Vegetative Growth and Yield of *Arachis hypogea* and *Vigna radiata* in Response to Region Specific *Rhizobium* Biofertilizer Treatment

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Effect of *Rhizobium* inoculation on vegetative growth and yield of two different leguminous crops *Arachis hypogea* and *Vigna mungo* was investigated. Inoculation of crop specific rhizobial strains increased plant biomass, nodule number, height of plant, leaf number, and flower number. The yield of *Arachis hypogea* and *Vigna radiata* due to *Rhizobium* inoculation was higher by 22% and 29% respectively over control.

Key words: *Rhizobium*, region specific strain, *Arachis hypogea*, *Vigna radiata*, growth, yield.

The nitrogen requirement for crops is well known and cropping in low fertility soils, especially those poor in nitrogen, contributes to the lower yield. This element is usually supplied to the crop as the commercially available urea, but heavy amount of the urea-nitrogen is lost through different mechanisms causing environmental problems[1]. In some regions crop production has stagnated or even declined due to depletion in bioavailability of nitrogen[2]. Reports have shown that utilization of biological nitrogen fixation (BNF) technology can decrease the use of urea-N and reduce the environmental problems to a considerable extent. *Rhizobia* are the important soil microorganisms form symbiotic association with leguminous crops and contribute significant amount of fixed nitrogen. Increasing number of reports has also shown that *Rhizobia* can act as PGPR in leguminous as well as non-leguminous plants[3]. Since *Arachis hypogea* and *Vigna radiata* are the major crops of many region of India, the present work was undertaken to assess the effect of inoculation of region specific and crop specific *Rhizobium* on the vegetative growth and yield of these two leguminous crops.

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MATERIAL AND METHODS

Thirty days old *Arachis hypogea* and *Vigna radiata* plants were uprooted washed in distilled water and the well-formed, healthy pinkish nodule on the tap roots were carefully cut out. The nodules were immersed in 95% ethanol for 10 sec, sterilized for 5 minutes in 0.1% acidified mercuric

Table 1. The growth pattern, colony type, colour of colony, Congo red and Bromothymol blue test, and EPS production by isolates of *Rhizobium* sp. from *Vigna radiata* and *Arachis hypogea*

Strain no.	Place of collection	Isolated from host plant	Growth pattern	Colony type and colour	Congo red test production	Bromothymol blue test	Growth after 3 days	EPS
UU-1	IARI culture strain-Delhi	*Vigna radiata*	Slow, alkali producer	White, translucent, round shape	+ ve, white colony	Blue	Good growth	+
UU-2	Gobindapur, Surada block, Ganjam	*Vigna radiata*	Fast, acid producer	White, gummy, oval shape	+ ve, white colony	Yellow	Good growth	+++
UU-4	Maniakati, Surada block, Ganjam	*Vigna radiata*	Moderate, alkali producer	White, gummy, slimy, round	+ ve, white colony	Pale yellow	Good growth	++
UU-7	Paduraisuni, Surada block, Ganjam	*Vigna radiata*	Fast, acid producer	White, translucent, elongated shape	+ ve, white colony	Yellow	Abundant growth	++
UU-10	Lathipada, Surada block, Ganjam	*Vigna radiata*	Moderate, acid producer	White, gummy, flat shape	+ ve, white colony	Pale yellow	Good growth	+
UU-13	Asurabandha Surada block, Ganjam	*Vigna radiata*	Fast, acid producer	White, round shape	+ ve, white colony	Yellow	abundant growth	+
UU-16	IARI culture strain-Delhi	*Arachis hypogea*	Slow alkali producer	White, translucent and round shape	+ ve, white colony	Blue	good growth	+
UU-17	Maniakati, Surada block, Ganjam	*Arachis hypogea*	Slow, alkali producer	White, gummy, slimy, elongated shape	+ ve, white colony	Blue	poor growth	++
UU-18	Maniakati, Surada block, Ganjam	*A.rachis hypogea*	Fast, acid producer	White, gummy, slimy, flat shape	+ ve, white colony	Yellow	Abundant growth	+++
UU-19	Amrutulu, Surada block, Ganjam	*Arachis hypogea*	Slow, alkali producer	White, gummy, slimy, flat shape	+ ve, white colony	Blue	Poor growth	+++
UU-20	Buguda, Surada block, Ganjam	*A.rachis hypogea*	Moderate, alkali producer	White, translucent, round shape	+ ve, white colony	Pale yellow	Good growth	++
UU-21	Khilabadi, Surada block, Ganjam *hypogea*	*Arachis*	Fast, acid	White, producer growth	+ ve, translucent, oval shape	Yellow	Good white colony	++
UU-22	Surada, Ganjam	*Arachis hypogea*	Fast, acid producer	White, translucent, round shape	+ ve, white colony	yellow growth	Abundant	+

* EPS= (+) = Less, (++) = Moderate, (+++) = High

chloride ($HgCl_2$-1g L^{-1}, Conc. HCl- 5ml.L^{-1}) and washed six times with sterile distilled water to get rid of the chemical[4]. Each nodule was crushed using a sterile glass rod in a aliquot of sterile distilled water. Serial dilutions of the suspension were made and an aliquot of appropriate dilution was plated on Yeast-Extract Mannitol Agar medium (YEMA) and incubated at 28±2°C for 4-7 days[5]. Distinct colonies were picked up and transferred to agar slants for further purification. Confirmation the *Rhizobia* were ascertained by streaking on YEMA medium supplemented with congored 0.025% (w/v)[6]. The *Rhizobia* stand out as white, translucent colonies[7]. One week old Rhizobial colonies kept on YEM agar media (1.5% agar) were used for preparation as inoculants. For this purpose loops of the respective colonies were inoculated in sterile YEMA media containing $K_{-2}HPO_4$-0.5g/l, $MgSO_4.7H_2O$-0.2g/l, NaCl-0.1g/l, Yeast Extract-0.4g/l, Mannitol-10.0g/l and pH 7.8, and the concentration of rhizobial suspension was 10^5 C.F.U/ml. Strains were routinely maintained on YEMA slants and kept at 4°C (8).

For field experiments, healthy seeds of *A. hypogea* and *V. radiata* were surface sterilized with 95% ethanol for 3-5 minutes followed by rinsing six times with sterile water. The seeds were then steeped in the respective rhizobial suspension. Seeds treated with distilled water were used as the control. All seeds were mixed gently in shade to bring the seeds and bacteria into close contact for 30 min. The treated and control seeds were sown immediately in 3' dia, 10" high circular cement pots containing non sterile soil from the forest with

Table 2. Vegetative growth and yield of *Arachis hypogea* in response to *Rhizobium* biofertilizer treatment

	Days after sowing					
	30		60		90	
Parameters	Control	Treated	Control	Treated	Control	Treated
Height of plant	14.2±3.5	16.6±2.9 (16)	32.2±9.4	51.5±17.8 (59)	91.9±9.9	110.5±31.8 (21)
Leaf number	30.3±6.7	33.2±7.9 (9.5)	88.9±13.1	96.4±17.9 (8.4)	144.6±31.2	176.8±67.1 (23)
Flower number	4±0.8	7±7.9 (94)	38±13.1	46±17.9 (81)	23±31.2	36±67.1 (56)
Nodule number/ plant					89±9.9	109±10.5 (22)
Nodule fresh wt/ Plant (mg)					273.5±20.4	317.7±28.6 (16)
Root weight (dry, g)					2±0.4	2.6±0.5 (30)
Shoot weight (dry, g)					128±22.1	144±26.1 (13)
Shoot wt/Root wt					64	56
Total harvested Pods/plant					14	19 (36)
Total harvested Seeds/plant					25±4.6	34±8.8 (36)
Weight of 100 dry Pods (g)					55±5.6	3±8.9 (14)
Weight of 100 dry Seeds (g)					28±4.6	32±2.2 (14)
Total yield of Seeds/acre (kg)					198	243 (22)

Values in parenthesis indicate percent increase (+) or decrease (-) over control.

less microbial load. The experiments were conducted during Kharif season of 2007. Height of plant, leaf number, initiation of flowering, flower number, nodule number, dry weight of root and shoot of these two crops, and in addition the total number of pods, weight of pods and seeds were recorded. Value of minimum 10 plants ± S.D was calculated. Taking the yield data per the area in the circular pits, the yield per acre has been calculated and presented.

RESULTS AND DISCUSSION

Totally six rhizobial strains from *Vigna radiata* and seven from *Arachis hypogea* were identified on the basis of morphological and physiological characteristics (Congored test, Bromothymol Blue test, and EPS production) (Table-1). The colonies produced were gummy, translucent, circular and convex with entire margins. They showed a mean generation time varying from 24-72h in still culture. Those rhizobial strains turned the YEM agar medium with Bromothymol Blue (BTB) to yellow were fast growing and acid producers having mean generation time of 24 h. where as those produced blue colorations of the medium with BTB were alkali producers; slow growing having mean generation time of 48-72h.

Rhizobium strains UU-22 and UU-13 for *A. hypogea* and *V. radiata* respectively were the fast growing strains isolated from the region. These

Table 3. Vegetative growth and yield of *Vigna radiata* in response to *Rhizobium* biofertilizer treatment

	Days after sowing					
	30		60		90	
Parameters	Control	Treated	Control	Treated	Control	Treated
Height of plant	20.3±2.7	21.7±2.9 (7)	25.9±5.3	32.9±4.5 (21)	34.8±6.2	41.0±4.6 (15)
Leaf number	10.2±1.6	11.3±2.1 (10)	18.6±7.1	26.8±4.9 (31)	34.0±8.6	36.0±7.1 (6)
Flower number	8.1±1.8	8.6±2.7 (6)	19.9±3.5	20.7±9.5 (43)	8.3±3.4	11.3±4.6 (27)
Nodule number/ Plant					66±9.9	89 ±10.5 (34)
Nodule fresh wt/ Plant (mg)					162.3±20.4	188.4±28.6 (16)
Root weight (dry, g)					1.2±0.2	1.9±0.4 (36)
Shoot weight (dry, g)					18.8±0.9	22.4±1.8 (19)
Shoot wt/Root wt					7.3	5.7
Fruit (pod) Number					11.7±4.2	13.3±4.5 (13)
Weight of 100 dry Pods (g)					20±3.2	26±4.2 (23)
Weight of 100 dry Seeds (g)					5.5±0.8	6.2±0.4 (12)
Total pod yield/ Plant (g)					56.4±2.8	72.4± 4.3 (28)
Total seed yield/ Plant (g)					25.8±6.9	34.8±6.2 (36)
Total yield/acre (kg)					789	1023 (29)

Values in parenthesis indicate percent increase over control used as treatment showed a positive influence on the vegetative growth as well as the yield of both the leguminous crops. Upon treatment with *Rhizobium* increase of biomass, height of plant, number of leaf, number of flower, nodule number and pod weight per plant of *A. hypogea* increased over control, though there was not much difference in the height of the plant, number of flower in case of *V. radiata* and flower number in case of *A. hypogea* through out the experimental period (Table 2 and 3). The control had an average biomass and less number of nodules on their roots. However, with crop specific and region specific *Rhizobium* inoculation 22 and 34% enhancement of nodule number was recorded in *A. hypogea* and *V. radiata* respectively. Similarly increase of shoot and root biomass by 13 and 30% in *A. hypogea* and 19 and 36% in *V. radiata* and yield by 22 and 29% in *A. hypogea* and *V. radiata* respectively due to *Rhizobium* biofertilizer treatment over the respective control was recorded.

Success of inoculation of *Rhizobium* at field levels has been reported several times in the past (7, 9-14). In these reports effect of crop specific *Rhizobium* species immobilized in different carrier materials and inoculated in different types of soils, and also in combination with other nitrogen fixing microorganisms in consortia on the yield attributes has been demonstrated. In many other cases failure of *Rhizobium* inoculation has also been observed. Failure to obtain desired response has been attributed to (I) presence of native ineffective strains which could not be displaced by the introduced ineffective strains, (II) the presence of effective native rhizobial strains in large number which compete and over power the inoculated ones, or (III) the soil conditions of the inoculated field quite different from that the locations from where the inoculated strains were isolated that limit symbiosis caused by acidity, alkalinity and other factors relating to physico-chemical properties of the soil. To overcome the possible barriers efficiency of region specific and crop specific *Rhizobium* species were tested in the field in the present work. The results clearly demonstrated that inoculated *Rhizobium* isolated from local environments enhanced the growth, nodulation as well as the yield of legume crops.

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