STEM NODULES ON THE TROPICAL LEGUME, Sesbania rostrata

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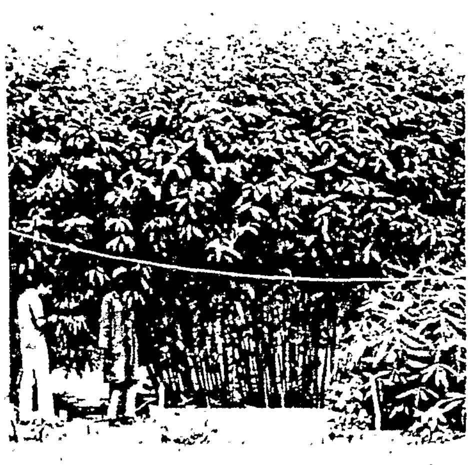
Sesbania rostrata, a tropical legume colonizing water-logged soils in the Sahel region of west Africa, forms nitrogen-fixing nodules with Rhizobium sp. on both the roots and the stems. S. rostrata differs from the other known stem-nodulated legumes Aeschynomene indica (1) and Neptunia oleracea (2) by its profuse stem nodulation. We report here some of the characteristics of the stem nodules of S. rostrata and their endophytic rhizobia.

Materials and Methods

Strains of Rhizobium sp. were isolated from stem nodules using standard procedures (3), and the stock cultures were maintained on YMA medium. The strains were cultured in YLS liquid medium containing per 1000 ml : Na-lactate, 5 g ; Na-succinate, 5 g; Na-glutamate, 0.5 g; K_2HPO_4, 1 g; NaCl, 0.05 g; $MgSO_4.7H_2O$, 0.1 g; $CaCl_2$, 40 mg; $FeCl_3$, 4 mg; yeast extract, 1 g; trace elements, pH 6.8. All strains were tested for their ability to form nodules on the stems and roots of S. rostrata grown under bacteriologically-controlled conditions. Re-isolates from nodules were tested for nitrogenase activity in culture (see below). Field-grown plants were used to determine total nitrogenase activity of nodules. To examine the effect of combined nitrogen on nodulation, surface-sterilised seeds were sown into test-tubes containing Jensen's liquid medium. The roots were inoculated 7 days after sowing, and the stems sprayed with a dilute culture (10^7/ml) of rhizobia 3 days later. For assays on the cultured rhizobia, a N-free medium was used (LSO); this medium differed from YLS in that Na-glutamate and yeast extract were omitted. The assays were done under argon with various levels of O_2 added.

Results and Discussion

Field-grown plants of S. rostrata can grow up to 4 m (Fig. 1), and in many instances display heavy nodulation (15-40 g F.W./plant) along the lines of the stem lenticles (Fig. 2) up to 2.5 m above ground level. When inoculated by spraying plants grown in tubes, nodules appeared 4-5 days later. In the field, their $N_2(C_2H_2)$-reducing activity was 400-600 µmoles C_2H_4/plant/h at 3 months (15 g nodules, 35 µmoles C_2H_4/g.F.W./h); their root nodules showed 42 µmoles C_2H_4/plant/h from 2.4 g nodules.



Cross-inoculation tests showed that strains isolated from stem nodules were able to nodulate, effectively, the shoots of S. rostrata, but they formed only ineffective nodules on the roots of other Sesbania spp. (e.g. S. pachycarpa, S. aculeata); Siratro (Macroptilium atropurpureum) was not nodulated. Young plants of S. rostrata grown hydroponically in test-tubes were supplied with 3 mM NH_4NO_3 at 7 days, and the nutrient solution replaced every 2 days thereafter. At 4 weeks, it was obvious that root nodulation of the N-supplied plants was seriously retarded, although stem nodulation was increased relative to the 'No-N' controls. A similar effect was evident in the C_2H_2 reduction data. These results are in agreement with the theory that inhibition of nodulation by combined N is localised to the region of uptake; they also indicate that the effect of combined N on nitrogenase activity is similarly localised, but this conclusion may be influenced by the presence of chlorophyll in the cortex of the nodules.

One strain (ORS 571) isolated from stem nodules was grown in YLS medium to late exponential phase, then centrifuged and resuspended in LSO medium to a concentration of approx. 10^7 cells/ml. 25 ml samples of this suspension were transferred to 145 ml serum flasks and incubated for 24 h under an O_2/Ar (3:97) mixture with shaking (100 rpm). Strain ORS 571 showed nitrogenase activity of 1500 nmoles C_2H_4/mg protein/h; the dissolved O_2 concentration was approx. 1.0 µM in these assays containing 10% C_2H_2. Three per cent O_2 during incubation gave maximum activity (O_2 range examined, 1-11%).

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2. Schaede, R., 1940. Planta (Berl.) 31, 425.
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