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Potency of Rhizobial Strains from Different Environments to Increase Economic Productivity in Some Legumes

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Rhizobial strains from different environments were isolated and characterized through root nodule-trap method. The isolates were employed as biofertilizer to three tropical legumes. Application of these strains as bio-inoculants increased economic productivity such as number of pods, total dry weight of pods, dry weight of seeds, dry weight of pod wall and dry weight of 100 seeds in *Vigna mungo* (L.) Hepper, *V. radiata* (L.) Wilczek and *V. unguiculata* (L.) Walpers. Among, the different rhizobial strains isolated virgin soil isolate performed well compared to others regarding economic productivity. The virgin soil rhizobia also belong to 'cowpea miscellany group' and they are more virulent and vigorous compared to other strains.

Key words: *Vigna mungo*, *Vigna radiata*, *Vigna unguiculata*, root nodule- trap method, Bio-inoculants, Biofertilizer, Extreme environments

Indian soil is mostly impoverised in nitrogen. The role of legumes in enriching soil fertility is known through centuries. The pulse crops capable of fixing atmospheric nitrogen symbiotically occupy the second position next to cereals (Pandey 1980). The seeds of these plants contain more proteins. The high protein content is related to the presence of root nodules containing nitrogen fixing bacteria. Microbes play many important roles in agricultural biotechnology; one of these is biological nitrogen fixation (BNF) (Das 1991). In view of spiralling rise in the cost of synthetic fertilizers, dwindling of cultivable land, year after year and increasing saline and drought-prone lands for cultivation; biofertilizer application to soils under different agroclimatic condition is being given a serious thought. The present study aims at screening drought prone, saline, virgin and garden and polluted soils (Tannery effluent) for naturally occurring efficient and stress tolerant strains of rhizobia for improvement of economic productivity of three summer legumes.

Materials and Methods

Soil samples were collected from sub-soil surface from different environments (locations) such as local botanical garden (Bharathidasan University) polluted land (Tannery effluent), virgin land and coastal saline land. The pH of the tannery polluted soil was more alkaline with highest electrical conductivity (EC) values of 4.41 $mScm^{-1}$. Total organic matter, nitrates and nitrites were higher in the garden, virgin soils, compared to the saline and polluted soils. Sodium was higher in the tannery polluted soil. Elements such

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as magnesium, nitrogen, iron were lower in both the polluted and saline soils compared to either virgin or garden soil. The collected soil samples were filled in polythene bags (30 cm x 20 cm) and uniform sized *Vigna mungo* (L.) Hepper var Vamban *V. radiata* (L.) Wilczek var Vamban and *V. unguiculata* (L.) Walpers var Vambam seeds were sown after surface sterilization. After 4 weeks of growth the plants were uprooted carefully and the roots washed with tap water to remove the soil particles. Fully formed and healthy pinkish nodules were selected. The surface sterilized nodules were crushed and the milky juicy, extract was allowed to mix with the sterile distilled water. From this, up to 10^{-6} serial dilutions were made and 0.5 ml sample from six-fold diluted series, was plated into yeast extract mannitol agar (YEMA) medium incorporated with congo red (Vincent 1970). The petri plates were incubated at 26°C for 48 to 72 h (BOD incubator, Chennai, India) for development of rhizobial colonies. The single colonies which did not absorb congo red were picked out and streaked successively on YEMA plates. The rhizobial strains were identified at the Centre for Advanced Studies (CAS) in Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, as "cowpea miscellany group."

The infectivity and nodulation tests were performed through Leonard jar experiment. 100 ml of the respective rhizobial cultures from different soils were independently mixed with a slurry (gum arabic), and seeds of *Vigna mungo*, *Vigna radiata* and *Vigna unguiculata* were added to the slurry so as to uniformly coat the seeds with the inoculant. Finely ground calcium carbonate sieved through 300 mesh was added to the freshly inoculated wet seeds in a container and mixed rapidly until seeds were evenly coated. The lime-coated seeds appeared as white tablets (pelleted seed).

Healthy and uniform-sized bio-inoculated seeds were sown in the field. After 100 days of growth, the plants were uprooted carefully to investigate economic productivity on the basis of number of pods, total dry weight of pods, dry weight of seeds, dry weight of pod wall and dry weight of 100 seeds per plant. Each experiment was triplicated and standard error as well as students t-test were applied to find out the significance of the results .

Results and Discussion

Soil samples from subsoil surface of different environments were collected and the plants of *Vigna mungo, V. radiata and V. unguiculata* plants were raised using respective soils. The nodules of the plants were collected from the respective soils and rhizobia were isolated. The isolated rhizobia from the respective soil were used as inoculum for all the three species of Vigna. Thus, each plant was treated with four different rhizobial isolates independently for assaying economic yield.

The number of pods increased significantly in virgin soil rhizobia treated plants of Vigna mungo by 58% over the control and in other treatments, the increment was only marginal (Table 1). In *V. radiata*, application of virgin soil rhizobia and saline soil rhizobia increased the number of pods compared to the control (Table 2). In *V. unguiculata*, application of saline soil rhizobia exhibited a significant increase in the number of pods by 42% over the control (Table 3). Total dry weight of pods (per plant) considerably increased in all the treatments, most significantly in virgin soil rhizobia-inoculated plants. A similar trend was observed in *Vigna radiata* and *V. unguiculata*. The reason for the better performance of virgin soil rhizobia is not clear. In one of our earlier studies (Ghouse Basha 2000) the virgin soil rhizobia was superior in nodulating vigna species compared to other rhizobial strains.

Regarding total dry weight of seeds, a significant increase (57%) was observed in *V. mungo* due to virgin soil rhizobial application. Similarly, in *V. radiata* virgin soil rhizobial application led to striking increase in dry weight of seeds by 87%. In *V. unguiculata*, application of saline soil rhizobia led to 43% improvement over the control. The total dry weight of pod wall increased most strikingly in *Vigna mungo* by application of virgin soil rhizobia (94%) over the control. In *V. radiata* the increase in dry weight of pod wall was two-fold over the control in all rhizobial treatments. However, in *V. unguiculata*, the increase in dry weight of pod wall was only marginal. Dry weight of seeds increased significantly (68%) in V. radiata by application of virgin soil rhizobia. Application of saline soil rhizobia in *V. unguiculata* increased seed weight by 35% over the control. In *V. mungo*, the increment in seed weight was almost uniform in all rhizobial treatments.

The above findings are supported by several observations reported in literature. Seed inoculation with rhizobium increased the number of nodules and pods/plant and 100 seed weight, and gave higher yield in groundnut and soy bean (Joshi et al. 1989). Similarly, Namdeo et al. (1991) reported that seed inoculation with rhizobial strains significantly increased nodulation, dry matter and yield of pigeon pea cultivars over uninoculated control. Similar observation was made by Khalafallah et al. (1984) when the soil was inoculated with rhizobium resulting in higher dry weight and yield. Rhizobium and/or arbuscular inoculations was also found to enhance the growth, biomass and yield status under experimental conditions (Nibha Gupta & Rahangdale 1999). It has also been observed that rhizobial treatment was found to evoke better response than VAM inoculation.

Table 1. Economic yield characters of 100-day old plants of *Vigna mungo* raised from seeds inoculated with different rhizobial strains.

Economic Yield Characters	1	2	3	4	5
Number of Pods/Plant	14.4 ± 0.25	18.5 ± 0.22* (128)	22.8 ± 0.34** (158)	15.9 ± 0.25 (110)	16.4 ± 0.24 (113)
Total dry weight of pods/plant (g)	4.58 ± 0.03	6.50 ± 0.05* (142)	8.52 ± 0.09** (186)	6.12 ± 0.05 (134)	6.20 ± 0.05* (135)
Total dry weight of seeds/plant (g)	3.80 ± 0.02	4.76 ± 0.02* (125)	5.99 ± 0.02** (157)	4.50 ± 0.05 (118)	4.86 ± 0.06* (127)
Total dry weight of podwall/plant (g)	1.45 ± 0.02	2.31 ± 0.05* (155)	2.8 ± 0.03* (194)	1.86 ± 0.01* (128)	1.83 ± 0.01 (125)
Dry weight of 100 seeds (g)	3.47 ± 0.07	4.67 ± 0.05* (134)	4.82 ± 0.02* (138)	4.31 ± 0.04 (124)	4.76 ± 0.02* (137)

Data are mean value of 20 different plants of three different experiments with ± SE.
Students t-test *P<0.05, **P<0.01.
Values in parenthesis indicate percentage over the control.
1 - Seeds without inoculum (Control)
2 - Seeds inoculated with garden soil nodule rhizobia
3 - Seeds inoculated with virgin soil nodule rhizobia
4 - Seeds inoculated with polluted soil nodule rhizobia
5 - Seeds inoculated with saline soil nodule rhizobia

Table 2. Economic yield characters of 100-day old plants of *Vigna radiata* raised from seeds inoculated with different rhizobial strains.

Economic Yield Characters	1	2	3	4	5
Number of Pods/Plant	15.2 ± 0.39	18.6 ± 0.55* (122)	21.1 ± 0.43** (138)	16.4 ± 0.32 (107)	19.8 ± 0.40* (130)
Total dry weight of pods/plant (g)	3.91 ± 0.05	4.50 ± 0.05 (115)	7.12 ± 0.04** (182)	5.13 ± 0.06 (131)	5.56 ± 0.05* (142)
Total dry weight of seeds/plant (g)	2.67 ± 0.02	3.88 ± 0.03* (145)	5.01 ± 0.04** (187)	3.38 ± 0.06 (126)	3.71 ± 0.05* (138)
Total dry weight of podwall/plant (g)	1.02 ± 0.07	1.47 ± 0.02 (144)	2.58 ± 0.02* (252)	2.43 ± 0.02 (238)	2.60 ± 0.15* (254)
Dry weight of 100 seeds (g)	2.46 ± 0.06	3.19 ± 0.02 (129)	4.14 ± 0.11** (168)	3.33 ± 0.08* (135)	3.62 ± 0.04* (147)

Data are mean value of 20 different plants of three different experiments with ± SE.
Students t-test *P<0.05, **P<0.01.
Values in parenthesis indicate percentage over the control.
1 - Seeds without inoculum (Control)
2 - Seeds inoculated with garden soil nodule rhizobia
3 - Seeds inoculated with virgin soil nodule rhizobia
4 - Seeds inoculated with polluted soil nodule rhizobia
5 - Seeds inoculated with saline soil nodule rhizobia

Table 3. Economic yield characters of 100-day old plants of *Vigna unguiculata* raised from seeds inoculated with different rhizobial strains.

Economic Yield Characters	1	2	3	4	5
Number of Pods/Plant	12.8 ± 0.34	14.2 ± 0.39 (110)	16.0 ± 0.37* (125)	15.8 ± 0.40* (123)	18.2 ± 0.34** (142)
Total dry weight of pods/plant (g)	20.83 ± 0.10	22.76 ± 0.15 (109)	26.26 ± 0.24** (126)	24.48 ± 0.17* (117)	28.29 ± 0.17* (136)
Total dry weight of seeds/plant (g)	14.79 ± 0.09	16.17 ± 0.08 (109)	18.21 ± 0.08* (123)	18.18 ± 0.11* (122)	21.22 ± 0.14** (143)
Total dry weight of podwall/plant (g)	5.86 ± 0.07	6.46 ± 0.07 (110)	7.01 ± 0.09* (119)	6.24 ± 0.04 (106)	7.30 ± 0.10* (124)
Dry weight of 100 seeds (g)	9.15 ± 0.12	9.75 ± 0.06 (106)	11.36 ± 0.12* (124)	10.30 ± 0.10 (112)	12.42 ± 0.16* (135)

Data are mean value of 20 different plants of three different experiments with ± SE.
Students t-test *P<0.05, **P<0.01.
Values in parenthesis indicate percentage over the control.
1 - Seeds without inoculum (Control)
2 - Seeds inoculated with garden soil nodule rhizobia
3 - Seeds inoculated with virgin soil nodule rhizobia
4 - Seeds inoculated with polluted soil nodule rhizobia
5 - Seeds inoculated with saline soil nodule rhizobia

It may be inferred from the above observations that the increase in primary yield components such as number of pods and dry weight of seeds may be attributed to increased above ground and below ground biomas, leaf area and higher rate of photsynthesis and increased supply of nitrogen through N_2 fixation.

From the investigation, it is obvious that the virgin soil rhizobial isolates performed better when compared to other rhizobial strains. Our findings are supported by literautre of Zahran (1999) who pointed out that some strains of rhizobia form effective N_2 fixing symbiosis with their host legumes under salt heat and acid stresses and can also perform better in arid lands. The application of nodulating strains of rhizobia from extreme environments such as the one attempted in the present study may be an ideal solution for improvement of soil fertility and rehabilitation of arid lands.

Summary

Generally, application of rhizobia resulted in an increase in aerial as well as under ground biomass coupled with nodule formation and economic yield. From the overall perusal of the results, it is evident that the virgin and saline soil rhizobial isolates have potency for higher N_2 fixation and better economic productivity in terms of pod yield, number of seeds per pod as well as seed weight.

References

Das H.K. 1991. Biological nitrogen fixation in the context of Indian agriculture. Curr. Sci. 60 (9.10) : 551 - 555.

Ghouse Basha M. 2000. Isolation and characterization of rhizobial strains from different environments to improve morphometric parameters nodulation and economic productivity in some legumes. Ph.D. Thesis, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

Joshi SS, Thorve PV & Nagre KT. 1989. Effect of Rhizobium and nitrogen on the yield and quality groundnut and soybean. P.K.V. Research Journal 13 (2) : 152 - 155. Dept of Agronomy. Punjabrao Krishi Vidyapeeth. Akola, Maharastra, India.

Khalafallah MA, Abd-El-Maksoud & Saber MSM. 1984. A preliminary comparative study on the effect of seed inoculation (Rhizobium), Phyllosphere enrichment (Azotobacter) and or Foliar application of micro-nutrients on soy bean. Egypt. J. Microbiol. 19 (2) : 165 - 169.

Namdeo SL, Bangar KS & Gupta SC. 1991. Response of Pigeon pea (Cajanus cajan (L.) Mill sp.) cultivars to Rhizobium strains. Indian J. Agron. 36 (1) : 29 - 31.

Nibha Gupta & Rahangdale R. 1999. Response of Albizia lebbeck and Dalbergia sissoo towards dual inoculation of Rhizobium and arbuscular mycorrhizal fungi. Indian J. Exptl. Biol. 37 : 1005 - 1011.

Pandey BP. 1980. Economic Botany. Published by S. Chand, New Delhi.

Vincent JM. 1970. A manual for the practical study of the root nodules bacteria. Black well scientific publications. Oxford and Edinburg, p. 164.

Zahran HH. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev. 63 (4) : 968 - 989.