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Influence of native bacterial isolates on growth and yield of velvet bean (*Mucuna Pruriens* L.)

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Abstract

This study was conducted to evaluate the influence of native plant growth promoting (PGP) potential of bacterial isolates rhizosphere, rhizoplane and root nodules of velvet bean (*Mucuna pruriens* L.), a medicinal legume valued for its therapeutic and agricultural applications. While chemical elicitors, radiation and heavy metal rhizobia were extensively studied on the enhancement of L-DOPA content, native microbes associated with velvet bean remain largely unexplored. 3 bacterial isolates, namely *Bradyrhizobium* sp. (NYRL1), *Sporosarcina newyorkensis* (SPB1) and *Sporosarcina psychrophila* (NPB2) including rhizobial and non-rhizobial strains, were obtained through standard microbiological techniques. Screening for PGP activities demonstrated capabilities such as phosphorus and potassium solubilization, siderophore and ammonia production and nitrogen fixation. Notably, the combination of 3 isolates exhibited the potential for enhancing plant growth, seed yield and metabolite (L-DOPA) content. This study highlights the importance of native microbes associated with velvet bean in promoting plant growth. The findings highlight the role of microbial isolates as potential candidates for biofertilizer development.

Keywords: L-DOPA content, native associated microbes, pgp traits, velvet bean

Introduction

Velvet bean is important medicinal crop, having promising pharmaceutical and cosmeceutical bioactive compounds among which, L-DOPA is the major metabolite present in all parts of the plant. Traditionally, it has been used for treating many ailments, including neurodegenerative disease namely Parkinson's disease, snake and scorpion bites, diabetes, as aphrodisiac in correcting male infertility and many more (Sathiyanarayanan *et al*., 2007) [33]. The velvet bean belongs to the family Fabaceae and subfamily Papilionaceae, comprises approximately 150 species of annual and perennial legumes. Among the various underutilized wild legumes, the velvet bean is widely distributed across tropical and subtropical regions worldwide. In addition to its diverse therapeutic applications, it is recognized as a valuable source of dietary protein (23–35%) (Janardhanan *et al*., 2003; Pugalenthi *et al*., 2005) [12, 27]. Its protein content is comparable to that of other pulses such as soybean, rice bean, and lima bean (Gurumoorthi *et al*., 2003) [8].

In recent years, there has been a shift from allopathic medicines to compounds derived from natural therapeutic sources due to increasing health concerns. In this context, the demand for mucuna is rising steadily because of its vast pharmaceutical potential.

Currently, conventional agriculture relies heavily on chemical fertilizers to improve crop yield and quality. However, their extensive and prolonged use has led to environmental and ecological disadvantages, such as soil degradation, pollution apart from enhanced production costs. Additionally, the extensive use of chemical fertilizers limits the export of such crops. Therefore, there is a growing global interest in exploring alternative sources that can naturally boost crop productivity to promote agricultural sustainability. In this regard, bio-stimulants which are comprised natural products derived from biological sources, are being employed to enhance the productivity (Sharanya *et al.,* 2022) [35]. However, their formulation involves processing of natural products, which again adds to the production costs. Thus, there is pressing need for natural sources requiring minimal or no processing, with multiple mechanisms for promoting plant growth to improve the plant productivity.

Plant Growth Promoting Rhizobacteria (PGPR) are natural biological sources known for their direct or indirect plant growth promoting mechanisms have been reported to positively influence the yield and quality of medicinal plants. Therefore, this study focuses on the isolation, PGP characterization and identification of native bacteria and their impact on the growth and yield of leguminous medicinal crop velvet bean.

Materials and methods

Sample collection

Rhizosphere soil, root and root nodules were collected from velvet bean plant grew wild at Krishi Vignana Kendra (KVK), Hadonahalli, Doddaballapura, Bangalore, India (13.37ºN, 77.55ºE). Root nodules were immediately transferred into an ice box and brought to the laboratory for isolation of bacteria associated with root nodules.

Isolation of bacteria

Isolation of bacteria was done from rhizosphere soil (Nie *et al.,* 2025) [20], rhizoplane (Mergeay *et al.,* 1985) [17] and root nodules (Zhen *et al.,* 2011) [43]. Collected root and root nodules were washed thoroughly to remove adhering soil particles and dirt. Then, surface sterilized with 70 % ethanol for 1 min and then with 3 % sodium hypochlorite for 1 min followed by serial washing with sterile distilled water for 7 consecutive washes. Sterilized rhizoplane and root nodules were crushed in small quantity (10 ml) of sterile distilled water and aliquot of 1 ml was serially diluted in 9 ml sterile distilled water up to 10^{-7}. An aliquot of 0.1 ml was spread on petri plates having Yeast Extract Mannitol Agar with Congo Red (CRYEMA) for isolation of rhizobium while an aliquot of 0.1 ml from was spread on petri plates having

Pikovskaya's agar (PA), Aleksandrov's agar (AA), Jensen's agar (JA) and Nutrient agar (NA) media for isolation of phosphate solubilizing, potassium solubilizing, nitrogen fixing and other non-fastidious bacteria respectively. Plates were incubated at 30°C for 24 - 96 h.

Screening for plant growth promoting (PGP) activities

Phosphate solubilization

Screening for phosphate solubilization ability of bacterial isolates was done by a plate assay method using Pikovskaya's agar medium (Pikovskaya, 1948) [25] supplemented with tricalcium phosphate. The plates were spot inoculated with 1.5 µL of three days old bacterial cultures ($OD_{600} = 0.8$) and incubated for upto 7 days at 30 °C. The formation of clear zone around bacterial colonies indicates positive for solubilizing ability of the isolates and the results were expressed as phosphorus solubilization index (PSI) (Tariq *et al.,* 2022) [40].

$$\text{Phosphorus solubilization index } (PSI) = \frac{\text{Colony diameter} + \text{zone diameter}}{\text{Colony diameter}}$$

Potassium solubilization

Bacteria isolates were screened for potassium solubilization following a plate assay method using Aleksandrov's agar medium supplemented with insoluble mica as a potassium bearing mineral. The plates were spot inoculated with 1.5 µL of three days old bacterial cultures ($OD_{600} = 0.8$) and incubated for up to 7 days at 30°C. Formation of clear zone around bacterial colonies indicate positive for solubilizing ability of the isolates and the results were expressed as potassium solubilization index (KSI) (Hu *et al.,* 2006) [10].

$$\text{Potassium solubilization index } (KSI) = \frac{\text{Colony diameter} + \text{zone diameter}}{\text{Colony diameter}}$$

Siderophore production

Bacterial isolates were screened of for siderophore production which was carried out by following method given by Schwyn and Neilands (1987) [34] in Chrome Azurol S (CAS) agar medium. The plates containing bluish green CAS agar were spot inoculated with 1.5 µL of bacterial cultures ($OD_{600} = 0.8$) and incubated at 30 °C for 3–5 days. Change in bluish green colour of the CAS agar medium around the bacterial colony to yellow, blue, orange or red indicated as positive for production of siderophore. Siderophore producing index (SPI) was calculated as the ratio of (colored zone diameter + colony diameter)/colony diameters (Desai *et al*., 2012) [6].

Ammonia production

Ammonia production ability of the bacterial isolates was carried out by following the method given by Cappuccino and Sherman (1992) [4]. 1.5 µl of bacterial cultures (OD_{600}=0.8) were inoculated in 10 ml peptone broth and incubated at 30°C for 48–72 h in an orbital shaking incubator. After incubation, 0.5 ml of Nessler's reagent was added in each test tube. Development of a yellow to dark brown colour indicated the production of ammonia. For quantification, 2 ml of the broth was taken in an eppendorf centrifuge tube and centrifuged at 10,000 rpm for 5 min. Then, 0.5 ml of Nessler's reagent was added to each tube containing the supernatant of bacterial isolates and the absorbance was read at 450 nm. The concentration of the NH_4^+ was calculated using the standard curve of ammonium sulphate solution and expressed as µg/mL (Mukherjee *et al.*, 2017) [18].

Identificaion of bacterial isolates using 16s rRNA sequencing

DNA isolated was PCR amplified using 16S rRNA with universal primers (27F and 1492R). The amplicons were sequenced by Barcode Biosciences Pvt. Ltd., Bengaluru, Karnataka. The sequence data received was analysed for homology using NCBI database.

Compatibility test

The microbial cultures were cross streaked on nutrient agar plates and incubated at 28±2°C for 48 h for testing their compatibility with each other. The zone of inhibition was observed and recorded. Isolates were considered compatible if there is no inhibition area and incompatible if there is inhibition zone (Prasad and Babu, 2017) [26].

Effect of PGPR on growth and yield of Velvet bean

Seed source and Variety

Arka Dhanwanthri variety of velvet bean seeds were collected from Zonal Agricultural Researh Station (ZARS), UAS, GKVK, Bengaluru.

Treatment details

The velvet bean seeds were treated with bacterial isolates in combination of two and three isolates under the influence of 50 and 75 % nitrogen content (Urea application).

Sowing

Sowing was done in the month of June, 2023 during *kharif* following Randomized Block Design (RBD). Bold and well-developed seeds were pre-treated as per the treatments and shade dried. Treated and untreated seeds were dibbled at a depth of about 2 cm in the furrows by maintaining spacing of 60 × 60 cm at the rate of one seed per hill and were covered with the soil.

Staking of plants

Velvet bean is a twining annual hence, artificial support was provided to the vines at 30 days after sowing in staking modules using plastic wires tied to eucalyptus poles of about 10 ft height.

Growth parameters

Plant length

The length of the vine was recorded by using meter scale from the base to the tip of the main shoot of the five randomly labeled plants at 50,100 and 150 days after sowing and at harvesting and the mean was worked out and expressed in centimeter (cm).

Number of branches per plant

The number of branches arising from the main stem was counted at 60 and 120 DAS and mean number of branches per plant was recorded.

Number of leaves per plant

Number of trifoliate leaves produced per plant was counted at 50,100 and 150 DAS, at flowering, at harvest and average was computed.

Yield parameters

Number of inflorescences per plant

Total numbers of inflorescence of 5 tagged plants in each treatment were counted at peak flowering period and the average was computed.

Number of pods per inflorescence

The total numbers of pods per inflorescence produced in five tagged plants was counted and the mean value was calculated.

Number of seeds per pod

Number of seeds present in randomly selected pods from five plants was counted and mean values for each treatment was calculated.

Seed weight per pod

Seeds extracted from five pods of randomly selected plants were weighed and mean values per pod for each bio-stimulant treatment module was calculated and expressed in grams.

Test weight of seeds

The observation was recorded by weighing 100 seeds from each treatment and the mean weight was calculated and expressed in grams (g).

Estimation of L-DOPA content in seeds

Ultra High-Performance Liquid Chromatographic (UHPLC) analysis was carried out to estimate the L-DOPA content of seeds from eleven treatments of the experiment as per the protocol standardized by Shivananda and Vasanthakumar (2003) [36]. Shade dried deeds from each treatment were used in L-DOPA extraction. The seeds were powdered, 50 mg of powdered seed was transferred to 50 ml volumetric flask and 15 ml of 0.1 M orthophosphoric acid was added and continuously shaken for 15 minutes to extract the L-DOPA in to the solution. The sol orthophosphoric acid was added and continuously shaken for 10 minutes to extract the remaining L-DOPA in to the solution. The volume was made upto 50 ml using double distilled water and filtered through 0.42 µ. The filtrate (20 µl) was used for the estimation of the L-DOPA in UHPLC. The L-DOPA peak was observed between 3.2-3.3 minutes of retention time. The L-DOPA (%) in the sample was calculated using the following formula.

L-DOPA yield

L-DOPA yield was calculated by multiplying L-DOPA content with seed yield per hectare and expressed in kilo gram per hectare.

Statistical analysis

The data was analyzed following a one-way ANOVA test and Duncan's Multiple Range Test (DMRT) was applied to separate the means at a significance level of $P<0.05$, using WASP v2.0 software (https://ccari.icar.gov.in/wasp2.0/index.php) (Jangam and Thali, 2004) [13].

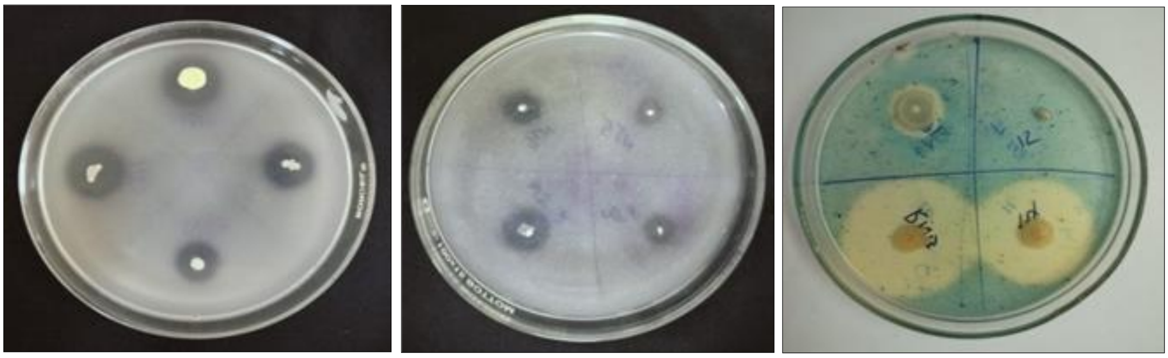
Results and discussion

Isolation and screening of bacterial isolates

The velvet bean samples were collected from KVK, Hadonahalli, Doddaballapura, Bangalore (13.37ºN, 77.55ºE). For isolation, Standard serial dilution and plate count (SDPC) method was followed. The bacterial isolates were isolated on five different media namely CRYEMA, PA, AA, JA and NA. A total of 127 bacterial isolates were obtained from rhizosphere rhizoplane and root nodules and were screened for PGP traits on different media *viz.,* PA, AA, JA and CAS agar. Isolates named NYRL1, SPB1 and NPB2 showed positive for multiple PGP activities. Further qualitatively estimated on the same media (PA, AA, JA and CAS agar) and ammonia production by isolates was quantitatively estimated on peptone broth.

Qualitative tests for Plant Growth Promoting (PGP) traits

P-solubilization indices and efficiencies were recorded as 3.70 and 269.76 % by isolate SPB1, 3.94 and 293.52 % by isolate NPB2, 1.71 and 70.83% by NYRL1 respectively (Plate 1). Whereas, K-solubilization indices and efficiency was recorded to be 3.79 and 278.57 % by isolate SPB1, 3.32 and 231.67 % by isolate NPB2. Similarly, siderophore production on agar plates was recorded as 2.71 and 170.83 by isolate SPB1 and 1.90 and 89.54 % by isolate NPB2. But isolate NYRL1 showed negative or negligible amount of K-solubilization and siderophore production. Ammonia production by the isolates recorded as 27.59, 11.44 and 53.11 µmol/ml by SPB1, NPB2 and NYRL1 isolates respectively. Plant growth promoting activities such as, phosphorus and potassium solubilization, nitrogen fixation helps in providing available form of nutrients such as N_2, P and K for uptake by the plants. Siderophore production by isolates aids in chelating iron in soil and making it available for plants and thereby enhances the plant growth as well as yield as reported by Cao *et al.* (2024) [3], where they isolated bacterial isolates from root nodules of *Abrus mollis* which exhibited PGP traits and enhanced the seedling growth upon inoculation.



(A) **(B)** **(C)**

Plate 1: PGP traits of bacterial isolates**,** a. Phosphorus solubilization, b. Potassium solubilization and c. Siderophore production.

Table 1: Qualitative PGP traits of bacterial isolates

Sl. No	Isolates	Phosphate Solubilization		Potassium Solubilization		Siderophore Production		NH_3 Production (µmol/ml)
		Index	Efficiency (%)	Index	Efficiency (%)	Index	Efficiency (%)	
1	SPB1	3.70	269.76	3.79	278.57	2.71	170.83	27.59
2	NPB2	3.94	293.52	3.32	231.67	1.90	89.54	11.44
3	NYRL1	1.71	70.83	-	-	-	-	53.11

Molecular identification of bacterial isolates

The isolates were identified molecularly using 16S rRNA sequencing and analyzed using BLAST software with the available published sequences at National Centre for Biotechnology Information (NCBI) database and results revealed that SPB1 is identified as *Sporosarcina newyorkensis*, NPB2 as *Sporosarcina newyorkensis* and NYRL1 as *Bradyrhizobium* sp.

Plant height

Significantly higher plant height was recorded in plants treated with bacterial isolates with reduced nitrogen supply (50 and 75% N) over the control plants (Table 2). At 50 DAS, the highest plant height of 209.41, 223.50 and 322.13 cm at 50, 100 and 150 DAS, was recorded in treatment T_{11}: 75% N + 100% P and K + *Bradyrhizobium* sp. + *S. newyorkensis* + *S. psychrophila.* The increased plant height of velvet bean treated with efficient bacterial isolates might be due to enhanced supply of nutrients through nitrogen fixation, P and K-solubilization by the bacterial isolates, which has enhanced the metabolic activity of plant by increasing meristamatic activity and chlorophyll synthesis in turn increased cell division and elongation intern resulted in maximum plant height. These findings are in line with Desai *et al.* (2018) [5] in alfa alfa, Patil *et al.* (2014) [23] in kalmeg and Nadukeri (2010) [19] in ashwagandha.

Table 2: Effect of efficient PGP bacterial isolates on plant height of Velvet bean at different stages under field condition

Treatments	Plant height (cm)		
	50 DAS	100 DAS	150 DAS
T_1: Absolute control	121.50^{i}	149.77^{i}	193.70^{f}
T_2: Recommended dose of fertilizer (RDF)	187.97^{e}	192.33^{e}	283.33^{c}
T_3: RDF + *Sinorhizobium meliloti* (Ref.)	209.41^{a}	223.50^{a}	320.55^{a}
T_4: 50% N + 100% PK + A	161.17^{h}	186.37^{h}	277.73^{e}
T_5: 50% N + 100% PK + A + B	161.93^{gh}	188.10^{g}	279.20^{de}
T_6: 50% N + 100% PK + A + C	162.5^{g}	189.73^{f}	279.67^{d}
T_7: 50% N + 100% PK + A + B + C	169.47^{f}	193.20^{e}	280.57^{d}
T_8: 75% N + 100% PK + A	194.27^{d}	219.21^{d}	315.67^{b}
T_9: 75% N + 100% PK + A + B	196.33^{c}	220.83^{c}	316.17^{b}
T_{10}: 75% N + 100% PK + A + C	196.40^{c}	220.67^{c}	317.20^{b}
T_{11}: 75% N + 100% PK + A + B + C	207.43^{b}	222.13^{b}	322.13^{a}

Note: DAS - days after sowing; Mean values (n=3) with the superscript in each column represent significance different as determined by DMRT ($p<0.05$). A-*Bradyrhizobium* sp. strain NYRL1, B- *Sporosarcina newyorkensis* strain SPB1 and C- *Sporosarcina psychrophila* strain NPB2

Number of branches per plant

At 50 DAS, the maximum number of branches were recorded in T_{11}: 75% N + 100% P and K + *Bradyrhizobium* sp. + *S. newyorkensis* + *S. psychrophila* (9.67) (Table 3). At 60 and 90 das similar trend was observed where T_{11}: 75% N + 100% P and K + *Bradyrhizobium sp.* + *S. newyorkensis* + *S. psychrophila* plants recorded the highest branches per plant (12.67 and 12.33). Increased branches could be due to combined effect of different bacterial isolates treatment in the experiment. By the multiple PGP mechanisms these bacterial isolates release nutrients into the soil in available forms, thereby increasing the easily forms of nutrients to the crop root to absorb more nutrients from the soil. This might have helped the crop for better utilization of photosynthates coupled with better meristamatic activity with the production of phytohormones and to put forth new vegetative growth. These findings are in good agreement with Patil *et al.* (2014) [23] in fenugreek, Osman *et al.* (2009) [22] in soybean, maize and cotton and Nadukeri (2010) [19] in ashwagandha.

Table 3: Effect of efficient PGP bacterial isolates on number of branches per plant of Velvet bean at different stages under field condition

Treatments	Number of branches $plant^{-1}$		
	50 DAS	100 DAS	150 DAS
T_1: Absolute control	4.67^{d}	6.67^{d}	9.12^{e}
T_2: Recommended dose of fertilizer (RDF)	7.33^{bc}	9.67^{c}	11.67^{cd}
T_3: RDF + *Sinorhizobium meliloti* (Ref.)	9.67^{a}	12.67^{a}	15.33^{a}
T_4: 50% N + 100% PK + A	6.00^{cd}	7.67^{d}	10.33^{de}
T_5: 50% N + 100% PK + A + B	6.33^{cd}	7.33^{d}	10.67^{de}
T_6: 50% N + 100% PK + A + C	6.33^{cd}	7.67^{d}	10.67^{de}
T_7: 50% N + 100% PK + A + B + C	6.67^{bc}	7.67^{d}	11.33^{d}
T_8: 75% N + 100% PK + A	7.01^{bc}	9.67^{c}	13.33^{bc}
T_9: 75% N + 100% PK + A + B	7.33^{bc}	10.67^{bc}	13.33^{bc}
T_{10}: 75% N + 100% PK + A + C	8.33^{b}	11.33^{ab}	13.67^{ab}
T_{11}: 75% N + 100% PK + A + B + C	9.33^{a}	12.33^{ab}	15.02^{a}

Note: DAS - days after sowing; Mean values (n=3) with the superscript in each column represent significance different as determined by DMRT ($p<0.05$). A-*Bradyrhizobium* sp. strain NYRL1, B- *Sporosarcina newyorkensis* strain SPB1 and C- *Sporosarcina psychrophila* strain NPB2

Number of leaves per plant

Significantly higher number of leaves per plant was recorded in plants treated with bacterial isolates with reduced nitrogen supply (50 and 75% N) over the control (Table 4). At 50 DAS, the highest number of leaves per plant (94.00, 101.33 and 116.67 at 50, 100 and 150 DAS respectively) was recorded in T_{11}: 75% N + 100% P and K + *Bradyrhizobium* sp. + *S. newyorkensis* + *S. psychrophila*. This improved leaf production in integrated treatments indicates better photosynthetic surface area, which is crucial for biomass accumulation and subsequent reproductive success in velvet bean. These findings are in accordance with the reports of Raza *et al.* (2019) [31], who reported that biofertilizers application improved the vegetative parameter of legumes by increasing the uptake of essential nutrients and promoting better root development. Similarly, Kumawat *et al* (2020) [14] observed increased leaf production in cowpea upon inoculation with PGPR and Rhizobium. The same results are also supported by the study of Meena *et al.* (2017) [16], where the integration of bioinoculants and reduced chemical fertilizers increased the number of leaves and overall plant growth in pearl millet.

Table 4: Effect of efficient PGP bacterial isolates on number of leaves per plant of Velvet bean at different stages under field condition

Treatments	Number of leaves plant^{-1}		
	50 DAS	**100 DAS**	**150 DAS**
T_1: Absolute control	44.67	68.11^{d}	79.33^{d}
T_2: Recommended dose of fertilizer (RDF)	84.67^{c}	93.33^{b}	109.67^{b}
T_3: RDF + *Sinorhizobium meliloti* (Ref.)	93.67^{a}	102.03^{a}	116.33^{a}
T_4: 50% N + 100% PK + A	75.00^{e}	83.42^{c}	97.01^{c}
T_5: 50% N + 100% PK + A + B	75.33^{de}	84.12^{c}	96.67^{c}
T_6: 50% N + 100% PK + A + C	75.33^{de}	83.33^{c}	96.67^{c}
T_7: 50% N + 100% PK + A + B + C	76.00^{d}	84.67^{c}	97.12^{c}
T_8: 75% N + 100% PK + A	92.67^{b}	96.67^{ab}	113.67^{ab}
T_9: 75% N + 100% PK + A + B	92.00^{b}	98.33^{ab}	115.00^{ab}
T_{10}: 75% N + 100% PK + A + C	92.67^{b}	99.01^{ab}	114.67^{ab}
T_{11}: 75% N + 100% PK + A + B + C	94.00^{a}	101.33^{a}	116.67^{a}

Note: DAS - days after sowing; Mean values (n=3) with the superscript in each column represent significance different as determined by DMRT ($p<0.05$). A-*Bradyrhizobium* sp. strain NYRL1, B- *Sporosarcina newyorkensis* strain SPB1 and C- *Sporosarcina psychrophila* strain NPB2

Inflorescence attributes

The study evaluated the impact of various treatments involving native bacterial isolates and fertilizer doses on inflorescence and pod production in Velvet bean are presented in Table 5. The number of inflorescences per plant was highest in treatment T_{11}:75% N + 100% P and K + *Bradyrhizobium* sp. + *S. newyorkensis* + *S. psychrophila* with 11.27 inflorescences. These values were significantly higher than the absolute control (T_1), which had 5.87 inflorescences. The number of pods per inflorescence was greatest in T_{11} and T_3, at 17.73 and 17.53 pods, respectively, whereas the control recorded only 6.87 pods. The symbiotic enhancement of nitrogen fixation and improved phosphorus and potassium availability by these microbes supports reproductive growth and yield components (Singh *et al.*, 2021; Hasanuzzaman *et al.*, 2022) [9, 39]. Moreover, PGPR-mediated production of phytohormones, including auxins and gibberellins, likely plays a role in stimulating flower initiation and pod set, further amplifying yield potential (Yasin *et al.*, 2023; Zhang *et al.*, 2022) [41, 42]. The maintenance of high pod and inflorescence numbers with 75% nitrogen along with bio-inoculants (T_{11}) highlights an eco-friendly nutrient management strategy compatible with sustainable agriculture goals by limiting chemical inputs while sustaining productivity (Gupta *et al.*, 2023; Ramesh *et al.*, 2022) [7, 30].

Seed variables

The influence of native bacterial isolates along with fertilizer treatments on seed variables (number of seeds, Seed weight per pod and test weight) of Velvet bean are presented in Table 5. The number of seeds per pod was highest in treatment T_{11}:75% N + 100% P and K + *Bradyrhizobium* sp. + *S. newyorkensis* + *S. psychrophila* with 5.40 seeds. Seed weight per pod (g) showed a similar trend, with T_{11} recording the highest weight (5.60 g). The test weight (g), an important indicator of seed quality and vigor, was significantly greater in T_{11} (116.00 g). The enhanced seed traits observed under treatments involving *Sinorhizobium meliloti* and PGPR consortia reflect their influential role in improving seed yield and quality in Velvet bean. The superior seed number per pod, seed weight, and test weight in treatments T_3 and T_{11} highlight the beneficial effects of microbial inoculants on nutrient uptake and seed development (Singh *et al.*, 2024; Alami *et al.*, 2023) [2, 38]. Nitrogen fixation by rhizobia along with phosphorus and potassium solubilization by PGPR improves the availability of essential nutrients, which is critical during seed filling stage for increasing seed size and weight (Oliveira *et al.*, 2022; Abdelrahman *et al.*, 2023) [1, 21]. Moreover, PGPR-induced production of growth regulators such as cytokinins and gibberellins positively affects seed development and vigor (Shu *et al.*, 2023) [37]. These sustainable nutrient management strategies with reduced chemical fertilizers combined with bio-inoculants demonstrated here are in line with findings by Huang *et al.* (2023) and Liu *et al.* (2024) [15], who reported similar improvements in seed attributes and yield performance in legumes.

Seed L-DOPA content

L-DOPA content and yield varied significantly among treatments (Table 34 and Fig. 12). The highest L-DOPA content was recorded in T_{11}:75% N + 100% P and K + *Bradyrhizobium* sp

Table 5: Effect of efficient PGP bacterial isolates on inflorescence attributes and pods per plant in Velvet bean

Treatments	Number of inflorescences per plant	Number of pods per inflorescence	Number of seeds per pod	Seed weight per pod (g)	Test weight (g)
T$_1$: Absolute control	5.87^{e}	6.87^{f}	2.67^{e}	2.45^{d}	60.52^{h}
T$_2$: Recommended dose of fertilizer (RDF)	8.13^{c}	12.60^{c}	4.47^{cd}	3.52^{c}	83.62^{e}
T$_3$: RDF + *Sinorhizobium meliloti* (Ref.)	10.73^{a}	17.53^{a}	5.13^{b}	5.42^{b}	111.98^{b}
T$_4$: 50% N + 100% PK + A	7.07^{d}	9.27^{e}	4.20^{d}	3.18^{c}	62.62^{g}
T$_5$: 50% N + 100% PK + A + B	6.87^{d}	9.93^{de}	4.4^{cd}	3.13^{c}	64.02^{f}
T$_6$: 50% N + 100% PK + A + C	6.93^{d}	10.60^{de}	4.33^{cd}	3.39^{c}	64.68^{f}
T$_7$: 50% N + 100% PK + A + B + C	7.27^{d}	11.47^{cd}	4.53^{cd}	3.29^{c}	65.02^{f}
T$_8$: 75% N + 100% PK + A	9.01^{b}	15.67^{b}	4.8^{bcd}	4.45^{b}	106.00^{d}
T$_9$: 75% N + 100% PK + A + B	8.47^{bc}	16.03^{ab}	4.67^{bcd}	4.49^{b}	109.06^{c}
T$_{10}$: 75% N + 100% PK + A + C	9.13^{b}	16.01^{ab}	4.93^{abc}	4.52^{b}	108.31^{c}
T$_{11}$: 75% N + 100% PK + A + B + C	11.27^{a}	17.73^{a}	5.40^{a}	5.60^{a}	116.00^{a}

Note: DAS - days after sowing; Mean values (n=3) with the superscript in each column represent significance different as determined by DMRT ($p<0.05$). A-*Bradyrhizobium* sp. strain NYRL1, B- *Sporosarcina newyorkensis* strain SPB1 and C- *Sporosarcina psychrophila* strain NPB2

+ *S. newyorkensis* + *S. psychrophila* with 5.86%. Correspondingly, L-DOPA yield was highest in T$_{11}$ (257.46 kg/ha). The lowest L-DOPA content and yield were observed in the absolute control (T$_1$) with 3.12% and 86.07 kg/ha, respectively. The significant enhancement of L-DOPA under treatments involving consortia of PGP bacterial inoculants can be linked to increased synthesis of the precursor amino acid tyrosine by bacteria. PGPR are known to stimulate amino acid biosynthesis pathways, including those for tyrosine, which is a direct precursor for L-DOPA biosynthesis in plants (Raghavendra *et al.,* 2021). The inoculation with PGP bacteria likely elevates enzymatic activities associated with L-DOPA production, enhancing secondary metabolite accumulation.

Table 6: Effect of efficient PGP bacterial isolates on L-DOPA content of Velvet bean seeds

Treatments	L-DOPA Content (%)	L-DOPA yield (kg/ha)
T$_1$: Absolute control	3.12^{i}	86.07^{i}
T$_2$: Recommended dose of fertilizer (RDF)	4.69^{e}	168.42^{f}
T$_3$: RDF + *Sinorhizobium meliloti* (Ref.)	5.42^{b}	234.47^{b}
T$_4$: 50% N + 100% PK + A	3.42^{h}	110.12^{h}
T$_5$: 50% N + 100% PK + A + B	3.45^{h}	111.97^{h}
T$_6$: 50% N + 100% PK + A + C	3.56^{g}	115.61^{h}
T$_7$: 50% N + 100% PK + A + B + C	3.96^{f}	131.20^{g}
T$_8$: 75% N + 100% PK + A	5.23^{d}	202.32^{e}
T$_9$: 75% N + 100% PK + A + B	5.36^{c}	211.68^{d}
T$_{10}$: 75% N + 100% PK + A + C	5.41^{c}	222.90^{c}
T$_{11}$: 75% N + 100% PK + A + B + C	5.86^{a}	257.46^{a}

Note: DAS - days after sowing; Mean values (n=3) with the superscript in each column represent significance different as determined by DMRT ($p<0.05$). A-*Bradyrhizobium* sp. strain NYRL1, B- *Sporosarcina newyorkensis* strain SPB1 and C- *Sporosarcina psychrophila* strain NPB2

Conclusion

Comparatively, other approaches to enhance L-DOPA such as heavy metal elicitation, radiation treatments, and precursor feeding have been reported but often involve abiotic stresses that may impair plant growth or yield (Rakesh and Praveen, 2022; Pattinson *et al.*, 2001 and Suryavanshi *et al.,* 2022) [29]. While these methods can induce L-DOPA biosynthesis, they often come with environmental and safety concerns. In contrast, the use of bioinoculants represents a sustainable and growth-promoting strategy to increase L-DOPA content by simultaneously enhancing nutrient availability, phytohormone production, and plant metabolic activity. The microbial consortium treatment T$_{11}$ showed a synergistic effect resulting in the highest L-DOPA content and yield, outperforming treatments with individual inoculants or chemical fertilizers alone. Thus, PGP bacterial inoculation emerges as an effective biostimulant strategy with dual benefits: increasing valuable secondary metabolites like L-DOPA and improving overall plant growth and yield, offering a safer alternative to physical or chemical elicitors traditionally used for L-DOPA enhancement.

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