

## Isolation of *Rhizobium* from moisture stress efficient groundnut nodules

### ABSTRACT

Groundnut is an important oil seed crop accounts for a less than half of the major oilseeds produced in the country. Oil content of Groundnut varies from 44 % to 50 % depending upon the varieties and agronomic practices. It is also called as 'king of oil seeds' More than 85% of the crop is grown under rainfed conditions in low fertile soils. Biological nitrogen fixation (BNF) has the capacity to reduce the usage of chemical nitrogenous fertilizers by 0.160 billion tons/ year (Lesueur *et al.*, 2016). *Rhizobium* is a well-known symbiotic nitrogen fixing bacteria. It can fix 200-300 kg of N ha<sup>-1</sup> year<sup>-1</sup> in legumes (Peoples *et al.*, 1995). Inoculation of *Rhizobium* improves plant growth, pod kernel and Oil yield. Besides its inoculation enhances the activity of other beneficial bacteria such as *Bacillus amyloliquefaciens*, *Pseudomonas denitrificans*, and *Arthrobacter simplex* and also improves soil fertility (Palai *et al.*, 2021). *Rhizobium* species help in improving root nodulation and nitrogen fixation, enhancing plant growth and yield of leguminous plants including groundnut under moisture stress conditions. Hence the present study is to isolate *Rhizobium* species from groundnut nodules which can be used for improving nodulation efficiency and plant growth promotion under moisture stress conditions.

**Key words:** *Arachis hypogea*, *Pseudomonas denitrificans*, *Arthrobacter simplex*, crop legume; *Rhizobium*.

### 1. INTRODUCTION

Groundnut belonging to Fabaceae family (earlier Leguminosae), is commonly known as peanut. It is an important crop in the tropical and subtropical regions. Groundnut is a major pulse crop which plays an important role in Indian economy, and it is grown as an oil seed crop. Groundnut seeds contain 22-30% of protein and almost 44-56% of oil. During the year 2023, Gujarat state has highest area (16.35 lakh ha) followed by Rajasthan (8.69 lakh ha), Madhya Pradesh (5.40 lakh ha), Karnataka (3.14 lakh ha) and Andhra Pradesh (2.93 lakh ha). In Telangana, groundnut is sown in approximately 17,148 acres. The Gadwal district had more area under groundnut (7,562 acres) followed by Wanaparthy (4,665 acres), Kothagudem (1,769 acres), Warangal (1,018 acres) and Suryapet (364 acres) ([www.agricoop.gov.in](http://www.agricoop.gov.in) 2023).

Legume-rhizobia symbiosis is a well-known nitrogen fixing microorganism-plant interaction that improves soil fertility (Mylona *et al.*, 1995). Groundnut is a legume nodulated by various rhizobia of cowpea cross inoculation group (Graham and Donawa, 1982) unlike other legumes that rely on a distinct group of rhizobia (Allen and Allen, 1981). Groundnut has more nitrogen requirement (150-200 kg ha<sup>-1</sup>) than cereals however it can meet 60-80 % of its N requirements through symbiotic nitrogen fixation via root nodules and only 20-40 % from soil applied nitrogen (Singh *et al.*, 2003). Water is the most important factor for Agriculture production in arid and semi-arid areas (Maheswari *et al.*, 2017). Now a days, rainfed areas are experiencing

**Comment [p1]:** Basic sections are missing in this abstract : material and methods, results and conclusion.

**Comment [p2]:** No references in the abstract

**Comment [p3]:** Those key words are not adapted for this study

**Comment [p4]:** <introduction did not present the aim of this study et in addition to there is no information treating rhizobium association with studied plant. References used are outdated.

frequent drought conditions during the cropping season. It's essential for the isolation of drought tolerant *Rhizobium* isolates in groundnut in order to increase the plant growth as well as yield of groundnut plants under semi-arid production system.

## 2.MATERIALS AND METHODS

### Study area and Nodule collection

Present study was conducted in ICAR-CRIDA, Santhoshnagar, Hyderabad. Nodules were collected by uprooting the groundnut plants which were given 5, 10, 15 and 20 days of stress. *Rhizobium* was isolated by crushing the nodules according to the method described by Vincent(1970). Large, pink, healthy nodules on the taproot of groundnut plants exposed to moisture stress for 5,10,15 and 20 days at flowering phase were selected. The nodules were thoroughly washed with water to remove any surface soil. The washed nodules were placed in a sterile beaker and surface sterilized with 0.1 % mercuric chloride and 70 % ethylalcohol for 3 minutes and 30 seconds, then washed with sterile distilled water six times. The nodules were crushed with a sterile glass rod to create nodule suspension. A loop of nodule suspension was streaked onto Congoed Yeast Extract Mannitol Agar (CRYEMA). The plates were incubated at 28 °C until small, round, colorless or white colonies with a center red dot and an entire margin was developed, after which the separated colonies were restreaked onto a new CRYEMA plate to get pure culture. Pure cultures on YEMA plates were preserved and maintained by subculturing for further use. For long term preservation 60 % glycerol stocks were prepared and stored at -20 °C.

Comment [p5]: Locations of areas?

Comment [p6]: There is no explanation about stress test experience protocol...

### Morphological characterization

Morphological characterization of *Rhizobium* isolates was examined on Yeast Extract Mannitol Agar Media according to the standard procedures given by Cappuccino and Sherma (1992). Different properties like shape, elevation and margin were recorded.

Chart 1: Composition of Yeast Mannitol Agar media :

Components	Amounts used
Mannitol	10g/l
K <sub>2</sub> HPO <sub>4</sub>	0.5g/l
MgSo <sub>4</sub> .7H <sub>2</sub> O	0.2g/l
Nacl	0.1g/l
Yeast Extract	0.5 g/l
Agar	15 g/l

Distilled water	1000 ml
-----------------	---------

### Gramstaining

A drop of sterile distilled water was placed in the middle of the glass slide. A loop of inoculum from the log phase culture was taken and mixed with water to create a homogeneous mixture. A thin smear was made and air dried followed by heat fixing by quickly passing over a flame. The smear was then flooded with crystal violet solution for 30 seconds to 1 minute before being gently rinsed under running tap water. The slide was then flooded with iodine solution. After 30 seconds incubation at room temperature, the iodine solution was drained and washed with 95 % ethanol. After that, it was cleaned with water for 15 to 30 seconds. The smear was flooded with safranin solution for one minute. The slide was gently washed with tapwater and air-dried. The slide was observed under a microscope at 100 X power with oil immersion and data was recorded.

### 3. Results and discussions

*Rhizobium* was isolated from nodules which were given stress at 5,10,15 and 20 days and named as IS1,IS2,IS3,IS4,IS5,IS6,IS7,IS8,IS9,IS10,IS11,IS12,IS13,IS14,IS15,IS16,IS17,IS18,IS19,IS20,IS21,IS22,IS23,IS24,IS25,IS26,IS27,IS28,IS29,IS30,IS31,IS32,IS33,IS34,IS35,IS36,IS37,IS38,IS39,IS40,IS41,IS42, IS43,IS44,IS45,IS46,IS47,IS48,IS49,IS50. The results were presented in Fig 1.

**Table1 Information of nodules obtained from 5,10,15 and 20 days of stress**

<b>5 days</b>	IS1, IS2, IS3, IS4, IS5, IS6, IS7, IS8, IS11, IS12, IS13, IS14, IS15, IS16, IS17, IS21, IS22, IS23, IS24, IS25, IS26
<b>10days</b>	IS27, IS28, IS29, IS30, IS31, IS32, IS33, IS34, IS35, IS36
<b>15days</b>	IS9, IS10, IS18, IS19, IS21, IS46, IS47, IS48, IS49, IS50
<b>20days</b>	IS20, IS37, IS38, IS39, IS40, IS41, IS42, IS43, IS44, IS45

### Morphological characteristics of *Rhizobium* isolates

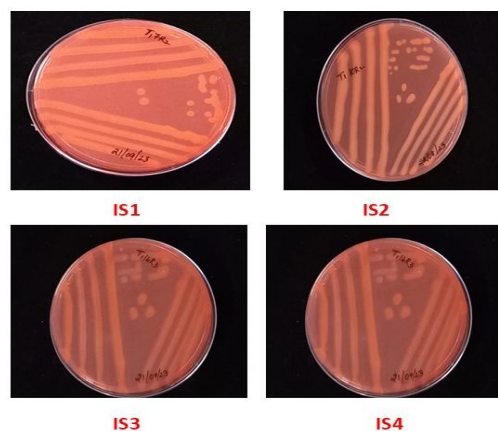
**Table-2 Morphological characteristics of *Rhizobium* isolates**

Name of the Isolate	Configurations	Elevations	Margins
IS1	Round with raised margin	Drop like	Smooth (Entire)

<b>IS2</b>	Round	Drop like	Smooth (Entire)
<b>IS3</b>	Round	Drop like	Smooth (Entire)
<b>IS4</b>	Round	Convex	Smooth (Entire)
<b>IS5</b>	Round	Drop like	Smooth (Entire)
<b>IS6</b>	Round	Drop like	Smooth (Entire)
<b>IS7</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS8</b>	Round	Convex	Smooth (Entire)
<b>IS9</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS10</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS11</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS12</b>	Round	Drop like	Smooth (Entire)
<b>IS13</b>	Round	Drop like	Smooth (Entire)
<b>IS14</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS15</b>	Round	Drop like	Smooth (Entire)
<b>IS16</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS17</b>	Round	Drop like	Smooth (Entire)
<b>IS18</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS19</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS20</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS21</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS22</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS23</b>	Round	Convex	Smooth (Entire)
<b>IS24</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS25</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS26</b>	Round	Drop like	Smooth (Entire)

<b>IS27</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS28</b>	Round	Drop like	Smooth (Entire)
<b>IS29</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS30</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS31</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS32</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS33</b>	Round	Drop like	Smooth (Entire)
<b>IS34</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS35</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS36</b>	Round	Convex	Smooth (Entire)
<b>IS37</b>	Round	Convex	Smooth (Entire)
<b>IS38</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS39</b>	Round	Drop like	Smooth (Entire)
<b>IS40</b>	Round	Drop like	Smooth (Entire)
<b>IS41</b>	Round with raised margin	Convex	Smooth (Entire)
<b>IS42</b>	Round	Drop like	Smooth (Entire)
<b>IS43</b>	Round	Drop like	Smooth (Entire)
<b>IS44</b>	Round	Drop like	Smooth (Entire)
<b>IS45</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS46</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS47</b>	Round	Convex	Smooth (Entire)
<b>IS48</b>	Round with raised margin	Drop like	Smooth (Entire)
<b>IS49</b>	Round	Drop like	Smooth (Entire)
<b>IS50</b>	Round with raised margin	Convex	Smooth (Entire)

Based on these morphological characteristics it was confirmed that it was *Rhizobium* and same results was supported by Manasa *et al.* (2016) in which groundnut nodules were collected from El-Gadarif, El-Gezira and El- Obeid and after series of streaking pure cultures of isolated strains it was observed that the colonies were circular, viscous and easily stuck to the inoculating loop.



**Fig1 :Pure cultures of *Rhizobium* isolates obtained after crushing of nodules**

#### **Gram staining**

All the *Rhizobium* isolates (IS1-IS50) were found to be gram negative which are found to be in accordance with the results of Chandrakanta *et al.*2024, observed pink colour of *Rhizobium* isolates, indicating that they were gram negative.

#### **Conclusion**

Present study revealed that a total of 50 *Rhizobium* isolates were isolated from groundnut nodules which were given 5, 10, 15 and 20 days of stress, they were morphologically characterized and gram staining was also done. They can be allowed for seed treatment with groundnut seeds to Improve the plant growth and yield of the crop after conducting necessary screening experiments. In future field study can also be conducted to improve the yield of the crop to survive under moisture stress conditions.

#### **BIBLIOGRAPHY**

- Allen, O. N., and Allen, E. K. 1981. *The Leguminosae, a source book of characteristics, uses, and nodulation*. University of Wisconsin Press.
- Cappuccino, J.G and Sherman, N. 1992. Biochemical activities of microorganisms. *Microbiology. A Laboratory Manual*. 188-247.
- Graham, R. A., and Donawa, A. L. 1982. Greenhouse and Field Evaluation of *Rhizobium* Strains Nodulating Groundnut (*Arachis hypogaea* L.). *Tropical Agriculture*.59(3): 254-255.

- Lesueur, D., Deaker, R., Herrmann, L., Bräu, L., Jansa, J. 2016. The Production and Potential of Biofertilizers to Improve Crop Yields. In: Arora, N., Mehnaz, S., Balestrini, R. (eds) *Bioformulations: for Sustainable Agriculture*. Springer, New Delhi, 71-92.
- Maheswari, M., Varalaxmi, Y., Yadav, S.K., Jyothilakshmi, N., Vanaja, M., and Venkateswarlu, B. 2017. Enhanced tolerance of transgenic sorghum expressing mtID gene to water-deficit stress. *Indian Journal of Biotechnology*.16: 63-67.
- Manasa, K., Reddy, R. S., and Triveni, S.2016. Isolation and Characterization of Rhizobial Isolates for their Multiple Beneficial Activities. *Research Journal of Agricultural Sciences*. 7(1): 172-176.
- Mylona, P., Pawlowski, K., and Bisseling, T. 1995. Symbiotic nitrogen fixation. *The Plant Cell*. 7(7): 869.
- Palai, J., Malik, G., Maitra, S., & Banerjee, M. 2021. Role of Rhizobium on growth and development of groundnut: A review. *International Journal of Agriculture, Environment and Biotechnology*. 14: 63-73.
- Peoples, M. B., Herridge, D. F and Ladha, J. K. 1995. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. *Plant and Soil*. 174:3–28.
- Singh, A. L., Basu, M. S., and Singh, N. B. 2003. Iron-chlorosis and its management in groundnut. *National Research Centre for groundnut (ICAR), Junagadh, India*, 30.
- Vincent, J.M. 1970. A Manual for the Practical Study of Root-Nodule Bacteria. IBP Hand Book, No.15, Oxford and Edinburgh, Blackwell Scientific Publication.

[www.agricoop.gov.in](http://www.agricoop.gov.in) 2023

UNDER PEER REVIEW

