
Wel-come

PRESENTATION ON

STEM NODULATING BACTERIA AND THEIR APPLICATION IN AGRICULTURE

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FLOW OF PRESENTATION

Introduction

Inoculation

Infection Process And Nodule Formation.

Factors Affecting Symbiosis

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INTRODUCTION

- The symbiosis between leguminous plants and soil bacteria of the Rhizobiaceae leads to the formation of nitrogen-fixing nodules, generally exclusively appearing on the roots.
- A few legume species, however, form nodules not only on their roots, but also at stem-located root primordia.
- The first example of this phenomenon was first reported in **1928** in *Aeschynomene aspera* L. by **Hagerup**.

- 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.

- Up to now, **26** different stem-nodulating legume species have been reported belonging exclusively to the genera *Aeschynomene*, *Neptunia*, and *Sesbania*.

❖The most distinctive characteristic of stem-nodulating legumes is the presence of **predetermined nodulation sites on the stems.**

❖The formation of these sites is independent of infection with **rhizobia** They comprise primordia of adventive roots that are able to grow out under waterlogged conditions .

❖ These primordia can be distributed over the whole length of the stem or appear only on lower stem portions. They are arranged in **straight vertical rows** *(Sesbania)* or in spiral-shaped rows winding around the stem *(Aeschynomene), giving* the appearance of random distribution

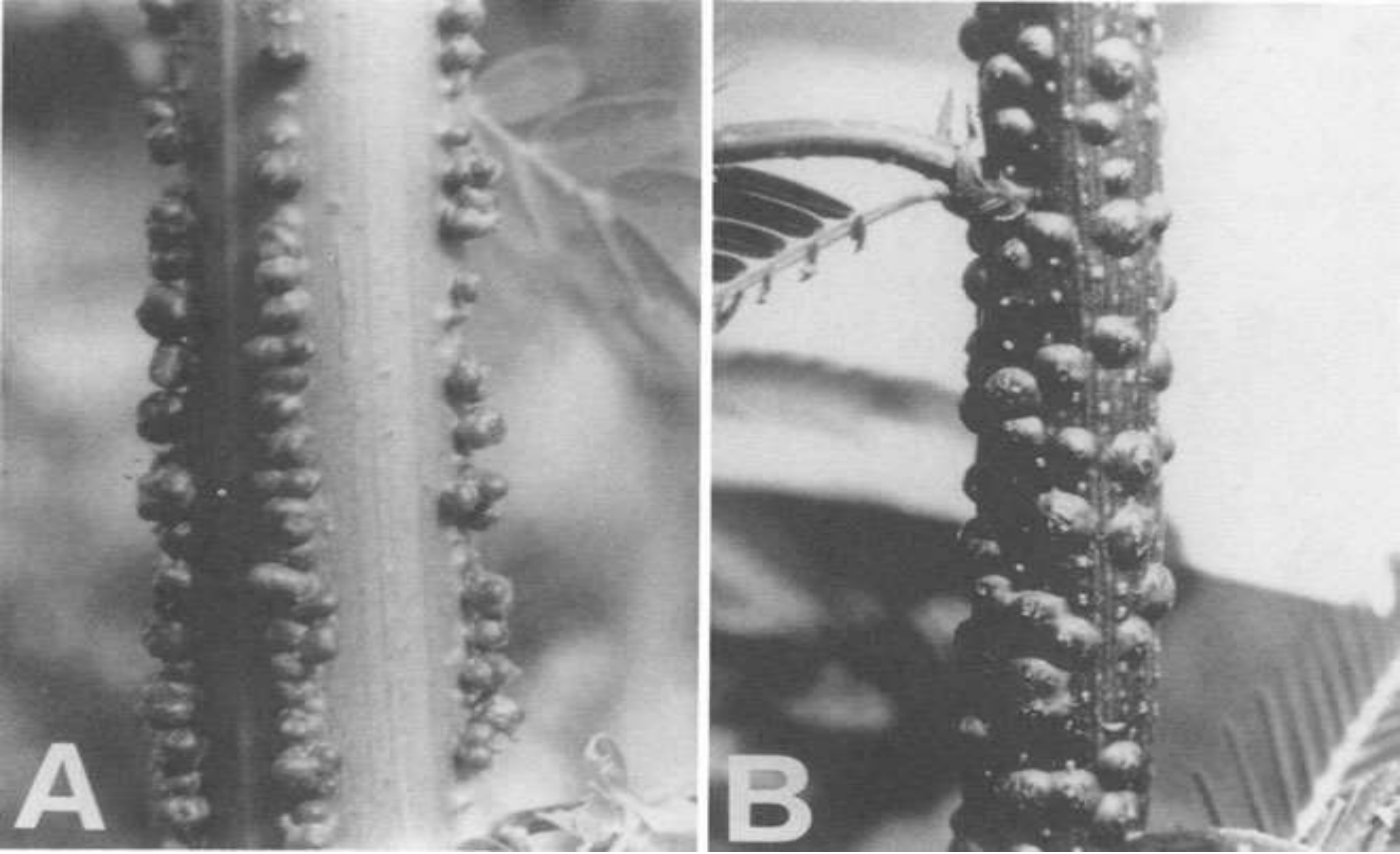


Fig 1. Nodulated stems of 50-day-old *Sesbania rostrata (A)* *and Aeschynomene afraspera (B)*.

Table1:Stem-nodulating legumes, their geographic distribution.

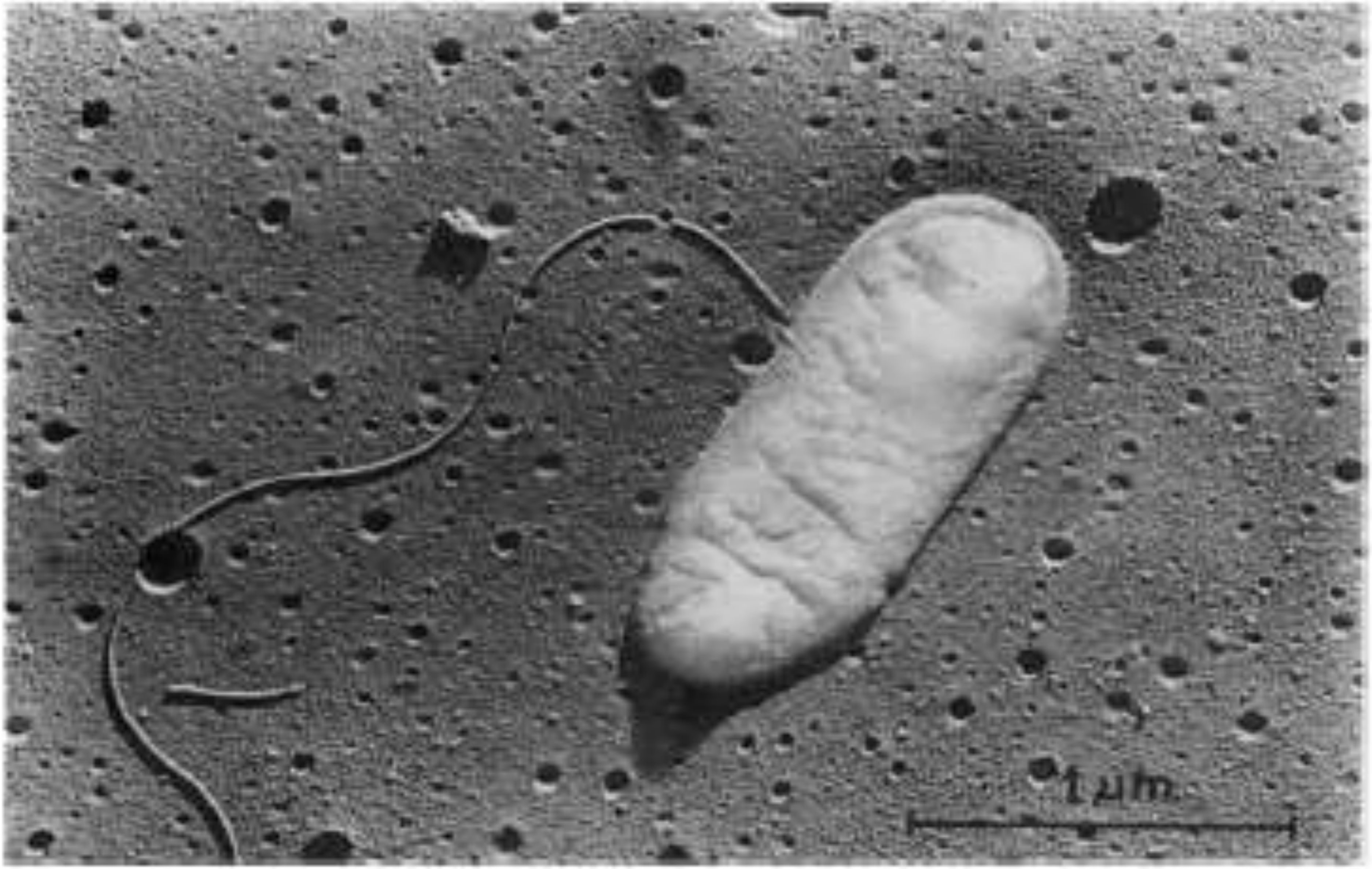
	Distribution
Aeschynomene	
aspera	Africa, S. Asia
afraspera	Africa
ciliata	Africa, S. America
crassicaulis	Africa
cristata	Africa, Madagascar
denticulata	S. America
elaphroxylon	Africa
evenia	S. America
fluminensis	S. America
indica	Pantropical
nilotica	Africa
paniculata	S. America
pfundii	Africa
pratensis	S. America
rudis	S. America
scabra	S. America
schimperi	Africa
sensitiva	Pantropical
tambacoundensis	W. Africa
uniflora	Africa
villosa	S. America
Sesbania	
punctata	Madagascar
rostrata	West Africa
speciosa	Asia, W. Africa
javanica	S.E. Asia
Neptunia	
oleracea	Pantropical

Azorhizobium caulinodans

- ✓The fast growing *Rhizobium strain ORS571,now called* ***Azorhizobium caulinodans***, was isolated from stem nodules of the tropical legume ***Sesbania rostrata.*** The strain can grow in the free-living state at the expense of molecular nitrogen.

- ✓The nitrogenase, purified from cells grown in a fermenter, was shown to be composed of two components, a **MoFe-**protein and a **Fe-**protein.

- ✓The enzyme activity was found to be subject to"switch-off" when **ammonia** was added to a N2-fixing culture.



Electron micrograph of negatively stained *Azorhizobium caulinodans ORS 571T grown in liquid medium*.

Inoculation

- For stem inoculation, shoots can be sprayed with a suspension containing about **10^8** bacteria/ml, using either a liquid culture of rhizobia, a colloidal suspension obtained by mixing entrapped *rhizobia* in a phosphate buffer (0.06m, pH 6.8), or a suspension of crushed stem nodules with water passed through a filter.

Fig 2. Typical appearances of stem nodules formed by mutants categorized by type.

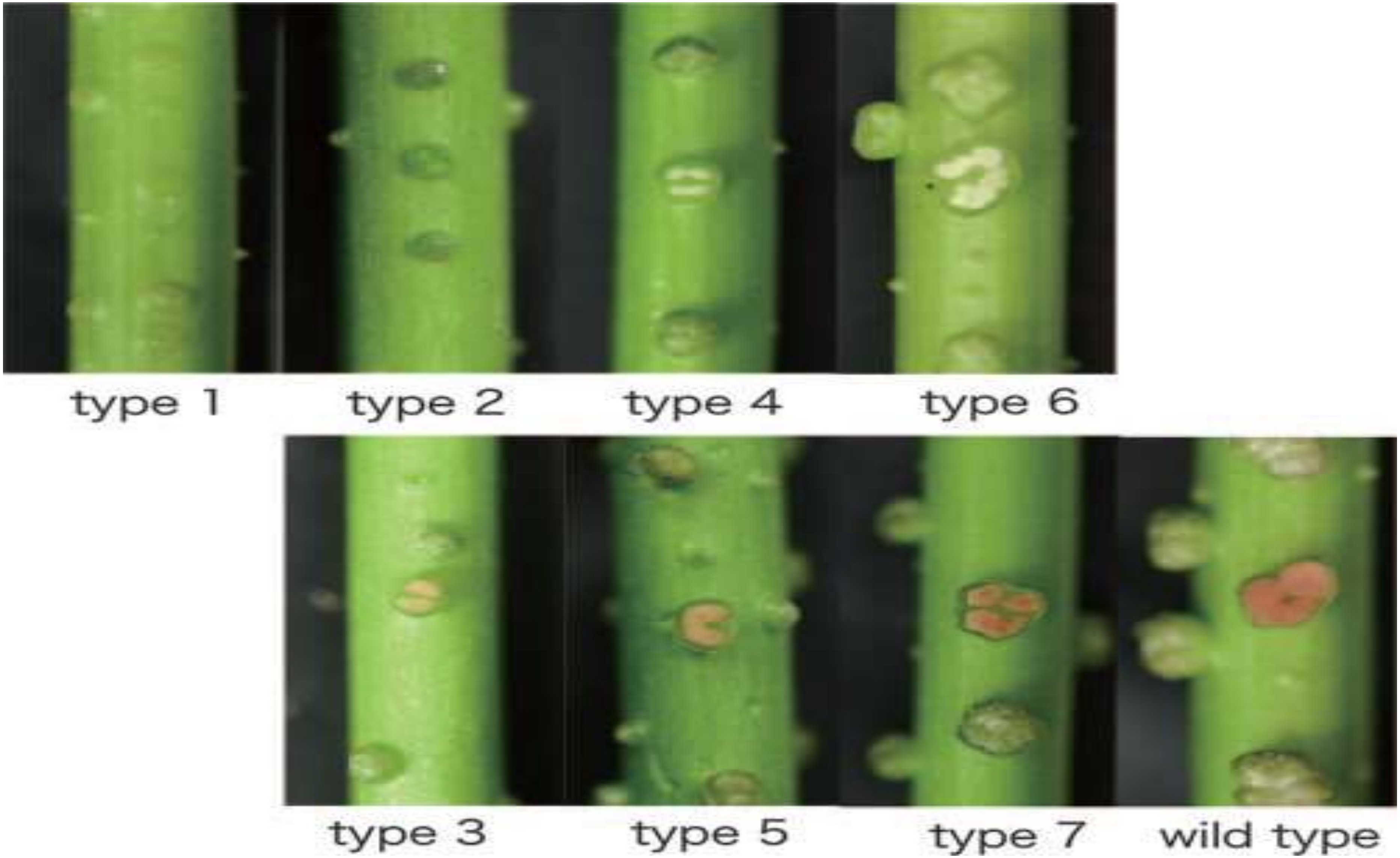


Table:2 Effects of *A. caulinodans* inoculation on N, P and K accumulation of *S. rostrata* on 60 DAS

Treatment	N (mg plant^{-1})	P (mg plant^{-1})	K (mg plant^{-1})
Seed inoculation	140.96 ab	16.36 a	131.45 ab
Stem inoculation	187.92 a	20.61 a	163.68 a
Seed and stem inoculation	97.51 b	10.42 a	89.32 b
No inoculation	78.64 b	10.78 a	82.14 b

Tukey–Kramer HSD test (*P<0.05).*

Infection Process, Nodule Formation, and Fine Structure of Stem Nodules

- Stem-nodulating rhizobia are true soil bacteria; without the appropriate macrosymbiont they thrive saprophytically on soil organic matter.
- Due to their **unipolar** flagellum they are highly mobile in the aquatic phase.
- Besides the soil, the **phyllosphere** of the host plant seems to be an alternate ecological niche for nonsymbiotic growth of stem-nodule rhizobia.

- Inoculum for stem infection can come from two sources: the **soil** and the **phyllosphere.** In both cases, **rain** seems to play major role for stem inoculation.
- soil splash due to rain may bring rhizobia to the lower stem.
- **ants** as a possible vector to inoculate primordia on the stem of *S. rostrata under nonflooded conditions.*
- **Wind** may be another important factor; often a high nodulation rate can be observed on stems of stem nodulating legumes along dusty roads.

- Wind may transport contaminated soil particles on above-ground plant parts thus favoring stem inoculation.
- After the rhizobia have reached the nodulation site, they penetrate into the cortical tissues of the stem.

- Upon penetration of rhizobia in the intercellular space, **infection pockets** are formed into the basal layers of the root primordium, which then resume their **meristematic activity.** As a result, **cell division** starts in the infective center and the nodule begins to form.
- intracellular infection threads are formed.
- At the infective stage, rhizobia change in shape and size. They become **bacteroids** and are surrounded by a membrane envelope.

- As the growing infective center forms the nodule, the majority of the cells in stem nodules are infected.
- The formation of stem nodules becomes macroscopically visible within **5 to 7 days** after inoculation and the nodules reach their full size in **15 to 20 days.**
- In *S. rostrata*, stem nodules are spherical protrusions, 5 to 12 mm in diameter. They can easily be detached from the stem, as the basal portion of the nodule forms a narrow neck.

- In *Aeschynomene flattened* hemispheric nodules are formed. Because **no** neck formation is found, the nodules are hard to remove.

Factors Affecting Symbiosis

- **Survival of Rhizobia :** Like root-nodule bacteria, stem-nodulating bacteria are true soil bacteria. A **carbon** source is critical for their survival in the soil.
- **Phyllospheric or Epiphytic Survival :** contain nutritional substances like amino acids and carbohydrates.

➢Mineral Nitrogen :

- Normally, crop plants respond to fertilizer nitrogen in the field since most of the cultivated soils worldwide are deficient in nitrogen.
- For reasons of energy legumes prefer soil or fertilizer nitrogen rather than biologically fixed nitrogen.
- **Mineral N** can reduce both nodulation and N2 fixation in stem nodulating legumes; the degree of inhibition varies with the compound and its concentration.

APPLICATION IN AGRICULTURE

Agronomic Use of the Stem-Nodulating Legumes

- Leguminous GM crops are potential **N** sources with relatively high efficiency, but also have diverse non-N effects such as the improvement of soil properties and control of **weeds**, pests, and diseases. Positive effects on soil properties include,
- maintenance or improvement of soil organic matter.
- conservation and recycling of nutrients.

- increased mineralization.
- increased biological activity.
- increased cation exchange capacity.
- reduction in soil erosion, and reduction in fertilizer N losses.

Potential Rice Environments for GM Use

- The irrigated lowland environment, characterized by an assured water supply, has a high cropping intensity.
- The main problem of this environment is crop competition for time, space, and labour. In areas where the gap is very short or where early-season waterlogging occurs, **fast-growing *Sesbania*** or ***Aeschynomene*** would fit between the main crops. S. rostrata may be used as GM before wet season rice, during the long-day period (April/May) and A. afraspera before the dry season rice (November/December) during the short-day period.

- **Growing GM as an Intercrop and on Bunds/Wastelands.**
- **Optimum Time for GM Incorporation and Rice Transplanting.**

***Sesbania* green manure is added to field**

Table 3:Onion yield under different treatments

Treatments	Yield (t/ha)
T_1 No N fertilizer application	20.3c
T2 Urea 50%N	25.0b
T_3 Urea 100 %N	25.1b
T4 Urea 150%N	25.2ab
T5 Urea 50%N+ *Sesbania rostrata 50%N*	26.8ab
T6 Urea 50%N and *Crotalaria juncea 50%N*	27.9a
T7 Urea50%N + *Sesbania rostrata 25%N+ Crotalaria juncea 25%N*	24.7b
T8 urea 25%N + *Sesbania rostrata 75%N*	25.3ab
T9 Urea 25%N+ *Crotalaria juncea 75%N*	26.5ab


Thank You