APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1985, p. 732–734 Vol. 50, No. 3
0099-2240/85/090732-03$02.00/0

Stem and Root Nodulation in *Aeschynomene* spp.

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Received 21 March 1985/Accepted 21 June 1985

Nodulation ability of 15 *Rhizobium* strains isolated from root and stem nodules of tropical *Aeschynomene* species was studied on 20 different *Aeschynomene* species and four other legumes—*Arachis hypogaea*, *Stylosanthes guianensis*, *Macroptilium atropurpureum*, and *Sesbania rostrata*. The results of this investigation showed that *Aeschynomene* species could be divided into three groups according to the cross-inoculation group concept.

Stem nodulation in the genus *Aeschynomene* was first reported on *A. aspera* by Hagerup in 1928 (8). Since then, stem nodulation was also found in the following species: *A. paniculata* (15), *A. indica* (2, 16), *A. evenia*, *A. filosa* (3), *A. denticulata*, *A. pratensis*, *A. rudis*, *A. scabra*, and *A. sensitiva* (7). The ability of legumes to form stem nodules, restricted to only one species in *Sesbania*, *S. rostrata* (6), and one species in *Neptunia*, *N. oleracea* (12), is much more widespread in *Aeschynomene* spp.

In nature, stem nodules are usually localized on the lower parts of the stem which have been temporarily or permanently submerged. However, waterlogging is not a prerequisite for the formation of stem nodules (7). Nodulation on the stems occurs on predetermined sites identified as adventitious root initials. Early infection of the stems by *Rhizobium* spp. depends on the accessibility of the root primordium to bacterial invasion (2, 5).

The genus *Aeschynomene* consists of about 160 species of primarily tropical legumes (10). This genus is included in the tribe *Hedysareae*, which also includes the genera *Arachis* and *Stylosanthes*. Date and Halliday (4) placed *Aeschynomene* spp. in the "promiscuous but often ineffective" group of plants which frequently nodulate with a wide range of *Rhizobium* strains, but many of which are ineffective in nitrogen fixation.

The present paper reports the results of a cross-inoculation study between *Rhizobium* strains isolated from species of *Aeschynomene* native to West Africa and several native and introduced species, including *Aeschynomene* spp., *Arachis hypogaea*, *Stylosanthes guianensis*, *Macroptilium atropurpureum*, and *Sesbania rostrata*.

The following *Aeschynomene* species were used as host plants: (i) species native to West Africa (*A. afraspera*, *A. americana*, *A. crassicaulis*, *A. elaphroxylon*, *A. indica*, *A. nilotica*, *A. pfundii*, *A. sensitiva* subsp. 1 and subsp. 2, and *A. tambacoundensis*); (ii) species from the IFAS collection, University of Florida (*A. ciliata*, *A. denticulata*, *A. evenia*, *A. falcata*, *A. fluminensis*, *A. histrix*, *A. pratensis*, *A. rudis*, *A. scabra*, and *A. villosa*). In addition the following well-known leguminous plants were included: *Arachis hypogaea*, *Stylosanthes guianensis*, *Macroptilium atropurpureum* cv. Siratro, and *Sesbania rostrata*.

Fifteen of the *Rhizobium* strains used in this study were isolated from *Aeschynomene* plants growing in West Africa. *Rhizobium* strains ORS301 and ORS302 were isolated from root nodules of *A. americana* and *A. pfundii*, respectively. Strains ORS304 and ORS326 were isolated from nodules of the lower and immerged part of *A. elaphroxylon* stems. ORS318 and ORS328 were isolated from root nodules of *A. sensitiva* subsp. 1 and subsp. 2, respectively. Other strains were isolated from stem nodules: ORS303, ORS315, and ORS322 were isolated from *A. afraspera*; ORS306 and ORS310 were isolated from *A. indica*; ORS320 was isolated from *A. sensitiva* subsp. 1; ORS319 and ORS330 were isolated from *A. sensitiva* subsp. 2; and ORS334 was isolated from *A. tambacoundensis*. ORS571 was isolated from stem nodules of *Sesbania rostrata* (6). Except ORS301, all strains were intermediate- or fast-growing rhizobia (generation time, 5 to 8 h), forming small colonies (ca. 1-mm diameter) in 3 to 5 days and producing alkali yeast extract and mannitol broth.

Seeds were superficially sterilized with concentrated sulfuric acid. The duration of sterilization was 30 min for *A. afraspera*, *A. ciliata*, *A. denticulata*, *A. evenia*, *A. fluminensis*, *A. indica*, *A. nilotica*, *A. pfundii*, *A. pratensis*, *A. rudis*, *A. scabra*, *A. sensitiva* subsp. 1 and subsp. 2, *A. tambacoundensis*, and *Sesbania rostrata*; 15 min for *A. americana*, *A. falcata*, *A. histrix*, and *A. villosa*; and 3 min for *Macroptilium atropurpureum* and *Stylosanthes guianensis*. *Arachis hypogaea* seeds were surface sterilized with 0.1% acidified mercuric chloride (14).

Seeds were then rinsed 10 times with sterile water and allowed to soak for 4 h in the final change of water. Surface-sterilized seeds were germinated at 30°C on sterile 1% water agar in petri dishes for 24 to 48 h. Then they were transferred into glass tubes (150 by 15 mm) containing Jensen medium (14) for root nodulation trials and into pots (15-cm diameter) containing sterile waterlogged soil for stem nodulation trials. Plants were grown in the greenhouse at 35 to 23°C (day-night temperatures) under 12-h photoperiods. *Rhizobium* cultures were developed on yeast extract and mannitol broth (14) for 5 days at 30°C. To induce root nodulation, a drop of culture (10^8 cells per ml) was added to each test tube. Stems were inoculated by being sprayed with a 10-fold dilution of the *Rhizobium* culture. There were three replicates in each inoculation treatment, and in each there was one uninoculated control.

Stem and root nodules appeared ca. 5 to 7 days after inoculation, and 3 weeks later they were fully developed. One month after inoculation, nodulation effectiveness was assessed by evaluating plant vigor and color of foilage and by estimating the N_2-fixing ability of root and stem nodules by the acetylene reduction assay (9).

Aeschynomene species studied here fell into three cross-inoculation groups (Table 1). Group 1 comprised *A. americana*, *A. crassicaulis*, *A. elaphroxylon*, *A. falcata*, *A. fluminensis*, *A. histrix*, *A. pfundii*, and *A. villosa*. Incidentally, this group was shown to include *Arachis hypogaea*,

TABLE 1. Cross-inoculation specificities in the genus *Aeschynomene* and four other legumes[a]

Test plant	Isolation hosts and *Rhizobium* strains															
	A. americana (ORS-301)	*A. pfundii* (ORS-302)	*A. elaphroxylon*		*A. afraspera*			*A. indica*		*A. sensitiva* subsp.					*A. tambacoundensis* (ORS-334)	*Sesbania rostrata* (ORS-571)
			ORS-304	ORS-326	ORS-303	ORS-322	ORS-315	ORS-306	ORS-310	ORS-318	ORS-319	ORS-320	ORS-328	ORS-330		
A. americana	E	E	E	E	–	–	–	–	–	–	–	–	–	–	–	–
A. crassicaulis	E	E	E	E	–	–	–	–	–	–	–	E	–	–	–	–
A. elaphroxylon	E	E	E	E	–	–	–	–	–	–	–	–	–	–	–	–
A. falcata	E	E	E	E	–	–	–	–	–	–	–	–	–	–	–	–
A. fluminensis	E	E	E	E	–	–	–	–	–	–	–	–	–	–	–	–
A. histrix	E	E	E	–	–	–	–	–	–	–	–	–	–	–	–	–
A. pfundii	E	E	E	e	–	–	–	–	–	–	–	E	–	–	–	–
A. villosa	–	E	E	–	–	–	–	–	–	–	–	–	–	–	–	–
Arachis hypogaea	E	I	–	E	–	–	–	–	–	–	–	–	–	–	–	–
Stylosanthes guianensis	E	E	I	E	–	–	–	–	–	–	–	–	–	–	–	–
Macroptilium atropurpureum	E	E	E	E	I	I	–	–	–	–	–	–	–	–	–	–
A. afraspera	E	E	E	E	E	E	I	–	–	–	–	E	–	–	–	–
A. nilotica	E	E	E	E	E	E	I	–	–	–	–	E	–	–	–	–
A. ciliata	–	–	–	–	E	E	–	E	E	E	E	E	E	E	E	–
A. denticulata	–	–	–	–	E	E	–	E	E	E	E	E	E	E	E	–
A. evenia	–	–	–	–	E	E	–	E	E	E	E	E	E	E	E	–
A. indica	–	–	–	–	e	e	–	E	E	E	E	E	E	E	E	–
A. pratensis	–	–	–	–	E	I	–	E	E	E	E	E	E	E	E	–
A. rudis	–	–	–	–	E	e	–	E	E	E	E	E	E	E	E	–
A. scabra	–	–	–	–	–	E	–	E	E	E	E	E	E	E	E	–
A. sensitiva subsp. 1	–	–	–	–	E	E	–	E	E	E	E	E	E	E	E	–
A. sensitiva subsp. 2	–	–	–	–	I	I	–	E	E	E	E	E	E	E	E	–
A. tambacoundensis	–	–	–	–	–	I	–	E	E	E	E	E	E	E	E	–
Sesbania rostrata	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	E

[a] E, Effective root and stem nodulation: E, effective root nodulation; e, partially effective root and stem nodulation; e, partially effective root nodulation; I, ineffective root and stem nodulation; I, ineffective root nodulation: –, no nodules produced.

Stylosanthes guianensis, and *Macroptilium atropurpureum*, a test plant of the cowpea group. Group 2 comprised only two species, *A. afraspera* and *A. nilotica*. Group 3 contained the following *Aeschynomene* species: *A. ciliata*, *A. denticulata*, *A. evenia*, *A. indica*, *A. pratensis*, *A. rudis*, *A. scabra*, *A. sensitiva* subsp. 1 and subsp. 2, and *A. tambacoundensis*.

No cross-inoculation could be found between the *Aeschynomene* species tested and *Sesbania rostrata*.

All plants of groups 2 and 3 could bear stem nodules. So far, we have only found one stem-nodulated *Aeschynomene* sp. in group 1, *A. elaphroxylon*. Nodules formed on this species were restricted to the lower part of the stem and appeared only when the soil was kept in waterlogged conditions.

Most of the rhizobia isolated from plants of group 1 (strains ORS302, ORS304, and ORS326) effectively nodulated both roots and stems of group 2 plants; however, ORS301, the only slow-growing *Rhizobium* strain of our collection, nodulated the roots of group 1 and 2 species, but not the stems.

Strains ORS303 and ORS322 isolated from *A. afraspera* failed to nodulate stems and roots of group 1 plants. The behavior of these strains largely varied with the host-plant species of group 3. In Table 1, we have included strain ORS315, an ineffective isolate from stem nodules of *A. afraspera*, which easily formed ineffective nodules on both roots and stems of group 2 plants. *Rhizobium* strains isolated from group 3 plants nodulated only plants of the homologous cross-inoculation group, except isolate ORS320 which also nodulated stems and roots of *A. afraspera* and *A. nilotica* and roots of *A. crassicaulis* and *A. pfundii*.

By the above classification, *Rhizobium* strain BT Ai1 isolated from stem nodules of *A. indica* should have the same host specificity as strains ORS306 and ORS310 isolated from the same host. This hypothesis was actually fulfilled because, according to Legocki et al. (11), strain BT Ai1 earlier failed to nodulate *Arachis hypogaea* and plants in groups 1 and 2 (*A. americana*, *A. falcata*, *A. histrix*, *A. villosa*, and *A. afraspera*), but nodulated stems and roots of plants belonging to group 3 (*A. denticulata*, *A. evenia*, *A. indica*, *A. pratensis*, *A. rudis*, and *A. sensitiva*).

Rhizobia isolated from group 1 plants nodulated *Arachis hypogaea*, *Macroptilium atropurpureum*, and *Stylosanthes guianensis*, thus suggesting that these *Aeschynomene* plants should be referred to as the "cowpea group." It is noted that in a previous study reported by Allen and Allen (1), *A. americana* was placed in the "cowpea miscellany." In these conditions, isolate ORS301 from *A. americana* might be a nonspecific indigenous slow-growing *Rhizobium* strain of the cowpea group.

It is interesting to note that *Aeschynomene* species of groups 2 and 3 were able to form both stem and root nodules, and that none of the plants tested showed the presence of

stem or root nodules only. This indicates, as suggested earlier (11), that expression of plant genes involved in the nodulation process is not restricted to the root. With the precedent restriction about *A. americana*, the absence of stem nodulation observed in most of the group 1 plants might be in relation to the accessibility of rhizobia to the nodulation site. Stem nodulation sites differ among the different plant groups by the structure of root primordia on the stem; root primordia in groups 2 and 3 are protruded, whereas those in group 1 are hidden and embedded in the cortical tissue of the stem (5).

Like strain ORS571 isolated from *Sesbania rostrata* (5), all the stem-nodulating rhizobia isolated from *Aeschynomene* plants, including strain BT Ai1, were intermediate- or fast-growing rhizobia and produced alkali in yeast extract and mannitol medium. This result indicates, as previously suggested, that these strains are intermediate types of *Rhizobium* spp. with characteristics of both fast and slow growers (13). Such *Rhizobium* strains involved in stem symbiosis could probably be considered members of a particular group of *Rhizobium* spp. A taxonomic study is under way to verify this hypothesis.

This work was supported by grant 83V0400 fom the French Ministry of Industry and Research.

I thank A. E. Kretschmer (IFAS, University of Florida) for his generous gift of seeds, and I am very grateful to Y. R. Dommergues and B. L. Dreyfus for helpful comments and discussions.

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