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Short Communication

Diversity of Stem Nodulation Sites in *Aeschynomene* spp.

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Summary

Fourteen species of *Aeschynomene* growing in waterlogged soils of Western Africa develop nodules on both stems and roots. Stem nodules are formed at predetermined sites called nodulation sites, which always include a lateral root primordium.

Aeschynomene species fall into three groups according to the anatomy of their nodulation sites:

a) In the most evolved nodulation site, the root primordium breaks through the epidermal dome (e.g. *A. afraspera*).

b) In the least evolved nodulation site, the root primordium remains embedded in the cortical tissues of the stem (e.g. *A. elaphroxylon*).

c) In an intermediate type of nodulation site, the apex of the root primordium is overlayed with intact epidermal cells (e.g. *A. indica*).

The frequency of stem nodulation is related to the accessibility of the root primordium for rhizobia.

Key words: Aeschynomene, stem nodulation.

Introduction

Stem nodulation induced by *Rhizobium* has been reported in only three genera of legumes *Aeschynomene, Neptunia,* and *Sesbania* (Dreyfus et al. 1984).

The common characteristic of all stem nodulated legumes is the presence of predetermined nodulation sites on the stem. Nitrogen-fixing stem nodules always occur at the emergence of lateral root primordia and the ruptured tissues in this region constitute the site of entry for *Rhizobium* (Arora 1954, Duhoux and Dreyfus 1982, Schaede 1940).

Neptunia oleracea (Schaede 1940) and *Sesbania rostrata* (Dreyfus and Dommergues 1981) are the only stem nodulated species within their genus, whereas fourteen species of *Aeschynomene* developing stem nodules are known (Alazard 1985). The genus *Aeschynomene* (mainly tropical plants) belongs to the tribe *Aeschynomeneae*. It comprises about 160 species, half of which are hydrophytes, and is distributed throughout America, Africa, with a few species occurring in Southeast Asia and the Pacific Islands (Kretschmer and Bullock 1980).

We report here a comparative study of the anatomy of stem nodulation sites in 14 *Aeschynomene* species, the nodulation characteristics of which were previously studied (Alazard 1985).

Materials and Methods

Bacterial strains and culture

The *Rhizobium* strains used in this study were isolated from *Aeschynomene* plants growing in West Africa (Alazard 1985). *Rhizobium* strains ORS 322 and ORS 310 were isolated from stem nodules of *A. afraspera* and *A. indica* respectively. Strain ORS 322 induced root and stem nodules on *A. afraspera* and *A. nilotica*. Strain ORS 310 induced root and stem nodules on *A. indica, A. ciliata, A. denticulata, A. evenia, A. pratensis, A. rudis, A. scabra, A. sensitiva,* and *A. tambacoundensis*. Strain ORS 304, isolated from nodules of the lower and immerged part of the stem of *A. elaphroxylon*, nodulated the roots and the immerged part of the stems of *A. elaphroxylon, A. pfundii* and *A. crassicaulis*. Stock cultures were maintained on yeast and mannitol agar slopes at 4 °C (Vincent 1970). *Rhizobium* cultures were developed on yeast extract and mannitol broth for 5 days at 30 °C (about 10^9 cells per ml).

Plant materials and inoculation

The following species were used as host plants: *A. afraspera, A. nilotica, A. indica, A. ciliata, A. denticulata, A. evenia, A. pratensis, A. rudis, A. scabra, A. sensitiva, A. tambacoundensis, A. elaphroxylon, A. pfundii,* and *A. crassicaulis*.

Aeschynomene plants were grown in plastic pots containing 5 kg sandy waterlogged soil. The experiment was carried out during the

rainy season at the ORSTOM station in Dakar (Senegal). Stems were inoculated by applying a 10-fold dilution of the specific *Rhizobium* culture (10^8 cells per ml) using a small spray bottle or a sterile brush (Alazard 1985).

Preparation of stem samples

Three mm long segments of stem bearing nodulation sites were fixed overnight in 3 % glutaraldehyde in 0.2 M cacodylate buffer, pH 7.0, at 4 °C. Samples embedded in Paraplast (+) (Brunswich Co) were cut and stained with Regaud's iron – haematoxylin stain (Lison 1960).

Results and Discussion

The stems of fourteen species of Aeschynomene were inoculated. Stem nodules appeared c. 5 to 7 days after inoculation they were fully developed 2 weeks later (Fig. 1).

Stem nodules were distributed all along the shoot in *A. afraspera, A. nilotica, A. indica, A. ciliata, A. denticulata, A. evenia, A. pratensis, A. rudis, A. scabra, A. sensitiva,* and *A. tambacoundensis* (Fig. 1 A, B). Stem nodulation was more profuse in *A. afraspera* and *A. nilotica* than in other *Aeschynomene* species. Stem nodules were restricted to the lower and immerged part of the stem in *A. elaphroxylon* and *A. pfundii* (Fig. 1 C). Similarly, *A. crassicaulis* developed stem nodules only in waterlogged soils with its stem floating on the surface of the water (data not shown).

Stem nodulation sites of *Aeschynomene* species always appeared to comprise a dormant root primordium with an apical meristematic zone and typical vascular bundles (Fig. 2). The ability of the root primordia of stem nodulated plants to develop into an adventitious root reported by Duhoux and Dreyfus (1982) was confirmed by immerging cuttings of stems in water (data not shown). Nodulation sites were formed continuously as the stem grew.

Stem nodulated *Aeschynomene* sp. can be classified into three groups according to the anatomy of their nodulation sites. Group 1 comprises *A. afraspera* and *A. nilotica.* The root primordium of *A. afraspera* (Fig. 2 A) is well developed and breaks through an epidermal dome forming a circular cavity around it. The apex itself is overlayed by a thin layer of flattened epidermal cells. Group 2, the found in *A. indica* (Fig. 2 B), comprises 9 species: *A. indica, A. ciliata, A. denticulata, A. evenia, A. pratensis, A. rudis, A. scabra, A. sensitiva,* and *A. tambacoundensis.* The root primordium is less developed and just underlies the stem epidermis. Intact epidermal cells generally cover the root primordium without formation of an epidermal dome and there is no circular cavity at the base

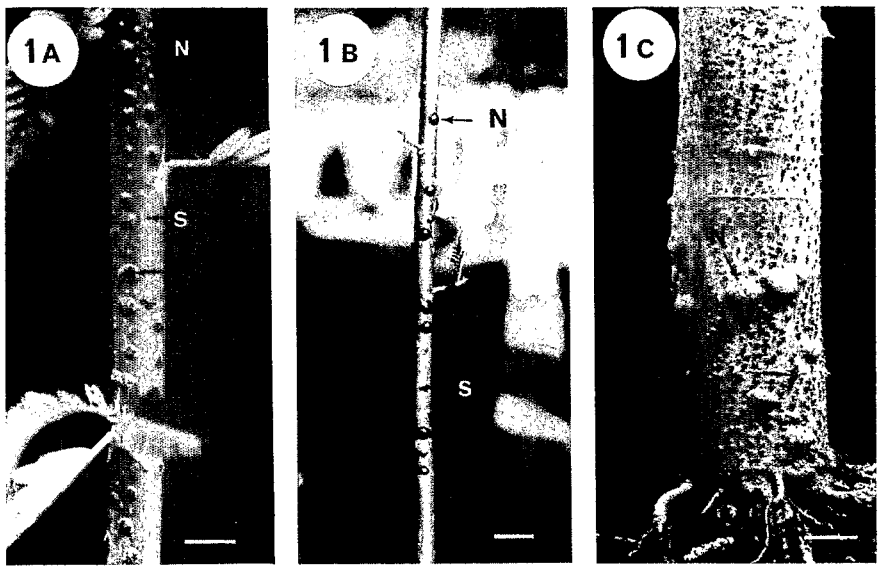

Fig. 1: Uninoculated nodulation sites (S) and nitrogen-fixing nodules (N) formed on the stems of *Aeschynomene* spp. after a single inoculation with the specific *Rhizobium.* – 1 A: Nine-week-old *A. afraspera* plants, 25 days after inoculation with *Rhizobium* strain ORS 322; sectioned nodule showing dark interior pigmentation (double arrowhead). – 1 B: Eight-week-old *A. indica* plants, 25 days after inoculation with *Rhizobium* strain ORS 310. – 1 C: Four-month-old *A. elaphroxylon* plants, 1 month after inoculation with *Rhizobium* strain ORS 304. – Scale bars = 1 cm.

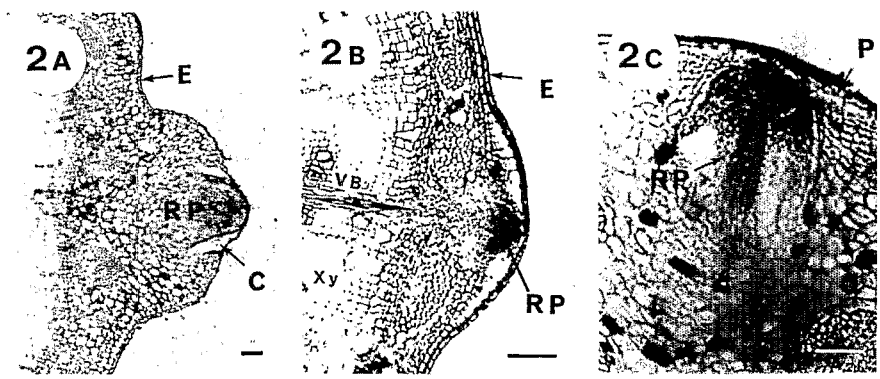

Fig. 2: Transverse sections of uninoculated nodulation sites of *A. afraspera* (2 A), *A. indica* (2 B) and *A. elaphroxylon* (2 C): Circular cavity (C), epidermis (E), periderm (P), root primordium (RP), vascular bundles (VB), xylem (Xy). – Scale bars = 0.1 mm.

of the root primordium. Group 3, type *A. elaphroxylon* (Fig. 2 C), comprises the following *Aeschynomene* species: *A. elaphroxylon, A. crassicaulis*, and *A. pfundii*. The root primordia are hidden and remain embedded in the cortical tissues of the stem as long as the dormancy of the root primordia is not broken by waterlogging.

Such differences in the structure of the nodulation site explain the variations observed in the stem-nodulating ability of the three groups of *Aeschynomene* species. *Aeschynomene* species of group 1 are readily infected since the root primordium is permanently exposed to rhizobial infection: the well developed root primordium is generally just covered by a layer of flattened epidermal cells. This layer may present discontinuities through which *Rhizobium* can enter. By contrast, the nodulation sites of species of group 3 cannot be infected by rhizobia unless the growth of adventitious root primordia is triggered by an external factor. This situation was described by Schaede (1940) who noted that flooding the stem of *Neptunia oleracea* was a prerequisite for the development of the root primordium into a rootlet susceptible to rhizobial infection.

Species of group 2, for example *A. indica*, nodulate more readily than species of group 3. The high level of humidity during the rainy season in Senegal is generally sufficient to break the dormancy of many nodulation sites on the stem. Consequently, except for the plants of the *A. elaphroxylon* group, waterlogging is not a prerequisite for stem nodulation (Eaglesham and Szalay 1983).

It can be noted that the classification of *Aeschynomene* species based on the anatomy of their nodulation sites is analogous to the classification based on the cross-inoculation group concept (Alazard 1985). The apparent relationship between the structure of nodulation sites and host compatibility needs further investigations.

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