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NITROGEN-FIXING NODULES INDUCED BY *RHIZOBIUM* ON THE STEM OF THE TROPICAL LEGUME *SESBANIA ROSTRATA*

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1. Introduction

The only recorded instances of legume species with aerial nodules are for *Neptunia oleracea* [1] and *Aeschynomene indica* [2,3]. This latter, which grows in flooded soils, has two types of nodules: root nodules like other legumes, and stem nodules. Stem nodules of *A. indica* usually are distributed sparsely along the lower stem and look more like small swellings than conventional *Rhizobium* nodules.

We report here on a tropical legume, *Sesbania rostrata* (Brem) [4], characterized by profuse stem nodulation and substantial nitrogen-fixing activity by the stem nodules. *Sesbania rostrata* is an annual plant which grows in flooded soils in the Sahel region of West Africa during the rainy season. It thrives in temporary ponds and paddy fields where it is considered as a weed. Stem nodules were found up to 2 m above the water level in plants 3 m tall; the weight of these nodules ranged from 15 to 40 g per plant. Nodules occurred solely at the sites of lenticels which, in *S. rostrata*, are arranged linearly along the stem (Fig. 1A). Nodules were green, usually spheroidal, and varied in diameter from 0.3 to 0.8 cm.

The aim of this paper is to describe briefly the structure of the stem nodules of *S. rostrata*, to evaluate their nitrogen-fixing activity and to characterize the bacterial strain isolated from the stem nodules.

2. Material and Methods

2.1. *Techniques for structural studies*

2.1.1. *Light microscopy*

Stem nodules were detached and fixed in formalin–acetic acid–50% ethanol (13 : 5 : 200 v/v for 72 h or longer. They were then embedded in paraffin, sectioned at 15 or 18 μm and stained with safranin and fast green. Photographs were taken with a Leitz "Orthomat" photomicroscope.

2.1.2. *Electron microscopy*

Nodules for study under the electron microscope were fixed in 2.5% glutaraldehyde in 0.025 M cacodylate buffer, post-fixed in 1% osmic acid an then embedded in resin. Sections were cut from the blocks on an ultramicrotome and stained with lead citrate.

2.2. *Assessment of acetylene reduction activity stems from field-grown plants*

Seven 3-month-old plants were sampled at flowering stage from a flooded field. Stems were cut into two 1-m long segments (upper and lower stem), taking care not to detach the adhering nodules. Segments and root systems were incubated separately in order to estimate their nitrogen-fixing capacity by the acetylene reduction method [5].

2.3. *Bacterial strains*

A strain of bacterium, designated ORS 551, was isolated from stem nodules, using standard procedures [6] and tested for its ability to form nodules on the stems and roots of *Sesbania rostrata*. Strains from root nodules of *Sesbania pachycarpa* and *Aeschynomene* sp., designated respectively ORS 507 and ORS 301, were isolated similarly. Strain CB 756 was kindly supplied by A.H. Gibson.

2.4. Culture of plants in greenhouse; assessment of nodulation and effectiveness

Seeds were pretreated and surface-sterilized with concentrated H_2SO_4. The times of treatment in H_2SO_4 were as follows: *Sesbania rostrata*, 30 min; *S. pachycarpa*, 30 min; *S. aculeata*, 10 min; *Aechynomene* sp., 3 min; *Macroptilium atropurpureum*, 5 min. The seeds were germinated in sterile petri dishes of water agar and then transferred to test-tubes containing nutrient medium [6]. The plants were grown with the shoots free above an aluminum-foil cap [7] in a greenhouse under natural light and daytime temperatures of 25° to 32°C. One drop of a 10^9 cells/ml liquid culture of the different strains was used for inoculation. Nodule appearance and effectiveness were assessed when the plants were 3–4 weeks old. Effectiveness was scored by visual observation of plant vigor.

3. Results

3.1. Structural study

Examination of a transverse section by light microscopy showed that the stem nodules resembled typical round or oval root nodules found on *Glycine max* or *Vigna unguiculata* [8] (Fig. 1B). Electron micrograph sections of the nodule tissue showed a dense development of bacteroids enclosed by the membrane envelope, as in cowpea nodules [9] (Fig. 1C). The outer cortex of the stem nodules contained cells with chloroplasts; the inner cortex, next to the bacterial zone, contained a number of newly invaded cells harboring both *Rhizobium* and chloroplasts (Fig. 1D). The effects, if any, of the proximity of chloroplasts on the nitrogenase activity of the stem nodules remains unknown.

3.2. Acetylene reduction activity

Stem nodules sampled from seven flowering plants of *S. rostrata* growing in a waterlogged field, were tested for nitrogen-fixing activity as measured by acetylene reduction activity (ARA). Table 1 shows that the mean total ARA per plant was 589 micromoles C_2H_4/h, a high value as compared to the total ARA of field-grown soybeans which ranges from 14 to 120 and, exceptionally, 290 μmol C_2H_4/h/plant [10,11]. The reduction of acetylene per g (dry weight) of nodules (specific ARA) of the upper stem nodules was higher than that of the lower stem nodules. This difference probably relfects the decreasing age of the nodules from bottom to top. The specific ARA of the stem nodules was comparable to that of root nodules of other legumes such as soybean [11–13] or cowpea [14].

All plants studied also had root nodules; these were smaller than the stem nodules (0.1 cm in diameter) and their total weight was lower (2 g per plant). The specific ARA of the root nodules was lower than that of the stem nodules, probably because waterlogging of the soil limited their activity.

3.3. Nodulating ability and effectiveness

Root systems of *S. rostrata* were inoculated at an early stage (1 week) with strain ORS 551 (twenty replicates). Nodules appeared on the roots of all plants within 1 week. Stems were inoculated as soon as lenticels appeared (3-week-old plants) by spraying the stem with a broth culture of the same strain. Nodules appeared on the stem of all plants within 1 week. These experiments indicated that strain ORS 551, isolated from the stem, was a strain of *Rhizobium* with the ability to nodulate both root and stem of *S. rostrata*.

Strain ORS 551 was then inoculated onto the roots of five plant species including *S. rostrata* (five replicates) and compared to three other strains of *Rhizobium* isolated from root nodules of different legumes (Table 2). Strain ORS 551 produced nodules on the roots of two different species of *Sesbania*, *S. pachycarpa* and *S. aculeata*, but these nodules appeared ineffective. No nodules were formed on the roots of *Aeschynomene* sp. and *Macroptilium atropurpureum*. Ineffective nodules were formed on *S.*

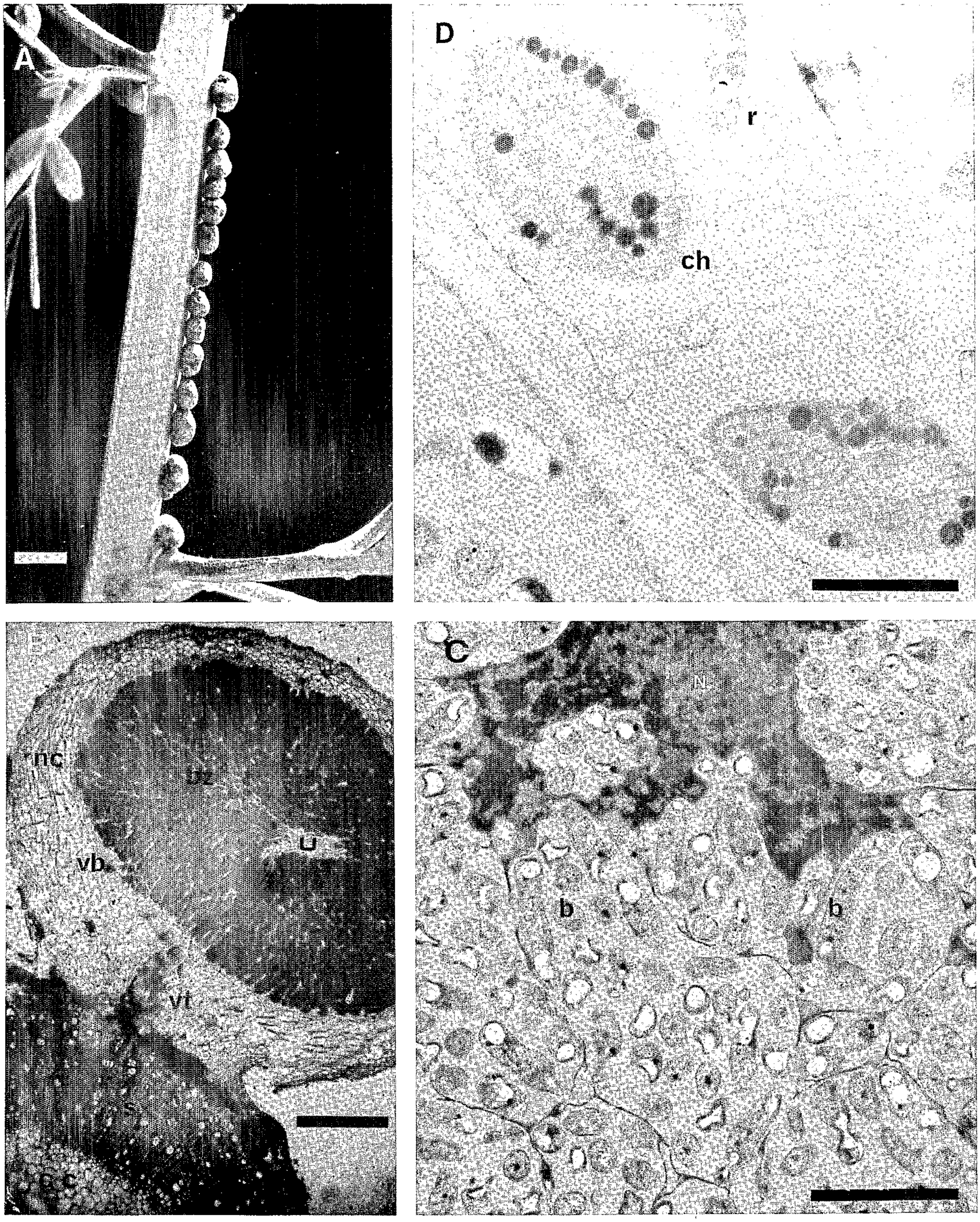

Fig. 1. Stem nodules of *Sesbania rostrata*. (A) Mature and young stem nodules. Mature nodules are distributed along a vertical line on the right side of the stem. Young nodules appear at the bottom of the line of lenticels. The plant was sampled in the field at the pod-filling stage. The bar represents 1 cm. (B) Transverse section through the stem and the attached nodule showing the central cylinder (cc) and the secondary xylem (sx) of the stem, and a large circular bacteroid zone (bz) surrounded by the nodule cortex (nc). Cells of the bacteroid zone are greatly enlarged, some of them remain uninvaded (u). A vascular trace (vt) divides at the base of the nodule and forms vascular bundles (vb) in the inner cortex, around the bacteroid zone. The bar represents 0.5 mm. (C) Electron micrograph of an invaded cell showing many mature bacteroids (b) enclosed by the membrane envelope, nucleus of the host cell (N). The bar represents 2.5 μm. (D) Newly invaded cells of the cortex adjacent to the bacteroid zone. Singly dispersed *Rhizobium* cells (r) and chloroplasts (ch) are present in the same cell. The bar represents 5 μm.

TABLE 1

The acetylene reduction activity (ARA) of stem and root of *Sesbania rostrata*

No C_2H_4 was produced by denodulated stems. Data are the mean of seven replicates.

	Nodule fresh weight (g)	ARA [a]	Specific [b] ARA
Upper stem segment (1.5 m) [c]	7.2 ± 0.5	377 ± 33	53.0 ± 3.5
Lower stem segment (0.5 m) [c]	7.8 ± 0.7	170 ± 23	22.6 ± 3.0
Root system	2.4 ± 0.3	42 ± 6	16.8 ± 1.3
Total per plant	17.4 ± 1.2	589 ± 35	

[a] ARA expressed as μmol C_2H_4 produced/h/stem segment or per root system.
[b] Specific ARA expressed as μmol C_2H_4 produced/h/g of nodules (fresh weight).
[c] Each stem segment was 1 m long; within brackets, mean distance of stem segments from water level.

rostrata roots by strain ORS 507 isolated from *S. pachycarpa*. Neither strain ORS 301 isolated from stem nodules of *Aeschynomene* sp., nor the cowpea strain CB756, formed nodules on *S. rostrata*.

4. Conclusion

Sesbania rostrata stems are infected by *Rhizobium*, which form five to ten times more nodules than the nitrogen-fixing crop plants such as soybean, peanut, or other root-nodulated *Sesbania* species of comparable size. Since the specific ARA of *S. rostrata* stem nodules is similar to that of the nodules of most legumes, *S. rostrata* exhibits an exceptionally high ability to use atmospheric nitrogen. Whether the profuse stem nodulation of *S. rostrata* results from the high susceptibility of stem tissues to *Rhizobium* infection is not known. Finally, one could hope that, by plant breeding, the character "stem nodulation" could be transferred to other *Sesbania* used as forage in the tropics and even to other crops, thus substantially increasing their nitrogen-fixing potential, not only in well drained but also in waterlogged soils, which are reputedly unfavorable for legumes.

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TABLE 2

Cross-inoculation test of root nodulation with four *Rhizobium* strains, including ORS 551 isolated from stem nodules of *Sesbania rostrata*

Uninoculated controls remainded free of nodules. Data are the mean of five replicates.

Plant species	*Rhizobium* strains			
	ORS 551 (*Sesbania rostrata*) [a]	ORS 507 (*Sesbania pachycarpa*) [a]	ORS 301 (*Aeschynomene* sp.) [a]	CB 756 [b]
Sesbania rostrata	E [c]	I	O	O
Sesbania pachycarpa	I	E	O	O
Sesbania aculeata	I	PE	O	O
Aeschynomene sp.	O	O	E	O
Macroptilium atropurpureum	O	O	O	E

[a] Within brackets, host plant from which strain was isolated.
[b] Cowpea collection strain.
[c] E, PE, I, O, denote effective, partially effective, ineffective and no nodulation, respectively.

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