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EFFECTS OF SEED INOCULATION WITH *PARARHIZOBIUM GIARDINII* ENPV2 ON NODULATION, GROWTH, AND YIELD COMPONENTS OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS

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ABSTRACT

In the 2025 growing season, a field experiment was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, to evaluate Pararhizobium giardinii (strain EnPv2) inoculation on three common bean cultivars: Algil, Florres, and Cannellino. Inoculation enhanced symbiotic performance and vegetative growth, increasing nodule number/plant by 70.33 %, green biomass by 27.23 %, and dry biomass by 30.54 % versus controls, with Algil exhibiting the highest response. While thousand-seed weight (TSW) showed a minor deviation (+1.96 %), inoculation markedly increased pods/plant (14.48 %), seeds/plant (14.99 %), and seed weight/plant (17.80 %). Consequently, average plot yield (14 m²) increased by 11.47 %, resulting in a 12.28 %/ha overall seed yield gain. Thus, Pararhizobium giardinii EnPv2 serves as an effective bio-inoculant for enhancing common bean productivity.

INTRODUCTION

Globally, driven by rising prices of nitrogenous fertilizers and government pressures to reduce emissions associated with synthetic nitrogen fertilization, there has been a resurgence in research evaluating the symbiotic relationship between legumes and atmospheric nitrogen-fixing bacteria, which could substitute, at least partially, mineral nitrogen inputs. Common bean (*Phaseolus vulgaris* L.) already holds a significant share in global nutrition and represents an essential component in crop rotation systems. The primary symbiotic relationship of the common bean is established with bacteria belonging to the genera *Rhizobium* and *Bradyrhizobium*.

Following recent studies, the genus *Pararhizobium* has been added to this list, identified as a potentially relevant partner in cultivation. Through the root nodules resulting from this symbiosis, the plant can meet a portion of its nitrogen requirements, thereby leading to a reduction in the use of synthetic nitrogen fertilizers and the emissions associated with their application. However, fixation performance can be inconsistent, varying as a function of the bacterial strain, the cultivar used, and local pedoclimatic conditions.The process by which molecular nitrogen (N_2) is converted into chemical forms directly accessible for plant nutrition is termed biological nitrogen fixation (BNF). This metabolic capability belongs strictly to prokaryotic microflora,

known as diazotrophic organisms, which are the only entities possessing the nitrogenase enzyme complex. From an agricultural perspective, the most important symbiotic system is formed between leguminous plants and bacteria of the family *Rhizobiaceae* (Postgate, 1998).

The symbiotic interaction between rhizobia and the host root system relies on bidirectional biochemical communication. Initially, the plant roots release flavonoid compounds into the rhizosphere to serve as signal molecules, inducing the expression of specific *nod* genes within compatible bacterial cells. The enzymes encoded by these genes catalyze the synthesis of Nod factors (lipochitooligosaccharide structures), which trigger the morphological response of the plant. As a result of this recognition, root hair curling occurs, followed by bacterial invasion via the infection thread, and ultimately, nodule organogenesis (Oldroyd, 2013). Within the nodular tissue, bacterial cells differentiate into bacteroids, a cellular stage in which the nitrogenase enzyme complex becomes functional. This enzyme, whose synthesis is controlled by *nif* genes, catalyzes the reduction of nitrogen atmospheric (N_2) to ammonia (NH_3), a form subsequently assimilated by the host plant into amino acids and other nitrogenous metabolites. In return, the host macroorganism provides the bacteroids with carbon sources, primarily in the form of dicarboxylic acids necessary to support the energetic demands of bacterial metabolism, thereby reinforcing the mutualistic nature of this interaction.

The efficiency of the biological fixation process is governed by a series of limiting factors, among which the genetic affinity between the bacterial strain and the legume genotype is prominent, alongside local pedoclimatic parameters (soil thermal and moisture regimes, pH, and mineral nitrogen reserves).

Under optimal environmental conditions, common bean exhibits a fixation capacity estimated between 30 and 80 kg N/ha. Although this quantitative contribution is lower compared to that recorded in soybean or alfalfa crops, it exerts a significant positive impact on the nitrogen balance within the agroecosystem (Hardarson and Atkins, 2003).

In current rhizobial taxonomy, the genus *Pararhizobium* was recently established following phylogenetic reconfigurations based on multilocus sequence analysis of housekeeping genes. The species *Pararhizobium giardinii* (initially classified as *Rhizobium giardinii*) was scientifically documented in 1998, having been primarily isolated from root nodules of *Phaseolus vulgaris* crops in France. Subsequently, its presence has been reported in various geographical regions, confirming its status as a specific microsymbiont of the common bean (Amarger et al., 1997). Recent investigations highlight that indigenous biotypes of *Pararhizobium giardinii*, isolated from local agricultural ecosystems, manifest superior symbiotic efficiency compared to allochthonous strains due to evolutionary adaptation to the pedoclimatic factors of their zones of origin. Consequently, the identification and biochemical characterization of local ecotypes are essential for developing microbial inoculants with specific applicability in Romanian agriculture.

The strain *Pararhizobium giardinii* EnPv2 was isolated in 2021 from nodules sampled from common bean plants cultivated in Dolj County, and its molecular identity was confirmed via partial sequencing of the 16S rRNA gene. Using PCR techniques, the presence of the target genes *nifH* and *nodC*, responsible for controlling dinitrogen fixation and initiating nodular organogenesis in legumes, respectively was demonstrated as immutable genetic markers of symbiotic potential. Given that preliminary testing indicated strain EnPv2 possesses a high root colonization capacity and induced the formation of active nodules across various

bean genotypes, evaluating its performance under real field conditions became highly warranted. This study aimed to evaluate the effects of *Pararhizobium giardinii* EnPv2 inoculation on common bean (*Phaseolus vulgaris* L.) cultivars (Algil, Cannellino, Florres) by quantifying nodulation at flowering, green/dry biomass, yield components (pods/plant, seeds/plant, seed weight/plant, and thousand-seed weight - TSW), and total yield per hectare.

MATERIAL AND METHODS

Experimental Site, Plant Material, and Crop Management

The biological material evaluated in this study comprised three common bean cultivars selected to represent diverse geographical origins and growth habits: Algil (Romania, IULS), Cannellino (Italy), and Florres (Netherlands). The field experiment was conducted during the 2025 growing season at the Experimental Field of the University of Agronomic Sciences and Veterinary Medicine (USAMV) Bucharest, utilizing a randomized complete block design (RCBD) with three replications.

Primary tillage consisted of deep autumn plowing at 30 cm, followed by seedbed preparation on March 25, 2025, using a disc harrow and a rotary tiller to achieve an optimal soil tilth. Sowing was performed manually on April 17, 2025, at a density of 50 seeds/m², with an inter-row spacing of 50 cm, an intra-row spacing of 6 cm, and a seeding depth of 4–5 cm. The area of each experimental plot was 14 m². Weed control was maintained at three-week intervals through two mechanical inter-row cultivations followed by two manual hoeings, completely avoiding herbicide application to adhere to sustainable agriculture principles. The vegetative period, from emergence to physiological maturity, lasted 83 days for Algil, 85 days for Cannellino, and 86 days for Florres (Table 1).

Table 1

Common bean cultivars: country of origin, growth habit, and phenological milestones (USAMV Bucharest, 2025)

Cultivar	Country of Origin*	Growth Habit**	Sowing Date	Emergence Date	Maturity Date
Algil	Romania (IULS)	I	17.04.2025	30.04.2025	22.07.2025
Cannellino	Italy	II	17.04.2025	02.05.2025	26.07.2025
Florres	Netherlands	II	17.04.2025	04.05.2025	28.07.2025

***IULS – Iasi University of Life Sciences*

*** I – determinate growth, erect stem; II – indeterminate growth, erect stem.*

Climatic conditions

According to NMA and meteoblue data, Bucharest experienced a positive mean annual temperature anomaly of +0.8 °C to +1.2 °C in 2025 relative to the 1991–2020 multi-annual baseline. This warming heavily impacted crops between June and August, when maximum temperatures exceeding 35 °C induced severe drought stress. Annual precipitation totaled 520–540 mm, falling slightly below the climatological average and exhibiting a highly uneven distribution.

A prolonged drought during spring and early summer was abruptly succeeded by short, torrential rain episodes characterized by convective phenomena, including hail and frequent electrical discharges. This rapid alternation

between severe moisture deficit and temporary excess further amplified agroecosystem stress (Figure 1).

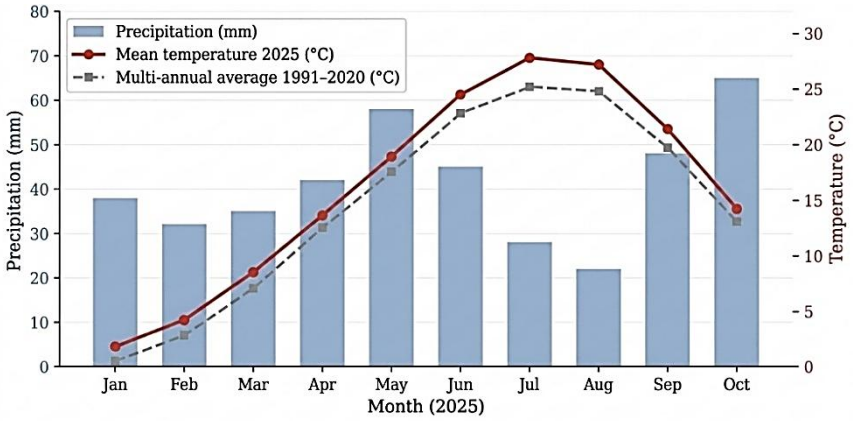


Figure 1. Variation of monthly mean temperatures and precipitation in Bucharest, January – October 2025.

Source: Own processing based on NMA (National Meteorological Administration) and meteoblue data (www.meteoblue.com).

Strain Characterization and Seed Inoculation

The indigenous strain *Pararhizobium giardinii* EnPv2 was isolated in 2021 from *Phaseolus vulgaris* nodules in Dolj County and validated via 16S rRNA sequencing and PCR detection of *nifH* and *nodC* genes. The strain was cultured on Yeast Mannitol Agar (YMA) medium at 28 °C for three days and stored at 4 °C. For inoculation, biomass was resuspended in sterile distilled water to a concentration of 4 × 10^9 CFU/mL (OD_{600} = 0.4). Seeds were treated on April 16, 2025, by applying 3 mL of bacterial suspension per 100 g of seeds. After manual homogenization and 10 minutes of rest, the seeds were air-dried on filter paper and sown the following day.

Data Collection

Nodule count, green biomass, and dry biomass were evaluated during the flowering phase from five randomly selected plants per replication. At maturity, yield parameters were recorded: pods per plant, seeds per pod, seeds per plant, thousand-seed weight (TSW, g), seed weight per plant, plot yield (kg/14 m^2), and total seed yield (kg/ha).

Data Processing and Descriptive Analysis

The experimental data obtained from the biometric and yield measurements were integrated and processed mathematically using Microsoft Excel 2021. For each evaluated parameter, mean values were calculated per replication, variant, and cultivar. The biostimulatory efficiency of the *Pararhizobium giardinii* EnPv2 inoculation was determined by calculating the relative percentage difference between the inoculated treatment (V1) and the non-inoculated control (V2).

RESULTS AND DISCUSSIONS

Throughout the 2025 growing season, significant physiological and phenotypic variations were observed between the common bean plants subjected to

Pararhizobium giardinii EnPv2 inoculation and the non-inoculated controls. These differences comprehensively manifested across vegetative growth parameters, root nodulation intensity, and yield components **(**Table 2, Table 3, and Table 4**).**

Table 2

Effect of *Pararhizobium giardinii* EnPv2 inoculation on green and dry biomass parameters of common bean cultivars

Cultivar	Treatment	Green Biomass (g/plant)	Relative Difference (%)	Dry Biomass (g/plant)	Relative Difference (%)
Algil	V1 – Inoculated	53.5	+25.88	13.0	+36.84
	V2 – Control	42.5		9.5	
Cannellino	V1 – Inoculated	52.5	+29.31	11.0	+23.60
	V2 – Control	40.6		8.9	
Florres	V1 – Inoculated	53.0	+26.49	12.2	+31.18
	V2 – Control	41.9		9.3	
MEAN			**+27.23**		**+30.54**

The biological efficiency of the rhizobial symbiosis is primarily reflected in the enhanced accumulation of dry matter and nitrogen within the host macroorganism (Kurlovich et al., 2004). The experimental data demonstrated that inoculation with *Pararhizobium giardinii* consistently promoted vegetative growth and significantly enriched biomass accumulation across all evaluated common bean cultivars. On average, dry biomass per plant increased by 30.54 % in inoculated variants compared to the non-inoculated controls. Genotypic variations were evident, with the Romanian cultivar Algil demonstrating the highest positive response to the bio-inoculant, achieving a 36.84 % increase in dry biomass. Conversely, cultivar Cannellino exhibited the lowest relative biomass gain (+23.60 %), indicating varying degrees of genetic affinity toward the EnPv2 strain.

Table 3

Effect of *Pararhizobium giardinii* EnPv2 inoculation on root nodule count during the flowering phenophase

Cultivar	Treatment	Nodule Number (nodules/plant)	Relative Difference (%)
Algil	V1 – Inoculated	5.2	+85.71
	V2 – Control	2.8	
Cannellino	V1 – Inoculated	4.6	+58.62
	V2 – Control	2.9	
Florres	V1 – Inoculated	5.0	+66.67
	V2 – Control	3.0	
MEAN			**+70.33**

Biometric assessments during the flowering stage confirmed that *Pararhizobium giardinii* EnPv2 successfully established functional symbioses, leading to a marked increase in root nodulation across all inoculated variants. The mean nodule count per plant rose by 70.33 % relative to the controls (Table 3). This substantial increase highlights the high infectivity and competitive colonization capacity of the indigenous strain under field conditions.

These findings align with previous literature demonstrating that effective rhizobial inoculation triggers early vegetative vigor and root development. Similar trends of biomass enhancement and increased nodulation profiling in *Phaseolus vulgaris* L. have been reported by Morad et al. (2013), who noted that optimized root colonization is highly correlated with shoot dry matter accumulation. Furthermore, the robust capability of indigenous isolates, such as the atypical rhizobia belonging to the *Pararhizobium* genus, to modulate early plant growth and establish functional nodules confirms the observations of Debnath et al. (2022), who underscored the high infectivity and competitive rhizosphere colonization behavior of *Pararhizobium giardinii* ecotypes.

Regarding yield components, thousand-seed weight (TSW) exhibited statistically negligible variations between the control and inoculated treatments, with an average relative increase of only 1.96 % (Table 4). The negligible variation observed in the thousand-seed weight (TSW) across all treatments corroborates the scientific consensus that seed size in common bean is a highly conserved genetic trait, heavily governed by the genotype rather than nutritional modifications. For instance, Vásquez Lozano et al. (2025) and Fageria et al. (2014) similarly noted that while bio-fertilization drastically improves reproductive sinks like pod and seed numbers per plant, it exerts minimal influence on individual seed weight (TSW), which remains stable under uniform grain-filling durations.

However, a strong positive response to *Pararhizobium giardinii* EnPv2 inoculation was observed across all other reproductive components: the number of pods per plant increased by 14.48 %, the number of seeds per plant rose by 14.99 %, and seed weight per plant was enhanced by 17.80 %. Consequently, plot yield increased by 11.47 %, resulting in a substantial overall seed yield gain of 12.28% per hectare compared to the non-inoculated controls (Table 4).

At the varietal level, the genotype Algil was distinguished by the highest productivity, yielding 1642 kg/ha in the inoculated treatment compared to 1428 kg/ha in the control variant. The cultivar Florres confirmed a stable responsiveness to interaction with the indigenous strain, recording a seed yield of 1257 kg/ha, which represents an increase of 115 kg/ha over the control lot.

For cultivar Cannellino, although yields were situated at a lower threshold, the impact of bacterization was remarkable in percentage terms, with productivity per hectare increasing from 900 kg/ha in the control to 1057 kg/ha in the treated variant (Table 4).

The recording of these yields, which remained below the actual genetic potential of the studied cultivars, was primarily determined by delayed and uneven seedling emergence. Low nocturnal temperatures immediately following sowing restricted the initial growth rate of the young plants and hampered their establishment process. These limiting factors were further compounded by severe drought stress between June and August, a climatic phenomenon that negatively affected the final harvest yield across all experimental variants.

Table 4

Structural yield components and productivity parameters of common bean cultivars under *Pararhizobium giardinii* EnPv2 inoculation (USAMV Bucharest, 2025)

Cultivar	Treatment	Pods /plant (nr.)	Seeds /plant (nr.)	Seed Weight /plant (g)	TSW (g)	Plot Yield (kg/14 m²)	Total Yield (kg/ha)
Algil	V1 – Inoculated	33.2	78.7	13.7	175.2	2.30	1642
	V2 – Control	28.9	67.2	11.5	172.5	2.00	1428
Cannellino	V1 – Inoculated	18.7	56.5	8.6	153.0	1.43	1057
	V2 – Control	16.5	46.6	6.9	150.2	1.26	900
Florres	V1 – Inoculated	28.9	69.5	9.7	139.6	1.76	1257
	V2 – Control	23.7	60.2	8.1	135.9	1.60	1142
Mean Relative Difference (%)		**+14.48**	**+14.99**	**+17.80**	**+1.96**	**+11.47**	**+12.28**

Overall, the agronomic seed yield (kg/ha) exhibited a general mean increase of 12.28 % under the influence of seed inoculation. These results demonstrate the high biological efficiency of strain EnPv2 in optimizing the nutritional balance of common bean plants, generating substantial yield gains and validating the potential for expanding the use of this indigenous bio-inoculant.

CONCLUSIONS

The field evaluation conducted during the 2025 growing season demonstrates the biostimulatory efficacy of the indigenous *Pararhizobium giardinii* EnPv2 strain, providing several key insights for sustainable common bean production: Seed inoculation successfully establishes highly active root symbioses across all evaluated cultivars, driving substantial improvements in both nodulation intensity and vegetative biomass accumulation. The bio-inoculant significantly optimizes reproductive sinks by increasing the number of pods and seeds per plant, as well as the total seed weight per plant. Conversely, thousand-seed weight (TSW) remains stable, confirming that individual seed size is a conserved genetic trait unaffected by bacterial treatment. Despite early-season thermal constraints delaying seedling emergence and mid-summer drought limiting absolute yields, the biological treatment generates secure agronomic yield gains per hectare, partially mitigating climate-induced stress.The Romanian cultivar Algil exhibits the highest physiological compatibility and responsiveness to the indigenous EnPv2 strain, identifying it as a premium genotype for bio-fertilized crop systems.

The experimental findings confirm that *Pararhizobium giardinii* EnPv2 represents a highly efficient biological tool. Its integration into low-input agricultural

practices offers a viable, eco-friendly strategy to reduce reliance on synthetic nitrogen fertilizers while optimizing common bean productivity in southern Romania**.**

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