

Rhizobium Biofertilizer for North-East Region of India



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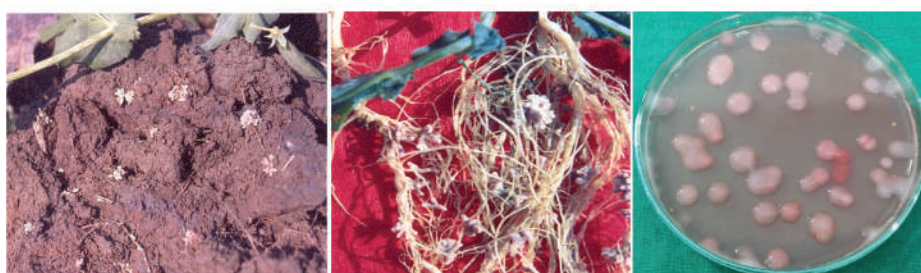
Background

The symbiotic systems are a major source of N in most legumes with an average of 80% of N derived from biological N-fixation (BNF). Besides N_2 -fixation, rhizobia have been increasingly associated with secretion of plant growth promoting substances, solubilization of organic and inorganic phosphate and antagonistic action against pathogen. The survival, growth and persistence of rhizobia largely depend on edaphic, biotic and abiotic factors. Soil acidity complex of arable land provides an array of constraints for size and effectiveness of rhizobial population owing to excess availability of Al and Mn to toxic levels and characterized by deficiency and poor availability of P, Ca, Mg and Mo.

ICAR- All India Network Project on Soil Biodiversity-Biofertilizers (AINP SBB) has developed *Rhizobium* biofertilizer technology for North East Region of India. Potential strain isolated after intensive screening of native isolates from nodules of commonly grown legumes in North East Region, and currently being used for mass production of biofertilizer. Performances of the biofertilizers were evaluated on legumes commonly grown in NEH regions. *Rhizobium* biofertilizers enhanced crops yield by 20-25% and saved nitrogenous chemical fertilizer significantly. The technology has also been transferred to 3 industries for mass production to meet the increasing demand by farmers. During last 3 years, about 550 farmers were benefitted and 10 KVK/Agricultural officers were trained to intensify use of this technology. State agriculture department and KVKs have adopted this technology and documented in the package of practices.

Rhizobium in North Eastern Region of India

AINP on Soil Biodiversity-Biofertilizers at Assam Agricultural University has been engaged in screening and evaluation of efficient *Rhizobium* from the root nodules of legume grown in NER. Twenty three *Rhizobium* strains consisting of field pea (07), lentil (03), greengram (03), blackgram (02), cowpea (03) and rajmah (05) were isolated through a process of nodulation status and plant infectivity tests from the respective legumes and has been utilized for preparation of biofertilizers.



Nodules in field

Washed nodules

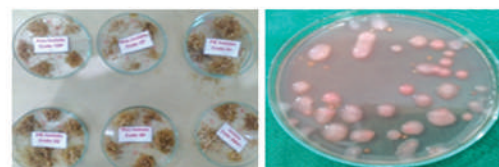
Isolated colonies of *Rhizobium*

Isolation of *Rhizobium*

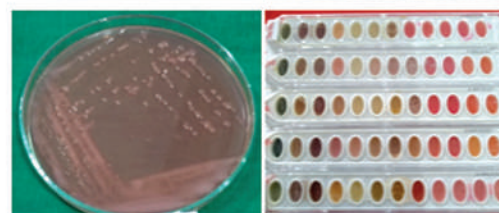
Step I : At early pod formation stage, rhizosphere containing plant roots was dug out and nodules and roots were separated by breaking the rhizosphere soil carefully in the field. The roots nodules were then washed.



Step II : Nodules were separated from roots, surface sterilized using 70% ethanol for 30 sec in 5% Na-hypochlorite for 5 min. The classical serial dilution technique was used for isolation of rhizobia in nodules, using differential yeast extract mannitol (YEM) agar media containing congo red (CR).



Step III : Single colonies showing white translucent/opaque were purified in the same YEM media. The purified prospective *Rhizobium* was then used for different biochemical tests



Step IV : Symbiotic effectiveness of *Rhizobium* was tested through plant infectivity tests in Leonard's Jar Assembly. *Rhizobium* mass multiplied in YEM broth for 48h to attain $>8.0 \text{ Log cfu/mL}$. Legume seeds were pregerminated.



Step V : Pre-germinated seed were planted in Leonard's Jar assembly at the centre. Seed was inoculated with 1000uL of inoculum of *Rhizobium*. N-free nutrient solution was filled in the bottom part of Leonard Jar Assembly. Plants were allowed to grow for 40 days. Nodules were separated and the nitrogenase activity tests were conducted in GLC. The nitrogenase positive isolates were utilized for preparation of *Rhizobium* biofertilizer.



Mass multiplication of *Rhizobium*

Step I : Mass multiplication of *Rhizobium* in YEM broth by inoculating the mother culture @1.0 % (mL). Allowed the *Rhizobium* to grow for 5-7 days in incubator shaker/BOD incubator at 30±1°C.

Step II : The carrier material (compost+charcoal,50:50) is powdered to a fine powder so as to pass through 212 micron IS sieve. The mixed carrier material is sterilized in an autoclave to eliminate the contaminants for three consecutive days at 24h of interval.

Step III : The sterilized carrier material is spread in a clean, dry, sterile metallic or plastic tray. The full grown (population approx 10⁹ cell/mL) *Rhizobium* culture is added to the sterilized carrier and mixed well by manual (by wearing sterile gloves). The culture suspension is added to a level of 40- 50% WHC of the carrier materials. Curing is done by spreading the inoculant on a clean floor/polythene sheet/ trays with polythene covering for 2 -3 hours at room temperature before packaging. Packaging of *Rhizobium* biofertilizer is carried out in polythene bags (thickness 50-75 micron) and sealed with electric sealer.

Step IV : Each packet is marked with the name of the manufacturer, name of the product, strain number, the crop to which recommended, method of inoculation, date of manufacture, batch number, date of expiry, price, full address of the manufacturer and storage instructions etc.

Step V : The packet is stored in a cool place away from the heat or direct sunlight. The population of inoculant in the carrier inoculant packet is determined at 15 days interval till its application. There should be more than 10⁹ cells / g of inoculant at the time of preparation and 10⁷ cells/g on dry weight basis before expiry date.



Mother Cultures



Growth of *Rhizobium* in broth



Mixed with sterilized carrier materials



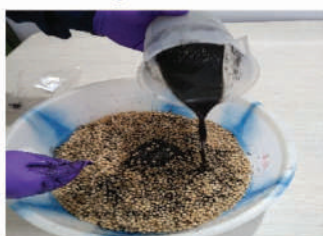
Packaging and Sealing

Benefits of *Rhizobium* inoculants

- ✿ Fixes 20-25 kg of N/ha/season in soil
- ✿ Increases grain yield by 20-25%
- ✿ Increases vegetative growth and leaf yield by 10-30%
- ✿ Secretes growth promoting and antibiotic like substances
- ✿ Can supplement more than 50-100% of nitrogenous fertilizer requirement by the crop.

Strategies for application of *Rhizobium* biofertilizers

The *Rhizobium* biofertilizer primarily used in Legume seeds as seed treatments. In this method 400gm of *Rhizobium* biofertilizers is suspended in 500mL of water to make slurry and mixed with 10-12kg of seed until seeds are uniformly coated. As the soils of NE Region are acidic in nature 10% lime or Calcium Carbonate may be used to form the seed pellet. After mixing the seed with biofertilizers properly, seeds need to be dried in shade for at least 1hour. The treated seeds to be sown immediately and cover the seed with soil.



Mixed with *Rhizobium*



Added lime @10%



Dried in shade for 1hour

Effect of *Rhizobium* biofertilizer on legumes

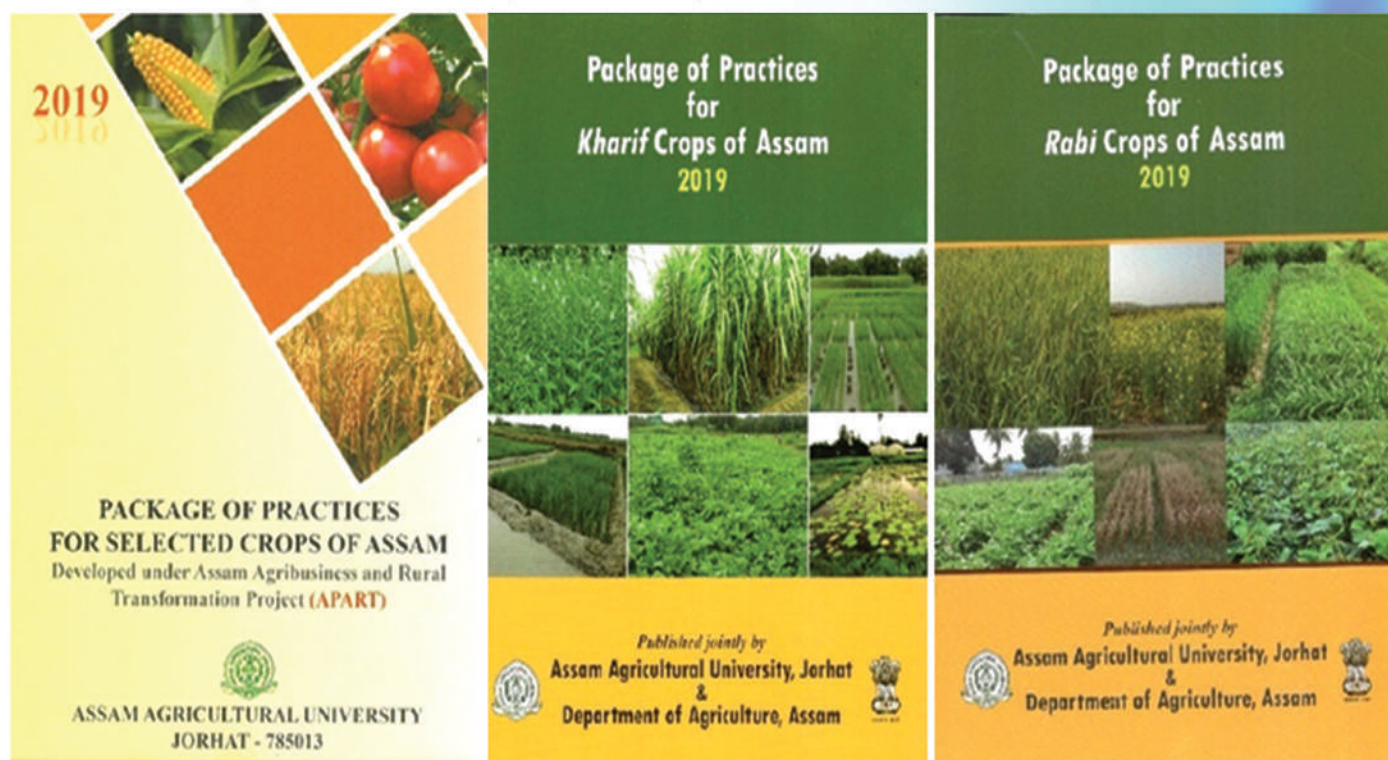
Crop	Application and crop response
Greengram	<ol style="list-style-type: none"> 1. Application of <i>Rhizobium</i> @500g/10kg as seed coating yielded 10.71 qt/ha 2. Application of full nitrogenous fertilizer (NPK@15:35:15 kg/ha) yielded 11.63qt/ha. 3. Application of full nitrogenous fertilizer (NPK@15:35:15 kg/ha +<i>Rhizobium</i> yielded 10.61qt/ha.
Blackgram	<ol style="list-style-type: none"> 1. Application of <i>Rhizobium</i> @500g/10kg as seed coating with reduced nitrogenous fertilizer (NPK@10:35:15 kg/ha) yielded 10.89 qt/ha 2. Application of <i>Rhizobium</i> @500g/10kg as seed coating with Full nitrogenous fertilizer (NPK@15:35:15 kg/ha) yielded 10.42qt/ha
Greengram	<ol style="list-style-type: none"> 1. Application of <i>Rhizobium</i> @500g/10kg as seed coating with reduced nitrogenous fertilizer (NPK@10:35:15kg/ha) +Pendimethalin @ 1 kg/ha (Pre emergence) + Hand weeding at 25 DAS yielded 13.59 qt/ha 2. Application of <i>Rhizobium</i> @500g/10kg as seed coating with full nitrogenous fertilizer(NPK@15:35:15 kg/ha) + One hand weeding at 20-25 DAS yielded 13.15qt/ha



Trainings / package of practices/recommendations

Farmers benefitted (2017-2020)	About 550 farmers of NEH regions and 10 KVK/state agriculture officers were trained.
Technology transfer	1. Technology transferred to State Agriculture Department, Assam.
Supply of biofertilizer strains to industries	1. Green Tech Ecosolutions Pvt Ltd, Manipur, Imphal, PIN: 795146 2. Green Agri Biotech, Moran, Abhaypur, P.O. Moran, Dibrugarh, 785670, Assam 3. Director of Agriculture, Mizoram Government, Aizwal: 796001
Revenue earned (2017-2020)	Rs.590730.00 (This includes biofertilizer strains and biofertilizer packets of <i>Azospirillum</i> , <i>Azotobacter</i> , <i>Rhizobium</i> and Phosphate solubilizing bacteria)
Package of practices	Package of practices developed and recommended to farmers of NEH regions. Published by AAU, Jorhat & State Agriculture Department, Assam as follows: <ul style="list-style-type: none">• Package of practices for kharif crops of Assam, 2019• Package of practices for Rabi crops of Assam, 2019• Package of practices for selected crops of Assam, 2019

Assam Agricultural University and State Agriculture Department - Package of practices



The information given in the document is based on the experiments carried out at the AINP centre- Department of Soil Science, Assam Agricultural University (AAU), Jorhat, Assam. For training, demonstration and other enquiries please contact Principal Investigator, AINP on Soil Biodiversity-Biofertilizers, AAU, Jorhat-13, Assam.

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