Acacia mellifera*, *A. seyal* and *A. senegal* gave the highest biomass implying a higher N fixing capacity and a higher amount of Ndfa.

To conclude, all the studied species have good potential for N fixation (% Ndfa between 50-60%), and selection of the suitable species for the right geographical area is important. Also, the inoculated plants performed better than the non-inoculated ones.

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قابلية بعض أنواع الأكاسيا فى السودان لتكوين العقد البكتيرية

وتثبيت النيتروجين الجوى

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الخلاصة

أجريت ثلاث تجارب في أصص لإختبار مقدرة بعض الأشجارالبقولية من جنس الأكاسيا (الطلح والكتر والهشاب والسنط والكاكاموت والسيال واللعوت) لتثبيت النيتروجين الجوى ولإختبار قابلية الباكتيريا من نوع الريزوبيا للمعيشة التكافلية مع أشجار الأكاسيا. كما تم تحديد كمية النيتروجين المثبتة بواسطة بعض الأنواع (الكتر والهشاب والطلح واللعوت) بإستعمال العرديب كنبات مرجعي لحساب كمية النيتروجين بإستعمال نظير النيتروجين 15N. تمت إضافة 20 (5% فوق المعدل الطبيعي) و 100 (1% فوق المعدل الطبيعي) كجم نيتروجين/ هكتار لكل من الأشجار البقولية والغير البقولية على التوالي. أخذت قراءات الوزن الجاف للكتلة الحية، عدد ووزن العقد البكتيرية كما تم تحليل النباتات لتحديد نسبة النيتروجين و المحتوى النيتروجيني للنبات. في التجربة الأخيرة تم تحليل أنسجة النباتات لتحديد محتوى نظائرالنيتروجين 15N و14N. في تجربتي مقدرة أشجار الأكاسيا علي تثبيت النيتروجين و تكافلية الباكتيرية العقدية للمعيشة معها أثبتت الدراسة أن هناك فروقاً معنوية في القراءات المختلفة بين أنواع الأكاسيا المختلفة. كما أثبتت الدراسة أن الكاكاموت والكتر والطلح أعطت أعلي القراءات. أثبتت التجارب أن الشتول الملقحة بالباكتيريا أعطت أعلي قراءات للوزن الجاف للكتلة الحية.كما أعطت الأنواع الأكثر وزنًا جافًا للكتلة الحية أعلي محتوى نيتروجيني. سجلت أنواع الأكاسيا المختلفة نسب تثبيت نيتروجين مختلفة (اللعوت 60% والهشاب 58% والكتر53% والطلح 50% من إحتياج النبات الفعلي) و كان وزن النيتروجين الكلي المثبت 0.063 و 0.104 و0.098 و 0.078 جم/نبات علي التوالي.