



Research article

Role of *Rhizobium* inoculation and mycorrhizal fungi in enhancing growth of soybean seedlings in sterilized gypsum soil fertilized with phosphorus

Wael Mohammed Mahdi

Biotechnology Department, Applied Sciences College, University of Samarra, Salah-AL-Din 34010, Iraq

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Abstract

Importance of the work: Soybean production in sterile gypsum soils is constrained by low fertility and poor nutrient availability, particularly of nitrogen and phosphorus, which are essential for plant growth. Although biological inoculants, such as *Bradyrhizobium japonicum* Kirchner and *Glomus mosseae* T.H. Nicolson & Gerd, are known to improve nitrogen fixation and phosphorus uptake, there is insufficient understanding of their combined effects under gypsum soil conditions, especially in relation to phosphate fertilization.

Objectives: To assess the impact of *Rhizobium*, mycorrhizal fungi and phosphorus fertilization on soybean growth in sterilized gypsum soil.

Materials and Methods: A field experiment was conducted using a $4 \times 2 \times 2$ factorial arrangement in a randomized complete block design with three replications. The experimental factors consisted of phosphorus applied at four levels (0 kg/ha (control), 80 kg/ha, 160 kg/ha, 240 kg/ha), two rhizobial treatments (with and without *B. japonicum* inoculation) and two mycorrhizal treatments (with and without *G. mosseae* inoculation).

Results: The analysis of variance revealed significant 3-way interaction effects for all measured traits. Single inoculation with *Rhizobium* or mycorrhiza and phosphorus application significantly increased plant height (up to 71.13 cm/plant), vegetative dry weight (up to 3.22 g/plant) and root dry weight (up to 0.58 g/plant). Co-inoculation enhanced phosphorus concentration in the vegetative part (up to 0.63%). Mycorrhizal infection rates reached 67.33%. Phosphorus application and biofertilizer co-inoculation significantly improved all growth parameters and symbiotic efficiency in early soybean development.

Main finding: The triple interaction of *Rhizobium*, mycorrhizal fungi and phosphorus fertilizer significantly boosted soybean early growth, offering a sustainable strategy for improving productivity in nutrient-poor soils.

E-mail address: wael79@uosamarra.edu.iq (W.M. Mahdi)

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Introduction

Soybeans are one of the world's most important crops, as their grains are an important protein source and a source of vegetable oils (Hartman et al., 2011). In addition to their vitamin and mineral contents, soybeans provide approximately 60% of the world's vegetable protein and 30% of its oil (Aker et al., 2013). Several factors influence soybean productivity. Low yields are attributed to the lack of varieties adaptable to specific environmental conditions and poor soil and nutrient management (Dankwa et al., 2021)). The scarcity of varieties adaptable to specific agro-ecological conditions has contributed substantially to the current knowledge gap regarding the relationship between traits and seed yield (Ngalamu et al., 2013). The indiscriminate use of chemical fertilizers and pesticides has serious environmental consequences, which have driven the search for bio-based fertilizers and soil conditioners.

Biofertilizers have become an important research focus due to their effectiveness and environmental friendliness in increasing nutrient availability in the soil, stimulating root growth and uptake and reducing the application of chemical fertilizers (Abd-Alla et al., 2014; Koskey et al., 2017). Consequently, they may increase beneficial bacteria in the soil, eliminate harmful bacteria and reduce crop diseases. Rhizobial bacteria, specialized for soybeans, are one such type of biofertilizer, facilitating nitrogen fixation and reducing nitrogen emissions (Wei et al., 2023). Inoculation of soybeans with *Rhizobium* increases growth and seed yield (Jalaluddin, 2005; Tahir et al., 2009). Mycorrhizal fungi form a widespread symbiotic relationship, described as the result of mutual interactions between fungi and plants, with both organisms benefiting from the exchange of nutrients (Bonfante and Genre, 2008). Inoculation with mycorrhizal fungi may improve substantially the growth of soybean plants by enhancing plant height, shoot and root biomass and phosphorus uptake (Mahdi et al., 2004). In conventional agriculture, phosphorus fertilizers are used widely worldwide, particularly in soybean production. However, they have negative effects on environmental resilience. Therefore, it is recommended to introduce agricultural techniques that reduce the use of inorganic fertilizers in crop production. Phosphorus deficiency is a limiting factor for root nodule formation, growth and yield in soybean; however, this can be compensated for with phosphorus fertilization (Dobo, 2022). In addition, inoculation with arbuscular mycorrhizal fungi (AMF) is believed to

provide soybeans with the required phosphorus. These findings indicate that the dual inoculation of *Rhizobium* and arbuscular mycorrhizal fungi provides plants with the two most important nutrients for plant growth and therefore, to produce and sustain high-quality soybeans, particularly in semi-arid environments, the use of *Rhizobium* spp. and mycorrhizal fungi is encouraged. The genus *Rhizobium* is well-known for its plant growth-promoting traits, such as biological nitrogen fixation, production of phytohormones (such as auxins and cytokinins) and enhancement of nutrient uptake (Igíehon and Babalola, 2018). *Rhizobium*, *Pseudomonas*, *Azospirillum* and *Bacillus* species have had positive impacts on crops by enhancing both above and belowground biomass and therefore, could play positive roles in achieving sustainable agriculture. Igíehon et al. (2021) reported an increase in nutrient uptake and yield in soybean inoculated with mycorrhizal fungi and *Rhizobium* spp. In addition, Yu et al. (2024) reported found that in eight legumes, dual inoculation with AMF and rhizobia substantially increased the yield compared with a single inoculation. Dual inoculation with AMF and rhizobia has been demonstrated to promote root AMF colonization and nodulation (Liu et al., 2023), increase N, amino acids and soluble protein levels and raise root nitrogenase activity (Xie et al., 2020; Kavadia et al., 2021). Dual inoculation had a greater effect than single inoculation (Liu et al., 2023; Yu et al., 2024). However, there is little information on the effects of co-inoculation with nodular bacteria and arbuscular mycorrhizal fungi on plant growth (Gorgia and Tsikou, 2025). Although several studies have reported positive effects of dual inoculation with rhizobia and arbuscular mycorrhizal fungi on legumes (Razakatiana et al., 2020; Xie et al., 2020; Kavadia et al., 2021; Primieri et al., 2022; Yu et al., 2024), information remains limited regarding their combined interaction with phosphorus fertilization in nutrient-poor soils, such as sterilized gypsum soils, especially during the early stages of soybean growth. This interaction is still not fully understood, necessitating further investigation to understand its impact on plant growth in these specific environments.

Materials and Methods

Location of experimental area

A field experiment was conducted during the summer in sterile gypsiferous soil in the fields of the College of Agriculture, Tikrit University, Salah al-Din, Iraq (34.6528° N,

43.6361° E), to study the effect of *Bradyrhizobium japonicum* L and the fungal mycorrhiza *G. mosseae* T.H. Nicolson & Gerdon the growth of soybean seedlings at different levels of phosphate fertilization in sterilized gypsum soil.

Inoculants used

B. japonicum inoculum

The bacterial inoculum was prepared from the local strain *B. japonicum*, obtained from the Department of Soil Sciences, College of Agriculture, University of Anbar, Ramadi, Iraq. The isolate was grown on yeast mannitol agar media and activated to produce viable young cultures for use in field experiments in the laboratories of the Department of Soil Sciences, College of Agriculture, Tikrit University, Iraq, before inoculating the seeds for planting.

Preparation of bacterial inoculant

A sterile loop was used to transfer an equal amount of colonies aged 24 hr from a single pure culture of *B. japonicum* to a 250 mL flask containing 150 mL of sterile yeast mannitol broth medium. Then, the pH was adjusted to 7 and the culture was maintained for 3-6 d in a shaking incubator at 28°C and 100 revolutions per minute.

The pre-prepared carrier, produced by the Food and Agriculture Organization was prepared by passing it through a 100 µm sieve. Next, 225 g of the carrier was distributed into polyethylene bags, which were then moistened to a relative humidity of 50-60%. The bags were steam sterilized at 121°C at 1.5 kg/cm² for 15 min. Then, a sample (1 g) was aseptically collected from the sterilized carrier and streaked onto yeast extract mannitol agar (YMA) plates to verify sterility. The plates were incubated at 28°C for 5 days, and the absence of any microbial growth was considered evidence of successful carrier sterilization. (Olsen et al., 1996). Next, 25 g of calcium carbonate were added to each bag to adjust the pH and the sterilized bags were transferred to a laminar flow hood, where 150 mL of the previously prepared bacterial solution at the desired density was injected into each bag. Then, 100 mL of sterile distilled water was added to each bag using a needle and the bags were tightly sealed to prevent contamination.

Finally, the bags were incubated in a shaking incubator for 2 d to ensure proper carrier inoculation (Younis et al., 2025).

Mycorrhizal fungus inoculum

A vesicular-arbuscular mycorrhizal fungus inoculum, whose spores belonged to the species *G. mosseae*, was used,

containing the following materials: spores, infected roots and dry soil. The inoculum was obtained from the Department of Soil Sciences and Water Resources, College of Agriculture, Tikrit University, Iraq. A sample of the inoculum was examined to ensure the presence of pure *G. mosseae* spores according to the method of (Gerdemann and Nicolson, 1963). The inoculum was propagated by planting onion plants (*Allium cepa* L.) in plastic pots containing 2 kg of autoclaved soil at 121°C and a pressure of 1.5 kg/cm² for 1.5 hr for sterilization (Vincent, 1970; Kormanik et al., 1980; Louis and Lime, 1988; Davies and Linderman, 1991). Then, the resulting inoculum was mixed with the soil surrounding the roots and the mixture was placed in sterile nylon (polyethylene) bags and stored in a cool place until use.

Application of bio-fertilizer and chemical fertilizer

Three factors were used in these experiments: inoculation with *B. japonicum* bacteria, inoculation with *G. mosseae* fungi and phosphate fertilizer. The biofertilizers used in the experiment were prepared to contain approximately 1×10^8 cells/g viable cells per gram of carrier material (not per gram of soil) and phosphate fertilizer at three levels of P (80 kg/ha, 160 kg/ha, 240 kg/ha) in addition to the control treatment (without added phosphate fertilizer). This resulted in 48 experimental units. A randomized complete block design (RCBD) was used, with three replicates for each treatment. The factors in the results were represented by: R = *B. japonicum* inoculation (R0 and R1), M = *G. mosseae* inoculation: M0 and M1, P = phosphate fertilization: P0, P1, P2 and P3.

Land preparation, soil sterilization and experimental layout

The experimental land was prepared by ploughing, levelling and dividing it into equal sections. The experimental soil was sterilized with 2% formaldehyde and sprayed using a 100 L knapsack pump. The use of 2% formaldehyde for soil sterilization was based on its high efficacy in reducing native soil microbial populations, including bacteria, fungi and actinomycetes, to provide a homogeneous starting point for the experiment and to minimize the influence of native microorganisms when studying the role of added bioinoculants. While this concentration was relatively strong, it is commonly used in greenhouse and controlled environments, as well as in open soils, as described by Richter et al. (2022). In these environments, there is a high need for effective sterilization that reduces background microbial variation without aiming for

absolute sterilization. This treatment was expected to produce a considerable reduction in the native microbial community, but not its complete eradication, which aligned with the experimental objective. A set of 150 mL plastic cups filled with a diluted water-based formaldehyde solution were distributed 75 cm apart. Then, the entire experimental area was covered with a sheet of polyethylene for 48 hr and then left uncovered for 10 d before planting.

The experiment was divided into three equal sectors, 1 m apart, with each sector divided into 16 experimental units, each with dimensions of 2 m × 2.5 m. The distance between experimental units was 0.75 m. Factorial treatments were distributed randomly. Then seeds were planted in five rows per sector, with 0.60 m between rows and 0.10 m between plants. For several physical, chemical and biological analyses, soil samples were taken before planting in the experimental plot from a depth of 5–20 cm after removing the surface layer.

The experiment was fertilized with triple superphosphate at 80 kg/ha, 160 kg/ha and 240 kg/ha of P. Urea fertilizer $\text{CO}(\text{NH}_2)_2$ was added at 80 kg/ha of N and potassium sulfate (K_2SO_4) was added at a rate of 100 kg/ha of K. The urea and potassium sulfate were divided into three equal applications and applied using the band method adjacent to the planting line. They were mixed thoroughly into the surface layer of the soil at a depth of 20 cm, with the first application made before planting.

The field sterilization and the use of formaldehyde were approved by the Institutional Safety and Environmental Committee at the University of Samarra under permit number C.AGR.2024-11. All procedures complied with Iraq's national regulations governing the use of hazardous chemicals in agricultural research.

Seed germination

Soybean seeds were incubated according to Zhou et al. (2019) and Mahdi et al. (2025) in Petri dishes (diameter = 9 cm) on two layers of medium-speed qualitative filter paper. Eight seeds were placed in each Petri dish and 20 mL of sterile water were added. The dishes were incubated at 25°C. The final germination rate was approximately 90%.

Biofertilizer application, seed inoculation and planting

The *G. mosseae* inoculum, based on propagation according to the treatments specified in the experiment, was added at

a rate of 500 g per treatment, or 100 g per row, in furrows dug along the planting lines to a depth of 10 cm. According to the previously specified treatments, the soybean seeds were inoculated with the *B. japonicum* inoculum 1 hr before planting (Alkurtany et al., 2018). The inoculated and uninoculated soybean seeds were sown in rows 0.60 m apart and 3 cm deep. The bacterially inoculated seeds were sown, then covered with fine soil after planting to ensure good seedling emergence. Drip irrigation was used immediately after sowing, based on a regular network of strip pipes along the planting rows. Water was applied to maintain soil moisture close to field capacity throughout plant growth. At 1 wk after germination and seedling emergence, empty areas were > using uniform 7-day-old soybean seedlings. The replacement seedlings were raised concurrently and subjected to the same Rhizobium inoculation, AMF inoculation, and phosphorus treatments as those applied to the corresponding experimental units, thereby ensuring experimental integrity and uniform plant density. and plants were thinned in dense areas to 0.10 m between plants, with 18 plants per row. Crop maintenance operations, such as hoeing, weeding, fertilizing and washing plants after exposure to dust, were carried out before sampling (Singh et al., 2023).

Measurements and analyses

According to the methods described, physical, chemical and biological analyses were performed on the experimental soil before planting (Alkurtany et al., 2018). The results are presented in Table 1.

Table 1 Physical, chemical and biological characteristics of experimental soil before planting

Soil characteristic	Unit	Value
EC electrical conductivity	Ms/cm	2.18
Soil pH	-	7.83
Cation exchange capacity CEC	Cmol (+)/kg	22.10
Organic matter OM	g/kg	15.57
Gypsum CaSO_4	g/kg	153
Available nitrogen N	mg/kg	26.23
Available phosphorus P	mg/kg	8.33
Available potassium K	mg/kg	89.50
Sand	g/kg soil	516
Loam	g/kg soil	190
Clay	g/kg soil	294
Texture class	-	Sandy clay loam
Biological characteristics (before sterilization)		
Total numbers of fungi	CFU/gm soil	5×10^8
Total numbers of bacteria	CFU/gm soil	102×10^6

Growth evaluation

Five plants were randomly sampled from each experimental unit 1 mth after planting for measurement and analyses of: plant height (centimeters per plant), shoot dry weight (grams per plant) and root dry weight (grams per plant). In addition, mycorrhizal colonization was assessed, according to Begoude et al. (2016), Spore density was determined in the laboratories of the College of Agriculture and the College of Samarra Education, Tikrit University, Iraq.

Statistical analyses

The experiment was arranged in a factorial design ($2 \times 2 \times 4$) within an RCBD with three replications. The factors consisted of *Rhizobium* inoculation (two levels), mycorrhizal inoculation (two levels) and phosphorus fertilization (four levels). Treatment factors and their interactions were considered fixed effects, while blocks (replications) were treated as a random effect. Data were analyzed using analysis of variance in the SAS software (version 9.1.3; SAS Institute Inc, 2004). When significant differences were detected, treatment means were compared using Duncan's multiple range test at $p < 0.05$. Plot means were considered the experimental units for statistical analysis (Shome et al., 2022).

Results and Discussion

The analysis of variance showed that the main effects of phosphorus fertilization (P), mycorrhizal inoculation (M) and rhizobial inoculation (R) affected plant height, dry weight of vegetative mass, dry weight of the root system and percentage of mycorrhizal infection (Table 2). Likewise, all interaction effects, ($P \times M$, $P \times R$, $M \times R$ and $P \times M \times R$) were significant for all studied traits. Based on these results, each factor independently influenced soybean growth and mycorrhizal

infection, while their combined effects further modified the magnitude and direction of these responses.

Plant height

Although the main effects of inoculation with *B. japonicum*, inoculation with *G. mosseae* and phosphate fertilization and their two-way interactions showed significant effects on soybean plant height, the three-way interaction was also significant. This implied that the effect of each factor on plant height depended on the level of the other two factors, indicating a combined and interdependent influence of *B. japonicum*, *G. mosseae* and phosphate fertilization on soybean growth.

Based on the results, the *B. japonicum* inoculated treatments significantly outperformed the uninoculated treatments, producing an average height of 59.52 cm/plant, compared to the 54.63 cm/plant for the uninoculated treatments (Table 3), an increase of 8.95%. The increase in this trait may have been due to the positive effect of nodular bacteria in fixing atmospheric nitrogen, thus achieving a state of balanced plant nutrition that led to improved growth, especially in height. These results were consistent with the findings of Egamberdieva (2018).

Inoculation with mycorrhizal fungi also significantly affected plant height, with the inoculated treatments outperforming the uninoculated ones. The inoculated treatments had an average plant height of 62.08 cm/plant (Table 3), while the uninoculated treatments increased 19.22% (52.07 cm/plant). The reason for this increase may have been due to the ability of the mycorrhizal fungi to extend a network of hyphae outside the plant roots, thus improving the absorption of essential nutrients, especially P, and increasing the plant's ability to absorb nutrients, thus increasing its growth (Musyoka et al., 2020). In addition, mycorrhizal fungi may secrete growth regulators that contribute to plant growth (Barker and Tagu, 2000). This was consistent with Wahab et al. (2023).

Table 2 p -Values from three-way analysis of variance for effects of phosphate level (P), *Bradyrhizobium japonicum* inoculation (R) and *Glomus mosseae* inoculation (M) and their interactions, on soybean growth and mycorrhizal infection traits

Main effect	Plant height	Dry weight of vegetative mass	Dry weight of the root system	Percentage of mycorrhizal infection
P	0.012	0.0003	0.0021	0.018
M	0.004	<0.0001	<0.0001	<0.0001
R	0.021	0.0012	0.0184	0.009
Interaction				
P×M	0.087	0.041	0.033	0.047
P×R	0.318	0.214	0.276	0.221
M×R	0.064	0.073	0.091	0.039
P×M×R	0.412	0.389	0.447	0.366

Table 3 Main effects of phosphate level (P), *Glomus mosseae* inoculation (M) and *Bradyrhizobium japonicum* inoculation (R) on measured traits of soybean grown in sterile gypsum soil

	Plant height	Dry weight vegetative mass (g)	Dry weight of the root system (- g)	Mycorrhizal infection (%)
P				
P0	52.10±1.95 ^b	1.86±0.16 ^c	0.23±0.03 ^c	42.67±3.49 ^a
P1	57.20±2.03 ^a	2.13±0.17 ^b	0.31±0.04 ^b	53.67±2.65 ^a
P2	60.21±2.19 ^a	2.36±0.18 ^{ab}	0.36±0.04 ^{ab}	46.17±3.01 ^a
P3	58.29±1.94 ^a	2.41±0.17 ^a	0.41±0.04 ^a	33.11±3.12 ^b
M				
M0	52.07±1.33 ^b	1.62±0.04 ^b	0.22±0.02 ^b	32.83±2.11 ^b
M1	62.08 ±1.97 ^a	2.77±0.08 ^a	0.44±0.02 ^a	55.50±1.67 ^a
R				
R0	54.63±1.66 ^b	1.93±0.12 ^b	0.28±0.03 ^b	39.41±2.45 ^b
R1	59.52±2.09 ^a	2.44±0.14 ^a	0.37±0.04 ^a	48.91±2.39 ^a

M0 = without mycorrhizal inoculation; M1 = with mycorrhizal inoculation; R0 = without rhizobial inoculation; R1 = with rhizobial inoculation; P = triple superphosphate fertilizer; P0 = control (0 kg/ha of P); P1 = 80 kg/ha of P; P2 = 160 kg/ha of P; P3 = 240 kg/ha of P.

Mean ± SD values within same column under each treatment factor (P, M, R) followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Phosphate fertilization also significantly affected plant height. Addition at levels of 80 kg/ha of P, 160 kg/ha of P and 240 kg/ha of P led to increased height (9.78%, 16.52% and 11.8%, respectively) in the fertilized treatments compared to the unfertilized control treatment. At the same time, there were no significant differences between the three fertilization levels (80 kg/ha of P, 160 kg/ha of P and 240 kg/ha of P) added to the soil (Table 3). The increase in plant height in the fertilized treatments may have been due to the vital role played by P in the formation of energy molecules (ATP) that are involved in the synthesis of nucleic acids (DNA and RNA), which enhance the efficiency of photosynthesis, thus increasing the size of

living cells and improving plant growth (Khan et al., 2023).

In the two-way interaction between *B. japonicum* inoculation and phosphate fertilization, the inoculated and fertilized treatments outperformed either rhizobial inoculation alone or phosphate fertilization alone (Table 4). The inoculated and fertilized treatment at the level of 160 kg/ha of P had a significantly greater mean plant height (62.76 cm) than the uninoculated and unfertilized treatment (49.54 cm). The increase in plant height may have been due to the formation of root nodules by rhizobial bacteria and their clear activity in nitrogen fixation, together with balanced P nutrition (Tomar et al., 2004). These results were consistent with Menaria et al. (2003).

Table 4 Two-way interaction effects of phosphate level (P), *Glomus mosseae* inoculation (M) and *Bradyrhizobium japonicum* inoculation (R) on measured traits of soybean grown in sterile gypsum soil

		Plant height	Dry weight vegetative mass (g)	Dry weight of the root system (g)	Mycorrhizal infection (%)
P0	M0	47.63±1.88 ^c	1.29±0.08 ^c	0.13±0.02 ^f	29.66±2.79 ^{cd}
	M1	56.56±2.01 ^{bcd}	2.43±0.14 ^b	0.33±0.03 ^c	55.33±2.11 ^a
P1	M0	53.8±1.51 ^{cde}	1.53±0.09 ^{de}	0.20±0.03 ^{ef}	44.33±3.49 ^b
	M1	63.2±2.35 ^{ab}	2.73±0.16 ^{ab}	0.43±0.04 ^{bc}	63.00±3.65 ^a
P2	M0	53.8±1.51 ^{cde}	1.71±0.08 ^{cd}	0.24±0.04 ^{de}	35.33±1.84 ^a
	M1	67.63±2.62 ^a	3.01±0.18 ^a	0.47±0.08 ^{ab}	57.00±3.24 ^a
P3	M0	55.64±1.40 ^{cd}	1.92±0.07 ^c	0.31±0.05 ^d	22.00±4.54 ^d
	M1	60.95±2.50 ^{abc}	2.89±0.16 ^a	0.52±0.06 ^a	46.66±3.42 ^b
P0	R0	49.54±2.05 ^c	1.58±0.11 ^d	0.19±0.06 ^c	38.0±5.09 ^d
	R1	54.66±1.90 ^{bc}	2.15±0.14 ^{bc}	0.27±0.05 ^{bc}	47.00±4.41 ^{bc}
P1	R0	54.61±1.92 ^{bc}	1.85±0.13 ^{cd}	0.26±0.06 ^{de}	50.33±5.37 ^{ab}
	R1	59.79±2.13 ^{ab}	2.41±0.15 ^{ab}	0.37±0.06 ^b	57.00±4.31 ^a
P2	R0	58.66±2.16 ^{ab}	2.13±0.14 ^{bc}	0.31±0.07 ^{de}	41.33±3.90 ^{cd}
	R1	62.76±2.33 ^a	2.58±0.16 ^a	0.40±0.09 ^a	51.00±4.52 ^{ab}
P3	R0	55.7±1.87 ^{abc}	2.17±0.11 ^{bc}	0.38±0.08 ^{bc}	28.00±5.48 ^c
	R1	60.89±2.40 ^{ab}	2.64±0.17 ^a	0.45±0.08 ^a	40.66±3.52 ^{cd}
M0	R0	50.44±1.52 ^c	1.34±0.08 ^d	0.19±0.02 ^d	27.66±2.76 ^d
	R1	53.70±1.69 ^c	1.88±0.05 ^c	0.25±0.02 ^c	38.00±1.38 ^c
M1	R0	58.82±2.06 ^b	2.53±0.06 ^b	0.38±0.02 ^b	51.16±1.93 ^b
	R1	65.35±2.40 ^a	3.00±0.11 ^a	0.50±0.04 ^a	59.83±2.21 ^a

M0 = without mycorrhizal inoculation; M1 = with mycorrhizal inoculation; R0 = without rhizobial inoculation; R1 = with rhizobial inoculation; P = triple superphosphate fertilizer; P0 = control (0 kg/ha of P); P1 = 80 kg/ha of P; P2 = 160 kg/ha of P; P3 = 240 kg/ha of P.

Mean ± SD values within same column for each treatment factor combination (P×M, P×R, M×R) followed by different lowercase superscripts are significantly ($p < 0.05$) different.

The interaction effect between *G. mosseae* inoculation and phosphate fertilization significantly affected plant height (Table 4). The inoculated and fertilized treatment at 160 kg/ha of P significantly outperformed most treatments, with an average plant height of 67.63 cm/plant, representing a 41.99% increase over the uninoculated and unfertilized treatment recorded (47.63 cm/plant). There was no significant difference between the inoculated and fertilized treatments at the three fertilization levels. These results were similar to those reported by Techapinyawat et al. (2002).

The treatment inoculated with *Rhizobium* and mycorrhizal fungi was significantly superior with an average height of 65.35 cm/plant compared to the treatments inoculated with mycorrhizal fungi alone and *Rhizobium* alone and the uninoculated control (58.82 cm/plant, 53.70 cm/plant and 50.44 cm/plant, respectively). The superiority may be attributed to the formation of a dual symbiotic system involving rhizobial bacteria and *G. mosseae*, which supplied the plant with nitrogen through biological nitrogen fixation by rhizobial bacteria and enhanced P uptake through the mycorrhizal fungus (Koegel et al., 2013; Shen and Feng, 2024).

Notably, plant height was also affected by the three-way interaction of the study factors (Table 5). The treatment inoculated with *Rhizobium* and mycorrhiza and fertilized at 160 kg/ha of P outperformed most treatments (except P1M1R1 and P3M1R1), yielding the highest average plant height of 71.13 cm/plant and highlighting the synergistic effect of phosphate fertilizer application with the inoculation of both *B. japonicum* and *G. mosseae*.

Dry weight of vegetative mass

The main effect of inoculation with *B. japonicum*, *G. mosseae*, or phosphate fertilization, was significant for the dry weight of the vegetative mass of soybean plants. Based on these results, inoculation with *Rhizobium* alone had a significant effect and the inoculated treatments outperformed the uninoculated

treatments, yielding an average dry weight of the vegetative mass of 2.44 g/plant. In comparison, the average dry weight of the uninoculated treatments was 1.93 g/plant, an increase of 26.42%. The positive role of the symbiotic relationship between rhizobial bacteria and legumes may explain the increase observed in the inoculated treatments, as these bacteria provide the plant with the nitrogen it needs through biological fixation of atmospheric nitrogen, which in turn contributes to the construction of amino acids and proteins in the plant and enhances growth, thereby possibly affecting the dry weight (Yeremko et al., 2025). The results were also consistent with Sajjad et al. (2024), who reported significant increases in fresh root and shoot weights and dried root and shoot weights, after seed inoculation with *B. japonicum* root strains.

Inoculation with AMF resulted in the inoculated treatments significantly outperforming the uninoculated ones, with average dry weights of 2.77 g/plant and 1.62 g/plant, respectively (an increase of 70.98%). The significant increase in the dry vegetative weight may be attributed to the role of infection with mycorrhizal fungi in stimulating the absorption of major nutrients, especially P, and increasing the rates of photosynthesis resulting from the increase in leaf area, as well as the absorption of some micronutrients, such as copper and zinc, in addition to a possible hormonal influence and nitrogenase activity (Huey et al., 2020; Balestrini et al., 2020).

The additions of 80 kg/ha of P, 160 kg/ha of P and 240 kg/ha of P all led to significant increases in the dry weight of the vegetative system of the fertilized treatments compared to the unfertilized treatments (Table 3). The fertilized treatment at the 240 kg/ha of P level had the highest average dry weight (2.41 g/plant), with an increase of 29.56% compared to the lowest average dry weight of the unfertilized treatment (1.86 g/plant). The increase in the dry weight of the vegetative system continued to the level of 240 kg/ha of P, although there was no significant difference between it and the level of 160 kg/ha of P. The increase in the dry weight of the vegetative system due to the increase in phosphate fertilization levels may have been

Table 5 Effect of combined treatments of phosphate level (P), *Bradyrhizobium japonicum* inoculation (R) and *Glomus mosseae* inoculation (M) on plant height (cm/plant) of soybean grown in sterile gypsum soil

P	M0		M1	
	R0	R1	R0	R1
P0	43.90±2.03 ^f	51.36±2.6 ^{def}	55.17±2.96 ^{cde}	57.95±2.56 ^{bcd}
P1	49.96±2.40 ^{ef}	52.43±2.36 ^{ef}	59.26±2.06 ^{bcd}	67.15±5.15 ^{ab}
P2	53.20±1.36 ^{def}	54.39±1.04 ^{cdef}	64.13±2.75 ^{abcd}	71.13±5.92 ^a
P3	54.68±0.85 ^{cdef}	56.60±2.15 ^{bcd}	56.72±1.61 ^{bcd}	65.19±6.75 ^{abc}

M0 = without mycorrhizal inoculation; M1 = with mycorrhizal inoculation; R0 = without rhizobial inoculation; R1 = with rhizobial inoculation; P = triple superphosphate fertilizer; P0 = control (0 kg/ha of P); P1 = 80 kg/ha of P; P2 = 160 kg/ha of P; P3 = 240 kg/ha of P.

Mean ± SD values followed by different lowercase superscripts are significantly ($p < 0.05$) different.

due to the positive correlation between the increase in Plant height and the number of branches, as reflected in the increase in the dry weight of the vegetative system, as explained by Al-obaidi et al. (2010), or it may be attributed to the encouragement of P to form a deep and dense root system, which helped to absorb water and nutrients. These results were consistent with the Feng et al. (2021) and Khan et al. (2023).

The interaction between *Rhizobium* inoculation and phosphate fertilization significantly affected this trait and the interaction values were higher than those of *Rhizobium* inoculation alone or phosphate fertilization alone (Table 4). The inoculated and fertilized treatment at the 240 kg/ha of P level was superior, with an average of 2.64 g/plant. In contrast, the average of this trait in the uninoculated, unfertilized treatment was 1.58 g/plant. This increase may be attributed to the role of P in improving the plant's vegetative growth and encouraging the nitrogen fixation process, which requires P as an energy source. This was positively reflected in the dry weight of the vegetative mass of the soybean plant.

The interaction between inoculation with AMF and phosphate fertilization resulted in significant differences in the dry weight of the vegetative mass and the interaction values were better than the values for inoculation with mycorrhiza alone or phosphate fertilization alone. The average dry weight of the vegetative mass for the inoculated and fertilized treatment at 160 kg/ha of P was 3.01 g/plant. In contrast, the uninoculated and unfertilized treatment within this interaction was 1.29 g/plant.

The inoculated and unfertilized treatment also significantly outperformed all uninoculated and fertilized treatments and the control treatment and had an average dry weight of the vegetative mass of 2.43 g/plant, while the uninoculated and fertilized treatment at the level of 240 kg/ha of P had an average vegetative dry weight of 1.92 g/plant, followed by the average dry weight of the vegetative mass of the control treatment (1.29 g/plant). Furthermore, the uninoculated treatment fertilized at the 240 kg/ha of level significantly outperformed the uninoculated treatment fertilized at the 80 kg/ha of P level and the control

treatment. There was no significant difference between it and the uninoculated treatment fertilized at 160 kg/ha of P. The treatments inoculated with mycorrhizal fungi and not fertilized had higher levels of dry matter production than those not inoculated and fertilized at 240 kg/ha of P. This confirmed the importance of inoculation with efficient AMF to increase the production of leguminous crops in soils poor in available phosphorus, with the possibility of reducing all or part of the added fertilizers, as confirmed by Siqueira and de Paula (1986), who reported that inoculation with mycorrhizal fungi produced an increase in growth equivalent to adding 210 parts per million P_2O_5 .

The results of inoculation with *Rhizobium* and mycorrhizal fungi showed a clear and significant superiority in the dry weight of the plant's vegetative mass at this stage. The treatment inoculated with *Rhizobium* and mycorrhizal fungi was significantly superior to the treatments inoculated with mycorrhizal fungi alone, *Rhizobium* alone and the uninoculated treatment, with percentage increases of 18.57%, 59.57% and 123.88%, respectively. The increase may be attributed to the beneficial effect of mycorrhizal fungi in absorbing nutrients, especially P, which is important in the formation of energy molecules (ATP), which enter into the construction of nucleic acids (DNA and RNA), thus improving plant growth and enhancing the formation of root nodules, which was positively reflected in increasing the dry weight of the vegetative mass. This was consistent with the results reported by Demir and Akkopru (2007) that AMF and rhizobial bacteria were the two most important organisms in coexistence with plants, as they played a major role in natural ecosystems and in improving plant nutrition, disease resistance, growth and production.

The three-way interaction between *Rhizobium*, mycorrhiza and P significantly affected the dry weight of the vegetative mass (Table 6). The treatment inoculated with *Rhizobium* and mycorrhiza and fertilized at the level of 160 kg/ha of P tended to result in higher plant dry weight (3.22 g/plant), although it did not differ significantly from the treatment receiving 240 kg/ha of P with the same inoculation combination.

Table 6 Effect of combined treatments of phosphate level (P), *Bradyrhizobium japonicum* inoculation (R) and *Glomus mosseae* inoculation (M) on dry weight of soybean plant vegetative mass (g/plant) grown in sterile gypsum soil

P	M0		M1	
	R0	R1	R0	R1
P0	0.96±0.08 ^h	1.63±0.13 ^{efg}	2.20±0.20 ^{cde}	2.67±0.33 ^{abc}
P1	1.18±0.21 ^{gh}	1.87±0.11 ^{ef}	2.52±0.20 ^{bcd}	2.94±0.18 ^{ab}
P2	1.47±0.13 ^{figh}	1.94±0.15 ^{ef}	2.80±0.11 ^{ab}	3.22±0.16 ^a
P3	1.75±0.19 ^{ef}	2.09±0.10 ^{de}	2.59±0.10 ^{bcd}	3.19±0.28 ^a

M0 = without mycorrhizal inoculation; M1 = with mycorrhizal inoculation; R0 = without rhizobial inoculation; R1 = with rhizobial inoculation; P = triple superphosphate fertilizer; P0 = control (0 kg/ha of P); P1 = 80 kg/ha of P; P2 = 160 kg/ha of P; P3 = 240 kg/ha of P.

Mean ± SD values followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Root dry weight

The main effects were significant for all treatment factors (Table 3). The treatment inoculated with *Rhizobium* alone significantly outperformed those without inoculation, yielding an average root dry weight of 0.37 g/plant. In comparison, the uninoculated treatments had a dry weight of 0.28 g/plant, representing an increase of 32.14%. *Rhizobium* bacteria stimulate the formation of root nodules that convert atmospheric nitrogen into absorbable ammonia, improving plant metabolism and increasing root mass. Some *Rhizobium* strains also produce plant hormones such as auxins that promote root elongation and branching, increasing the surface area for nutrient absorption (Ngosong et al., 2022).

Inoculation with AMF also significantly affected the dry weight of the plant's root system. The inoculated treatments outperformed the uninoculated ones, as the inoculated treatments had an average dry weight of 0.44 g/plant which was twice that of the uninoculated treatments (0.22 g/plant). This may have been because the fungi improved the plant's absorption of nutrients and water and promoted root growth and branching, significantly increasing root mass. In other words, the fungi provided a more favorable environment for root growth and nutrition, thereby increasing root dry weight (Cruz and Husain, 2008; Zhou et al., 2025).

The effect of phosphate fertilization was also significant (Table 3). The dry weight of the plant's root system increased with the increase in the P level, with the highest value recorded at 240 kg/ha of P, which in turn significantly outperformed the other two P treatments, being 32.25% greater than the 80 kg/ha of P and 78.25% greater than the unfertilized treatment. Root growth and distribution are closely linked to P distribution in the soil, with P availability stimulating root growth and increased root density in soil layers containing higher phosphorus levels. In addition, Nunes et al. (2021) reported that increasing the phosphate fertilization rate led to increased root biomass and P uptake, positively impacting crop productivity.

Table 4 shows that the interaction between *Rhizobium* inoculation and phosphate fertilization significantly affected the dry weight of the plant's root system. The two treatments inoculated with *Rhizobium* and fertilized at 160 kg/ha of P and 240 kg/ha of P significantly outperformed the other treatments. The treatment inoculated and fertilized at 240 kg/ha of P had an average dry weight of the root system of 0.45 g/plant, followed by the treatment inoculated and fertilized at

160 kg/ha of P (0.40 g/plant), whereas the treatment not inoculated with *Rhizobium* and not fertilized had an average dry weight of the root system of 0.19 g/plant. This increase may have been due to the role of P nutrition in increasing the dry weight of the plant through the production of sugar compounds that promoted greater root growth, which increased the surface area of the root hairs, which may have increased the chance of infection with root nodule bacteria (Purwaningsih et al., 2021).

Based on the results, the interaction between AMF and phosphorus had a significant effect, as the treatment inoculated with mycorrhizal fungi and fertilized at 240 kg/ha of P significantly outperformed all the two-way interaction treatments (except for the treatment inoculated and fertilized at 160 kg/ha of P), with the inoculated and fertilized treatment receiving 240 kg/ha of P having the highest mean root system dry weight (0.52 g/plant), compared with the significantly lower uninoculated and unfertilized control (0.13 g/plant). Notably, the response of the dry weight of the root system largely followed the response of the dry weight of the vegetative system, which aligned with Barznjy et al. (2025), who found that broad bean plants inoculated with mycorrhizal fungi and added P had increased dry weight compared to the control treatment. In their study, the highest root dry weight (7.00 g) was recorded with the addition of 160 kg/ha of P, while the lowest was in the treatment without phosphorus application. This may be attributed to the role of mycorrhizal fungi in lengthening the plant's root system, as well as increasing its characteristics such as root surface area, extent, diameter, density and branching. This, in turn, enhanced the plant's ability to absorb more nutrients, leading to increased plant growth, increased root biomass and higher root dry weight. (Banni et al., 2014; Spagnoletti et al., 2018).

Furthermore, inoculation with *Rhizobium* and mycorrhizal fungi significantly affected the dry weight of the plant's root system, outperforming the treatments inoculated with mycorrhizal fungi alone, *Rhizobium* alone and uninoculated, as the average dry weight of the root system for the treatment inoculated with *Rhizobium* and mycorrhizal fungi was 0.50 g/plant. In contrast, the treatments inoculated with mycorrhizal fungi alone, *Rhizobium* alone and uninoculated were 0.38 g/plant, 0.25 g/plant and 0.19 g/plant. These results were consistent with the results reported by Babajide et al. (2009) for soybean.

The three-way interaction between *Rhizobium*, mycorrhiza and P was significant for root dry weight (Table 7). The treatment inoculated with *Rhizobium* and mycorrhiza and fertilized at 240 kg/ha of P had the highest average dry weight of the root system (0.58 g/plant). In contrast, the uninoculated and unfertilized treatment significantly decreased the dry weight, reaching 0.10 g/plant. The synergistic effect between microbes and nutrients plays an important role in soil. An integrated system can be formed to enhance root growth through the interaction between *Rhizobium*, mycorrhizal fungi and P. Mycorrhizal fungi enhance P uptake through an extended fungal network, while *Rhizobium* fixes nitrogen, directly stimulating root growth (Zhang et al., 2023).

Percentage of mycorrhizal-infected roots

Table 8 indicates that inoculation with *Rhizobium* alone significantly affected the percentage of mycorrhizal-infected roots, with inoculated treatments outperforming uninoculated ones. Inoculated treatments yielded the highest percentage of mycorrhizal-infected roots (48.91%) compared to uninoculated treatments (39.41%). The positive effect may be attributed to inoculation with beneficial bacteria, which enhanced positive interactions between plants and fungi in the root zone, improving the fungi’s ability to colonize the roots and establish beneficial colonization (Ngosong et al., 2022).

Inoculation with AMF significantly affected the percentage of roots infected with mycorrhiza, with the inoculated treatments outperforming the uninoculated ones, since the inoculated treatments had the highest percentages of roots infected with mycorrhiza (55.50%) compared to the uninoculated treatments (32.83%). This may have been due to the increased biological efficiency of the fungal inoculum, which has a greater ability to colonize roots than uninoculated soil. For example, Abbott and Robson (1981) showed that using inocula containing *G. mosseae* could significantly increase the incidence of root infection compared to natural conditions.

In the current study, the addition of phosphate fertilizer significantly decreased the incidence of mycorrhizal fungi in the treatments fertilized at 240 kg/ha of P compared to the control, 80 kg/ha of P and 160 kg/ha of P, which were not significantly different (Table 3). The P concentration increases in plant tissues due to phosphate fertilization, which reduces the plant’s need for mycorrhizal fungi to uptake phosphorus (Ferrol et al., 2019). Specifically, plants can downregulate the signals that attract the fungi and limit fungal entry into the roots (Balzergue et al., 2011; Ho-Plágaro and García-Garrido, 2022). Excess phosphate may alter root physiology and reduce the production of certain compounds needed to maintain the symbiotic relationship. This regulation protects the plant from excessive fungal colonization, which could be costly when nutrient availability through fertilizer is sufficient.

Table 7 Effect of combined treatments of phosphate level (P), *Bradyrhizobium japonicum* inoculation (R) and *Glomus mosseae* inoculation (M) on dry weight of the root system (g/plant) of soybean grown in sterile gypsum soil

P	M0		M1	
	R0	R1	R0	R1
P0	0.10±0.03 ^j	0.16±0.02 ^{ghij}	0.28±0.10 ^{ef}	0.38±0.05 ^c
P1	0.15±0.03 ^{ij}	0.24±0.03 ^{fgh}	0.37±0.06 ^{de}	0.49±0.04 ^{abc}
P2	0.21±0.03 ^{hi}	0.27±0.08 ^{efg}	0.41±0.11 ^{cd}	0.53±0.13 ^{ab}
P3	0.30±0.06 ^{ghi}	0.31±0.08 ^{ef}	0.45±0.15 ^{abc}	0.58±0.12 ^a

M0 = without mycorrhizal inoculation; M1 = with mycorrhizal inoculation; R0 = without rhizobial inoculation; R1 = with rhizobial inoculation; P = triple superphosphate fertilizer; P0 = control (0 kg/ha of P); P1 = 80 kg/ha of P; P2 = 160 kg/ha of P; P3 = 240 kg/ha of P. Mean ± SD values followed by different lowercase superscripts are significantly ($p < 0.05$) different.

Table 8 Effect of combined treatments of phosphate level (P), *Bradyrhizobium japonicum* inoculation (R) and *Glomus mosseae* inoculation (M) on mycorrhizal infection percentage of soybean grown in sterile gypsum soil

Phosphate level	M0		M1	
	R0	R1	R0	R1
P0	24.00±3.06 ^{gh}	35.33±3.53 ^{efg}	52.00±4.16 ^{bcd}	58.66±0.67 ^{abc}
P1	42.00±7.57 ^{def}	46.66±1.76 ^{cde}	58.66±6.01 ^{abc}	67.33±4.81 ^a
P2	31.33±1.76 ^{fg}	39.33±1.33 ^{def}	51.33±4.37 ^{bcd}	62.66±4.06 ^{ab}
P3	13.33±3.33 ^h	30.66±3.06 ^{fg}	42.66±2.40 ^{def}	50.66±0.67 ^{bcd}

M0 = without mycorrhizal inoculation; M1 = with mycorrhizal inoculation; R0 = without rhizobial inoculation; R1 = with rhizobial inoculation; P = triple superphosphate fertilizer; P0 = control (0 kg/ha of P); P1 = 80 kg/ha of P; P2 = 160 kg/ha of P; P3 = 240 kg/ha of P. Mean ± SD values followed by different lowercase superscripts are significantly ($p < 0.05$) different.

This effect may be related to plant regulatory mechanisms that inhibit fungal reproduction when sufficient P is available (Balzergue et al., 2013). Alternatively, increased soil P concentrations could inhibit mycorrhizal fungi by reducing the availability of organic matter to the fungi (Cavagnaro et al., 2015).

Considering the two-way interaction (Table 4) between phosphate fertilization and inoculation with *Rhizobium* on the percentage of roots infected with mycorrhiza, the inoculated and fertilized treatment with 80 kg/ha of P was significantly superior and had the highest infection rate (57.00%) compared to the uninoculated and unfertilized treatment, which had the lowest infection rate (28.00%). AMF form a mutually beneficial partnership with plant roots, where the fungus relies on the host plant for carbon (sugars and lipids), while obtaining nutrients from the soil through its fungal network. In return, the fungus delivers P, N and other essential elements to the host root. Often, these nutrients are less physically and chemically accessible to the host plant; however, the fungus can absorb and actively transport them via its mycelia (Van't Padje et al., 2021)—this may also be related to the improved overall health of the plant resulting from phosphorus addition which stimulates proline production and chlorophyll formation and is reflected in the development and reproduction of mycorrhizal fungi (Timofeeva et al., 2023).

Considering the interaction effect between inoculation with AMF and phosphate fertilization, the treatments inoculated at levels of 0 kg/ha of P, 80 kg/ha of P and 160 kg/ha of P were significantly superior to the treatment inoculated and fertilized with a P level of 240 kg/ha and the treatments only fertilized. The control treatment (inoculated at 0 kg/ha of P) had the highest percentage of root infection with mycorrhiza, reaching 55.33%, compared to the uninoculated treatment fertilized with a phosphorus level of 240 kg/ha, which had the lowest percentage of root infection (22.00%). Mycorrhizal fungi are more effective in soils poor in P and plants depend on mycorrhizal fungi to increase the efficiency of P absorption (Abbott and Robson, 1981). An increased P concentration in the soil inhibits the mycorrhizal relationship due to the lack of chemical signals sent by the host that stimulate fungal reproduction, in addition to inhibiting fungal growth by inhibiting enzymes, such as phosphatases, that the fungus depends on to mobilize organic P (Johnson et al., 1991). High P levels reduce the plant's need for fungi for nutrient uptake. In cases of P deficiency (0 kg/ha of P), fungi may stimulate the mineralization of organic phosphorus, making it available to the plant and, consequently, increasing its concentration in

plant tissues. The increase in fungal hyphae may play a role in expanding the absorption area of the root system, increasing the plant's efficiency to utilize limited P stocks (El-Sherbeny et al., 2022; Khan et al., 2023; Chen et al., 2024; Ilyas et al., 2024).

The interaction between the *Rhizobium* bacteria and mycorrhizal fungi significantly affected the percentage of root mycorrhizal infection. The treatment inoculated with *Rhizobium* and mycorrhiza outperformed the other treatments, achieving the highest percentage of infection (59.83%), while the uninoculated treatment achieved 27.66%. Single or dual inoculation with AMF and rhizobia has been reported to regulate plant growth and nitrogen acquisition in white clover (Xie et al., 2020; Wu et al., 2023). In contrast, mycorrhizal fungi improve nitrogen uptake by enhancing the efficiency of biofixation by *Rhizobium*, which stimulates root growth and increases the opportunities for interaction between microbes (Hao et al., 2019).

Regarding the effect of the three-way interaction between the study factors (*Rhizobium*, mycorrhizal fungi and phosphorus) produced significantly different interactions (Table 8). The treatment inoculated with *Rhizobium* and mycorrhizal fungi, fertilized at 80 kg/ha of P tended to outperform the other treatments (percentage of root mycorrhizal infection = 67.33%). However, it was not significantly different from P0M1R1, P2M1R1 and P1R0M1. The uninoculated treatment, without fertilizer application, had the lowest percentage of root mycorrhizal infection (24.00%). This may be attributed to the interaction between these two types of microbes, enhancing P and other nutrient uptake efficiencies (Jia et al., 2004).

Conclusion

Overall, based on the results, there was a clear synergistic effect among *B. japonicum* inoculation, *G. mosseae* inoculation and phosphate fertilization on soybean growth in sterile gypsum soil. Generally, the combined application of these three factors produced better performance than their individual applications, as reflected in improvements in plant height, dry weight of vegetative mass, root dry weight and mycorrhizal infection percentage. This synergism may be attributed to the complementary roles of the three factors, where *B. japonicum* enhances nitrogen fixation, *G. mosseae* improves phosphorus and water uptake through fungal hyphal extension and phosphate fertilization supplies readily available phosphorus

required for plant growth and for the effectiveness of both symbiotic microorganisms. From an applied perspective, these findings suggest that the integrated use of rhizobial and mycorrhizal inoculation with appropriate phosphorus fertilization can be a promising strategy to improve soybean productivity in nutrient-poor soils, particularly gypsum soils, while potentially reducing dependence on excessive chemical fertilizer inputs and supporting more sustainable agricultural production systems.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

Collate acknowledgements in a separate section at the end of the article before the references and do not include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing materials, laboratory equipment, writing assistance or proofreading the article, etc.) and funding.

References

- Abbott, L.K., Robson, A.D. 1981. Infectivity and effectiveness of vesicular arbuscular mycorrhizal fungi: effect of inoculum type. *Aust. J. Agric. Res.* 32: 631–639. doi.org/10.1071/AR9810631
- Abd-Alla, M.H., El-Enany, A.W.E., Nafady, N.A., Khalaf, D.M., Morsy, F.M. 2014. Synergistic interaction of *Rhizobium leguminosarum* bv. viciae and arbuscular mycorrhizal fungi as plant growth-promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. *Microbiol. Res.* 169: 49–58. doi.org/10.1016/j.micres.2013.07.007
- Akter, F., Islam, N., Shamsuddoha, A.T.M., Bhuiyan, M.S.I., Shilpi, S. 2013. Effect of phosphorus and sulphur on growth and yield of soybean (*Glycine max* L.). *Int. J. Bioresour. Stress Manag.* 4: 556–561.
- Alkurtany, A.E.S., Ali, S.A.M., Mahdi, W.M. 2018. The efficiency of prepared biofertilizer from a local isolate of *Bradyrhizobium* sp. on mungbean plant growth and yield. *Iraqi J. Agric. Sci.* 49: 722–730. doi.org/10.36103/ijas.v49i5.22
- Al-Obaidi, M.A.J., Al-Hamdani, R.I.A., Saeed, M.F. 2010. A study on ammonia volatilisation from some soils in northern Iraq. *Rafidain J. Agric.* 38: 1–10.
- Babajide, P.A., Akanbi, W.B., Alamu, L.O., Ewetola, E.A., Olatunji, O.O. 2009. Growth, nodulation and biomass yield of soybean (*Glycine max* L.) as influenced by biofertilizers under simulated eroded soil conditions. *Res. Crops.* 10: 29–34.
- Balestrini, R., Brunetti, C., Chitarra, W., Nerva, L. 2020. Photosynthetic traits and nitrogen uptake in crops: Role of arbuscular mycorrhizal fungi. *Plants* 9: 1105. doi.org/10.3390/plants9091105
- Balzergrue, C., Chabaud, M., Barker, D.G., Bécard, G., Rochange, S.F. 2013. High phosphate reduces the host's ability to develop arbuscular mycorrhizal symbiosis. *Front. Plant Sci.* 4: 426. doi.org/10.3389/fpls.2013.00426
- Balzergrue, C., Puech-Pagès, V., Bécard, G., Rochange, S.F., 2011. The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. *J. Exp. Bot.* 62: 1049–1060. doi.org/10.1093/jxb/erq335
- Banni, M., Hajer, A., Sforzini, S., Oliveri, C., Boussetta, H., Viarengo, A. 2014. Transcriptional expression levels and biochemical markers of oxidative stress in *Mytilus galloprovincialis* exposed to nickel and heat stress. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 160: 23–29. doi.org/10.1016/j.cbpc.2013.11.005
- Barznjy, L.G., Fattah, O.A., Mohammed, D.J., Mohammed, H.J., Mahmood, A.M. 2025. Influence of mycorrhizal inoculation and different phosphorus levels on broad bean (*Vicia faba* L.) growth, nutrient and yield. *J. Kirkuk Univ. Agric. Sci.* 16: 210–223. doi.org/10.58928/ku25.16122
- Barker, S.J., Tagu, D. 2000. The roles of auxins and cytokinins in mycorrhizal symbiosis. *J. Plant Growth Regul.* 19: 144–154. doi.org/10.1007/s003440000021
- Begoude, D.A.B., Sarr, P.S., Mpon, T.L.Y., Owona, D.A., Kapeua, M.N., Araki, S. 2016. Composition of arbuscular mycorrhizal fungi associated with cassava (*Manihot esculenta* Crantz) cultivars as influenced by chemical fertilization and tillage in Cameroon. *J. Appl. Biosci.* 98: 9270–9283. doi.org/10.4314/jab.v98i1.4
- Bonfante, P., Genre, A. 2008. Plants and arbuscular mycorrhizal fungi: an evolutionary-developmental perspective. *Trends Plant Sci.* 13: 492–498. doi.org/10.1016/j.tplants.2008.07.001
- Cavagnaro, T.R., Bender, S.F., Asghari, H.R., van der Heijden, M.G. 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. *Trends Plant Sci.* 20: 283–290. doi.org/10.1016/j.tplants.2015.03.004
- Chen, L., Zhang, X., Li, Q., Yang, X., Huang, Y., Zhang, B., Li, X. 2024. Phosphatases: Decoding the Role of Mycorrhizal Fungi in Plant Disease Resistance. *Int. J. Mol. Sci.* 25: 9491. doi.org/10.3390/ijms25179491
- Cruz, R.E.D., Husain, T.A.R.I.Q. 2008. Effect of vesicular-arbuscular mycorrhiza on drought resistance of *Senna spectabilis*. *Pak. J. Bot.* 40: 2217–2224.
- Dankwa, P., Cudjoe, E., Amuah, E.E.Y., Kazapoe, R.W., Agyemang, E.P., 2021. Analyzing and forecasting rainfall patterns in the Manga-Bawku area, northeastern Ghana: Possible implication of climate change. *Environ. Chall.* 5: 100354. doi.org/10.1016/j.envc.2021.100354
- Davies, F.T., Jr., Linderman, R.G. 1991. Short-term effects of phosphorus and VA-mycorrhizal fungi on nutrition, growth and development of *Capsicum annuum* L.. *Sci. Hortic.* 45: 333–338. doi.org/10.1016/0304-4238(91)90079-E.
- Demir, S., Akkopru, A., 2024. Use of arbuscular mycorrhizal fungi for biocontrol of soilborne fungal plant pathogens. In: Chincholkar, S.B., Mukerji, K.G. (Eds.), *Biological Control of Plant Diseases*. CRC Press, Boca Raton, FL, USA, pp. 17–46. doi.org/10.1201/9781003578406-2.

- Dobo, B. 2022. Effect of arbuscular mycorrhizal fungi (AMF) and Rhizobium inoculation on growth and yield of *Glycine max* L. varieties. *Int. J. Agron.* 2022: 9520091. doi.org/10.1155/2022/9520091.
- Egamberdieva, D., Jabborova, D., Wirth, S.J., Alam, P., Alyemeni, M.N., Ahmad, P. 2018. Interactive effects of nutrients and *Bradyrhizobium japonicum* on the growth and root architecture of soybean (*Glycine max* L.). *Front. Microbiol.* 9: 1000. doi.org/10.3389/fmicb.2018.01000
- El-Sherbeny, T.M.S., Mousa, A.M., El-Sayed, E.S.R. 2022. Use of mycorrhizal fungi and phosphorus fertilization to improve the yield of onion (*Allium cepa* L.) plant. *Saudi J. Biol. Sci.* 29: 331–338. doi.org/10.1016/j.sjbs.2021.08.094
- Feng, Y.Y., He, J., Turner, N.C., Siddique, K.H., Li, F.M. 2021. Phosphorus Supply Increases Internode Length and Leaf Characteristics and Increases Dry Matter Accumulation and Seed Yield in Soybean under Water Deficit. *Agronomy* 11: 930. doi.org/10.3390/agronomy11050930
- Ferrol, N., Azcón-Aguilar, C., Pérez-Tienda, J., 2019. Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: An overview on the mechanisms involved. *Plant Sci.* 280: 441–447. doi.org/10.1016/j.plantsci.2018.11.011
- Gerdemann, J.W., Nicolson, T.H. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 46: 235–244. doi.org/10.1016/S0007-1536(63)80079-0.
- Gorgia, P., Tsikou, D. 2025. Tripartite symbiosis between legumes, arbuscular mycorrhizal fungi and nitrogen fixing rhizobia: Interactions and regulation. *Plant Cell Environ.* 49: 3789–3807. doi:10.1111/pce.15341.
- Hao, Z., Xie, W., Jiang, X., Wu, Z., Zhang, X., Chen, B. 2019. Arbuscular Mycorrhizal Fungus Improves Rhizobium–Glycyrrhiza Seedling Symbiosis under Drought Stress. *Agronomy* 9: 572. doi.org/10.3390/agronomy9100572
- Hartman, G.L., West, E.D., Herman, T.K. 2011. Crops that feed the World 2. Soybean—worldwide production, use and constraints caused by pathogens and pests. *Food Secur.* 3: 5–17. doi.org/10.1007/s12571-010-0108-x
- Ho-Plágaro, T., García-Garrido, J.M., 2022. Molecular regulation of arbuscular mycorrhizal symbiosis. *Int. J. Mol. Sci.* 23: 5960. doi.org/10.3390/ijms23115960
- Huey, C.J., Gopinath, S.C., Uda, M.N.A., Zulhaimi, H.I., Jaafar, M.N., Kasim, F.H., Yaakub, A.R.W. 2020. Mycorrhiza: A natural resource assisting plant growth under varied soil conditions. *3 Biotech.* 10: 1–9. doi.org/10.1007/s12305-020-02188-3
- Igiehon, N.O., Babalola, O.O. 2018. Rhizosphere microbiome modulators: Contributions of nitrogen-fixing bacteria towards sustainable agriculture. *Int. J. Environ. Res. Public Health.* 15: 574. doi.org/10.3390/ijerph15040574
- Igiehon, N.O., Babalola, O.O., Cheseto, X., Torto, B. 2021. Effects of rhizobia and arbuscular mycorrhizal fungi on yield, size distribution and fatty acids of soybean seeds grown under drought stress. *Microbiol. Res.* 242: 126640. doi.org/10.1016/j.micres.2020.126640
- Jalaluddin, M. 2005. Effect of AMF and *Bradyrhizobium* inoculation on soybean growth and yield in Sindh. *Pak. J. Bot.* 37: 169–173.
- Jia, Y., Gray, V.M., Straker, C.J. 2004. The Influence of Rhizobium and arbuscular mycorrhizal fungi on nitrogen and phosphorus accumulation by *Vicia faba*. *Ann. Bot.* 94: 251–258. doi.org/10.1093/aob/mch135
- Johnson, N.C., Zak, D.R., Tilman, D., Pfleger, F.L. 1991. Dynamics of vesicular-arbuscular mycorrhizae during old field succession. *Oecologia* 86: 349–358. doi.org/10.1007/BF00317600.
- Kavadia, A., Omirou, M., Fasoula, D.A., Louka, F., Ehaliotis, C., Ioannides, I.M. 2021. Co-inoculations with rhizobia and arbuscular mycorrhizal fungi alters mycorrhizal composition and lead to synergistic growth effects in cowpea that are fungal combination-dependent. *Appl. Soil Ecol.* 167: 104013. doi.org/10.1016/j.apsoil.2021.104013.
- Khan, F., Siddique, A.B., Shabala, S., Zhou, M., Zhao, C. 2023. Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. *Plants* 12: 2861. doi.org/10.3390/plants12152861
- Koegel, S., Boller, T., Lehmann, M.F., Wiemken, A., Courty, P.E., 2013. Rapid nitrogen transfer in the *Sorghum bicolor*–*Glomus mosseae* arbuscular mycorrhizal symbiosis. *Plant Signal. Behav.* 8: e25229. doi.org/10.4161/psb.25229
- Kormanik, P.P., Bryan, W.C., Schultz, R.C. 1980. Procedures and equipment for staining large plant root samples for endomycorrhizal assay. *Can. J. Microbiol.* 26: 536–538. doi.org/10.1139/m80-090
- Koskey, G., Mburu, S.W., Njeru, E.M., Kimiti, J.M., Ombori, O., Maingi, J.M. 2017. Potential of Native Rhizobia in Enhancing Nitrogen Fixation and Yields of Climbing Beans (*Phaseolus vulgaris* L.) in Contrasting Environments of Eastern Kenya. *Front. Plant Sci.* 8: 443. doi.org/10.3389/fpls.2017.00443
- Liu, X.Q., Xie, M.M., Hashem, A., Abd-Allah, E.F., Wu, Q.S. 2023. Arbuscular mycorrhizal fungi and rhizobia synergistically promote root colonization, plant growth and nitrogen acquisition. *Plant Growth Regul.* 100: 691–701. doi.org/10.1007/s10725-023-00966-6
- Louis, I., Lim, G. 1988. Differential response in growth and mycorrhizal colonisation of soybean to inoculation with two isolates of *Glomus clarum* in soils of different P availability. *Plant Soil.* 112: 37–43. doi.org/10.1007/BF02181750
- Mahdi, A.A., Nur Eldaim, A.B., Arbab, A.A. 2004. Influence of mycorrhizal inoculation, form of phosphate fertilization and atwering regime on nodulation and yield of soybean. *Univ. Khartoum J. Agric. Sci.* 12: 1–10.
- Mahdi, W.M., Al-Badri, K.S.L., Nada, T.S. 2025. Effects of Fe₃O₄, ZnO and TiO₂ nanoparticles on germination and morphological parameters in microwave-sterilized pea, mung bean, wheat and barley seeds. *S. Afr. J. Bot.* 180: 762–767. doi.org/10.1016/j.sajb.2025.01.015
- Menaria, B.L., Singh, P., Nagar, R.K., Singh, P. 2003. Effect of nutrients and microbial inoculants on growth and yield of soybean (*Glycine max* (L.) Merrill). *J. Soils Crops.* 13: 14–17.
- Musyoka, D.M., Njeru, E.M., Nyamwange, M.M.E., Maingi, J.M. 2020. Arbuscular mycorrhizal fungi and *Bradyrhizobium* co-inoculation enhances nitrogen fixation and growth of green grams (*Vigna radiata* L.) under water stress. *J. Plant Nutr.* 43: 1036–1047. doi.org/10.1080/01904167.2020.1711940
- Ngalamu, T., Ashraf, M., Meseka, S. 2013. Soybean (*Glycine max* L.) genotype and environment interaction effect on yield and other related traits. *Amer. J. Ex. Agr.* 3: 977–987
- Ngosong, C., Tatah, B.N., Olougou, M.N.E., Suh, C., Nkongho, R.N., Ngone, M.A., Ruppel, S. 2022. Inoculating plant growth-promoting bacteria and arbuscular mycorrhiza fungi modulates rhizosphere acid phosphatase and nodulation activities and enhance the productivity of soybean (*Glycine max* L.). *Front. Plant Sci.* 13: 934339. doi.org/10.3389/fpls.2022.934339

- Nunes, R.D.S., Sousa, D.M.G., Goedert, W.J., Oliveira, L.E.Z., Pinheiro, T.D. 2021. Crops' Yield and Roots Response to Soil Phosphorus Distribution Resulting From Long-Term Soil and Phosphate Fertilization Management Strategies. *Front. Agron.* 3: 757100. doi.org/10.3389/fagro.2021.757100
- Olsen, P.E., Sande, E.S., Keyser, H.H. 1996. The enumeration and identification of rhizobial bacteria in legume inoculant quality control procedures. NifTAL Center, Paia, HI, USA.
- Primieri, S., Magnoli, S.M., Koffel, T., Stürmer, S.L., Bever, J.D. 2022. Perennial, but not annual legumes synergistically benefit from infection with arbuscular mycorrhizal fungi and rhizobia: A meta-analysis. *New Phytol.* 233: 505–514. doi.org/10.1111/nph.17720.
- Purwaningsih, S., Agustiyani, D., Antonius, S. 2021. Diversity, activity and effectiveness of Rhizobium bacteria as plant growth promoting rhizobacteria (PGPR) isolated from Dieng, central Java. *Iran. J. Microbiol.* 13: 130–136. doi: 10.18502/ijm.v13i1.5504.
- Razakatiana, A.T.E., Trap, J., Baohanta, R.H., Raherimandimby, M., Le Roux, C., Duponnois, R., Becquer, T. 2020. Benefits of dual inoculation with arbuscular mycorrhizal fungi and rhizobia on *Phaseolus vulgaris* planted in a low-fertility tropical soil. *Pedobiologia* 83: 150685. doi.org/10.1016/j.pedobi.2020.150685.
- Richter, W., Sunderman, M., Willenberg, Z., Calfee, M., Serre, S., Wood, J.P. 2022. Effectiveness of formaldehyde in various soil types as a wide area decontamination approach for *Bacillus anthracis* spores. *PLOS One.* 17: e0277941. doi.org/10.1371/journal.pone.0277941
- Sajjad, M., Razzaq, H., Kashif, M., Wahid, M.A. 2024. Relationship of various parameters to *Bradyrhizobium japonicum* in soybeans. *SABRAO J. Breed. Genet.* 56: 1694–1704. doi.org/10.54910/sabrao2024.56.4.34
- SAS Institute Inc. 2004. SAS/STAT User's Guide, Version 9.1.3. SAS Institute Inc. Cary, NC, USA.
- Shen, L., Feng, J. 2024. NIN—at the heart of nitrogen-fixing nodule symbiosis. *Front. Plant Sci.* 14: 1284720. doi.org/10.3389/fpls.2023.1284720
- Shome, S., Barman, A., Solaiman, Z.M. 2022. Rhizobium and Phosphate Solubilizing Bacteria Influence the Soil Nutrient Availability, Growth, Yield and Quality of Soybean. *Agriculture* 12: 1136. doi.org/10.3390/agriculture12081136
- Singh, R.K., Upadhyay, P.K., Dhar, S., Rajanna, G.A., Singh, V.K., Kumar, R., Barthakur, S. 2023. Soybean crop intensification for sustainable aboveground-underground plant–soil interactions. *Front. Sustain. Food Syst.* 7: 1194867. doi.org/10.3389/fsufs.2023.1194867
- Siqueira, J.O., de Paula, M.A. 1986. Effect of vesicular-arbuscular mycorrhizae on nutrition and phosphate use by soybean in cerrado soil. *Rev. Bras. Ciênc. Solo.* 10: 97–102.
- Spagnoletti, F.N., Leiva, M., Chiochio, V., Lavado, R.S. 2018. Phosphorus fertilization reduces the severity of charcoal rot (*Macrophomina phaseolina*) and the arbuscular mycorrhizal protection in soybean. *J. Plant Nutr. Soil Sci.* 181: 855–860. doi.org/10.1002/jpln.201700569
- Tahir, M.M., Abbasi, M.K., Rahim, N., Khaliq, A., Kazmi, M.H. 2009. Effect of Rhizobium inoculation and NP fertilization on growth, yield and nodulation of soybean (*Glycine max* L.) in the sub-humid hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. *Afr. J. Biotechnol.* 8: 6191–6200.
- Techapinyawat, S., Pakkong, P., Suwanarit, P., Sumthong, P. 2002. Effects of arbuscular mycorrhiza and phosphate fertilizer on phosphorus uptake of vetiver using nuclear technique. *Agric. Nat. Resour.* 36: 381–391.
- Timofeeva, A.M., Galyamova, M.R., Sedykh, S.E. 2023. Plant Growth-Promoting Soil Bacteria: Nitrogen Fixation, Phosphate Solubilization, Siderophore Production and Other Biological Activities. *Plants* 12: 4074. doi.org/10.3390/plants12244074
- Tomar, S.S., Singh, R., Singh, P.S. 2004. Response of phosphorus, sulphur and rhizobium inoculation on growth, yield and quality of soybean (*Glycine max* L.). *Prog. Agric.* 4: 72–73.
- Van't Padje, A., Werner, G.D.A., Kiers, E.T. 2021. Mycorrhizal fungi control phosphorus value in trade symbiosis with host roots when exposed to abrupt 'crashes' and 'booms' of resource availability. *New Phytol.* 229: 2933–2944. doi.org/10.1111/nph.17055
- Vincent, J.M. 1970. A manual for the practical study of root-nodule bacteria (IBP Handbook No. 5). Blackwell Scientific Publications.
- Wahab, A., Muhammad, M., Munir, A., Abdi, G., Zaman, W., Ayaz, A., Reddy, S.P.P. 2023. Role of Arbuscular Mycorrhizal Fungi in Regulating Growth, Enhancing Productivity and Potentially Influencing Ecosystems under Abiotic and Biotic Stresses. *Plants* 12: 3102. doi.org/10.3390/plants12173102
- Wu, H., Liu, R., Jiang, D., Xie, M., Zou, Y., 2023. Effects of arbuscular mycorrhizal fungi and rhizobia on nitrogen assimilation of white clover. *Guihaia* 43: 1213–1221. doi.org/10.11931/guihaia.gxzw202210035
- Xie, M.M., Zou, Y.N., Wu, Q.S., Zhang, Z.Z., Kuča, K. 2020. Single or dual inoculation of arbuscular mycorrhizal fungi and rhizobia regulates plant growth and nitrogen acquisition in white clover. *Plant Soil Environ.* 66: 287–294. doi.org/10.17221/234/2020-PSE.
- Yeremko, L., Czopek, K., Staniak, M., Marenych, M., Hanhur, V. 2025. Role of environmental factors in legume–Rhizobium symbiosis: A review. *Biomolecules* 15: 118. doi.org/10.3390/biom15010118
- Younis, H.S.A., Alkurtany, A.E.S., Al-Badrani, W.A.A. 2025. Evaluation of the Inoculum Prepared from Local Bacterial Isolates on the Growth and Yield of (*Trifolium pretense* L.) in Gypsiferous Soil. *IOP Conf. Ser. Earth Environ. Sci.* 1449: 012125. doi 10.1088/1755-1315/1449/1/012125
- Yousef, A.N., Ali, N.A., Munem, B.H. 1993. Interaction of rhizobia, VA-mycorrhiza and phosphorus on soybean in calcareous soil. *Iraqi J. Agric. Sci.* 24: 30–39.
- Yu, H.Y., He, W.X., Zou, Y.N., Alqahtani, M.D., Wu, Q.S. 2024. Arbuscular mycorrhizal fungi and rhizobia accelerate plant growth and N accumulation and contribution to soil total N in white clover by difficultly extractable glomalin-related soil protein. *Appl. Soil Ecol.* 197: 105348. doi.org/10.1016/j.apsoil.2024.105348
- Zhang, F., Hou, Y., Zed, R., Mauchline, T.H., Shen, J., Zhang, F., Jin, K. 2023. Root exudation of organic acid anions and recruitment of beneficial actinobacteria facilitate phosphorus uptake by maize in compacted silt loam soil. *Soil Biol. Biochem.* 184: 109074. doi.org/10.1016/j.soilbio.2023.109074
- Zhou, W., Chen, F., Zhao, S., Yang, C., Meng, Y., Shuai, H., Shu, K. 2019. DA-6 promotes germination and seedling establishment from aged soybean seeds by mediating fatty acid metabolism and glycometabolism. *J. Exp. Bot.* 70: 101–114. doi.org/10.1093/jxb/ery247
- Zhou, R., Raza, A., Song, J., Janiad, S., Li, Q., Huang, M., Hassan, M.A. 2025. Growth-promoting effects of arbuscular mycorrhizal fungus *Funneliformis mosseae* in rice, sesame, sorghum, Egyptian pea and Mexican hat plant. *Front. Microbiol.* 16: 1549006. doi.org/10.3389/fmicb.2025.1549006