

***Sesbania rostrata* and other stem-nodulated legumes**

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INTRODUCTION

For several centuries, many nitrogen-fixing plants have been used as green manure, notably in Asia. Legumes, such as *Aeschynomene americana*, *A. indica*, *Astragalus sinicus*, *Crotalaria juncea*, *C. stricta*, *Indigofera tinctoria*, *Lablab purpureus*, *Medicago hispida*, *M. officinalis*, *Sesbania aculeata*, *S. cannabina*, *S. paludosa*, *Vicia cracca* (14,17,18) or non legumes, such as *Coriaria sinica* (Watanabe, pers. comm.) have been used in this way.

Recent progress has been made in this field with the discovery of stem-nodulated legumes whose dual nodulation confers upon them an unusually high N₂-fixing potential. Only a few legume species bear nodules both on their roots and stems. They belong to the three genera *Sesbania* (one species), *Aeschynomene* (about 15 species) and *Neptunia* (one species). These plants have in common the ability to grow in waterlogged soils and are potential candidates for green-manuring in paddy fields.

1. CLASSIFICATION OF STEM-NODULATED LÉGUMES ACCORDING TO THE ANATOMY OF THEIR NODULATION SITES.

The common characteristic of all stem-nodulated legumes is the presence of predetermined nodulation sites on the stem. These sites are formed by lateral root primordia, whose development stage varies with the host plant. The most evolved sites have protruding root primordia, which are readily infected by rhizobia, whereas the least evolved ones have hidden primordia much less susceptible to rhizobial infection. According to the development stage of their nodulation sites, stem-nodulated legumes can be divided into three subgroups (3,6).

1.1. Infection sites of *Sesbania* type

Subgroup 1, which comprises species with the most evolved infection site is that of *Sesbania rostrata* (8) The root primordium always pierces the stem epidermis, emerging 0.1 to 0.3 cm from an epidermal dome and forming a circular fissure through which the rhizobia penetrate.

Infection sites of *Aeschynomene afraspera* and *A. nilotica* belong to this type, in spite of their less developed structure.

1.2. Infection sites of Aeschynomene elaphroxylon type

Subgroup 2 comprises species with the least evolved sites, with root primordia remaining embedded in the cortical tissues of the stems. Rhizobial infection occurs only when the growth of the root primordia is triggered by flooding. A. crassicaulis and A. pfundii also belong to this type.

Flooding is also necessary to induce the formation of nodules in Neptunia oleracea, whose infection sites are located nearby the stem nodes (15).

1.3. Intermediate infection sites

This third subgroup can be considered as intermediate between subgroup 1 and 2. In this case the apex of the root primordia is covered with a layer of intact epidermal cells. The dormancy of the primordia is much more easily broken by external factor (such as high humidity) than in the case of Aeschynomene elaphroxylon. Two typical species of this subgroup are A. indica and A. scabra.

2. STEM-NODULATING BACTERIA

Bacteria which form nitrogen-fixing nodules on legumes are currently divided into two genera, Rhizobium and Bradyrhizobium (11). The genus Rhizobium comprises four species, Rhizobium leguminosarum, R. meliloti, R. loti and R. fredii. The species R. leguminosarum regroups the former three species, Rhizobium trifolii, R. phaseoli and R. leguminosarum. All species of the genus Rhizobium are fast-growing bacteria. The genus Bradyrhizobium comprises one well defined species, Bradyrhizobium japonicum and includes all the bacteria previously referred as slow-growing rhizobia (9,10).

2.1. Bacteria nodulating Sesbania rostrata

Sesbania rostrata is nodulated by three groups of rhizobia :

(i) The first group comprises strains, such as strain ORS 571, which nodulates both roots and stems. These strains have been recently shown to form a new genus named Azorhizobium (7). Azorhizobium associated with Sesbania rostrata has been called Azorhizobium caulinodans. Azorhizobium caulinodans markedly differs from other Rhizobiaceae (Rhizobium sensu stricto and Bradyrhizobium) by the fact that they can grow ex planta at the expense of atmospheric N₂ (5). Interestingly the genus Azorhizobium is closely related to the genus Xanthobacter, which includes hydrogen-oxidizing bacteria able to grow on atmospheric N₂ in autotrophic conditions. Strains of Azorhizobium nodulate many species of Sesbania other than S. rostrata, but form ineffective nodules except in S. rostrata and S. paludosa.

(ii) The second group comprises strains of Rhizobium sensu stricto (i.e. fast-growing rhizobia) which effectively nodulate only the roots of Sesbania rostrata. Unlike Azorhizobium strains, these Rhizobium strains do not grow ex planta at the expense of atmospheric N₂. They are usually able to form effective nodules on a large spectrum of Sesbania species.

(iii) The third group comprises a few strains of Rhizobium sensu stricto which effectively nodulate both roots and stems of Sesbania rostrata (13). Like Rhizobium of group (ii) they belong to the Sesbania inoculation group and nodulate several species of Sesbania.

2.2. Bacteria nodulating Aeschynomene

Stem-nodulated Aeschynomene are nodulated by fast- and slow- growing rhizobia.

(i) **Fast growing rhizobia.** Despite the fact that they are fast- growers, these strains also present some characteristics of slow- growers (Subpolar flagellation, alkali production). They could be classified as an intermediate group of rhizobia sharing physiological and nutritional characteristics with both fast- and slow-growers (Alazard, unpublished results, 4,16).

These fast-growing rhizobia exhibit high nitrogenase activity ex planta but, unlike Azorhizobium, they do not grow on N₂ as sole nitrogen source (Alazard, unpublished results). They fall into two cross-inoculation subgroups (1):

- subgroup 1, comprises strains nodulating Aeschynomene afraspera and A. nilotica.
- subgroup 2, comprises strains nodulating Aeschynomene ciliata, A. denticulata, A. evenia, A. indica, A. pratensis, A. rudis, A. scabra, A. sensitiva, and A. tambacoundensis.

(ii) **Slow-growing rhizobia.** They are typical Bradyrhizobium and closely related to the strains nodulating Arachis, Stylosanthes and Macroptilium. They have not been shown to exhibit any nitrogenase activity *in vitro*. The host range of these strains comprises the Aeschynomene species with the less evolved nodulation sites : A. crassicaulis, A. elaphroxylon and A. pfundii.

2.3. Bacteria nodulating Neptunia

The Rhizobium of Neptunia are very closely related to Rhizobium meliloti but they form ineffective nodules on Medicago sativa. Like R. meliloti, they harbor a megaplasmid (J. Dénarié, unpublished results).

3. UTILIZATION OF STEM-NODULATED LEGUMES AS GREEN MANURE

3.1. N₂-fixing potential of stem nodulated legumes

Using ¹⁵N methods, we have compared the N₂-fixing potential of Sesbania rostrata and Sesbania sesban (a non stem-nodulated Sesbania) grown in waterlogged or drained conditions. N₂ fixation by Sesbania rostrata was 0.7 - 0.8 g N₂ per plant in waterlogged conditions and 0.6 g in drained conditions during a period of 60 days (12). N₂ fixation by Sesbania sesban was only 0.05 - 0.06 g N₂ per plant in waterlogged conditions and 0.13 g in drained conditions during the same period of 60 days.

Similarly N₂-fixation by Aeschynomene afraspera estimated by the difference method, was 1.2 g N₂ per plant during a period of 10 weeks (2). These results indicate that like Sesbania rostrata, Aeschynomene afraspera has a high N₂-fixing potential which should be exploited in rice-based agriculture.

Similarly to that has already been shown in *Sesbania rostrata*, stem nodulation and N₂-fixation by field-grown *Aeschynomene afraspera* is practically not affected by combined nitrogen provided that the amount of nitrogen fertilizer is less than 100 kg N/ha (3).

3.2. Field experiments

In 1985 an experiment was carried out at the farm level in Casamance (South Senegal) using a randomized block with 40m² plots device with three treatments (8 replications): control, addition of 60 kg N-fertilizer, and *Sesbania rostrata* green-manuring.

The yield of rice on one hectare basis, was 2.0 tons in the control, 2.9 tons in the fertilizer plot and 4.9 tons in the green-manured plot.

In 1986, a second experiment using a simpler device carried out at the farm level by a farmer in Casamance showed that the rice yield was increased from 1.7 t/ha in the control, to 3.2 t/ha in the green-manured plot.

In 1987 a one hectare experiment increased the rice yield from 2.2 t/ha in the control, to 4.4 t/ha in the green manured plot.

Because *Sesbania rostrata* grows very fast, the amount of biomass incorporated 45 days after sowing was high, c.a. 25 tons fresh weight/ha. In addition to N input into the soil, there is a major input of organic C, which favorably affects the soil structure, and also other elements specially that have been applied as P and K fertilizers at sowing time. These elements are then returned to the soil and are used by the subsequent rice crop.

CONCLUSION

The fast-growing stem nodulated legume *Sesbania rostrata* was shown to contribute much to rice yield, boosting grain production by 1-2 t/ha and improving the nutrient quality of paddy. Our recent investigation have shown that two *Aeschynomene* species, *A. afraspera* and *A. nilotica*, were also promising stem-nodulating legumes, suggesting that more research should be devoted to these plants.

Since stem-nodulated legumes have the unusual superiority over other legumes to fix N₂ actively and to tolerate large amounts of combined nitrogen in the soil, it is tempting to try transferring the stem-nodulating character to non stem-nodulating legumes.

Using chemical mutagenesis with EMS (Ethyl Methanesulfonate), we recently obtained a plant mutant without stem nodulation sites. The second step would be to identify the plant genes coding for these specific structures and to tentatively transfer these genes to other species of the same genus devoid of stem nodulation sites. These last species should be chosen among those which are known to be the best adapted species to different climatic or edaphic conditions, or interesting for agriculture (e.g. *Sesbania sesban*, *S. aculeata*) or agroforestry (*S. grandiflora*).

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