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Research Article

CROSS INOCULATION STUDIES: RESPONSE OF *ARACHIS HYPOGAEA* TO INOCULATION WITH *RHIZOBIUM NEPOTUM* ISOLATED FROM STEM NODULES (COLLER) OF *AESCHYNOMENE INDICA*

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ABSTRACT

Cross inoculation experiments were conducted to test *Rhizobium nepotum* isolated from stem nodules (coller) of *Aeschynomene indica* and their effectiveness on the growth of *Arachis hypogaea*, crop plant. *Rhizobium nepotum* forms nodules on roots of *Arachis hypogaea*. Dry weight of shoot, root and number of nodules increased significantly in response to cross inoculation as compared to uninoculated control. The *Rhizobium nepotum* may be used to inoculate crops. The cross infection is agriculturally important isolated from wild legumes may prove a useful means of increasing nitrogen contents within cultivated crops.

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INTRODUCTION

The most cheap and common source of proteins are cereals and it is a part of diet for millions of people in the world. Seeds are commonly consumed and contain 17.34% of protein (Gour,1993). The cross inoculation of agricultural important crop with wild Rhizobial isolates result into increase in dry matter and total nitrogen contents of cross inoculated plant (Zaharan *et.al.*, 1999). Awareness of benefits of cross inoculation comparing with symbiotic effectiveness of wild Rhizobial strains with cultivated strains has increased (Vessey *et.al.*, 2004). The experiment was conducted to test *Rhizobium nepotum* isolated from stem nodules (coller) of *Aeschynomene indica* and its effectiveness on *Arachis hypogaea*. The impact of cross inoculation on nodulation, dry weight and total nitrogen content of *Arachis hypogaea* was studied.

MATERIAL METHODS

Nodules (stem) coller were collected from *Aeschynomene indica* from Ratnagiri Konkan region of Maharashtra state. Rhizobia were isolated from the nodules described by Somasegaram and Hoben, (1994). Cultures were maintained on Congo Red Yeast Extract Mannitol Agar (CRYMA) slant. The

cultural and biochemical characteristics were studied and the culture was confirmed by using 16s rRNA sequence technique. Cross inoculation experiments were performed by using polyethylene bags. The seeds of *Arachis hypogaea* were surface sterilized by washing for 4-5 minutes with 10% (w/v) HgCl₂ solution and also washed with sterile water. The seeds were soaked in the suspension of *Rhizobium nepotum* containing 10⁸ cell/ml and sowed on polyethene bag containing sterile soil and sand. The uninoculated controls were kept.



Fig 1 Effect on growth of *Arachis hypogaea* without inoculation of *Rhizobium nepotum*

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Fig 2 Effect on growth of *Arachis hypogaea* with inoculation of *Rhizobium nepotum*

Diameter (20 cm) and width (15 cm) polyethylene bags were disinfected with CuSO₄ 5% (w/v) solution and filled with sterilized soil and sand were used. In present studies a) length and weight of roots and shoot were measured in cm after dry weight of root and shoot were measured in gram unit after 15, 25, 45 days cultivation of *Arachis hypogaea*.

b) Root nodules were counted after 15,25,45 days cultivation of *Arachis hypogaea*.

c) Root colonization study of *Rhizobium nepotum* were studies after 15, 25, 45 days cultivation of *Arachis hypogaea*.

Nodules were surface sterilized and crushed and streaked on Congo Red Yeast Extract Mannitol Agar (CRYMA) medium and the population was counted by using CFU/strain of plant.

RESULT AND DISCUSSION

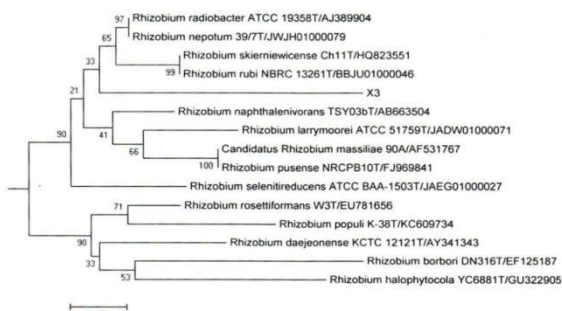
Isolation and Identification of Rhizobia

Stem (coller) nodules Rhizobia were isolated from *Aeschynomene indica* plant.

>X2

GCCAATGCCCGTGAAGTGAATGCCTTAGGGTTGTAAGCTCTTCCACCGGAGAAGATAATGACGGTATCCGG
AGAAGAAGCCCGGTAACCTTCGTCCAGCAGCCCGGTAATACGAAGGGGGTAGCGTTGTCGGAATTAAGT
GGCGTAAAGCCGACGTAGCGGATTTAAGTCAGGGGTAAATCCAGAGCTCAACTCGGAAGCTGCTTTGAT
ACTGGGTATCTGAGTATGGAAGAGTAAAGTAAATCCGAGTGTAGAGGTGAAATCGTACATATCGGAGGA
ACACCAAGTGGCGAAGGCGCTTACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAACAGGATT
AGATACCTGGTAGTCCACGCCGTAACGATGAATGTTAGCCGTCGGGCGATATACTTTCGGTGGCGCAGCTAA
CGCATTAAACATTCGCGCTGGGAGTACGGTCCGCAAGATTAACCAAGGAATTGACGGGGCCCGCACAAAG
GGTGGAGCATGTGGTTAATCGAAGCACGCGGAGAACCTTACAGCTCTTGACATTCGGGGATGGGGCATTN
GAGACGATGCTCAGTATAGGTGGCCCAAGCAAGGTCCTGATGGCTGCTCAGCTCGTGTCTCGGAAT
GTTGGATTAAGTCCCGCAACGAGCGCAACCCCTCCCTTAAATTTGCCAGCATTNATTTGGGCGCTCAAGGGG
ACTGCGGGT

Strain Designation	Closest phylogenetic affiliation	Max ident
X2	<i>Rhizobium nepotum</i> 39/7 (T) 16S ribosomal RNA gene partial sequence (JWJH01000079)	97.59%



Strain was found Gram negative and does not absorb congo red on Yeast Extract Mannitol Agar containing congo red. We have

performed biochemical test for characterization of Rhizobia and Siderophore producing capacity and found to be positive. By using 16s rRNA sequence method it was confirmed as *Rhizobium nepotum* (LT560376).

Effect of *Rhizobium nepotum* on *Arachis hypogaea*

We have measured effect of *Rhizobium nepotum* on *Arachis hypogaea* after 15, 25, 45 days of cultivation respectively along with control. In this period, we observed for different changes in root, shoot and nodules of plants. In first 15 days we observed increase in length of root and shoot and number of nodules. Length and shoot were measured in terms of dry weight of plant. After 45 days of cultivation showed good results. Results are shown in Table

Table No 1 Effect of *Rhizobium nepotum* on plant growth of *Arachis hypogaea* after 15 days.

Name of isolate	Length in cm		Dry weight in gm		Number of nodule
	Root	Shoot	Root	Shoot	
<i>Rhizobium nepotum</i>	4.5	16.2	0.015	0.23	40

Table No. 2 Effect of *Rhizobium nepotum* on plant growth of *Arachis hypogaea* after 25 days.

Name of isolate	Length in cm		Dry weight in gram.		Number of nodule
	Root	Shoot	Root	Shoot	
<i>Rhizobium nepotum</i>	5.0	30.2	0.020	0.31	64

Table No. 3 Effect of *Rhizobium nepotum* on plant growth of *Arachis hypogaea* after 45 days.

Name of isolate.	Length in cm		Dry weight in gram.		Number of nodule
	Root	Shoot	Root	Shoot	
<i>Rhizobium nepotum</i>	5.6	36.1	0.026	0.36	126

Colony formulation in roots of *Arachis hypogaea*

Arachis hypogaea cultivated crop showed higher colonization of bacteria in roots. In this method we treated plant with *Rhizobium nepotum* with regular intervals of 25 and 35 days. In after 35 days of cultivation plant showed higher colonization in roots as compare to 25 days of cultivation. Results are shown in the table

Table No.1 Root colonization of *Rhizobium nepotum* on the growth of *Arachis hypogaea* after 25 days.

Name of the Rhizobium treated	After 25 days population (CFUX10 ⁵)
<i>Rhizobium nepotum</i> + sterilized soil + <i>Arachis hypogaea</i> seeds	1.9

Table No.2 Root colonization of *Rhizobium nepotum* on the growth of *Arachis hypogaea* after 35 days.

Name of the Rhizobium treated	After 35 days population (CFUX10 ⁵)
<i>Rhizobium nepotum</i> + sterilized soil + <i>Arachis hypogaea</i> seeds	2.7

Camille *et.al.* (2004) worked and evaluated the symbiotic compatibility and nodulation efficiency of Rhizobia isolated

from *Desmodium intecanum* and *L. glaber* plant by cross inoculation.

Ramachandran *et.al.* (1960) studies effect of composite Rhizobial culture inoculation on cowpea. Balaji *et.al.* (1985) studied responses of certain tree legume to Rhizobial inoculation.

Ahmad *et.al.* (2008) treated seeds of *V. radiata* with Rhizobacteria exhibiting Acc. deaminase activity significantly enhanced the root length (up to 50%) and number of roots (up to 47%) over water treated control.

In this connection we studied and confirmed the effect of inoculation of *Rhizobium nepotum* isolated from stem nodules of plant *Aeschynomene indica* on the growth of *Arachis hypogaea* and it was confirmed in terms of dry weight of shoot and root and number of nodules increased which indicated that it promoted the growth of plant and increase the yield.

References

1. Ahmad F., Ahmad I., Khan M.S. (2008) Screening of free living Rhizospheric bacteria for their multiple plant growth promoting activities Microbiol Res. 163: 173-181.
2. Balaji S. and Rangarajan M. (1985) Responses of certain tree legumes to Rhizobial inoculation, Abstract XXXVI Annual conference of association of Microbiologists India, Madras, 69.
3. Camille E. Granada, Marcos Sterochem (2014) Genetic Diversity and Symbiotic Compatibility among Rhizobial strain Genet Mol. 1415-4757.
4. Gour Y.D. (1993) Microbiology, physiology and agronomy of nitrogen fixation: Legume-Rhizobium symbiosis, Proc. Indian Nat. Sci. Acad., B 59: 33-358.
5. Ramachandran K., Menon M.R. and Ayer R.S. (1980) Effect of composite Rhizobial culture inoculation on cowpea (*Vigna unguiculata* (L) wild) Ind. J. Microbiol 20: 220-224.
6. Somasegaram P., Hoben H.J. (1994) Handbook of rhizobia: Method is Legume- *Rhizobium* Technology Springer-Verleag, New York.
7. Vessey J.K., Pawlowski K. and Bergman B. (2004) Root based N₂-fixation symbiosis, Legumes, actinorhizal plants, *Parasponia sp.* And Cycads plant and soil 266:205-230.
8. Zahran H.H., Ahmad M.S., Abdel- Fattah M., Zaki A.Y. (1999) Phenotypic characterization, cross nodulation and nitrogen fixation of root nodule bacteria isolated from wild leguminous plants in Egypt. Proc. Int. Symp. Biol. Nit. Fix and Crop Prod., 77-90.

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