

Nitrogen fixation and chemical composition of wild annual legumes at Beni-Suef governorate, Egypt.

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ABSTRACT

Wild herb legumes play an important role in agriculture because of their nitrogen fixing ability and genetic resources. Wild annual legumes at Beni-Suef Governorate, Egypt, were studied for their nodulation status, nitrogen fixation and their chemical composition. The nodulation percentage of the recorded wild legumes was between 70 % and 100 %. The highest nodule formation rate (nodule number) occurred in *Trifolium resupinatum* and *Melilotus indicus*, about 92 and 53 nodules per plant, respectively. *Melilotus indicus*, *Vicia sativa* and *Medicago intertexta* produced root nodules with higher mass (fresh weight). The most effective N₂-fixing (acetylene reducing) species were *Medicago intertexta* and *Melilotus indicus*. The structure of nodules from most of the wild herb legumes showed the characteristic features of indeterminate nodules (with an apical meristematic tissue). All studied wild legume species showed higher content of both vitamin B₁ and B₂ compared to cultivated species. From our results we recommend the cultivation of these wild legumes in reclaimed areas in Egypt because they will improve soil fertility and can be used as animal fodder.

Keywords: wild legumes, nitrogen fixation, chemical composition, Egypt.

INTRODUCTION

The family Leguminosae is one of the largest families of flowering plants, consisting of about 463 genera and 18000 species (Sprent 1999), with almost cosmopolitan distribution: it has played a very important role in human civilization. Nitrogen acquisition and assimilation is second to photosynthesis in terms of importance for plant growth and development, and nitrogen is perhaps the single most important factor currently limiting crop yields (Vance 1997). Waggoner (1994) reported that individual protein consumption of the 5.3 billion people on earth averages about 70 g of protein per day, or 23 million tones of nitrogen per annum. To maintain this level of intake in the face of a doubling of the earth's population over the next 40 years, a doubling or tripling of crop production is necessary (Vance 1997).

Nitrogen fixation by legumes plays an important role in sustaining crop productivity and increasing fertility of marginal lands and in smallholder systems of the semiarid tropics (Serraj *et al.* 2004). The measurement of N₂ fixation by legumes is necessary for gaining an understanding of their contribution to the nitrogen economies of agricultural and forestry systems, and for their management in those systems (Herridge & Peoples 2002). Biological nitrogen fixation accounts for 65% of the nitrogen currently utilized in agriculture, and will be increasingly important in future crop productivity (Vance 1997).

Legume root nodules are "organs" in which the soil bacterium *Rhizobium* grows endosymbiotically and is capable of reducing or fixing molecular or atmospheric nitrogen (Sprent 2001). The economic importance of wild legumes, and the significance of wild herb and tree legumes to soil fertility is well known, and some leguminous plants that are wild in some countries are cultivated in others (e.g. *Vicia sativa*: Sanchez-Grion *et al.* 2004). The ability of wild herb legumes to fix nitrogen makes them an important nitrogen source in

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agroecosystems (Zahran 1998). The amount of nitrogen fixed by forage legumes varies greatly with species, management, soil nitrogen supply and the presence of grasses, but it can reach 275 Kg N ha⁻¹ yr⁻¹ in the Northcentral USA (Mallarino *et al.* 1990).

The aim of the present study is to estimate the nitrogen fixing ability and chemical composition of annual wild legumes in Egypt, with a view to cultivating them as forage.

MATERIALS AND METHODS:

The concentration of the minerals Na, Ca, Mg, P, Fe, Cu, Zn, Ni, Pb and Mo in plant material (shoot system) and soil were determined by using atomic absorption spectrophotometry (using a Perkin 403: Perkin-Elmer 1983). The content of C, H and N was determined in plant dry matter of the shoot system of leguminous plants by using an elemental analyzer (Perkin-Elmer ICP 400). Protein content was determined following the method of Lowry *et al.* (1951). The nitrogen-fixing activity (nitrogenase activity) of the legume–*Rhizobium* symbiosis was determined according to the methods described by Witty & Minchin (1988).

Nodulation status depends on many factors, including the legume stage and edaphic factors, especially moisture content. In the present study, leguminous plants were collected at the vegetative stage: 20 plants were sampled for determining nodulation percentage, and 5 plants for counting nodule number and determination of fresh and dry weights of nodules.

Nodules were fixed in a solution containing 4 % glutaraldehyde (w/v) overnight and rinsed in buffer for 30 minutes, then passed through a graded ethanol: water series to pure ethanol, embedded in LR white resin (Nap *et al.* 1989), and left overnight at 70 °C for polymerization. Blocks were sectioned (1-4 µm thick) in a Sorval Inc. Norwalk, (USA). Sections were heat-fixed on clean slides and stained with 0.1 % toluidine blue in 1.0 % (w/v) sodium borate. The stained sections were then examined and photographed.

The vitamin B₆ content was measured according to the method described by Bergaentzle *et al.* (1995). Amino acids were measured using the method described by Lee & Takahashi (1966).

RESULTS

The nodulation percentage of the recorded wild herb legumes was between 70 % and 100% (Table 1), with great variation among species collected from different habitats. *Melilotus indicus* in cultivated land was highest, and *Trigonella hamosa* the lowest. The highest number of nodules occurred in *Trifolium resupinatum* and *Melilotus indicus*. Three plants had relatively heavy total nodule weights (*Melilotus indicus*, *Vicia sativa* and *Medicago intertexta*), but their nodules were also very small in size. Although *Trifolium resupinatum* formed the highest nodule number, the fresh weight of these nodules was relatively low. Some of the species found in salt-affected soils (e.g. *Melilotus indicus* and *Medicago intertexta*) were characterized by a reduced nodulation percentage and nodule number, but nodules found in this habitat were larger in mass, and less pigmented or lacking pigment altogether. It is noticeable (Table 1) that legumes from canal-bank habitats had higher nodulation percentages and nodule numbers, probably due to the availability of water.

Wild herb legumes exhibited high rates of nitrogen-fixing activity compared to cultivated crops (Table 2). Nitrogenase activity (acetylene reduction) ranged between 0.05 and 3.3 µmole C₂H₄ pl⁻¹ hr⁻¹, with the most effective plant species being *Medicago intertexta* and *Melilotus indicus*. There was some variation within species collected from

different localities (e.g. *Vicia sativa*). The wild forage legume *Lotus corniculatus* appears to be more salt-tolerant than its cultivated forage equivalent *Trifolium alexandrinum*, and also a more effective nitrogen-fixing legume. The least effective legume with the lowest nitrogen-fixing ability was *Trigonella hamosa*.

Table 1: Nodulation percentage, nodule number, nodule fresh weight (mg) and nodule dry weight (mg) of the recorded wild herb legumes. Values are cited as the mean \pm 1 standard error.

| Species | Habitat | Nod. % | Nodule no. per plant | Nodule f.wt | Nodule dry wt |
|----------------------------------|---------------|--------|----------------------|--------------|---------------|
| <i>Melilotus indicus</i> All. | Cultivated | 100 | 53 \pm 3.3 | 460 \pm 70 | 98 \pm 7.0 |
| <i>Melilotus indicus</i> All. | Salt-affected | 70 | 12 \pm 2.9 | 115 \pm 60 | 24 \pm 1.0 |
| <i>Melilotus siculus</i> Jacks. | Salt-affected | 80 | 17 \pm 3.0 | 76 \pm 2.0 | 16 \pm 2.0 |
| <i>Trifolium resupinatum</i> L. | Canal bank | 100 | 92 \pm 5.0 | 55 \pm 3.0 | 19 \pm 1.0 |
| <i>Trifolium resupinatum</i> L. | Cultivated | 90 | 35 \pm 2.1 | 18 \pm 4.0 | 06 \pm 0.2 |
| <i>Medicago intertexta</i> Mill. | Cultivated | 95 | 15 \pm 2.4 | 199 \pm 50 | 49 \pm 1.0 |
| <i>Medicago intertexta</i> Mill. | Salt-affected | 75 | 03 \pm 1.0 | 38 \pm 9.0 | 10 \pm 0.3 |
| <i>Medicago polymorpha</i> L. | Cultivated | 90 | 17 \pm 2.5 | 175 \pm 20 | 40 \pm 4.0 |
| <i>Vicia sativa</i> L. | Canal bank | 100 | 29 \pm 4.0 | 258 \pm 10 | 53 \pm 5.0 |
| <i>Vicia sativa</i> L. | Cultivated | 85 | 14 \pm 2.0 | 93 \pm 1.0 | 14 \pm 2.0 |
| <i>Vicia monantha</i> Retz. | Cultivated | 85 | 16 \pm 1.8 | 123 \pm 30 | 19 \pm 3.0 |
| <i>Trigonella hamosa</i> L. | Cultivated | 70 | 09 \pm 1.2 | 45 \pm 1.0 | 09 \pm 0.9 |
| <i>Lotus corniculatus</i> L. | Salt-affected | 95 | 21 \pm 3.5 | 105 \pm 50 | 18 \pm 3.0 |
| <i>Lathyrus hirsutus</i> L. | Canal bank | 100 | 17 \pm 2.0 | 62 \pm 3.0 | 17 \pm 4.0 |

Table 2: Nitrogen fixation (nitrogenase activity) of some wild herb legumes collected from different habitats of Beni-Suef Governorate compared to that of some cultivated legumes.*

| Species of Cultivated legumes | Location | Habitat | Activity (μ mole C ₂ H ₄ p1 ⁻¹ h ⁻¹) |
|----------------------------------|-----------|-----------------------------|--|
| <i>Vicia faba</i> | Somosta | Cultivated soil | 0.56 |
| <i>Trifolium alexandrinum</i> | Somosta | Salt-affected (10%) soil | 0.07 |
| <i>Arachis hypogaea</i> | Somosta | Cultivated soil | 0.61 |
| <i>Glycine max</i> | Somosta | Cultivated soil | 0.04 |
| Wild herb legumes | | | |
| <i>Medicago intertexta</i> Mill. | Beba | Cultivated soil | 3.30 |
| <i>Medicago polymorpha</i> | Beba | Cultivated soil | 0.86 |
| <i>Medicago polymorpha</i> | Somosta | Cultivated soil | 1.08 |
| <i>Melilotus indicus</i> | Beba | Cultivated soil | 1.14 |
| <i>Melilotus indicus</i> | Somosta | Cultivated soil | 2.32 |
| <i>Trifolium resupinatum</i> | Somosta | Canal bank soil | 0.52 |
| <i>Trigonella hamosa</i> | Somosta | Reclaimed soil | 0.05 |
| <i>Vicia sativa</i> | Somosta | Canal bank soil | 1.90 |
| <i>Vicia sativa</i> | Beba | Canal bank soil | 0.70 |
| <i>Lotus corniculatus</i> | Ehnasia 1 | Salt-affected (12%) soil | 0.15 |
| <i>Lotus corniculatus</i> | Ehnasia 2 | Slightly saline (0.2%) soil | 0.24 |

* Most of the collected legumes were in vegetative stage except *A. hypogaea* and *G. max* which were in flowering and fruiting stages, respectively.

All nodules were elongate and meristematic, i.e. indeterminate, except those of *Lotus corniculatus*, the only plant with determinate, spherical or globose nodules. Most were branched, except those of *Trifolium resupinatum*, *Trigonella hamosa* and *Lathyrus*

hirsutus. Nodules of *Medicago intertexta* were collected from plants in salty soil (electrical conductivity 2.99 mS/cm); infected cells appeared healthy

We determined the chemical composition of two legumes, *Melilotus indicus* and *Medicago intertexta*, from salt-affected and cultivated land, and also *Trifolium resupinatum* from cultivated land and canal banks (Table 3). The microelements Fe, Cd, Al and Zn were slightly higher from salt-affected soil, and Na concentration was greatly increased. In *T. resupinatum*, the microelement concentrations did not show much variation, but Na content was higher in plants from cultivated lands. *Medicago intertexta* and *Melilotus indicus* from cultivated land accumulated relatively high amounts of shoot protein, but protein content of all species appeared to decrease with increasing soil salinity.

Table 4 shows the measured amounts of some amino acids. The results show that, for example, *Melilotus indicus* from salt-affected soil contained higher proline, glycine, threonine and methionine than plants from cultivated land, usually very much higher. In *Trifolium resupinatum* there were few differences between habitats. Great variation was found among species from the same habitat.

The vitamin content (B₁ and B₆) was compared to those of the cultivated legume clover, *Trifolium alexandrinum* (Table 5). All wild legume species showed higher content of both vitamins compared to the cultivated species, sometimes substantially higher. This underlines the nutritional value of these plants for animal feed.

Table 3: Chemical composition (mg/kg dry matter) and protein content of some wild legumes collected from different habitats of Beni-Suef. Values are means ± 1 s.e.

| Species | <i>M. indicus</i> | <i>M. indicus</i> | <i>M. intertexta</i> | <i>M. intertexta</i> | <i>T. resupinatum</i> | <i>T. resupinatum</i> |
|----------|--------------------------|--------------------------|------------------------|----------------------|-----------------------|-----------------------|
| Habitats | Cultivated | Salt-affected | Salt-affected | Cultivated | Canal bank | Cultivated |
| Location | Somosta | El-Wasta | El-Wasta | Somosta | Beba | Somosta |
| Fe mg/Kg | 4.102 ± .887 ± .883 ± .8 | 7.8.9 ± .7.9 ± .75 ± 3.7 | .53 ± .5.7 ± .5.3 ± .5 | 3 ± .7 ± .5.778 ± .3 | .78 ± .89.3 ± .9 | 7 ± .5 ± .3 |
| A | 3.8 ± .3 | 9.8 ± 7 | 3.89 ± .9 | 5 ± .389 | .573 ± . | 7.3 ± .3 |
| Z | 9.7 ± .7 | .3 ± . | 5.8 ± .5 | 3 ± . | .93 ± .57 | 5.7 ± .8 |
| N | ± . | .5 ± . | .7 ± . | 8 ± . | .77 ± 5. | ± 9. |
| % | ± . | .5 ± . | ± 7 | 8 ± 5 | 3855 ± | 3.3 ± 9 |
| % | 35 ± .5 | 5 ± .5 | 5.5 ± . | 5 ± .5 | 5. ± . | 3.58 ± .5 |
| N% | .5 ± .5 | 5 ± .5 | 3.55 ± .5 | 35 ± .5 | 7 ± . | 55 ± .5 |
| T DW | 79.5 ± .5 | 5 ± 3. | 33 ± . | 53 ± .3 | 3 | ± 5.55 |

Table 4: Amino acid content (%) in some wild herb legumes.

| Legume species | Habitat | ProL. | Glyc. | Ther. | Val. | Meth. |
|-----------------------|---------------|-------|-------|-------|-------|-------|
| <i>M. indicus</i> | Salt-affected | 1.414 | 0.946 | 1.892 | - | 0.362 |
| <i>M. indicus</i> | Cultivated | 0.375 | 0.347 | 0.151 | 0.19 | 0.174 |
| <i>M. intertexta</i> | Salt-affected | 0.786 | 1.104 | - | 0.782 | - |
| <i>M. intertexta</i> | Cultivated | 0.343 | 0.302 | - | 0.172 | - |
| <i>T. resupinatum</i> | Canal banks | 0.343 | 0.282 | - | 0.257 | - |
| <i>T. resupinatum</i> | Canal banks | 0.343 | 0.258 | 0.359 | 0.094 | 0.617 |

Table 5: Content (mg/100 g dry matter) of B₁ and B₆ vitamins in shoots of some wild herb legume species. Values are means \pm 1 s.e.

| Species | Habitat | B ₁ (mg/100 g) | B ₆ (mg/100 g) |
|--|----------------|------------------------------|------------------------------|
| <i>Trifolium alexandrinum</i> (clover crop) | Cultivated | 0.314 \pm 0.05 | 0.244 \pm 0.01 |
| <i>Trifolium resupinatum</i> | Cultivated | 0.536 \pm 0.05 | 0.821 \pm 0.11 |
| <i>Melilotus indicus</i> | Cultivated | 0.484 \pm 0.05 | 0.494 \pm 0.05 |
| <i>Melilotus indicus</i> | Salt- affected | 0.492 \pm 0.11 | 0.397 \pm 0.01 |
| <i>Medicago polymorpha</i> | Cultivated | 0.500 \pm 0.11 | 0.829 \pm 0.08 |
| <i>Medicago intertexta</i> | Salt-affected | 0.537 \pm 0.05 | 0.387 \pm 0.05 |

DISCUSSION

In the present study, wild herb legumes were characterized by a higher rate of nodulation and higher nitrogen-fixing ability than their cultivated relatives. Sprent (1999) reported that nodulation is a robust taxonomic character, both at the presence/absence level and the structural / physiological level. However, the nodulation patterns of individual species varied among different habitats in this study. For example, *Trifolium resupinatum*, *Vicia sativa* and *Lathyrus hirsutus* produced plenty of nodules and showed higher rates of nodulation in the canal bank habitat than in other habitats, indicating perhaps that these plants prefer this habitat. Nodulation is greatly affected by the prevailing environmental conditions, but it may also regulated by plant-dependent factors. Walsh (1995) reported that some plants develop greater nodule mass when mineralized nitrogen is limiting, and when the specific rate of N fixation is decreased. Yousef & Sprent (1983) reported an increase in nodule mass of *Vicia faba* treated with 100 mM NaCl.

The nitrogen fixation ability of the studied herb legumes was higher than that of the cultivated legumes (Table 2). Zahran (1998) suggested that four wild herb legumes could be significant nitrogen-fixers, especially when grown under drought conditions: estimates of fixation rates of *Lotus* in North America range from 50 to 205 kg N ha⁻¹ yr⁻¹ (Mallarino *et al.* 1990). Many wild herb legumes exist in the studied area as weeds. There are about 50 annual or perennial species of *Medicago*, of which about one half have been researched for agriculture (Langer & Hill 1985), most importantly *Medicago sativa* (alfalfa or lucerne). There are two *Medicago* species in the studied area in Egypt, *M. intertexta* and *M. polymorpha*; the former showed the higher rate of nitrogen fixation (Table 2) and grows in salty habitats. Such salt-tolerant legumes could be cultivated in areas that are neglected because of salinization.

Only about ten species of *Trifolium* are of agricultural importance, although the genus contains probably more than 250 species. In this study, *T. resupinatum* favours canal banks where soil is saturated, but also occurs as well as in other habitats. Other species of *Trifolium* (e.g. *T. repens* - white clover) is often absent from saturated soils, and is said not to be adapted to them (Burdon 1983). *T. resupinatum* can be cultivated in soils sometimes flooded by water, such as the Nile islands and the north coast.

Vicia sativa also exhibited high rates of nitrogen fixation (Table 2). Known as common vetch, this species is used as a protein-rich forage for cattle (Langer & Hill 1985).

Individuals of the salt-tolerant *Melilotus indicus* and *Medicago intertexta* accumulate more Na⁺ ions in their tissues when grown in salty ground than in cultivated land, due to the high concentration of Na in the root zones. They also accumulate more K⁺ ions under salt

conditions, an effect which enables these plants to tolerate moderate salinity. Plants take up K^+ under salinity to adjust the osmotic potential of their tissues (Zahran 1999). Potassium is known to be an essential factor determining the resistance to water stress and water balance of white clover (Robin *et al.* 1989).

Accumulation of compatible solutes under salt stress is one of the basic mechanisms of adaptation of plants to saline conditions. Proline accumulates consistently in numerous plant species under a wide range of environmental conditions, including water stress, but levels cannot be used as a diagnostic feature of different ecological groups (Batanouny *et al.* 1985), including drought resistance (Atteya 1996), because accumulation rates vary widely. Thus a simple positive correlation between proline accumulation potential and drought resistance in plants may be an error. However, proline accumulation is an indicator of stress in plants and may be considered as a biochemical response to different environmental stresses. Snehal (1984) found that proline content increased in pigeonpea (*Cajanus cajan*) at low salt levels (2.5 and 5.0 mmhos cm⁻¹ NaCl), but was reduced at higher levels (15 mmhos cm⁻¹). Negative relations between salt tolerance and proline accumulation have been also reported (Mendelssohn 1987).

Belesky & Wilkinson (1984) reported that proline as a proportion of the total amino acids decreased with N fertilization at adequate soil water levels, but increased with N fertilization when soil water was inadequate: the opposite effect was observed for glycine. The accumulation of amino acids associated with water stress may be a result of disrupted metabolism, or may actually be part of an adaptive protein complement synthesized in response to stress (Dungey & Davies 1982). In addition to their role in adaptation to salt and water stress, the accumulation of certain amino acids in the tissues of wild herb legumes indicates the nutritive value of these plants, and supports the idea of their cultivation as forage.

In conclusion, we strongly recommend the cultivation of these wild herb legume species for forage in Egypt. The species recorded in this study showed high rate of N fixation under normal as well as unfavourable soil conditions (salt-affected soil). Their performance was better than that of cultivated crop legumes, and they also contain a satisfactory amount of protein and vitamins, underlining their nutritive value for animal feedstuff.

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الملخص العربي

تثبيت النيتروجين و التركيب الكيميائي للنباتات البقولية الحولية البرية بمحافظة بنى سويف، مصر.

عماد الشريف؛ حمدى زهران ، عطية مصطفى

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تلعب النباتات البرية البقولية دورا هاما فى الزراعة و ذلك لقدرتها على تثبيت النيتروجين الجوى و ايضا كونها مصدر للجينات الوراثية. تمت دراسة هذه النباتات البرية (العشبية) بمحافظة بنى سويف (مصر) من حيث قدرتها على تكوين العقد الجذرية و تثبيت النيتروجين الجوى و تركيبها الكيميائى فى بيئاتها الطبيعية. و قد وجد ان نسبة تكوين العقد الجذرية فى هذه النباتات تتراوح بين ٧٠ و ١٠٠% و سجل نبات البرسيم البرى و نبات الحنقون أعلى عدد عقد جذرية (٩٢ و ٥٣ عقدة جذرية لكل نبات على التوالى). كما سجل نبات الحنقون *M. indicus* و نبات *M. intertexta* أعلى قدرة على تثبيت النيتروجين فى التربة العادية و التربة المصابة بالأملاح. و بينت الدراسة التشريحية للعقد الجذرية ان نبات *L. Corniculatus* عقد محدودة النمو (لا تحتوى على خلايا ميرستيمية) بينما باقى النباتات ذات عقد جذرية غير محدودة النمو (تحتوى على خلايا ميرستيمية). كما وجد ان نسبة البروتين و فيتامين ب_١ و فيتامين ب_٢ عالية مقارنة بالأصناف الزراعية الموجودة بنفس البيئات. و من خلال هذه الدراسة نوصى باستزراع هذه النباتات فى الأراضى المصابة بالأملاح و المستصلحة حديثا لتحسين خصوبة التربة و استخدامها كعلف حيوانى.