

## Nitrogen Fixing Legumes in the Plant Communities

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**Abstract: Problems statement:** Numerous authors have used energetic to explain the ecological success of N-fixing plants. Legume biodiversity assessment, species dynamics, nitrogen fixation monitoring and environment impact assessment of these ecological events in Al-Hassa Oasis, Saudi Arabia are rare and need to be continuous and more frequent. **Approach:** Thus the objectives of this study were to analyze legume abundance within and outside Al-Hassa Oasis and relate it to the distribution of the different genera. **Results:** Thirty two legume plant species from 20 genera have been recorded within and outside the Oasis. The largest genera were *Cassia* (4 species), *Indigofera* (4) and *Acacia* (3). Annual herbs were the dominant growth form (34% of species recorded), followed by shrubs (28%), perennial herbs (19%) and trees (19%). Eighteen alien plant species were recorded (maybe an underestimated number). The nitrogen fixation of the legume plant species in Al-Hassa Oasis was estimated/analyzing the fixing potentiality of these species and nonfixing reference species (*Panicum turgidum*) using the  $^{15}\text{N}$  natural abundance method. Species with great nitrogen fixing capacity in Al-Hassa include: *Medicago sativa*, *Vicia faba*, *Vicia sativa*, *Melilotus indicus*, *Dolicus lablab*, *Melilotus alba* and *Cliforia ternate*. The mean biological fixation contribution of most of the recorded legume plants were high, varying from 3.9% (*Indigofera argentea*) to 64.6% (*Medicago sativa*). **Conclusion:** Al-Hassa Oasis is richer than expected based on its location within the desert zone. This study confirms the importance of the Oasis for national flora conservation in the Kingdom. results showed a good potential for use of the  $^{15}\text{N}$  natural abundance methodology for evaluating the nitrogen fixation ability of the legume plants under field conditions as well as for the estimation of %Ndfa.

**Key words:** Nitrogen fixing, legume plant, Global Position System (GPS), Geographical Information System (GIS), data analysis, Least Significant Difference (LSD), Analysis Of Variance (ANOVA)

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### INTRODUCTION

The family Fabaceae (Leguminosae) is by far the most diverse and widespread group of plants that have the capacity to host N-fixing bacteria. Ranking behind only the Asteraceae and Orchidaceae in size, the Fabaceae is the third largest family of flowering plants with about 650 genera and 18,000 species (Sprent, 1995; Akran *et al.*, 2009). The Fabaceae is broken up into sub-families: the Caesalpinoideae, the Mimosoideae and the Papilionoideae (Sprent, 1995). The Caesalpinoideae and Mimosoideae mainly consist of woody shrubs and trees that are largely confined to tropical and subtropical regions. The Papilionoideae is made up of woody shrubs and trees as well as perennial and annual herbs; this sub-family is distributed worldwide with the woody members being largely concentrated in the tropics and sub-tropics (Kass *et al.*, 1997; Al-Taweil *et al.*, 2009).

Only 20% of all legume species and about half of legume genera have been examined for nodulation (Ardakani, 2009; Ezrin *et al.*, 2010; Onyango *et al.*, 2011) and far less have been actually tested for nitrogen fixing activity. Members of the three sub-families have been found to have different capacities to support nodulation. Of the species examined, 97% of the Papilionoideae, 90% of the Mimosoideae and 23% of the Caesalpinoideae have been found to nodulate. The N-fixing bacteria that infect the roots of Fabaceae belong to one of two genera, *Rhizobium* or *Bradyrhizobium*.

Nitrogen fixation from the gaseous form in the atmosphere to forms that plants and other organisms can use (either  $\text{NH}_4^+$  or  $\text{NO}_3^-$ ) is mediated either by bacteria in symbiotic relationships with vascular plants, or by symbioses between cyanobacteria and fungi (lichens) or plants, or by free living heterotrophic or autotrophic bacteria that are typically associated with soil (Garten, 1993; Mirzakhani *et al.*, 2009; Ezrin *et al.*,

2010). The three sources of fixation are more global in occurrence, however, the bacteria/vascular plant symbiotic relationship usually sustain the highest rates of nitrogen fixation per unit area where they occur (Mirzakhani *et al.*, 2009).

Ecological constraints that may limit the success of N-fixing plants include the availability of soil nutrients other than N (especially P or Mo) (Aranibar *et al.*, 2003), the existence of poor edaphic conditions such as high acidity, alkaline or aridity (Diabate *et al.*, 2005), removal of N-fixing species by preferential grazing (Du Toit, 2003) and removal of woody dicots (including woody legumes) by fire (Aranibar *et al.*, 2003; Akran *et al.*, 2009).

Desert communities that evolved from tropical floristic elements, tend to have a significant legume presence. The Sonoran desert (The United States and Mexico) is an example of a tropically-derived desert flora (Brown, 1994; Akran *et al.*, 2009; Onyango *et al.*, 2011), with a strong legume (herbaceous and woody) component (Turner *et al.*, 2005). Numerous authors have used energetics to explain the ecological success of N-fixing plants (Hogberg and Alexander, 1995). When adequate soil N is available, the nonfixers will have high relative growth rates assuming that they can invest more in aboveground, photosynthetic tissues and less in belowground, N-acquiring tissues (Dawson *et al.*, 2002). Legume biodiversity assessment, species dynamics, nitrogen fixation monitoring and environment impact assessment of these ecological events in Al-Hassa Oasis, Saudi Arabia are rare and need to be continuous and more frequent. Thus the objectives of this study were to analyze legume abundance within and outside Al-Hassa Oasis and relate it to the distribution of the different genera.

## MATERIALS AND METHODS

**Study site:** Al-Hasa lies in the south of the Kingdom's Eastern region and is bounded by the Al-Dahna and the Al-Daman deserts. The Al-Hasa oasis is the largest oasis in the Kingdom of Saudi Arabia and the municipality of Al-Hasa constitutes the largest administrative area in the Kingdom. In ancient times, Al-Hasa was at the center of the trade routes which traders followed between the east of the Arabian peninsula and India, Persia and the Far East. It enjoys the benefit of copious reserves of underground water which has allowed the area to develop its agricultural potential. Nevertheless, Al-Hasa has to deal with tons of sand which the wind carries and deposits over the land. To counter this problem, the Kingdom has planted large barriers of trees to prevent the wind-borne sand from damaging inhabited and agricultural areas.

**Climate:** The climate of Al-Hassa is characterized by long, hot, rainless summers with northerly winds, often bringing sandstorms. Average rainfall is about 75 mm, falling mainly in December and January, but it is unpredictable and sometimes falls as localized heavy downpours. Typical annual values vary from 45-150 mm. The mean annual rainfall in Al-Hassa is 70 mm. The temperature ranges from 28-47.5°C with a mean of 34.1°C although peaks of 50°C can be recorded. The humidity is exceptionally high, often reaching 90% in summer.

**Vegetation survey:** In the field, sites were located accurately using a Global Position System (GPS) and Geographical Information System (GIS) on a lap-top computer. At each site, tape measures were used to delineate 50×50 m quadrates.

The field survey was conducted in 2009. Field trips were made to the different sites in Al-Hassa Oasis and botanical notes and observations were registered. Extra information related to vegetation parameters was recorded using line-transect method. Plant species were collected and taken to the laboratory for identification. Herbarium specimens were collected, pressed, numbered and deposited at the herbarium of the college of Science, King Faisal University, in Al-Hofuf, KSA.

**Data analysis:** Prior to any data analysis, species were assigned to major plant groups based on life-form and life-span (trees, shrubs and herbs, annual or perennial) using descriptions contained in Cunningham *et al.* (1993). This produced a total of four major plant groups including (1) trees (2) shrubs, (3) annual herbs and (4) perennial herbs. An index of total cover was calculated for each species at each site by summing the cover percentages recorded in all ten 50×50 m quadrats. This was deemed necessary to avoid working with percentages greater than 100 resulting from vertically overlapping plant layers. Species richness was calculated as the total number of species recorded within each 50×50 m quadrat.

Initial inspection of the data showed that transformation,  $[\log_{10} (x+1)]$ , of all variables was necessary to meet the assumption of normality required by Analysis Of Variance (ANOVA) (Zar and Zar, 1999). The effects of the 2 sites (within and outside the Oasis) on species richness and total cover within each of the sites were then tested using ANOVA. Means which differed significantly ( $p < 0.05$ ) were then determined using the Least Significant Difference (LSD) test. All univariate analysis were conducted in SPSS (SPSS Version 17).

**Nitrogen fixation:** Species potentially able to fixate N<sub>2</sub> (target species), or not (reference species) were sampled in each plot. The target species were legumes with a known nodulation capacity or without information on nodulation (Allen *et al.*, 1981; De-Faria *et al.*, 1998; Akran *et al.*, 2009). Samples were collected from all the target species found in each site and also from a reference species. This reference species was (*Panicum turgidum*). It was selected because of their wide distribution and high density in Al-Hassa. Leaf material from different branches was collected from adult plants with diameters at breast height larger than 3 cm. After collection, completely expanded healthy green leaves were selected, forming a composite sample of 200 g of leaves per plant.

**Determination and statistical analysis:** The leaf material was oven dried at 60°C for 72 h and then ground to fine powder. Total N was analyzed by micro Kjeldhal methodology and <sup>15</sup>N abundance by mass spectrometry and expressed in “delta” notation, which is the deviation per thousand (%) of <sup>15</sup>N abundance of the sample in relation to the standard:

$$\delta = (R_{\text{sample}} / R^{\text{standard}} - 1) \times 1000$$

where, R<sub>sample</sub> and R<sub>standard</sub> are the ratio <sup>15</sup>N:<sup>14</sup>N of the sample and the standard (atmospheric N<sub>2</sub>), respectively (Dawson *et al.*, 2002).

The data were submitted to a statistical analysis to compare the mean isotopic signals of the target legume species with the reference species signals, in each site, using the values of each individual of the same species as replications. Significant differences were established considering the 0.01 level of probability of the LSD test. The species whose mean were significantly impoverished in <sup>15</sup>N in relation to the reference species were considered as being capable of atmospheric N<sub>2</sub> fixation (fixing species).

**Estimation of BNF:** For the N<sub>2</sub>-fixing species, estimates of percentage of plant nitrogen derived from the air (%Ndfa) were made, using the formula recommended by Shearer and Kohl (1986):

$$\%Ndfa = \delta^{15}N_{(\text{reference})} - \delta^{15}N_{(\text{N-fixing})} / \delta^{15}N_{(\text{reference})} - B) \times 100$$

Where,  $\delta^{15}N_{(\text{reference})}$  is the mean value of the <sup>15</sup>N of the reference species of each site,  $\delta^{15}N_{(\text{N-fixing})}$  is the mean <sup>15</sup>N value for the plants of each N<sub>2</sub>-fixing species and B is the <sup>15</sup>N value for fixing plants cultivated in the

absence of soil mineral N supply. With the absence of data for the studied species, values of 0% and -2‰ were used which are commonly found in studies of tree legumes (Raddad *et al.*, 2005; Akran *et al.*, 2009; Ezrin *et al.*, 2010). The quantities of fixed N (kg/ha/year) were estimated using %Ndfa and %N content results, obtained in this study and the annual production of leaf mass of the fixing species.

## RESULTS AND DISCUSSION

Vegetation is characterized by a thin sprinkling of shrubs, grass tussocks and occasionally tree growth such as *Tamarindus*. Variety is limited, with a small number of plants dominating the land. Sub-Sudanian vegetation association of *Hammadetea salicornici*, *Haloxylotea salicornici* and *Sauedetea deserti* are common in and around Al-Hassa Oasis. The most widespread shrubs are *Rhanterium epapposum*, *hammada elegans* and *Calligonum comosum*. The two main grasses are *Panicum turgidum*, *Stipa capensis* and *Cyperus conglomerates*.

Thirty two species from the Fabaceae family were recorded during the vegetation survey (Table 1). Cassia in the perennial herb plant groups, was the most commonly occurring genera and *Acacia* in the tree plant group, was the most abundant genera. Most of the species found in study area were woody perennials (46.9%) and 15.6% herbaceous perennials. With 37.5%, the annual herbaceous making up another large group. These species flower irregularly from year to year, depending on the timing and amount of irrigation and rainfall, but most blossoms appear from January to March. The thirty two species belong to 20 genera within the Fabaceae family. The number of species per genus in the study area averages about 1.6. This is a common feature of the desert flora. It is no indication that only a few of the large number of species which belongs to the Fabaceae family have adapted themselves and survived in the harsh environment of Al-Hassa.

Thirty species were recorded from sites within Al-Hassa Oasis and seventeen species were recorded at sites outside the Oasis in Al-Hassa region. Five species, including three tree and shrub plant species, were restricted to sites outside Al-Hassa Oasis. Twelve species were recorded at both locations, while eighteen species are restricted to sites within Al-Hassa Oasis most of them were shrubs and perennial herb plant groups.

Species richness and total cover both decreased significantly (p<0.05) in sites outside Al-Hassa Oasis as compared to the within Oasis values.

Table 1: List of species belonging to the Fabaceae family recorded in the vegetation survey of Al-Hassa Oasis

| Species                                 | Plant group | Recorded % of the total number of plants |
|---|-------------|--|
| <i>Acacia farnesiana</i> (Lam) Wild     | Tr          | 2.96                                     |
| <i>Acacia gerradi</i> Benth.            | Tr          | 2.88                                     |
| <i>Acacia tortilis</i> Hayne            | Tr          | 5.67                                     |
| <i>Alhagi camelorum</i> Fisch.          | Sh          | 1.21                                     |
| <i>Allbizia lebbek</i> (L.) Benth       | Tr          | 0.17                                     |
| <i>Anagyris foetida</i> L.              | Sh          | 0.25                                     |
| <i>Astragalus tribuloides</i> Del.      | A. He       | 4.75                                     |
| <i>Cassia holosericea</i> Fres          | P. He       | 8.20                                     |
| <i>Cassia italica</i> (Miller) spreing  | P. He       | 7.14                                     |
| <i>Cassia occidentalis</i> L.           | P. He       | 6.14                                     |
| <i>Cassia senna</i> L.                  | P. He       | 9.04                                     |
| <i>Clitoria ternatea</i> L.             | P. He       | 0.29                                     |
| <i>Crotalaria ertusa</i> L.             | Sh          | 9.23                                     |
| <i>Crotalaria microphylla</i> Vahl      | A. He       | 1.12                                     |
| <i>Delonix elata</i> (L.) Gambe         | Tr          | 0.29                                     |
| <i>Dolichus lablab</i> L.               | A. He       | 0.25                                     |
| <i>Indigofera argentea</i> L.           | Sh          | 3.29                                     |
| <i>Indigofera oblongifolia</i> Forssk   | Sh          | 2.25                                     |
| <i>Indigofera spinosa</i> Forssk        | Sh          | 2.17                                     |
| <i>Indigofera articulata</i> Gouan      | Sh          | 1.42                                     |
| <i>Lupinus albus</i> L.                 | A. He       | 0.75                                     |
| <i>Medicago sativa</i> L.               | P. He       | 18.31                                    |
| <i>Melilotus indicus</i> (L.) All.      | A. He       | 0.54                                     |
| <i>Melilotus alba</i> L.                | A. He       | 0.71                                     |
| <i>Tamarindus indicus</i> L.            | Tr          | 0.33                                     |
| <i>Taverniera lappacea</i> (Forssk) DC. | Sh.         | 0.17                                     |
| <i>Tephrosia purpurea</i> (L.) Pers     | A. He       | 0.33                                     |
| <i>Tephrosia uniflora</i> L.            | Sh          | 0.12                                     |
| <i>Trigonella foenum-graecum</i> L.     | A. He       | 0.09                                     |
| <i>Vicia faba</i> L.                    | A. He       | 6.13                                     |
| <i>Vicia sativa</i> L.                  | A. He       | 1.87                                     |
| <i>Vigna unguiculata</i> L.             | A. He       | 1.93                                     |

Tr = Tree; Sh = Shrub; P. He = Perennial herb; A. He = Annual herb

Within the Oasis sites, the high soil water values could be due to frequent irrigations in the nearby farms, had significantly high total cover in all of the major plant groups with the exception of the tree plant group (Table 2). Total cover of annual herbs was significantly higher in sites from within the Oasis and the total cover of perennial herbs and shrubs also tended to decrease significantly in the outside the Oasis sites (Table 2). Conversely, total cover of the tree plant group exhibited an inverse relationship (Table 2). Within the shrub plant group, the *Indigofera* exhibited shifts in total cover between the two locations (the within and outside the Oasis).

The annual herb plant group consisted of species that belong to the genera *Astragalus*, *Crotalaria*, *Lupinus*, *Melilotus*, *Tephrosia*, *Trigonella*, *Vicia* and *Vigna*. The annual herbs were only present within the Oasis. However, the perennial herbs and shrubs also exhibited significant variations between the two sites and were significantly higher within the Oasis.

Table 2: F-values obtained from ANOVA of species richness and total cover of the legume major plant groups in the two studied sites in Al-Hassa Oasis

| Parameter        | Al-Hassa Oasis sites (d.f = 1,31) |         |
|------------------|-----------------------------------|---------|
|                  | Within                            | Outside |
| Species richness | 6.155*                            | 3.851   |
| Total cover      | 21.701*                           | 5.956   |
| Annual herb      | 14.069*                           | 0.102   |
| Perennial herb   | 17.471*                           | 1.576   |
| Shrub            | 25.953*                           | 7.085   |
| Tree             | 1.021                             | 4.353*  |

\*: Significant at p<0.05

In the tree plant group, only four genera were present: *Acacia*, *Allbizia*, *Delonix* and *Tamarindus* (Table 2) most of them exhibited significant variability between the two sites (Table 2). *Acacia* and *Tamarindus* were mainly present outside the Oasis.

Spatial variation in legume plant community composition and structure in Al-Hassa Oasis appears to be closely related to soil water, despite the high hydrological variability. The results of this study indicate that the within Oasis legume plants community are structured primarily on a spatial gradient of water availability.

**Identification of Nitrogen-fixing species:** In the two sites studied, species of legumes with isotopic signals ( $\delta^{15}\text{N}$ ) similar to the mean of the reference species (*Panicum turgidum*), as well as species with significantly inferior signals were identified (Table 3). The species with statistically lower signals were considered as species that were able to obtain part of their N supply through biological fixation of atmosphere  $\text{N}_2$ . All the legume plants presented 15N impoverished leaves in relation to the reference species. The plant *I. argentea*, *I. oblongifolia*, *I. spinosa*, *T. lappacea*, *T. purpurea* and *T. uniflora* collected in Al-Hassa had isotopic signals similar to those of the reference species and thus were considered as non-fixers in Al-Hassa region.

The mean biological fixation contribution for plant nitrogen was according to calculations that were done considering  $B = -2$  or  $B = \%$ . Respectively (Table 3). Species with great nitrogen fixation capacity ( $\text{Ndfa} > 50\%$ ) were recorded in Al-Hassa (Table 3), even considering the most conservative estimates ( $\% \text{Ndfa}$  calculated considering  $B = -2\%$ ). The most remarkable ones were *Medicago sativa*, *Vicia faba*, *Melilotus indicus*, *Melilotus alba* and *Dolichus lablab*. The lowest N content of leaves was found in the reference species. Among the legumes, the N leaf content differed significantly ( $p < 0.01$ ) between the two sites. The within Al-Hassa Oasis legumes had a mean content of 3.1%, while the outside had a mean of 2.19% in leaves.

Table 3: List of species belonging to the Fabaceae family recorded in the vegetation survey

| Species                                 | $\delta^{15}\text{N}$ | %Ndfa |      |
|---|-----------------------|-------|------|
|   |                       | a     | b    |
| <i>Acacia farnesiana</i> (Lam) Wild     | 8.04 ± 0.73*          | 22.7  | 19.0 |
| <i>Acacia gerradi</i> Benth.            | 6.33 ± 1.43**         | 39.1  | 32.8 |
| <i>Acacia tortilis</i> Hayne            | 4.46 ± 0.82**         | 54.1  | 47.9 |
| <i>Alhagi camelorum</i> Fisch.          | 9.86 ± 1.21 ns        | 5.4   | 4.4  |
| <i>Allbizia lebbek</i> (L.) Benth       | 9.06 ± 0.78 ns        | 13.1  | 10.8 |
| <i>Anagyris foetida</i> L.              | 4.27 ± 1.01**         | 58.9  | 47.9 |
| <i>Astragalus tribuloides</i> Del.      | 9.30 ± 1.48 ns        | 10.6  | 8.9  |
| <i>Cassia holosericea</i> Fres          | 4.22 ± 0.86**         | 59.4  | 49.8 |
| <i>Cassia italica</i> (Miller) spreng   | 4.96 ± 1.06**         | 52.3  | 43.9 |
| <i>Cassia occidentalis</i> L.           | 5.28 ± 0.93**         | 49.2  | 41.3 |
| <i>Cassia senna</i> L.                  | 5.96 ± 0.53**         | 42.7  | 35.8 |
| <i>Clitoria ternatea</i> L.             | 4.18 ± 1.57**         | 59.8  | 50.2 |
| <i>Crotalaria ertusa</i> L.             | 5.12 ± 1.16**         | 50.8  | 42.6 |
| <i>Crotalaria microphylla</i> Vahl      | 5.43 ± 1.32**         | 47.8  | 40.1 |
| <i>Delonix elata</i> (L.) Gambe         | 8.86 ± 1.27 ns        | 14.8  | 12.4 |
| <i>Dolichus lablab</i> L.               | 3.94 ± 0.93**         | 62.1  | 52.1 |
| <i>Indigofera argentea</i> L.           | 9.92 ± 0.65 ns        | 4.6   | 3.9  |
| <i>Indigofera articulate</i> Gouan      | 4.03 ± 0.69**         | 61.3  | 51.4 |
| <i>Indigofera oblongifolia</i> Forssk   | 8.73 ± 0.36 ns        | 16.1  | 13.5 |
| <i>Indigofera spinosa</i> Forssk        | 8.21 ± 0.49 ns        | 21.1  | 17.7 |
| <i>Lupinus albus</i> L.                 | 7.09 ± 0.84**         | 31.8  | 26.7 |
| <i>Medicago sativa</i> L.               | 2.39 ± 0.27**         | 77.0  | 64.6 |
| <i>Melilotus indicus</i> (L.) All.      | 3.74 ± 1.02**         | 64.0  | 53.7 |
| <i>Melilotus alba</i> L.                | 4.00 ± 0.69**         | 61.5  | 51.6 |
| <i>Panicum turgidum</i> (reference)     | 10.4 ± 10.3           |       |      |
| <i>Tamarindus indicus</i> L.            | 6.71 ± 0.93           | 48.5  | 40.5 |
| <i>Taverniera lappacea</i> (Forssk) DC. | 8.28 ± 1.11 ns        | 20.4  | 17.1 |
| <i>Tephrosia purpurea</i> (L.) Pers     | 9.48 ± 0.77 ns        | 8.8   | 7.4  |
| <i>Tephrosia uniflora</i> L.            | 9.55 ± 0.63 ns        | 8.2   | 6.9  |
| <i>Trigonella foenum-graecum</i> L.     | 5.38 ± 0.94**         | 45.8  | 40.5 |
| <i>Vicia faba</i> L.                    | 3.05 ± 0.64**         | 70.7  | 59.3 |
| <i>Vicia sativa</i> L.                  | 3.29 ± 0.71**         | 68.4  | 57.3 |
| <i>Vigna unguiculata</i> L.             | 3.75 ± 1.05**         | 63.9  | 53.6 |

A: Ndfa calculated with B value of 0%; b: Ndfa calculated with B value of -2% \* : Significantly different (p<0.05) from the reference mean; \*\*: Highly significantly different (p<0.01) from the reference mean; ns: Not significantly mean

Among the 32 target species, the studied species ( $\delta^{15}\text{N}$ ) isotopic signals significantly inferior to the reference species, being identified as fixing species. The behavior of these Fabaceae points to the fact that the occurrence of  $\text{N}_2$  atmospheric fixation in legumes with nodulating capacity is influenced by environmental and/or physiological conditions. In systems where the N stock is well conserved, fixing legumes may obtain small quantities of BNF (Boddey *et al.*, 2000). Therefore, it is probable that the high  $^{15}\text{N}$  signal of certain legume plants e.g., *I. argentea*, *A. camelorum*, *T. purpurea* and *T. uniflora* is due to some physiological and/or environmental restriction for the  $\text{N}_2$  fixation and not to lack of nodulating capacity.

Results of isotopic signals provided very promising conditions for calculating N derived from biological fixation. The signals of the reference plants was high in all the sites studied (Table 3) and in the fixing plants they were more than two units of  $\delta^{15}\text{N}\%$  lower than those of the reference plant. The differences varied between 0.48% (*I. argentea*) and 8.01% (*M. sativa*).

This condition, that permits safety in fixation studies (Akran *et al.*, 2009; Ardakani *et al.*, 2009). In semi-arid environments the differences between the  $\delta^{15}\text{N}$  signals of potentially fixing species and non fixing species can be ambiguous, generating doubts as to whether the fixing species are fixing a little or if the method does not work (Akran *et al.*, 2009). As a consequence, there are very few studies with clear evidence of fixation in native vegetations of dry ecosystems in the world (Mirzakhani *et al.*, 2009; Ezrin *et al.*, 2010).

High contributions of BNF were estimated for the cropped legumes of Al-Hassa, which had mean %Ndfa, in the minimum, of 51.6% and in the maximum, 64.6% considering  $B = -2\%$  (Table 3). If the calculations had been done with  $B = 0$ , these values would have increased to 61.5% and 77.0%, respectively. Comparison of these results with those found in literature, were done utilizing  $B = -2\%$ , a value that is commonly used in tree legume studies (Raddad *et al.*, 2005; Mirzakhani *et al.*, 2009; Ezrin *et al.*, 2010).

Although higher values of %Ndfa are found in some highly efficient species in symbiotic nitrogen fixation, as much under natural conditions as in plantations (Gathumbi *et al.*, 2002), the mean values for sites with natural vegetation are generally much lower than those found for Al-Hassa. Ndiaye and Ganry (1997) found very small contributions (Ndfa/4 10% and 20%) of BNF in two ecological zones localized in semi-arid regions of Senegal, while Schulze *et al.* (1991) found a mean of 32% of BNF contribution in an arid gradient in Namibia. Similar values (43-65%) to those found Al-Hassa were found in some desertic areas of California covered by *Prosopis* spp (Shearer *et al.*, 1983). Highly fixing species (Ndfa greater than 50%, even considering  $B = -2\%$ ) were found Al-Hassa, the most remarkable ones being *M. sativa*, *V. faba*, *V. unguiculata*, *V. sativa*, *M. Alba*, *M. indicus* and *C. ternatea*. The occurrence of these species could be related to the ecological advantage provided by N fixation regarding P uptake in P-limited. The lowest value of %Ndfa found in Al-Hassa could be related to its lower availability of water. Factors not evaluated in this study, such as nutrient and micorrhiza availability (Cardoso and Kuyper, 2006; Mirzakhani *et al.*, 2009; Akran *et al.*, 2009; Ezrin *et al.*, 2010), also affect the BNF and they could be more favorable in Al-Hassa.

The N content of the legume leaves differed from the non legume leaves (Table 4). Legumes had 3.1% of N in their leaves. This richness of N in leaves of legume species, is attributed to their growth habit (McKey, 1994), which demands large quantities of nitrogen and maximizes the rates of photosynthesis per leaf area.

Table 4: Mean leaf nitrogen concentrations (%) of legume plants collected at the two sites of Al-Hassa Oasis

| Sites   | Legumes      | Reference    | Overall mean |
|---------|--------------|--------------|--------------|
| Within  | 3.10 ± 0.83  | 1.81 ± 0.20  | 2.46 ± 0.52a |
| Outside | 2.19 ± 0.35  | 1.60 ± 0.15  | 1.90 ± 0.25b |
| Mean    | 2.65 ± 0.59a | 1.71 ± 0.18b | 1.71 ± 0.18b |

Means follows by the same small letter in the row and the same capital letters in the column are not significantly different at the 0.05 level according to the LSD test

Our results demonstrate that, in Al-Hassa, the capacity of BNF. These estimates indicate that BNF contribution to the leaf nitrogen outside Oasis is small, reaching a mean of 2.19%. The contribution to the whole plant would be higher considering the N translocated to branches and roots. However, most of the annual accumulation of N is directed to the leaves, whose biomass is renovated each year.

### CONCLUSION

The survey on the legume plant diversity in the flora of Al-Hassa Oasis, Kingdom of Saudi Arabia indicated that the Fabaceae family is presented in the flora with 32 species belonging to 20 genera. The Cassia and *Indigofera* have the highest number of species (4) of the legume plants. Annual herbs were the most common growth form (34%). The results showed a good potential for use of the <sup>15</sup>N natural abundance methodology for evaluating the nitrogen fixation ability of the legume plants under field conditions as well as for the estimation of % Ndfa. Some legume species of Al-Hassa Oasis have the potential of fixing high amounts of fixing high amounts of nitrogen, especially *Medicago sativa*, *Vicia faba*, *Vicia sativa*, *Melilotus indicus*, *Molilotus alba*, *Dolicus lablab* and *Cliforia ternate*. The mean contribution of nitrogen fixation for plant nitrogen were high for most of the species studied.

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