# Aeschynomene Aspera L., Nitrogen Fixing Stem Nodulation Plant From Manipur

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**Abstract :** Aeschynomene aspera L. is a wild annual legume growing in periodically waterlogged soils in Manipur, India with profuse stem nodulation. Nodules are formed on the stem at the emergence of lateral root primordia, called nodulation sites. They are distributed on vertical rows all along the stem and branches. Stem nodules are hemispherical in shaped, dark green outside and contain a red-pigmented central zone. Stem nodules exhibit high nitrogen-fixing potential being detected by micro Kjeldahl method. The amount of Nitrogen (N) content (%) found in term of kg N per kg dry weight was as 2.4x10-7 ± 1x10-7 kg from the 45-50 days old stem nodules. Because of nitrogen fixing potential and its ability to grow in waterlogged conditions, A. aspera L. could be introduced as organic manure into the rice cropping system in Manipur.

**Keywords:** Stem nodulation; Aeschynomene aspera L.; Manipur; Nitrogen fixation; micro Kjeldahl method

## 1 INTRODUCTION

STEM nodulation in Aeschynomene was first reported on A. aspera [1] and followed by A. paniculata [2]. Many scholars [3-11] had studied and published their findings on the stem nodulation of Aeschynomene spp. Stem nodulation is much more widespread within the genus Aeschynomene. Today 16 species of Aeschynomene are known having stem nodules [7]. Aeschynomene spp. fall into three cross-inoculation groups according to their effective nodulation response patterns with strains of Rhizobium isolated from stem and root nodules [7]. Earlier Rhizobium strains isolated from root nodules of A. aspera L. for nodulation ability on selected legumes had reported from India [12]. The present communication deals with the structure of A. aspera L. stem nodulation and its potential for use as nitrogen-fixer in Manipur rice cropping system.

## 2 MATERIAL AND METHOD

A Rhizobium strain (MTCC- 10038) was isolated from the stem nodules of A. aspera L. following Vincent's techniques [13]. The stock culture was maintained at 4°C on Yeast Extract Mannitol Agar (YEMA) slants and was also grown at 32ºC±1ºC in YM broth.

### 2.1 Plant culture and inoculation

For stem nodulation tests, seeds were surface sterilized with 0.1N sulphuric acid for 3 minutes and washed three times with sterile distilled water. Surface sterilized seeds were germinated at 30°C±1ºC on sterile water agar (1%) in the petri dishes for 24 to 48 hr. Then seedlings were transferred into plastic pots (15cm diameter) containing sterile water and poured more water to make the pots logged in 2kg sandy soil, pH 7.0 for stem nodulation trail in the Departmental greenhouse at 30°C±1ºC. Stems were inoculated by spraying with a 10-fold dilution of the *Rhizobium* (MTCC-10038) from the broth culture [7-9].


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### 2.2 Histology

Stem nodule containing and non-nodule twigs uninoculated were fixed overnight in 2.5% glutaraldehyde in 0.1M sodium-cacodylate buffer, pH 7.0, at 4°C. After washing in Na-cacodylate buffer, the samples were dehydrated in an ethanol series and embedded in BEEM capsules. Serial thin-sections were stained with Regaud's iron-hematoxylin stain.

### 2.3 Efficacy of N-fixing

Plants were grown with nutrient solution containing 30ppm [14]. Four weeks after sowing, the plants started normal growth until stem and root inoculation. After another four weeks of inoculation, nodule bearing stem portions and root systems were chopped off and assayed for nitrogen-fixing activity by micro Kjeldahl method [15].

### 2.4 N-Accumulation

Plants grown in plastic cylinders made of commercially available drainage tubes (diameter 0.3m, length 0.45m) was kept vertically into the experimental field of ICAR, North East Hill Region, Manipur. The bottom of cylinders were closed and filled with 30kg sterile sandy soil supplemented with 0.0016kg $K_2HPO_4$. Three seedlings of *A. aspera* L. were transplanted into already moistened soil and kept waterlogged. One week later, the entire plants roots were inoculated with *Rhizobium* strain. Half of the plants were stem inoculated with *Rhizobium* after 3 to 4 weeks of sowing. The other half was not stem inoculated (control). Four weeks after the last inoculation, all plant materials were harvested and oven dried for 48hr at 70°C±1ºC and weighed. The dry material was grounded and mixed, and the total nitrogen content was determined by micro Kjeldahl method [15].

## 3 RESULTS

### 3.1 Stem Inoculation of *A. aspera* L.

Regular stem nodulation was produced in *A. aspera* L. by the application of *Rhizobium* strain onto the stem (Plate A). In controlled condition, nodules appeared only on those parts of the stem where inoculums had been externally applied. Infection did not occur from inoculated parts of the plant to uninoculated parts through the stem vascular bundles. Repeated stem inoculation with *Rhizobium* strain (4 times over 2 months) into the growing plant until height of

1.5m resulted in a mean nodules weight of 0.032kg of fresh weight (Table 1). A number of aerial stem nodules were found distributed at random along the principal stem and lateral branches on *A. aspera* L. grown in natural habitat. Whereas few nodules occurred on the roots of the plants grown in controlled condition which seems that it was less than the number of stem nodules due to an alternative adaptation to wet environmental condition [1]. However, waterlogging was not a pre-requisite for stem nodulation [5].

**TABLE 1**
WEIGHT OF FRESH STEM NODULES OF AESHYNOMENE ASPERA L. AFTER INOCULATION WITH RHIZOBIUM STRAIN MTCC-10038.

| PLANT HEIGHT (M) | WEIGHT OF NODULES (KG) | MEAN WT. OF NODULES (KG) |
|---|---|---|
| 1.0 | 0.028 | |
| 1.3 | 0.031 | 0.032 |
| 1.5 | 0.035 | |

**Plate A.** Nitrogen-fixing nodules and uninoculated nodulation sites on the stem of *Aeschynomene aspera* L. (Bar = 1cm)

### 3.2 Nodulation Site

The stem of *A. aspera* L. bore small swelling (diameter of 0.001-0.002m) resembling small prints which were distributed evenly in 2 or 3 vertical lines along the stem. These swellings are known as the nodulation sites. Nodulation sites were gradually distributed more frequently at the base of the stem than at the apex of the plant. At half-height (1.3m long) plant bore about 10 sites /m². Stem nodulation sites in *A. aspera* L. include adventitious root primordiums located below the two upper internodes in mature for rhizobial infection. Rhizobium infection became susceptible only between the central root primordium and the surrounding cortical tissue when a circular cavity was formed (Plate B). Due to mechanical forces associated with the developing root primordium only a thin layer of flattened epidermal cells overlaid which allowed rhizobial access to inside stem nodulation site. The large base of the epidermal cells was continuous with the stem cortex. In immature sites, at the top of the plant, the root primordium was still embedded in the cortical tissues of the stem and nodule development was never observed. The stem or branches developed abundant adventitious roots when immersed in water (Plate C).

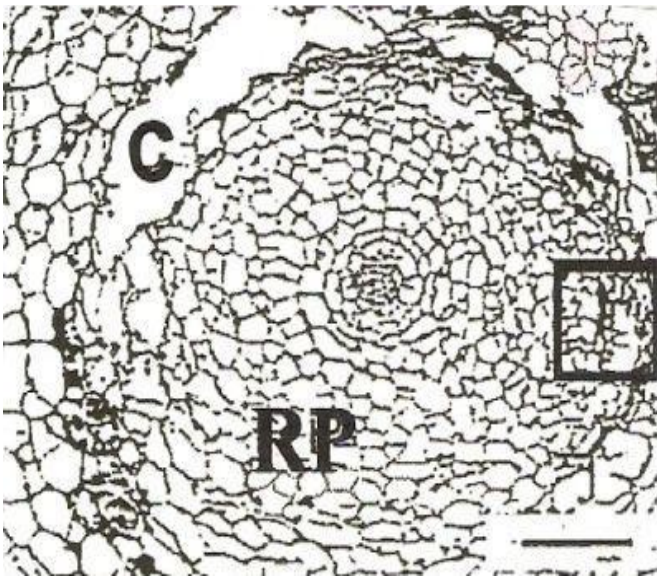


**Plate B.** Light micrograph of a TS of *A. aspera* L. through the inoculated nodulation site at a later stage of development showing the collapsed cell (Boxed area) located in the outer cortical cells of the root primordium. The circular cavity (C) encircling the root primodium (RP) in Iron-haematoxylin stain. x100 (Bar = 0.1 mm)

**Plate C.** A portion of *A. aspera* L. plant cultivated and immersed in water with root primordia evolved into adventitious rootlets. (Bar = 1 cm)

### 3.3 Morphology of Stem Nodules

Three-four days after *Rhizobium* inoculation, stem nodules were appeared. Prominent nodules on the stem epidermis were developed 18 days after *Rhizobium* inoculation. Numerous or sometimes contiguous nodules were developed having hemispherical in shaped. Mature nodules were found attaching broadly to the stem and appear flattened against the stem (diameter 0.004-0.006m and

0.002-0.003m high). In external appearance nodules were dark green in which central tissue contained red-pigments due to the presence of leghaemoglobin. Cross-sections of young nodules showed three distinct infected areas on both sides of vascular connections of the root primordium (Plate D). The central tissues of the nodule never contain any uninfected cells. Mature infected cells present prominent nucleus (Plate E).

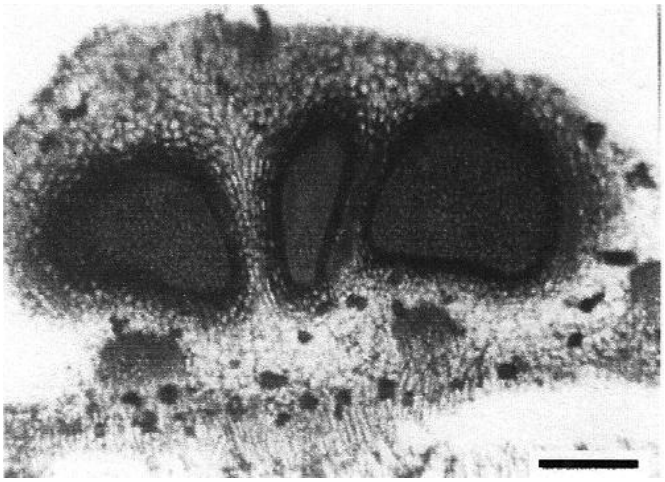

**Plate D.** Cross section of a 20 -day-old stem nodule showing two dark areas of infected tissue on both sides of remaining vascular bundles of the root primordium. x100. (Bar = 1mm)

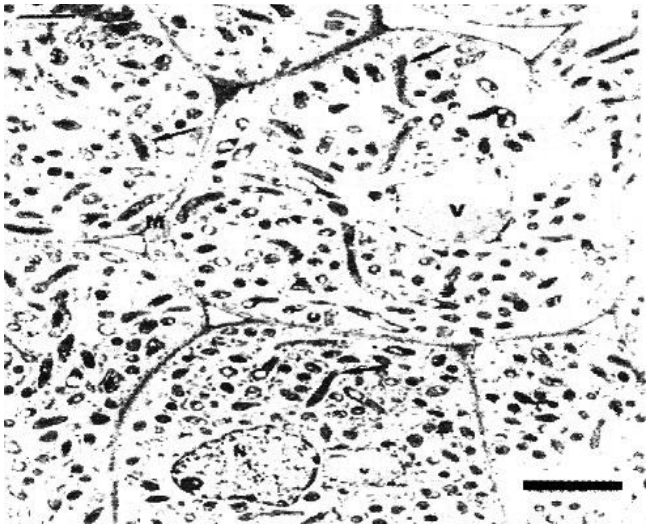


**Plate E.** TEM of infected cells from the upper stem nodule with one or two rod-shaped bacteroids per peribacteroid unit (PBU) (arrows). Host cell nucleus (N), mitochondria (M) and vacuoles (V). x 7000 (Bar = 2µm)

### 3.4 Nitrogen Fixing Potential

The value obtained gives the amount of Nitrogen (N) (%) content of the stem nodule in term of kg N per kg dry weight and estimated as $2.4x10^{-7} \pm 1x10^{-7}$ kg as mean of three (3) replications from the 45-50 days old stem nodules of *A. aspera* L. with ± standard deviation (Table 2).

**TABLE 2**
NODULATION AND NITROGEN ESTIMATION OF 9-WEEK OLD STEM NODULES OF AESCHYNOMENE ASPERA L. INOCULATED WITH RHIZOBIUM STRAIN MTCC-10038 (MICRO KJELDAHL METHOD).

| WT. OF NODULES (DRY WT. KG) | NITROGEN (KG) |
|---|---|
| 0.05 | $2.3x10^{-7} \pm 0.0$ |
| 0.05 | $2.4x10^{-7} \pm 1x10^{-8}$ |
| 0.05 | $2.5x10^{-7} \pm 2x10^{-8}$ |

## 4 DISCUSSIONS

These data suggest that *A. aspera* L. have an important agronomic potential as a green manure crop, particularly in the rice cropping system in Manipur. These finding has been communicated elsewhere. Furthermore, the plant is also able to fix nitrogen under waterlogged or flooded soil conditions which are usually unfavourable for other legumes. Because of nitrogen fixing potential and its ability to grow in waterlogged conditions, *A. aspera* L. could be introduced as organic manure into the rice cropping system in Manipur.

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## REFERENCES

[1]. O. Hagerup, “En hygrofil baelplante (*Aeschynomene aspera* L.) med bakterieknolde paa staengelen”, *Dan Bot Ark*. 15:1-9, 1928

[2]. K. Von Suessenguth and R. Beyerle, “Uber Bakterienknöllchen am Spross von *Aeschynomene paniculata*”, *Willd Hedwigia*, 75:234-237, 1936

[3]. S. Barrios and V. Gonzalez, “Rhizobia1 symbiosis on Venezuelan savannas”, *Plant and Soil*, 34:707-719, 1971

[4]. M. Yatazawa and S. Yoshida, “Stem nodules in *Aeschynomene indica* and their capacity of nitrogen fixation”, *Plant Physiology*, 45:293-295, 1979

[5]. A.R.J. Eaglesham and A.A. Szalay, “Aerial stem nodules on *Aeschynomene* spp”, *Plant Science Letter*, 29:265-272, 1983

[6]. M. Yatazawa, S. Yoshida and E. Maeda, “Fine structure of root nodules of *Aeschynomene indica* L”, *Soil Science and Plant Nutrition*, 30:405-16, 1984

[7]. D. Alazard, “Stem and root nodulation in *Aeschynomene* spp”, *Applied Environmental Microbiology*, 50:732-734, 1985

[8]. D. Alazard and E. Duhoux, (1987) “Nitrogen-fixing stem nodules on *Aeschynomene afraspera*”, *Biology and Fertility of Soils*, 4:61-68, 1987

[9]. D. Alazard and E. Duhoux, “Development of stem nodules in a tropical forage legume, *Aeschynomene afraspera*”, *J. Experimental Botany*, 41(230):1199-1206, 1990

[10]. M.F. Loureiro, E.K. James, J.I. Sprent and A.A. Franco, “Stem and root nodules on the tropical wetland legume *Aeschynomene fluminensisu*”, *New Phytol.*, 130:531-544, 1995

[11]. N.I. Singh, “Technology development and demonstration on the use of *Aeschynomene aspera* as green manure for rice cultivation in North East India”, Final technical report, CSIR, Govt. of India, New Delhi, 2004

[12]. N.S. Subba Rao, K.V.R. Tilak and C.S. Singh, “Root nodulation studies in *Aeschynomene aspera*”, *Plant and Soil*, 56:491-494, 1988

[13]. J.M. Vincent, “A manual for the practical study of root nodule bacteria. IBP Handbook N 15, Blackwell, Oxford, 1970

[14]. E.J. Hewitt, Sand and water culture methods used in the study of plant nutrition. Tec Comm N 22. Commonwealth Agricultural Bureaux, Farnham Royal, England, 1966

[15]. C.A. Lang, “Simple micro-determination of kjeldahl in biological materials”, *Anal Chem.*, 30:1692-1694, 1958