



Assessment of *Rhizobium* Strains and their Impact on Urdbean Productivity

R.Uma Sankareswari ^{a++*}, J.Prabhakaran ^{b#},
T.Sivasankari Devi ^{c†} and K.Sasikala ^{a‡}

^a Dr. MSS AC&RI, Eachangkottai, Thanjavur - 614 902, India.

^b AC&RI, Madurai, Othakadai – 625 104, India.

^c TRRI, Aaduthurai, Thanjavur- 612101, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author RUS collected the literature, compilation of the content, wrote the article and writing the review. Author JP suggesting his ideas to shape the review and author TSD helped in revising the article. All the authors read and approved the manuscript.

Article Information

DOI: <https://doi.org/10.9734/ajsspn/2024/v10i4450>

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/129160>

Original Research Article

Received: 24/10/2024
Accepted: 28/12/2024
Published: 29/12/2024

ABSTRACT

The study sought to assess the impact of inoculation by several *Rhizobium* strains on field-grown urdbean (Blackgram). *Rhizobia*, which promotes the growth of legume plants. The bacteria that live in the soil and generate the root nodules where symbiotic biological nitrogen fixing occurs are called *rhizobia*. Four AICRP centers viz., Kalaburagi (GUR5 & GUR8), Akola (WUR 12-1), Vamban (VUC), and Pantnagar (A-3, P-5 & PUR 34), provided a total of seven *Rhizobium* isolates, which were employed in the experiment together with the best *Rhizobium* BMBS 47. Urdbean seeds were

⁺⁺Associate Professor (Agrl. Microbiology);

[#]Associate Professor (SS&AC);

[†]Assistant Professor (Agrl. Microbiology);

[‡]Professor (Seed Science & Technology);

*Corresponding author: Email: umasankareswari@tnau.ac.in;

treated with the *Rhizobium* isolates and sown. Observations were recorded at 45th DAS, to assess their effect on plant growth parameters viz., number of nodules/plants, root length, shoot length, nodule dry weight, and yield parameters were recorded during harvest. Among the *Rhizobium* isolates, VUC (Vamban) recorded a greater number of nodules/plant (30 / plant) and nodule dry weight (0.038 g/plant), which is significantly higher than other isolates. The same isolate recorded the highest root length (13.50 cm). The treatment T₉ recorded the maximum shoot length of 27.26 cm. Crop was harvested at 65 DAS and yield parameters viz., number of pods per plant, plant dry weight and yield were recorded and tabulated (Table 2). Among the different *Rhizobium* isolates, VUC (Vamban) performed better and recorded the higher values for yield parameters. The highest yield of 1162 kg/ha was recorded by T₅ which received VUC through seed treatment followed by T₆ treatment A-3 (Pantnagar) which recorded 1077.6 kg/ha of seed yield.

Keywords: Yield; inoculation; symbiotic; *Rhizobium*.

1. INTRODUCTION

“Nitrogen (N) is the macronutrient that is required in the largest amount for plant growth, development, and yield” [1]. “Its deficiency in the soils adversely impacts the overall agricultural productivity. A quick method to supply N to plants is via synthetic industrial production of reactive N-fertilizers to maintain higher crop yields in highly agricultural intensification systems” [2,1]. “However, in low-input production systems, such as in many smallholder farms in Africa, food production is hampered by the use of little or no N” [3,4]. “Other impediments to high crop yields in sub-Saharan Africa (SSA) are the inherent low N status of soils, a high nutrient depletion rate (which is exacerbated by crop removals), and non-replenishment” [5,6,7]. “Increasing events of drought associated with uncertainties in climate change are also observed in many areas of West African regions, which will also likely aggravate the yield gaps, specifically for legume crops that are often grown toward the end of the rainy season” [8-11]. Therefore, exploration of alternatives to synthetic N sources and fertilization, such as the natural biological N₂ fixation (BNF), is paramount to increasing crop yields in smallholder farms, particularly in Ghana.

Unlike most non-legumes, legume crops establish a beneficial root association with bacteria in the α - and β -proteobacteria groups, where rhizobia convert abundant N₂ present in the atmosphere into usable N via specialized plant structures called “nodules” [12,13]. “Through this process, legumes satisfy their N demand and that of other nonlegume plants when grown in rotation or association. This process contributes to the agricultural sustainability of production systems for the benefit of millions of smallholder farmers”

[14-18]. However, various abiotic and biotic stress factors, such as drought [19], low phosphorus (P) availability (Suliman and Tran, 2015), non-competitive *Rhizobium* strains, and diseases [20], may affect the BNF process and limit legume productivity [9].

“Seed inoculation with effective *Rhizobium* at sowing is a recommended agronomic practice for pulse production technology. The soil contains many types of microorganisms such as bacteria, actinomycetes, fungi, and algae. These soil microorganisms are important because they affect the soil's physical, chemical, and biological properties through different processes” [21]. “Among the soil bacteria, there is a unique group called rhizobia that has a beneficial effect on the growth of legumes. Rhizobia are soil-inhabiting bacteria that form the root nodules where symbiotic biological nitrogen fixation takes place. In root nodules, the nitrogen-fixing rhizobia exist as irregular cells called bacteroids, which are often club and Y-shaped whereas regular rod-shaped when they are living freely in the soil” [21,22]. Different groups of “Rhizobia genera include *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, and *Azorhizobium*, which can form symbiotic associations with different legumes” [23]. The formation of root nodules [24] in symbiotic legumes involves complex molecular signaling between the legume host and the rhizobial microsymbiont (Jaiswal et al., 2021). “*Sinorhizobium* consists of all fast-growing acid-producing rhizobia while *Bradyrhizobium* contains the slow-growing, alkali-producing rhizobia” [24]. “Nowadays, global agricultural production increased considerably. But due to the global human population increase, the demand for higher crop production has also increased substantially. Because of this, to achieve higher agricultural yields, farmers have

adopted the extensive application of chemical fertilizers and pesticides, which causes soil degradation and a decrease in soil fertility” [25]. “Inoculants are products composed of living microorganisms capable of benefiting the development of different plant species. Rhizobia are the first microorganisms used as inoculants. Currently, the use of inoculants is widespread and indicated in agriculture, mainly for legumes such as soybeans, common beans, faba beans, and cowpea, but the production of inoculants for other legumes and nonlegumes has to be increased to obtain the greater yield” [26]. “*Rhizobium* inoculants are selected strains of beneficial soil microorganisms cultured in a laboratory and packed in with or without a carrier. They are host-specific, low-cost, and an environmentally friendly source of nitrogen. Rhizobia inoculants coated on legume seeds before planting enhance the growth, yield of legume crops and provide nitrogen and organic carbon for subsequent or associated crops. Seeds coated with rhizobia inoculants are not exposed to chemical nitrogen fertilizer. The coated seeds must be planted in moist soil as soon as possible” [27,28]. The present paper highlights the *Rhizobium* sp. effect on nodulation and productivity in urdbean under field conditions.

2. MATERIALS AND METHODS

To assess the performance of *Rhizobium* strains at various locations, a trial was laid out with

Rhizobium strains received from various AICRP centres. The experimental details are mentioned below.

The field experiments were conducted during kharif 2022 in red sandy loam soil of pH 7.3 and low in organic C, and available N and medium in available N and P. The ten treatments consisted of inoculation with different *Rhizobium* strains (T1-BMBS 47 (Coimbatore), T2-GUR 5 (Kalaburagi), T3-GUR8 (Kalaburagi), T4-WUR 12-1 (Akola), T5-VUC (Vamban), T6-A-3 (Pantnagar) T7-P-5 (Pantnagar), T8-PUR 34 (Pantnagar Ch. strain), T9- N as per RDF, T10-uninoculated control) were tested following Randomized Block Design in three replicates in plot of 4.0 x 3.0 m size. Urdbean seeds (VBN8) were treated with the required inoculants (200g/10 kg seeds) at the time of sowing and crop was raised as per the recommended agronomic practices.

Seven *Rhizobium* isolates were obtained from four AICRP centres viz., Kalaburagi (GUR5 & GUR8), Akola (WUR 12-1), Vamban (VUC), and Pantnagar (A-3, P-5 & PUR 34), along with the local best *Rhizobium* BMBS47 were used for the experiment. Urdbean seeds were treated with the *Rhizobium* isolates and sown. Observations were recorded at 45th DAS, to assess their effect on plant growth parameters viz., number of nodules/plants, root length, shoot length, nodule dry weight and yield parameters were recorded during harvest.



Fig. 1. Experimental field

3. RESULTS AND DISCUSSION

The plant growth parameters viz., root length, shoot length, number of nodules / plant and nodules dry weight (g/plant) were observed after vegetative growth and the results were tabulated in Table 1 & Fig. 2.

The below table concluded the findings in which among the *Rhizobium* isolates, VUC (Vamban) recorded a greater number of nodules/plant (30/plant), and nodule dry weight (0.038 g/plant), which is significantly higher than other isolates. The same isolate recorded the highest root length (13.50 cm). The treatment T₉ recorded the maximum shoot length of 27.26 cm. The findings corroborated with the findings of Jinwen et al., [29] indicated that the seeds treating with *Rhizobium* significantly increase root length, nodule number on a plant root, and plant biomass. Native rhizobial inoculation significantly affects the shoot dry weight of cowpea, this

improvement is because rhizobia increase plant growth and improve the plant biomass by providing nitrogen through symbiotic fixation. Inoculated common beans have a higher shoot dry weight compared to the control indicating that inoculation with native isolates improve the growth of plants [30]. Also compared with the findings of Mfilinge et al., [31] and concluded that *Rhizobium* inoculation significantly affects the growth and yield components like number of pod-bearing branches per plant, number of pods per plant, number of seeds per pod, and 1000-seed weight. The study on the effects of *Rhizobium* inoculation in *Vigna mungo* and *Vigna radiata* shows that inoculated plants possess improved height, fresh weight, number of roots, nodules, number of leaves, shoots, pods, length of pods, seed weight, over the uninoculated control. Tairo and Ndakidemi [32] on soybean found that plant height for field experiment increases with *Rhizobium* inoculation for the entire interval of the soybean growth.

Table 1. Multilocation testing of *Rhizobium* strains for Urd bean – Plant growth parameters

Treatments	Root length (cm)	Shoot length (cm)	Number of nodules/plant	Nodule dry weight (g/plant)
T1	10.00	18.73	19.30	0.024
T2	11.43	19.13	19.60	0.019
T3	10.00	19.33	16.33	0.025
T4	12.50	19.80	18.00	0.027
T5	13.50	27.26	30.00	0.038
T6	11.00	22.86	25.30	0.032
T7	9.80	19.53	19.00	0.028
T8	10.00	18.46	18.30	0.026
T9	10.86	17.33	12.60	0.023
T10	9.90	15.60	14.30	0.013
SE (d)	0.64	0.90	2.23	0.002
CD (0.05%)	1.35	1.88	4.68	0.003

Table 2. Multilocation testing of *Rhizobium* strains for Urd bean – Yield parameters

Treatments	Number of pods /plant	Plant dry weight (g/plant)	Yield (kg/ha)
T1	27.76	15.33	845.00
T2	23.00	13.20	813.30
T3	26.26	16.00	800.00
T4	22.00	11.10	766.60
T5	36.66	22.66	1162.00
T6	32.00	20.26	1077.60
T7	25.00	16.26	947.00
T8	21.50	14.26	881.00
T9	22.00	12.10	847.00
T10	20.66	10.83	653.30
SE (d)	3.74	2.01	54.12
CD (0.05%)	7.86	4.23	113.70

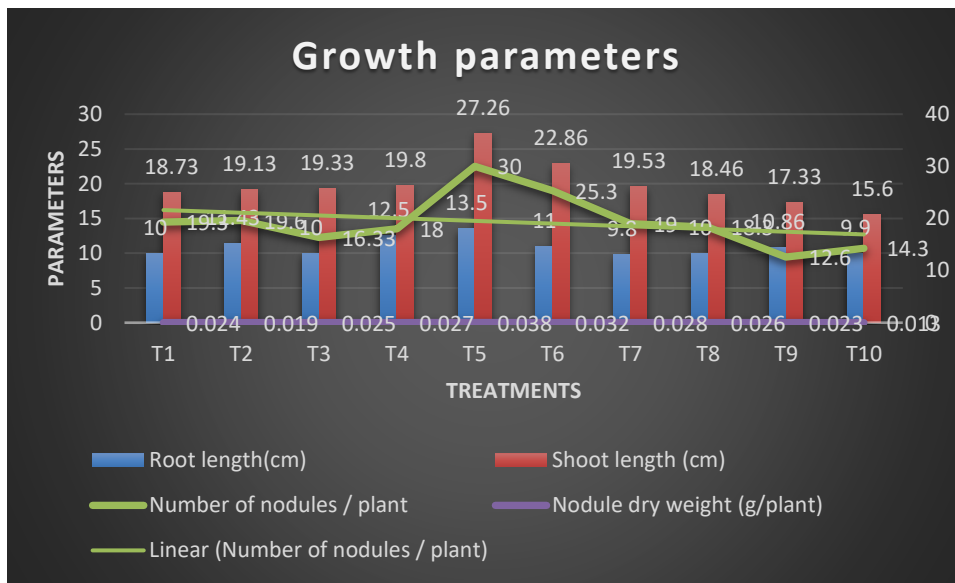


Fig. 2. Growth Parameters of various treatments of plant

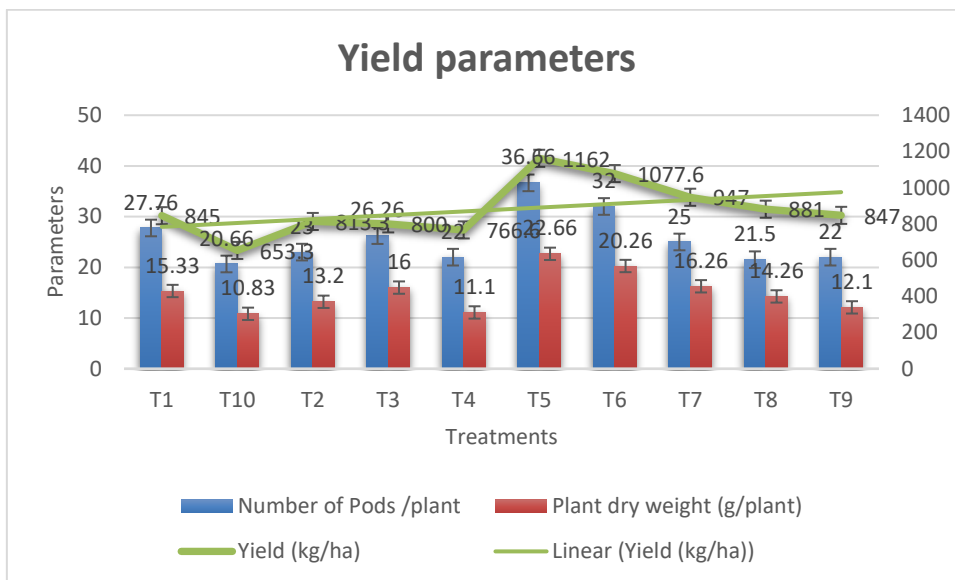


Fig. 3. Yield Parameters of Various treatments of plant

Crop was harvested at 65 DAS, and yield parameters viz., number of pods per plant, plant dry weight, and yield were recorded and tabulated (Table 2 and Fig. 3). Among the different *Rhizobium* isolates, VUC (Vamban) performed better and recorded the higher values for yield parameters. The highest yield of 1162 kg/ha was recorded by T₅, which received VUC through seed treatment followed by T₆ treatment A-3 (Pantnagar) which recorded 1077.6 kg/ha of seed yield. The above results agreed with the findings of Asante et al., [33], indicated that the Table 2 inoculation of legumes with rhizobia triggers plant growth, development, and yield and

it is used as a substitute for mineral nitrogen fertilizer which is often costly. However, the *Rhizobium* is host-specific, as certain species can only infect specific legumes. Inoculation of soybean seed with a mixture of bradyrhizobia strains increases nodulation and total plant nitrogen. Zennouhi O et al., [34] reported that the infectivity and efficiency are commonly used to evaluate the ecological and evolutionary relationships between *Rhizobium* strains and their hosts. Although all the retained strains induce nodule formation on the roots of the host plant as well as the degree of infectivity is clearly differentiate between treatments. Several

researchers have found that inoculation of soybean seeds before planting results in increases in nodulation, percentage N, plant growth, seed yield, and yield components compared to un-inoculated control [35]. Growth and yield of leguminous crops can be triggered by rhizobial inoculation directly by producing various metabolites/substances such as plant hormones, ACC deaminase enzymes, LCOs, siderophores, lumichrome, riboflavin, etc., and or by fixing as well as solubilizing and increasing the uptake of mineral nutrients [36]. Inoculated soybean plants show improvement in nodulation, growth, and yield (Zerihun and Lijalem, 2020).

4. CONCLUSION

Rhizobia inoculants are used in biological nitrogen fixation, which is a superior substitute for other N sources. It is a more economical and non-polluting method of increasing soil fertility. There are significant ecological, economic, and environmental advantages to using rhizobial inoculants in the production of legumes. Reducing the usage of chemical fertilizers and their negative effects on the environment can be achieved by expanding the use of biofertilizers like *Rhizobium*. Therefore, because *Rhizobium* inoculants are economical and environmentally friendly, using them to increase nitrogen fixation and grain legume productivity is highly recommended. Of the *Rhizobium* isolates, VUC (Vamban) had the longest root (13.50 cm), the most nodules per plant (30 per plant), and the highest nodule dry weight (0.038 g/plant). At 65 days after sowing, the crop was harvested, and the yield parameters viz., number of pods per plant, plant dry weight, and yield were noted (Table 2). VUC (Vamban) outperformed the other *Rhizobium* isolates and reported the highest yield of 1162 kg/ha.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the Ex Vice-Chancellor Dr.K.Ramasamy, Professor (Agrl. Microbiology), TNAU, Coimbatore for his valuable guidance to write this paper. I am grateful to all the scientists who

provided a qualitative digital environment for academic exploration and knowledge acquisition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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