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Response of *Sesbania sesban* (L.) Merr. to rhizobial inoculation in an N-deficient soil containing low numbers of effective indigenous rhizobia

E. T. Makatiani · D. W. Odee

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Abstract *Sesbania sesban* (L.) Merr. (sesbania) is a fast growing N_2-fixing widely used as an improved fallow species by smallholder farmers in eastern and southern Africa to restore fertility of their N-deficient soils. In order to establish the need for inoculation, the population of sesbania rhizobia in soil collected from a site where the species is intended for introduction was assessed using the most probable number (MPN) plant infection assay. The MPN of sesbania rhizobia was low (21, 6–81 fiducial limits at $P=0.05$ g^{-1}soil) but with N_2-fixation potential comparable to sesbania inoculant strain KFR 651. Evaluation of an indigenous sesbania rhizobial isolate GSS 1 from the MPN assay in potted field soil showed that it was more effective than strain KFR 651 in terms of plant growth and shoot dry matter (DM) accumulation at 9 and 12 weeks after planting, respectively. Total shoot N content was also higher for plants inoculated with isolate GSS 1 than inoculant strain KFR 651 and uninoculated control treatments 12 weeks after planting. These results demonstrate that it is better to inoculate with effective indigenous than exogenous rhizobia where the need for inoculation has been established.

E. T. Makatiani · D. W. Odee (✉)
Biotechnology Laboratory, Kenya Forestry Research Institute, P.O. Box 20412-00200, Nairobi, Kenya
e-mail: dodee@africaonline.co.ke

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Introduction

Sesbania sesban (L.) Merr. (sesbania) is a fast growing N_2-fixing tree with important agroforestry attributes, e.g., provision of fuelwood, fodder and high biomass for soil fertility replenishment. Smallholder maize farmers of eastern and southern African region are increasingly adopting the species as a major source of N input to their N-deficient soils to increase productivity (Niang et al. 1996). The number of farmers using sesbania-based improved fallow technology is estimated to reach 400,000 by the end of 2006 (Ajayi et al. 2003; Kwesiga et al. 2003). Utilization of sesbania, and other nitrogen fixing trees and shrubs (NFTs), for soil fertility restoration relies largely on the N inputs derived from biological N_2-fixation. Significant contribution of N by symbiotic N_2-fixation can only be achieved if the number of effective indigenous rhizobia is sufficient to nodulate the host and fix N_2 adequately or, if absent, seedlings are inoculated with an effective rhizobial strain.

The population densities of indigenous rhizobia capable of nodulating *Sesbania* species vary widely from site to site (Turk et al. 1993; Odee

et al. 1995; Bala 1999). Furthermore, the species are known to have specific rhizobial requirements, largely nodulating most effectively with homologous strains (Turk and Keyser 1992; Bala and Giller 2001). These factors, therefore, emphasize the need to determine the symbiotic status of *Sesbania* species wherever they are cultivated for the first time in order to make appropriate decisions on whether to inoculate or not.

In this study, we assessed the presence and symbiotic potential of indigenous rhizobia nodulating sesbania present in a N-deficient soil from a site located in the Kenyan coastal region where there are plans to introduce the species in soil fertility improvement trials. Our objectives were to (i) establish the presence and size of indigenous sesbania rhizobia, and (ii) evaluate their symbiotic N_2-fixation potential.

Materials and methods

Source, collection and preparation of soil samples

We used a sandy loamy soil collected from a site intended for *S. sesban* cultivation near Gede Forestry Research Station, Malindi, Kenya. The site is also the source of nursery soil for the station and was previously a *Casuarina equisetifolia* stand. Soil samples were obtained from the top 15–30 cm (~1 ha). A composite soil comprising at least 20 point samples was transported back to the laboratory within a day. Representative subsamples of 1 kg each were prepared for most probable number (MPN) assay and standard analyses. The rest of the soil was stored in a cold room (4°C) until used in a pot experiment evaluating symbiotic potential of indigenous rhizobia.

Soil properties

Properties of the soil were determined using the methods described by Anderson and Ingram (1989). They were 1.4% organic matter (Walkley-Black); 7.7 pH in $CaCl_2$; 0.18 mS cm^{-1} electrical conductivity; 0.07% N; 1.5 mg kg^{-1} P ($NaHCO_3$ extractable); 15 $cmol_c$ kg^{-1} K; 11.3 $cmol_c$ kg^{-1} Na; 6.4 $cmol_c$ kg^{-1} Ca; and 1.4 $cmol_c$ kg^{-1} Mg (NH_4-acetate extractable).

Seed source, pretreatment and germination

Sesbania seeds were obtained from the Kenya Forestry Seed Centre (P. O. Box 20412-00200, Nairobi) originally collected from a local provenance, Kakamega, western Kenya. For all the plant growth experiments, sesbania seeds were scarified, surface sterilized and germinated as described by Odee et al. (1995).

Most probable number (MPN) plant infection assay and symbiotic N_2-fixation potential

Indigenous sesbania rhizobia in the Gede soil were evaluated in modified plastic growth pouches (Weaver and Frederick 1972) by using locally available autoclavable bags (5 × 7) lined with absorbent cloth and sleeved in brown paper envelopes. The soil subsample for MPN was mixed thoroughly and quartered. Ten grams was suspended in 90 ml of sterile quarter strength N-free nutrient solution (Broughton and Dilworth 1971) in a 250 ml conical flask and shaken in orbital shaker at 100 rpm for 10 min. Tenfold serial dilutions were prepared (10^{-1}–10^{-6}), and 1 ml aliquots of the appropriate dilutions were inoculated onto the roots of 5-d old sesbania seedlings that were previously pretreated, germinated and planted axenically in pairs in plastic pouches containing N-free nutrient solution. This assay also doubled as symbiotic N_2-fixation potential assessment of the indigenous soil inoculum by including inoculation treatment of a sesbania inoculant strain KFR 651 pure culture grown to late log phase (~1×10^9 rhizobia ml^{-1}) in yeast mannitol broth (Vincent 1970). All pouches at each dilution, including uninoculated controls and standard KFR 651, were replicated 4 times. The whole assay was duplicated to check for reproducibility. The plants were grown for 8 weeks in a growth cabinet (Model MLR-350, Sanyo Electric Co., Ltd., Japan) at 28/18°C and 12 h photoperiod of 250 μE $(m^2)^{-1}$ s^{-1} photosynthetically active radiation at plant height generated by 40 W fluorescent bulbs. Population estimates were calculated using the most

probable number enumeration system (MPNES) program (Bennett et al. 1990). Uninoculated control plants did not nodulate. Nodules were detached from nodulated plants and counted, and shoots separated from roots at the cotyledon scar. Shoots were oven-dried at 60°C for 72 h and the mass of dry matter (DM) measured.

A representative sample of 20 nodules from nodulated plants was used for isolation. Rhizobial isolates were characterized by colony appearance (size and colour), pH reaction and intrinsic antibiotic resistance on yeast extract mannitol agar media as described by Odee et al. (1997). All isolates were identical (data not shown), indicating homogeneous indigenous sesbania rhizobia in the Gede soil. Consequently, one rhizobial isolate, designated as GSS 1, was used to evaluate symbiotic N_2-fixation potential in potted soil (below).

Evaluation of symbiotic N_2-fixation potential of indigenous isolate GSS 1 in potted field soil

Strong polyvinyl chloride (PVC) pots of 15 cm diameter, and 18 cm height with four drainage holes at the bottom were lined with appropriately sized holed black polyethylene bags and filled with about 2 kg of air-dried soil (oven dry weight basis). They were then watered to field capacity with N-free nutrient solution. Germinated *S. sesban* seedlings were transplanted to pots, two per pot, containing the soil supplied with N-free nutrient solution to field capacity.

Isolate GSS 1 and sesbania inoculant strain KFR 651 were grown in yeast mannitol broth to late log phase and each seedling was inoculated with one ml of the appropriate culture five days after transplanting. All treatments (two inoculated and one uninoculated control) were replicated seven times and laid out in a randomized complete block design. The plants were grown for 12 weeks in a glasshouse at 30/18°C (day/night) and *c.* 12 h natural light. They were replenished with N-free nutrient solution at weeks three, six and nine while watering was done regularly as required. Seedling height measurements were taken at 9 and 12 weeks after planting. At harvest, nodules were detached from roots and counted, and shoot and root separated at soil level. Plant shoots, roots and nodules were oven-dried and weighed as MPN assay. The plant tissues were also ground using a ball mill grinder and later used for N determinations (Anderson and Ingram 1989).

Data analysis

All parameters determined on plant growth, DM and tissue N contents were subjected to analysis of variance (ANOVA), and means compared by the Newman-Keuls test at $P < 0.05$ (Zar 1974).

Results

Indigenous population density

The duplicate MPN assays gave identical nodulation profiles: 3 and 2 nodulated seedlings at dilutions 10^{-1} and 10^{-2}, respectively, thus verifying reproducibility. This profile estimated a population density of 21 (6-81 fiducial limits at $P = 0.05$) indigenous sesbania rhizobia g^{-1} of soil. There was no significant difference ($P < 0.05$) in nodule numbers $plant^{-1}$, shoot and nodule dry matter (DM) between plants inoculated with the lowest soil dilution inoculum (10^{-1}) and strain KFR 651 (data not shown). These results indicate that despite their low numbers, the indigenous sesbania rhizobia are as effective as the inoculant strain.

Response to inoculation in Gede soil

There was no response to inoculation with respect to number of nodules $plant^{-1}$ but the uninoculated control had a significantly higher nodule DM than the inoculated treatments, a result we attributed to compensation for low numbers of indigenous sesbania rhizobia. Shoot DM was larger in plants inoculated with the indigenous strain GSS 1 than those inoculated with strain KFR 651 and uninoculated control (Table 1). Similarly, shoot N content was significantly higher in plants inoculated with strain GSS 1 than those inoculated with strain KFR 651 and uninoculated control treatments (Table 2). Inoculation with indigenous isolate also had significantly higher

Table 1 Nodulation and dry matter accumulation in sesbania seedlings grown in potted Gede soil 12 weeks after planting

Inoculated with:	No. of nodules plant^{-1}	Nodule dry wt (g)	Shoot dry wt (g)	Root dry wt (g)
GSS 1	359 ± 39.4a*	0.96 ± 0.078a	7.4 ± 0.389b	8.4 ± 0.587a
KFR 651	333 ± 39.1a	0.84 ± 0.081a	6.1 ± 0.251a	9.2 ± 0.461a
Uninoculated control	242 ± 23.6a	1.29 ± 0.057b	6.0 ± 0.493a	7.8 ± 0.779a

*Values within a column followed by the same letter are not significantly different according to Newman and Keuls test at $P < 0.05$

Values are means ± SE ($n = 7$)

total shoot N content than uninoculated treatment. The inoculant strain KFR 651 did not significantly increase any of the parameters measured relative to uninoculated control with the exception of nodule DM. In addition, indigenous strain GSS 1 showed better inoculation response than strain KFR 651 with most of the parameters assessed (Table 3). Table 4 also shows that plants inoculated with GSS 1 were taller at 9 and 12 weeks after planting than those inoculated with strain KFR 651 and uninoculated control.

Discussion

The magnitude and composition of indigenous rhizobia can be influenced by the trap host species or by the environmental conditions (Brockwell et al. 1995). Among the environmental conditions, awareness of the legume flora that occur in the area and the nature of rhizobial partners known to associate with them might give an indication on the composition and population density of rhizobia for the introduced legume. We determined low population density of sesbania rhizobia in Gede soil. The low population density in Gede soil contrasts sharply with high population density (≥10^5 rhizobia g^{-1} soil) found in soils from natural sesbania stands in western Kenya (Odee et al. 1995). Elsewhere in Africa, Latin America and Asia, Bala (1999) also reported low population densities of sesbania rhizobia (<50 g^{-1}) in soils which also haboured higher population densities (>10^3 g^{-1}) of alternative trap hosts namely; *Calliandra calothyrsus* Meissn., *Gliricidia sepium* (Jacq.) Steud., *Leucaena leucocephala* (Lam.) de Wit and *Macroptilium atropurpureum* (DC.) Urban. This study and the others (Odee et al. 1995; Bala 1999) underscore the specificity of sesbania rhizobia.

In spite of low indigenous sesbania rhizobia population density, the effectiveness of soil inoculum for N_2-fixation was comparable to inoculant strain KFR 651 suggesting higher symbiotic potential. The superiority of the indigenous isolate GSS 1 in the pot evaluation experiment was demonstrated by significantly higher shoot N and DM accumulation as compared to inoculant strain KFR 651 and uninoculated control. Similar pot studies using several NFTs and herbaceous legumes in field soils have indicated that response to rhizobial inoculation mostly occur when the indigenous population densities are <50 rhizobia g^{-1} (Singleton and Tavares 1986; Turk et al. 1993). The indigenous sesbania rhizobia population density in Gede soil was low and below the reported thresholds. However, the significant inoculation response in the pot experiment due

Table 2 Mean nitrogen (N) accumulated in shoots, roots, nodules and whole plants at 12 weeks after planting

Inoculated with:	Shoot N (mg)	Root N (mg)	Nodule N (mg)	Whole plant N (mg)
GSS 1	170 ± 11.88b*	149 ± 8.22a	61.00 ± 6.31ab	379 ± 17.98b
KFR 651	130 ± 5.01a	150 ± 7.88a	47.16 ± 4.90a	330 ± 14.10ab
Uninoculated control	136 ± 10.33a	134 ± 21.29a	46.16 ± 3.72a	282 ± 22.76a

*Values within a column followed by the same letter are not significantly different according to Newman and Keuls test at $P < 0.05$

Values are means ± SE ($n = 7$)

Table 3 Response to inoculation with indigenous isolate GSS 1 and exogenous inoculant strain KFR 651 in potted Gede soil (% increase mean parameter yield of inoculated [I] over non-inoculated [N] plants: [I–N]/N × 100)

Values are means ± SE (n = 7)

Variable	Inoculation response (%) due to inoculant:	
	GSS 1	KFR 651
Height	13.28 ± 3.30	0.026 ± 3.34
Shoot DM	23.89 ± 6.49	2.28 ± 4.22
Root DM	5.31 ± 7.32	15.29 ± 5.75
Total plant DM	15.82 ± 4.59	9.61 ± 3.66
Nodule number	48.35 ± 16.26	37.60 ± 16.14
Nodule dry wt	26.53 ± 10.25	11.33 ± 10.73
Shoot total N	–7.89 ± 2.53	–8.17 ± 4.96
Root total N	7.92 ± 3.50	–2.34 ± 4.79
Nodule total N	4.57 ± 5.23	–0.35 ± 4.30
Total plant N	1.11 ± 4.32	–2.74 ± 3.59

Table 4 Shoot growth response of *S. sesban* seedlings in Gede soil 9 and 12 weeks after planting to inoculation with rhizobia isolated from the same soil (indigenous isolate) GSS 1 and inoculant strain KFR 651

Inoculated with:	Week 9	Week 12
GSS 1	42.82 ± 1.26b*	71.25 ± 2.18b
KFR 651	37.75 ± 1.24a	64.57 ± 3.27a
Uninoculated control	37.74 ± 1.30a	63.61 ± 2.90a

*Values within a column followed by the same letter are not significantly different according to Newman and Keuls test at $P < 0.05$

Mean height of isolate GSS 1 inoculated seedlings was significantly higher than KFR 651 inoculated and uninoculated control seedlings according to the Newman-Keuls test ($P < 0.05$) at 9 weeks after planting. Values are means ± SE (n = 7)

to re-inoculation with an effective indigenous rhizobial isolate demonstrates the need to establish the effectiveness of such low indigenous rhizobia prior to considering inoculation with introduced strains. For a species with specific rhizobial requirements such as sesbania, re-inoculation would increase N_2-fixation and establishment of the host while concomitantly enriching the effective indigenous population density such that immediate subsequent planting of the host at the site may not be necessary.

Conclusion

Sesbania rhizobia were low in Gede soil but elicited better inoculation response than the exogenous strain KFR 651 when tested in the same soil. We therefore recommend the use of indigenous sesbania rhizobia as inoculant in sites where they are insufficient but containing effective populations.

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