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^{-1} $year^{-1}$ 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 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^{-1}) 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

frequent drought conditions during the cropping season. It'sessential 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

Presentstudy 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 Congored Yeast Extract Mannitol Agar (CRYEMA). The plates were incubated at 28 ℃ 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 □.

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_2HPO_4	0.5g/l
$MgSo_4.7H_2O$	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,I38,IS39,IS40,IS41, IS42, IS43,IS44,IS45,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

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

Morphological characteristics of *Rhizobium* isolates

Table-2 Morphological characteristics of *Rhizobium* isolates

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

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

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

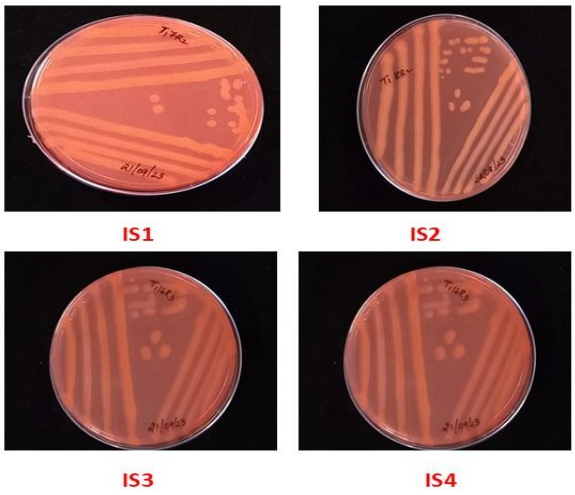

Fig1 :Pure cultures of *Rhizobium* isolates obtained after crushing of nodules

Gram staining

All the *Rhizobium i*solates (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.

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