African Journal of Biotechnology Vol. 4 (1), pp. 57-60, January 2005
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2004 Academic Journals

Full Length Research Paper

Nodulation and growth response of *Sesbania sesban* (L.) Merr. to increasing nitrogen (ammonium) supply under glasshouse conditions

S.A. Indieka and D.W. Odee*

Biotechnology Laboratory, Kenya Forestry Research Institute, P.O. Box 20412-00200, Nairobi, Kenya.

Accepted 15 November, 2004

A glasshouse experiment was carried out to study the effect of ammonium-N on the nodulation, growth and N-uptake of *Sesbania sesban* (L.) Merr. inoculated with an effective *Mesorhizobium* (*S. sesban*) strain. Ammonium-N was supplied twice weekly as 100 ml of nutrient solution at concentrations of 0, 100, 200, 400, 600 and 800 mg N/l. The seedlings were grown in pots with sterilized sand substrate and assessed at 5, 7 and 9 weeks after planting (WAP). Nitrogen supply significantly improved nodulation (number of nodules and nodule dry weight/plant) with treatment 100 mg N/l compared with 0 mg N/l at 5 and 7 WAP, thus indicating synergism for the N_2-fixation symbiosis. However, with treatments ≥ 200 mg N/l nodulation was either depressed or inhibited. Seedlings treated with 100-400 mg N/l generally exhibited better growth (shoot and root dry weight/plant) and N-uptake than the other treatments (0, 600 and 800 mg N/l). This study has demonstrated that S*. *sesban* seedlings are tolerant to relatively high levels of N, and that treatment with 100 mg N/l (20 mg N per week) is necessary to stimulate an early and effective N_2-fixing symbiosis.

Key words: Ammonium, improved fallow, *Mesorhizobium*, N_2-fixation, nodulation, *Sesbania sesban*.

INTRODUCTION

Sesbania sesban (L.) Merr. (Papilionoideae) is a N_2-fixing tree (NFT) widely used in East and Central Africa as a short duration fallow species to increase available nitrogen (N) in soil for subsequent crops. Recent field studies have shown that *S. sesban* can fix up to 59% of N_2 in improved fallow systems in western Kenya (Gathumbi et al., 2002). Other regional field studies have consistently demonstrated that following *S. sesban* fallows, yields from maize crops are comparable to those supplied with recommended levels of mineral N and that yields are significantly higher than those obtained from un-fertilized soils (Kwesiga and Ngugi, 1996; Niang et al., 1996; Ikerra et al., 2001; Ståhl et al., 2002).

The possible sources of N in soils available to plants are nitrates, ammonium, or simple organic compounds such as amino acid and urea. Generally, the major form of N in agricultural soils is nitrate, while in most uncultivated soils it is ammonium-N (Sprent, 1999). However, Ikerra et al. (2001) showed that ammonium- and nitrate-N were equally predominant in topsoil in southern Malawi two to three weeks after *S. sesban* fallow biomass incorporation and contributed up to 37 kg inorganic N/ha in the topsoil. It is not clear how such flushes of N affect nodulation and N_2-fixation of subsequent *S. sesban* fallows.

Nitrogen is capable of influencing nodulation, N_2-fixation and growth in various ways. The quantity and

*Corresponding author. E-mail: dodee@africaonline.co.ke Phone +254 66 33383. Fax: +254 66 32844.

form of accessible N determines whether its availability is stimulating or detrimental to nodulation and N_2-fixation (Marschner, 1995). Additionally, the genotypes of the host and rhizobia vary in their affinity for N-uptake and tolerance in low or high N conditions (Goi et al., 1992; Hansen, 1994; Muofhe and Dakora, 1999; O'Hara, 2001). Unlike nitrates, high concentrations of ammonium are generally considered to be toxic to plants irrespective of whether they can nodulate or not (Marschner, 1995).

The effect of ammonium-N on nodulation and growth of *S. sesban* needs to be understood since such information will be crucial in the management of conditions suitable for the re-establishment of effective symbiosis of *S. sesban* fallows. The aim of this study was therefore to evaluate the effect of increasing levels of ammonium-N (supplied as $(NH_4)_2SO_4$) on nodulation, growth and N-uptake of *S. sesban* seedlings grown under glasshouse conditions.

MATERIALS AND METHODS

Seed source, pretreatment and germination

Seed of *S. sesban* (Kakamega provenance) was obtained from the Kenya Forestry Research Institute Seed Centre, Muguga. Uniformly sized, healthy seeds were pretreated by immersion in lukewarm water for 2-3 min. They were then surface sterilized in 3% NaOCl for 3 min, rinsed in five changes of sterilized distilled water and soaked overnight. Soaked seeds were pre-germinated on 0.8% w/v agar plates at 28°C for 2 days.

Planting, rhizobial inoculation and plant maintenance

Germinated seeds with approximately 2 cm of radical were planted in 3.5 l polyvinylchloride (PVC) pots (two per pot) containing washed, sterilized, river sand. The characteristics of the sand were: pH (H_2O) 7.2, electrical conductivity 22 μ/cm and trace macro- and micro-elements.

Prior to planting, the substrate was wetted with 100 ml sterilized nutrient solution (Broughton and Dilworth, 1971) with N treatments applied in the form of $(NH_4)_2SO_4$ at 0, 100, 200, 400, 600 and 800 mg N/l. Three days after planting, one ml of late-log phase yeast mannitol broth culture (~1×10^9 cells/ml) of the effective *Mesorhizobium* (*S. sesban*) strain KFR 647 was used to inoculate the seedlings using the method described in Somasegaran and Hoben (1994). Two weeks after planting, seedlings were fed twice weekly with 100 ml of nutrient solution containing the respective N concentrations. Prior to nutrient addition the substrate was flushed with 500 ml of sterilized, de-ionized water.

Experimental layout and growth conditions

The treatments were replicated 4 times (2 seedlings per pot) and laid out in a randomized complete block design. The experiment was conducted over a period of 9 weeks under glasshouse conditions at Muguga (KEFRI) with approximate mean temperatures of 25/18°C (day/night) and a photoperiod of approximately 12 h (natural daylight).

Assessment and analysis

Plants were harvested and assessed at 3, 5, 7 and 9 weeks after planting (WAP). At each harvest, nodules were detached from roots and counted. Shoots, roots and nodules were oven-dried at 60°C for 72 h then weighed. The dried shoot and root samples were finely ground with a ball mill grinder and N content was determined using a Shimadzu NC analyzer (Sumika Chemical Analysis Service [SCAS]–Sumigraph NC–90A, Japan). Analysis of variance and the Newman-Keuls test were performed on all data to detect significant differences between means.

RESULTS

Analysis of variance

The results of analysis of variance indicated that nodulation, growth and N-uptake in both shoot and root tissue of plants were significantly ($P<0.05$) influenced by ammonium-N concentration and their interaction with time of harvest (i.e. [ammonium-N] × WAP, data not shown).

Nodulation

At 5 and 7 WAP, plants treated with 100 mg N/l had significantly ($P<0.05$) better nodulation (nodule number and nodule dry weight/plant) than all other N treatments. Overall, treatment 100 mg N/l gave the best nodulation throughout the growth period. Nodulation increased markedly between 7 and 9 WAP for plants treated with 0 and 100 mg N/l (Table 1). However, plants treated with ≥ 200 mg N/l exhibited depressed nodulation compared to treatments 0 and 100 mg N/l. Nodulation was inhibited at 5 WAP in plants treated with ≥ 400 mg N/l, and was either inhibited or severely depressed at 7 and 9 WAP on plants treated with 600 and 800 mg N/l.

Shoot and root dry weight

Plants treated with 100-400 mg N/l had significantly higher shoot dry weight than those treated with 0 mg N/l at 5, 7 and 9 WAP. Root dry weight showed a similar trend to that of the shoot (Table 2). The highest shoot and root dry weight was obtained with plants treated with 200 mg N/l at 9 WAP. Seedlings treated with 0 mg N/l had the lowest shoot and root dry weight followed by those treated with 800 and 600 mg N/l in increasing order of magnitude.

Nitrogen uptake

Nitrogen uptake increased with time and generally reflected the growth trends shown by shoot and root dry weight. Plants treated with 0 mg N/l showed the least N-

Table 1. Effect of N concentration on nodulation of *S. sesban* plants inoculated with *Mesorhizobium* strain KFR 647 at 5, 7 and 9 weeks after planting (WAP). Values in a column (means, *n* = 4) followed by the same letter are not significantly different according to the Newman-Keuls test at *P<0.05* (NA, not applicable).

N concentration (mg/l)	Number of nodules/plant			Nodule dry weight (g/plant		
	5 WAP	7 WAP	9 WAP	5 WAP	7 WAP	9 WAP
0	18c	44b	108b	0.017a	0.060b	0.211b
100	32d	86c	113b	0.053b	0.224c	0.251b
200	9b	25ab	37a	0.002a	0.059b	0.189b
400	0a	10a	11a	NA	0.006a	0.029a
600	0a	5a	9a	NA	0.001a	0.011a
800	0a	0a	2a	NA	NA	0.001a

Table 2. Effect of N concentration on shoot and root growth (dry matter accumulation) of *S. sesban* plants inoculated with *Mesorhizobium* strain KFR 647 at 5, 7 and 9 weeks after transplanting (WAP). Values in a column (means, n = 4) followed by the same letter are not significantly different according to the Newman-Keuls test at P<0.05.

N concentration (mg/l)	Shoot dry weight (g/plant)			Root dry weight (g/plant)		
	5 WAP	7 WAP	9 WAP	5 WAP	7 WAP	9 WAP
0	0.063a	0.189a	1.030a	0.019a	0.066a	0.273a
100	0.572b	1.563b	3.224bc	0.185c	0.544c	0.620ab
200	0.486b	2.004b	3.355bc	0.126bc	0.610c	0.803b
400	0.405b	1.991b	3.130bc	0.138bc	0.495bc	0.735ab
600	0.168a	1.666ab	3.036bc	0.072ab	0.350bc	0.722ab
800	0.123a	0.861a	1.784ab	0.024a	0.268ab	0.525ab

Table 3. Effect of N concentration on shoot and root N content of *S. sesban* plants inoculated with *Mesorhizobium* strain KFR 647 at 5, 7 and 9 weeks after planting (WAP). Values in a column (means, n = 4) followed by the same letter are not significantly different according to the Newman-Keuls test at P<0.05.

N concentration (mg/l)	Shoot N (mg/plant)			Root N (mg/plant)		
	5 WAP	7 WAP	9 WAP	5 WAP	7 WAP	9 WAP
0	2.62a	7.98a	35.95a	0.36a	1.02a	3.72a
100	16.60b	44.34b	109.72b	2.83a	8.52bcd	10.12ab
200	21.10b	60.66b	109.92b	2.84a	12.19d	11.76ab
400	22.88b	109.87c	113.93b	2.33a	13.73d	19.79bc
600	9.87a	68.10b	150.82b	1.63a	8.18cd	25.98c
800	7.52a	29.94ab	79.91ab	0.95a	4.85abc	8.71a

uptake, but were not significantly different from seedlings treated with 800 mg N/l (Table 3). At 7 and 9 WAP, seedlings treated with 100-600 mg N/l showed significantly better N-uptake than treatment 0 mg N/l. There was a marked decrease in N accumulated by plants treated with 800 mg N/l compared to all other N-treatments, indicating a reduced N-uptake.

DISCUSSION

This study revealed some interesting nodulation and growth trends as influenced by increasing levels of ammonium-N supply. The extent of nodulation (used as an indicator of N_2-fixation) in relation to N supply could be broadly categorized as follows: (1) moderately to highly

nodulated plants, without ammonium–N (0 mg N/l) but dependent on N_2-fixation; (2) optimally nodulated plants, treated with 100 mg N/l; (3) poorly nodulated plants, treated with 200 mg N/l, and, (4) seedlings either with severely depressed nodulation or without nodules, treated with ≥ 400 mg N/l. Although the best nodulation occurred in plants treated with 100 mg N/l, the rate of nodulation increased exponentially over the 9-week growth period for plants relying solely on N_2-fixation suggesting a strategy by the plants to compensate for the poor N growth conditions. The key question here is why did the best nodulation occur in plants treated with 100 mg N/l, even at the early stages of growth (assessed at 5 WAP)? *S. sesban* is a small seeded legume capable of forming nodules within 7 days after germination (Odee et al., unpublished data). As a fast growing species cotyledon N reserves tend to be exhausted rapidly and, with no external N sources, an early N stress period may be experienced with a subsequent negative effect on nodule development and function. Eaglesham et al. (1983) showed that the larger seeded cowpea exhausted cotyledon N reserves within 2-3 weeks of growth. In our study, treatment 100 mg N/l had a synergistic effect on nodulation and N_2-fixation of *S. sesban*.

However, this result is in contrast to that obtained with *Acacia auriculiformis* seedlings in which ammonium-N supplied at a slightly lower dose (7-14 mg N per plant per week) produced a significantly lower nodule number and dry weight/plant than those without N supply over a growing period of almost 6 weeks after planting (Goi et al., 1992). Similar studies with other N_2-fixing trees, using different forms of N source such as urea and ammonium nitrate (NH_4NO_3), supplied at low concentrations also showed negative effects on nodulation (Umali-Garcia et al., 1988; Muofhe and Dakora, 1999).

Shoot and root dry weight showed a slightly different trend compared to nodulation. Whereas nodulation in 0 and 100 mg N/l treatments were comparable, the corresponding shoot and root dry weights were significantly different. Shoot and root dry weight was optimal in plants treated with 100-400 mg N/l but poor for plants solely dependent on N_2-fixation.

The trend of N-uptake was similar to shoot and root dry weight which generally showed that optimal N-uptake occurred in plants supplied with N within the treatment range of 100-400 mg N/l thus demonstrating that *S. sesban* seedlings are tolerant to a relatively high N supply. The general decrease in N-uptake in plants that were treated with 600-800 mg N/l is attributed to toxicity of plants leading to interference of the regulatory plant root mechanisms responsible for nutrient uptake (Elmer, 1999). Similar to shoot and root dry weight, N-uptake in plants solely dependent on N_2-fixation compared very poorly with those which were supplied with optimal N treatments thus emphasizing the need to supply a limited amount of N (starter N) to stimulate an early and effective symbiosis in *S. sesban* seedlings for improved growth and N-uptake.

Optimal nodulation and N-uptake in plants treated with 100 ml N/l suggests that N derived from N_2-fixation in this treatment could be substantial or even higher than that of plants that were dependent on seed N reserve and N_2-fixation, i.e. 0 mg N/l treatment. Further studies using ^{15}N methodology are required to determine the proportions of N derived from N_2-fixation in seedlings supplied with N.

ACKNOWLEDGEMENT

This work was supported by the European Commission INCO-DEV Contract no. ICA4-CT-2000-30011.

REFERENCES

Broughton WJ, Dilworth MJ (1971). Control of leghaemoglobin synthesis in snake beans. Biochem. J. 125: 1075-1080.

Eaglesham AR, Hassouna S, Seegers R (1983). Nitrogen fixation by cowpea and soybean. Agron. J. 75: 61-66.

Elmer WH (1999). Influence of ammonium sulfate and rotational crops on strawberry black rot. Plant Dis. 83: 119-123.

Gathumbi SM, Cadisch G, Giller KE (2002). ^{15}N natural abundance as a tool for assessing N_2-fixation of herbaceous, shrub and tree legumes in improved fallows. Soil Biol. Biochem. 34: 1059-1071.

Goi SR, Sprent JI, James EK, Jacob-Neto J (1992). Influence of nitrogen form and concentration on nitrogen fixation of *Acacia auriculiformis*. Symbiosis 14:115-112.

Hansen AP (1994). N_2 fixation of leguminosae. In University Hohenheim Centre for Agriculture in the Tropics and Subtropics (eds) Symbiotic N_2-fixation of crop legumes: Achievements and Perspectives. Series: 2, Margraf-Verlag, Weikersheim, pp. 16-96.

Ikerra ST, Maghemebe JA, Smithson PC, Buresh RJ (2001). Dry-season Sesbania fallows and their influence on nitrogen availability and maize yields in Malawi. Agroforest. Syst. 52: 13-21.

Kwesiga FR, Ngugi DN (1996). Agroforestry Research in Miombo Ecozone: Experiences in Southern Africa. E. Afr. Agric. For. J. 62: 79-101.

Marschner H (1995). Mineral Nutrition of Higher Plants, 2 nd Edition; Academic Press, San Diego. P 889.

Muofhe ML, Dakora FD (1999). Nitrogen nutrition in nodulated field plants of the shrub tea legume *Aspalathus linearis* assessed using ^{15}N natural abundance. Plant Soil 209: 181-186.

Niang A, Gathumbi S, Amadalo B (1996). The potential of short duration improved fallow for crop productivity enhanced in the highlands of western Kenya. E. Afr. Agric. For. J. 62:103-114.

O'Hara GW (2001). Nutritional constraints on root nodule bacteria affecting symbiotic nitrogen fixation: a review. Aus. J. Exp. Agr. 41: 417-433.

Somasegaran P, Hoben HJ (1994). Handbook for rhizobia: methods in legume-*Rhizobium* technology. Springer-Verlag New York, Inc.

Sprent JI (1999). Nitrogen fixation and growth of non-crop legume species in diverse environments. Perspect. Plant Ecol. 2:149-162.

Ståhl L, Nyberg G, Högberg P, Buresh RJ (2002). Effects of planted tree fallows on soil nitrogen dynamics, above-ground and root biomass, N_2-fixation and subsequent maize crop productivity in Kenya. Plant Soil 143: 103-117.

Umali-Garcia M, Libuit JS, Baggayan RL (1988). Effects of Rhizobium inoculation on growth and nodulation of *Albizia falcataria* (L.) Fosh. and *Acacia mangium* Willd. in the nursery. Plant Soil 108: 71-78.