ФІТОЦЕНОЗ ЯК СТРУКТУРНИЙ КОМПОНЕНТ БІОГЕОЦЕНОЗУ

UDK 58

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EXPERIMENTS WITH DIFFERENT *RHIZOBIUM* ISOLATES ON GROWTH AND PRODUCTIVITY IN GREEN GRAM

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Beneficial roles of certain symbiotic nitrogen fixing bacteria such as *Rhizobium* on seed germination, growth, plant health, and yield of Green Gram are well known. Isolation of new strains of the bacteria from wild or other cultivated pulse species to inoculate legume crops has become an important strategy to improve the efficiency of *Rhizobium*-legume symbiosis. In order to find out a suitable *Rhizobium* strain to be applied along with Arbuscular Mycorrhiza in Green Gram, an experiment was carried out to examine the influence of six different isolates of the bacteria from widely different species of peas. Positive influences or effectiveness of these *Rhizobium* isolates on growth parameters and yield of Green Gram were examined in pot culture growth conditions. There were seven sets of pots including the control and six treatments, each set with eight replications each. Growth parameters such as root dry weight, shoot dry weight, total dry weight, total leaf area and total chlorophyll were analyzed at twenty day intervals till the final harvest on the sixtieth day. All the *Rhizobium* isolates tested were able to nodulate Green Gram, but the maximum positive influence on growth and yield of the crop was observed in treatment with a *Rhizobium* isolate from *Phaseolus multiflorus,* which was identified as the most suitable *Rhizobium* strain for Green Gram.

Keywords: Rhizobium, bacteria, soil, nitrogen, phosphorus.

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ДОСЛІДЖЕННЯ ВПЛИВУ РІЗНИХ ВИДІВ РІЗОБІЮ НА РІСТ ТА БІОЛОГІЧНУ ПРОДУКТИВНІСТЬ ЗОЛОТИСТОЇ КВАСОЛІ (PHASEOLUS AUREUS)

Представлені результати досліджень з виділення кращого ризобію, який розчиняє фосфор та зменшує кількість азоту. У ході експерименту були відібрані бактерії з 6 різних рослин, які відносяться до однієї родини золотистої квасолі, та які мають достатню кількість здорових бульбочок. Оцінка впливу різних бактерій на параметри росту та продуктивності показала, що ризобій, виділений від *Phaseolus multiflorus* є найбільш ефективним.

Ключові слова: ризобій, бактерії, ґрунт, азот, фосфор.

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ИЗУЧЕНИЕ ВЛИЯНИЯ РАЗЛИЧНЫХ ВИДОВ РИЗОБИЯ НА РОСТ И БИОЛОГИЧЕСКУЮ ПРОДУКТИВНОСТЬ ЗОЛОТИСТОЙ ФАСОЛИ (PHASEOLUS AUREUS)

Представлены результаты исследований по определению лучшего *ризобия*, растворяющего фосфор и уменьшающего количество азота. В ходе эксперимента были отобраны бактерии с шести различных растений, принадлежащих к одной и той же семье золотистой фасоли, и обладающих достаточным количеством здоровых клубеньков. Оценка влияния различных бактерий на параметры роста и продуктивности показала, что р*изобий*, отделенный от *Phaseolus multiflorus* является наиболее эффективным.

Ключевые слова: ризобий, бактерии, почва, азот, фосфор.

Beneficial roles of Rhizobium-Legume symbiosis are well known. However, all the legume crops are not sustaining the same strains of the bacteria and the influences of these different strains of bacteria on growth and yield of pulse crops may not be equal. Examination of the differential roles of different strains of *Rhizobium* isolates on each and every crop is therefore significant to understand the best suitable strain for the optimum growth and productivity of the crop under a particular environmental condition. Isolation of effective strains of *Rhizobium* from wild legumes to inoculate other legume crops is a new strategy to improve the efficiency of the *Rhizobium*-legume symbiosis (Zaharan, 1999). Once a suitable strain is experimentally isolated for a crop from among diverse strains available in the wild relatives of the pulse crop growing in the area, that strain can be used in large scale as the best *biofertilizer* under a particular field condition.

Nitrogen enrichment of soil and plant by *Rhizobium phaseoli* was reported by Ruschel et al. (1979). Graham (1981) in a review on the problems of nodulation and symbiotic Nitrogen (N_2) fixation in *Phaseolus vulgaris* emphasized the significance of specific strains of *Rhizobium* as critical to nitrogen fixation in crop genotypes. Perveen et al. (2002) studied the effect of Phosphorus (P) solubilizing and Nitrogen (N_2) fixing *rhizospheric* microorganisms on growth and yield of Green Gram and found that the P solubilizing and N_2-fixing organisms improve seed germination, plant health, nodulation and grain yield of green gram. In these contexts, in order to find out a suitable *Rhizobium* isolate that may be applied along with suitable Arbuscular Mycorrhiza (AM) in Green Gram, bacterial isolates from six different host plants belonging to the same family as that of Green Gram, with sufficient number of healthy effective nodules were selected. Evaluation of the influence of different *Rhizobium* isolates on the major growth and productivity parameters has revealed that the isolates differed in their effect on Green Gram. A *Rhizobium* isolate from *Phaseolus multiflorus* was found to be very effective in promoting seed yield as well as residual nitrogen in the soil.

MATERIALS AND METHODS

The variety of green gram (*P. aureus*) used in the present experiment was 'Co6', the seeds of which were supplied by the Tamilnadu Agriculture University, Coimbatore, Tamilnadu, India. The *Rhizobium* isolates used were taken from six different host plants belonging to *Fabaceae*. The criteria for the selection of host plants were (i) the plants belong to the same family as that of Green Gram, so that the *Rhizobium* isolates would nodulate Green Gram easily (ii) the number of healthy effective nodules on the respective hosts. The host plants and the numbers given to these isolates are shown in the Table 1.

Table 1

The host plants from which different *Rhizobium* isolates were collected

Sl. No.	Name of host plant	*Rhizobium* isolate
1.	*Crotalaria striata*	R1
2.	*Vigna wightii*	R2
3.	*Pueraria phaseoloides*	R3
4.	*Phaseolus aureus*	R4
5.	*Flemingia bracteata*	R5
6.	*Phaseolus multiflorus*	R6

The percentage of effective nodules (determined on the basis of nodule size and presence of *leghemoglobin*) on the hosts ranged from 40 to 55. The nodules were separated from the root system and surface sterilized with alcohol. They were crushed in sterile water and the *Rhizobium* inoculum was prepared in Yeast Extract-Manitol (YEM) medium (Dye, 1979). The *Rhizobium* cultures were incubated at 35^0 C for 48 hrs and used for inoculating the seedlings.

The *Rhizobium* isolates were screened for their effectiveness on Green Gram under pot culture conditions. 56 small pots, each of 10 cm diameters were used for the experiment. The pots were filled with double sterilized (using autoclave) pot mixture consisting of top soil (Gravelly Clay Loam on *Laterites* with a mechanical composition of about 40.1% coarse sand, 28.5% fine sand, 8.8% silt, 22.6 % clay) coarse sand and dried and powdered cow dung in the ratio 3:1:1 by weight. The nitrogen content of the pot mixture used for the experiment was found to be 196 kg ha^{-1}. The pots were arranged in seven sets having eight replications each. In each pot one surface sterilized and pre soaked seed was laid in a small pit made for the seed. The *Rhizobium* inoculation was made on the second day after sowing the seeds. 10ml of 48 hr old bacterial culture (containing approximately 10^9 cells per ml) was added to the root zone of each seedling using a pipette. The treatments were designated as follows:

R0 R2 R4 R6

R1 R3 R5

R0- without *Rhizobium*; R1 to R6- with respective *Rhizobium* isolate

The pots were arranged separately to avoid contamination. Since the growth period of the crop was sixty days, sampling was done on 20, 40 and 68 days after inoculation (DAI). Two plants each from a treatment were sampled at a time. Growth parameters such as root dry weight (RDW), shoot dry weight (SDW), total dry weight (TDW), total leaf area (TLA) and total chlorophyll (TC) were analyzed. The total leaf area was measured using a computer with the help of MATLAB software, employing the provision for image processing. The leaves were scanned and the images were stored in the computer. The measurement of the total leaf area was done using the computer software. The steps involved in the measurement of leaf area were; (1) First scanned the image of a leaf into the computer and saved it as 'leaf.tif', (2) MATLAB first displayed the original image on the monitor, then showed the black and white equivalent image on the screen, and finally displayed the complemented image, (3) The software then calculated the area and displayed it. The amount of total chlorophyll was calculated following Arnon (1949). Total number of nodules, total weight of nodules, and percentage of effective nodules were estimated for each plant. Leaf tissue nitrogen (%) was also determined for each treatment. The harvest data relating to each plant included total weight of pods and total weight of seeds.

The soil N was estimated by Micro-diffusion method (Sparks, 1996). The soil samples collected from the root zone of plants were dried, powdered and sieved. 2g of powered soil was taken in an injection bottle and 2ml of 0.32% $KMNO_4$ and 2ml of 40% NaOH solutions were added. After mixing them well, the bottle was kept inside a plastic bottle, which contained 10ml boric acid-mixed indicator. The plastic bottles were tightly closed and kept in an incubator for 18 hrs at 38^o C. The injection bottles were removed after the release of nitrogen as ammonia from the soil. The bottles were rinsed from outside with distilled water into the same plastic bottle. The ammonia absorbed by boric acid resulted in bluish colour. It was titrated against 0.01N H_2SO_4. From the titre value the amount of N was estimated. Micro-Kjeldahl method (AOAC, Washington, 1978) was employed for estimating tissue N.

RESULTS

No significant variations were observed in growth parameters from that of the control during the early stages of growth in all the treatments. At the final stage of harvest, the average of three observations on RDW did not show significant difference between the *Rhizobium* treated plants and the control. Significant increases from the control in SDW as well as TDW were noticed in plants treated with R4 and R6. The TLA was significantly higher in R4 and R6 treated plants in comparison with the control. The TC content in the treatment R6 was significantly higher than that in other treatments. Details of the growth parameters and yield of the crop at the final harvest stage of the crop are given in Table 2.

The data on nodulation showed that the rate of nodulation varied with different *Rhizobium* isolates. In R1 treated plants the root system was much branched and massive compared to control and the nodules were scattered on the lateral roots. In R2 the root system was less branched and the nodules were seen clustered at the root crown as well as on the lateral roots. The root system was less branched and the nodules were scattered on the lateral roots of R3 treated plants, whereas in R4 and R5 the lateral roots were few and the nodules were seen only towards tip of the lateral roots. In R6 the root system was less profusely branched and the nodules were seen clustered at the root crown region and scattered on the lateral roots. The nodules were assessed as effective based on their size and presence of leghemoglobin. Bigger nodules with leghemoglobin were considered as effective. The treatment R4 showed higher rate of nodulation, but the percentage of effective nodules was only 23. On the other hand, in R6 although the average number of nodules was 32, about 42% of the nodules were effective. The percentage of effective nodules was the least in R5 (18%). Few nodules were observed in the control, which may be due to the contamination from irrigation water. The final yield measured as total pod and seed weight per plant showed significant increase in plants treated with R6, compared to control.

Table 2

Growth and yield characteristics in green gram under different *Rhizobium* strains after 68 days of treatment

Treatment	Root Dry Weight (g)	Shoot Dry Weight (g)	Total Dry Weight (g)	Total leaf area (cm^2)	Total Chlorophyll (mg/g of Fresh Weight)	No. of nodules/ plant	Total weight of nodules (g)	% of effective nodules	Total weight of pods (g)	Total weight of seeds (g)
C	0.230	1.207	1.437	202	0.480	3	0.004	0	0.90	0.84
R1	0.327	1.603	1.930	246	0.675	56	0.26	29	2.75	2.32
R2	0.150	1.000	1.150	151	0.527	30	0.13	25	2.93	2.1
R3	0.223	1.820	2.043	253	0.728	78	0.29	34	3.31	2.27
R4	0.250	2.500	2.750	326	0.892	93	0.41	23	2.80	1.84
R5	0.207	1.763	1.970	227	0.838	28	0.21	18	2.50	1.83
R6	0.227	2.077	2.303	324	1.170	32	0.26	42	5.46	3.96
CD at 0.05 level	0.157	0.727	0.740	109	0.1172	8.25	0.030	6.70	4.037	1.653

The influence of *Rhizobium* isolates on nitrogen nutrition of the plant was revealed by the leaf tissue N content. All treatments except the R5 treatment showed significant increase in leaf tissue N content than the control (Table 3). The post harvest soil N was also significantly higher in all *Rhizobium* treatments except the R5.

Table 3

Effect of *Rhizobium* isolates on leaf tissue and the post harvest soil N

Treatment	Leaf tissue N %	Soil N kg ha^{-1}
C	2.123	196
R1	3.247	294
R2	2.597	254.8
R3	2.920	308
R4	3.110	254.8
R5	2.337	217.5
R6	3.443	333.25
CD at 0.05level	0.326	50.29

DISCUSSION

Isolation of effective rhizobia from wild legumes to inoculate other legume crops is significant to improve the efficiency of the *Rhizobium*-legume symbiosis. The results of the current experiments revealed that the *Rhizobium* isolates from different species of legumes have diverse effects on the different growth parameters and yield in Green Gram. This observation agrees with that of Graham (1981). In general, all the *Rhizobium* isolates showed obvious changes in the root morphology in green gram. The non inoculated control plants were having profusely branched root system, while plants which received bacterial inoculation showed less branching. This was in agreement of the observations of Perrine et al. (2001) who reported that *Rhizobium* affects the growth and development of root morphology. Change in root morphology has direct influence on nutrient accumulation and the resultant growth and productivity of crops. Bacterial root nodule formation is the result of a complex series of interactions between bacteria and the roots. Therefore, the differential influence of different bacterial strains on morphology of root system might be due to specificity of different strains in these interactions. Since the nodule forming bacterial interactions with plant roots includes many biochemical events, the current observations of differential influence of different bacterial isolates on root morphology provide the scope of further exploration of all biochemical influences on root morphological changes in association with specific nitrogen fixing bacterial strains.

It is also probable that profuse branching of root system in the control plant enables absorption of nitrogen from more soil volume. Inoculated plants with root-nodules have better nitrogen availability even with fewer branches. It was noted that in treatments with isolate from *P. aureus* (R4) and *P. multiflorus* (R6), there was significant increase in shoot biomass over that of the control (Table 2). Energy saved for lesser root growth was thus visible in the increased shoot biomass, which is beneficial to the farmer. However, *Rhizobium* treatments in general gave no significant effect on root biomass in the crop (Table 2).

The total leaf area showed significant increase in plants treated with *Rhizobium* isolates R4 and R6. Other *Rhizobium* treatments were not significant on the total leaf area. The differential influence of different stains on the shoot morphology such as leaf area might be out of the positive role of the strain in enhancing nitrogen availability of the plants. The effect of all the treatments except the treatment with the *Rhizobium* isolate from *Vigna wightii* (R2) on the total chlorophyll content was significant, indicating better nitrogen nutrition in the presence of this *Rhizobium* isolate. However, it may be noted that enhancement of nitrogen availability has differential impact on the shoot morphology or growth parameters of the plants. The percentage of effective nodules and leaf tissue N content were positively correlated, suggesting enhanced nitrogen nutrition in all the nodulated plants. It is well known that *Rhizobium* inoculation can increase nitrogen content of plants (Bagyaraj et al., 1979).

The isolate R6 was the most effective on Green Gram with respect to total chlorophyll content of leaves. Chlorophyll content is an index of photosynthetic ability and growth (Rajasekaran and Nagarajan, 2005). The nitrogen left in the soil after harvest was the highest and significantly different from other treatments in the sample R6. The percentage of effective nodules was also the highest in the same treatment. Enrichment of soil nitrogen after a crop is of great significance in soil fertility management (Misra and Misra, 1975). Enhancement of post harvest soil nitrogen is an important criterion to use a bacterial strain in Green Gram. Because, the crop is usually considered as an intercrop in South India in a cyclic rotation of different crops on a field aiming at improving fertility status of the soil after the pulse crop. The harvest data measured as total seed out put per plant showed highest numerical value in R6. One of the limitations of the current experiment was that only a single host variety (Green gram cultivar 'Co6') was used in all the treatments. But the experiments with different isolates were carried out in identical set up on this crop variety. In plants treated with the *Rhizobium* isolate R6, the chlorophyll content was significantly the highest. Tissue nitrogen content, seed yield, and the nitrogen left in the soil after the cultivation was also significantly the highest in this treatment.

It may be noted that all the *Rhizobium* isolates tested were able to nodulate green gram. However, the rate of nodulation varied significantly with different treatments. The position of nodules on the root system is more significant on nitrogen fixation than the number or fresh weight of the nodules; the crown nodules are significant during early stage of growth, while those on the lateral roots are important during the flowering season (Hardarson et al., 1989). Root nodules in R6 were both on crown and on lateral roots. Therefore, in this aspect also this isolate was found to be the most congenial for nitrogen fixation during all stages of growth and development of the crop. The nitrogen fixed towards mature stages of growth is important in increasing grain yield (Bagyaraj et al., 1979). Therefore, in the present experiment, among the six different *Rhizobium* isolates tested, R6 (isolate from *P. multiflorus*) was found to be the most effective isolate to enhance productivity in green gram. The results suggest that *Rhizobium* isolates from closely related host plants are more effective in promoting growth and seed yield in Green Gram than distantly related ones.

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Надійшла до редколегії 08.12.08