Response of Three Faba Bean (*Vicia faba* L.) Cultivars to Rhizobium Inoculation under Gypsiferous Soil Conditions

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Abstract

The present study was conducted at the Research Station of the Department of Soil Science and Water Resources, College of Agriculture, Tikrit University, during the 2025–2026 growing season to evaluate the effect of *Rhizobium* inoculation on the growth and productivity of three faba bean (*Vicia faba* L.) cultivars (Local, Italian, and Turkish) grown under gypsiferous soil. Gypsiferous soils are soils containing appreciable amounts of gypsum ($CaSO_4 \cdot 2H_2O$), which can substantially influence their physical, chemical, and biological properties.

The results demonstrated that *Rhizobium* inoculation significantly improved most of the growth and yield characteristics of all evaluated cultivars. Inoculated plants exhibited significant increases in plant height and the number of root nodules, confirming the establishment of the symbiotic association between the host plant and *Rhizobium*, which enhanced biological nitrogen fixation. Consequently, inoculated plants produced a significantly higher seed yield per plant compared with non-inoculated plant.

The physiological responses also showed marked improvement following inoculation, particularly in chlorophyll content, indicating enhanced photosynthetic efficiency as a result of increased nitrogen availability through biological nitrogen fixation. Furthermore, genetic analysis revealed considerable genetic variability among the studied cultivars. High broad-sense heritability estimates were recorded for several important traits, including the number of root nodules and seed yield per plant, suggesting that these traits can be effectively improved through direct selection in breeding programs.

Among the evaluated cultivars, the local cultivar consistently outperformed the Italian and Turkish cultivars in most of the investigated traits, demonstrating adaptation to gypsiferous soil conditions and a greater positive response to *Rhizobium* inoculation.

Overall, the findings indicate that *Rhizobium* inoculation represents an effective and environmentally sustainable strategy for improving the growth and productivity of faba bean cultivated in gypsiferous soils while reducing dependence on synthetic nitrogen fertilizers. The results also emphasize the importance of selecting cultivars with superior performance under the tested conditions to maximize crop performance and productivity under gypsiferous soil conditions.

Key words: Rhizobium, Vicia faba, Gypsiferous soil , Nitrogen fixation.

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Introduction

Faba bean (*Vicia faba* L.) is one of the most important legume crops due to its considerable nutritional, economic, and agricultural value. It represents an excellent source of plant protein, carbohydrates, vitamins, and essential minerals, making it an important component of both human and animal nutrition (4). In addition to its nutritional significance, faba bean plays a vital role in improving soil fertility through its symbiotic association with *Rhizobium* bacteria, which fix atmospheric nitrogen and convert it into plant-available forms. This biological process reduces the dependence on synthetic nitrogen fertilizers, lowers production costs, and minimizes the environmental impacts associated with excessive fertilizer application (1)(14).

Despite its importance, faba bean productivity is constrained by several environmental factors, particularly in arid and semi-arid regions. Among these constraints, gypsiferous soils represent a major challenge because they occupy extensive agricultural areas and are characterized by high calcium sulfate (gypsum) content, poor physical structure, and low water- and nutrient-holding capacity. These characteristics adversely affect root development and microbial activity in the rhizosphere, thereby limiting nutrient availability and reducing the efficiency of biological nitrogen fixation(16)(19). Consequently, plants grown under gypsiferous soil conditions often exhibit reduced vegetative growth and lower yield potential (2).

Rhizobium bacteria are among the most beneficial soil microorganisms associated with leguminous crops. They establish symbiotic root nodules that facilitate biological nitrogen fixation, thereby enhancing plant growth, improving nutrient-use efficiency, and increasing crop productivity (12). Numerous studies have demonstrated that *Rhizobium* inoculation significantly improves growth and yield traits in legume crops. However, the effectiveness of inoculation is influenced by several factors, including bacterial strain, environmental conditions, soil characteristics, and the genetic background of the host cultivar(20).

Genetic variation among faba bean cultivars is a key determinant of their response to *Rhizobium* inoculation and their ability to adapt to adverse environmental conditions, including those imposed by gypsiferous soils (7). Therefore, investigating the genetic variability associated with cultivar responses to *Rhizobium* inoculation provides valuable insights into the genetic control of growth, yield, and biological nitrogen fixation-related traits. Such information is essential for identifying cultivars with superior adaptation and productivity under challenging soil conditions (18). Genetic analysis is also fundamental to plant breeding programs, as it enables the estimation of genetic and environmental variance components, heritability, and expected genetic advance through selection. These parameters facilitate the development of high-yielding cultivars with enhanced responsiveness to biological inoculation and improved tolerance to unfavorable environmental conditions(10). Moreover, the application of *Rhizobium* inoculation represents a sustainable agricultural practice that contributes to improving crop productivity while reducing dependence on

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chemical nitrogen fertilizers and preserving soil fertility and environmental quality (14).

Based on these considerations, the present study was conducted to evaluate the response of three faba bean cultivars to *Rhizobium* inoculation under gypsiferous soil conditions. The study also aimed to investigate the genetic variability among these cultivars for selected agronomic and physiological traits related to growth and yield, estimate key genetic parameters, and identify the cultivar exhibiting the greatest responsiveness to *Rhizobium* inoculation and the highest level of adaptation to gypsiferous soils. The findings are expected to contribute to faba bean breeding programs and promote sustainable production of this economically important crop.

Materials and Methods

Experimental Site

The field experiment was conducted during the 2025–2026 growing season at the Research Station of the Department of Soil Science and Water Resources, College of Agriculture, Tikrit University, Iraq. The experimental site is located in a semi-arid climatic region characterized by high temperatures and limited rainfall, providing suitable conditions for evaluating crop performance under environmental stress. Prior to sowing, representative soil samples were collected from the experimental field at a depth of 0–30 cm for the determination of the physical and chemical properties of the experimental soil. Soil analyses were performed according to the standard procedures described by Allen and Hajek(3). The analyses included soil pH, electrical conductivity (EC), soil texture, and selected physical and chemical properties to characterize the gypsiferous soil used in the study. The physical and chemical characteristics of the experimental soil are presented in Table 1.

Table 1. Selected physical and chemical properties of the experimental soil.

Property	Unit	Value
EC	dS m^{-1}	2.41
pH	___	7.15
CEC	cmol kg^{-1} soil	17
Organic matter	g kg^{-1}	1.08
Gypsum	g kg^{-1}	100
Available nitrogen	mg kg^{-1}	54
Available phosphorus	mg kg^{-1}	7.1
Available potassium	mg kg^{-1}	101
Sand	g kg^{-1}	520
Silt	g kg^{-1}	280
Clay	g kg^{-1}	200
Texture	—	sandy clay mixture

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Plant Materials

Three faba bean (*Vicia faba* L.) cultivars were used in this study, namely the Local, Italian, and Turkish cultivars. These cultivars were selected to represent genetic variability in their response to *Rhizobium* inoculation and adaptation to gypsiferous soil conditions.

Rhizobium Inoculum and Seed Inoculation Procedure

A commercial *Rhizobium* inoculum specific to leguminous crops was obtained from the Microbiology Laboratory, Department of Soil Science and Water Resources, College of Agriculture, Tikrit University. The viability and effectiveness of the inoculum were verified prior to its application.

Immediately before sowing, faba bean seeds were inoculated with the *Rhizobium* culture. The seeds were first moistened with a dilute sucrose solution to enhance bacterial adhesion to the seed surface, after which the inoculum was thoroughly mixed with the seeds to ensure uniform coating. The inoculated seeds were then air-dried in the shade before planting. Seed inoculation was carried out following the standard procedures recommended for legume inoculation with *Rhizobium* (21)(22).

Experimental Design

The experiment was conducted using a randomized complete block design (RCBD) arranged as a two-factor factorial experiment with three replications. The first factor consisted of three faba bean (*Vicia faba* L.) cultivars (Local, Italian, and Turkish), while the second factor comprised two *Rhizobium* inoculation treatments (inoculated and non-inoculated). The combination of the two experimental factors resulted in a total of 18 experimental units.

Crop Management Practices

The experimental field was prepared using standard agronomic practices, including plowing, land leveling, and seedbed preparation prior to sowing. The field was subsequently divided into equal-sized experimental plots. Seeds were sown manually at the recommended planting spacing for faba bean cultivation. Irrigation, weed control, and all other crop management practices were carried out uniformly throughout the growing season to ensure optimal crop establishment and minimize the influence of non-experimental factors.

Traits Evaluated

The following agronomic and physiological traits were evaluated during the experiment:

Vegetative growth trait

Plant height (cm)

Nodulation trait

Number of root nodules per plant

Yield trait

Seed yield per plant (g)

Physiological trait

Chlorophyll content

Chlorophyll content was determined using the spectrophotometric method described by Marr *et al*.(16).

Statistical and Genetic Analyses

The experimental data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS) (or GenStat) according to the Randomized

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Complete Block Design (RCBD). Treatment means were compared using the Least Significant Difference (LSD) test at the 5% probability level(9).

Genetic parameters were estimated for all studied traits, including genotypic variance (σ^2g), environmental variance (σ^2e), and phenotypic variance (σ^2p). Broad-sense heritability (H^2) and the expected genetic advance were also calculated to evaluate the genetic control of the investigated traits and their potential for improvement through selection(5).

Results and Discussion

Effect of Cultivars and *Rhizobium* Inoculation on Plant Height

The results presented in Table 2 revealed significant variation among the three faba bean cultivars with respect to plant height. In addition, *Rhizobium* inoculation had a significant positive effect on this trait.

The local cultivar exhibited the greatest response to inoculation, with plant height increasing from 68.2 cm under the non-inoculated treatment to 78.4 cm following *Rhizobium* inoculation, representing an increase of 10.2 cm. This improvement may be attributed to the superior ability of the local cultivar to establish an effective symbiotic association with *Rhizobium*, thereby enhancing biological nitrogen fixation and promoting vegetative growth.

Similarly, plant height of the Italian cultivar increased from 63.5 cm to 72.1 cm, corresponding to an increase of 8.6 cm. In contrast, the Turkish cultivar showed the lowest response to inoculation, with plant height increasing from 58.7 cm to 66.3 cm, an increase of 7.6 cm, indicating relatively lower genetic efficiency in benefiting from *Rhizobium* inoculation under gypsiferous soil conditions.

All observed differences exceeded the LSD value (3.45 cm), indicating that the differences among treatments were statistically significant rather than attributable to random variation.

Plant height is considered an important indicator of vegetative growth and reflects the plant's response to environmental conditions and agronomic management practices, including biological inoculation with *Rhizobium* bacteria (6).

Table 2. Effect of faba bean cultivars and *Rhizobium* inoculation on plant height (cm).

Varieties	With Pollination	Without Pollination
Local	78.4	68.2
Italian	72.1	63.5
Turkish	66.3	58.7
LSD (0.05)	3.45	

Effect of Cultivars and *Rhizobium* Inoculation on the Number of Root Nodules

The results presented in Table 3 showed that *Rhizobium* inoculation significantly increased the number of root nodules in all faba bean cultivars compared

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with the non-inoculated treatment. The local cultivar produced the highest number of root nodules, increasing from 25.4 nodules plant^{-1} in the non-inoculated treatment to 42.6 nodules plant^{-1} following inoculation, representing an increase of 17.2 nodules plant^{-1}. This result indicates a high degree of compatibility between the host plant and *Rhizobium*, resulting in more effective nodulation.

Similarly, the Italian cultivar exhibited an increase in the number of root nodules from 22.1 to 37.8 nodules plant^{-1}, corresponding to an increase of 15.7 nodules plant^{-1}. In contrast, the Turkish cultivar produced the lowest number of nodules, increasing from 18.6 to 31.4 nodules plant^{-1}, an increase of 12.8 nodules plant^{-1}, suggesting relatively lower genetic potential for nodulation compared with the other cultivars.

Root nodule formation is considered one of the most reliable indicators of successful *Rhizobium* inoculation, as it reflects the ability of the bacteria to colonize plant roots and establish an effective symbiotic association for biological nitrogen fixation (12). The observed differences among cultivars indicate substantial genetic variation in their response to *Rhizobium* inoculation, highlighting the importance of the genetic background in determining symbiotic efficiency and nodulation capacity (8).

Table 3. Effect of faba bean cultivars and *Rhizobium* inoculation on the number of root nodules (nodules plant^{-1}).

Varieties	With Pollination	Without Pollination
Local	42.6	25.4
Italian	37.8	22.1
Turkish	31.4	18.6
LSD (0.05)	2.98	

Effect of Cultivars and *Rhizobium* Inoculation on Seed Yield per Plant

Seed yield per plant is considered one of the most important economic traits, as it represents the final outcome of the interaction among genetic factors, environmental conditions, and agronomic practices. The results presented in Table 4 demonstrated that *Rhizobium* inoculation markedly increased seed yield per plant in all evaluated faba bean cultivars.

The local cultivar recorded the highest seed yield after inoculation, reaching 38.7 g plant^{-1} compared with 28.3 g plant^{-1} in the non-inoculated treatment, representing an increase of 10.4 g plant^{-1}. This superior performance reflects the higher efficiency of this cultivar in utilizing available nutrients and converting them into biomass and grain production.

The Italian cultivar also showed a considerable increase in seed yield, rising from 25.6 to 34.2 g plant^{-1} following *Rhizobium* inoculation, with an increase of 8.6 g plant^{-1}. Meanwhile, the Turkish cultivar exhibited the lowest increase, with seed yield improving from 22.1 to 30.5 g plant^{-1}, corresponding to an increase of 8.4 g plant^{-1}.

The improvement in seed yield resulting from *Rhizobium* inoculation may be attributed to enhanced nitrogen nutrition through biological nitrogen fixation, which promotes vegetative growth, improves photosynthetic activity, and consequently increases assimilate production and final

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yield (11).

Table 4. Effect of faba bean cultivars and *Rhizobium* inoculation on seed yield per plant (g plant^{-1}).

Varieties	With Pollination	Without Pollination
Local	38.7	28.3
Italian	34.2	25.6
Turkish	30.5	22.1
LSD (0.05)	2.21	

Effect of Cultivars and *Rhizobium* Inoculation on Chlorophyll Content

Chlorophyll content is considered an important physiological indicator reflecting the nutritional status of plants, particularly nitrogen availability, which plays a crucial role in chlorophyll biosynthesis and photosynthetic performance. The results presented in Table 5 revealed that *Rhizobium* inoculation significantly increased chlorophyll content in all evaluated faba bean cultivars.

The local cultivar recorded the highest chlorophyll content following inoculation, reaching 2.41 mg g^{-1} FW compared with 1.82 mg g^{-1} FW in the non-inoculated treatment, with an increase of 0.59 mg g^{-1} FW. Similarly, the Italian cultivar showed an increase in chlorophyll content from 1.65 to 2.12 mg g^{-1} FW, representing an increase of 0.47 mg g^{-1} FW. The Turkish cultivar exhibited the lowest response, with chlorophyll content increasing from 1.51 to 1.96 mg g^{-1} FW, corresponding to an increase of 0.45 mg g^{-1} FW.

The enhancement in chlorophyll content following *Rhizobium* inoculation may be attributed to improved nitrogen availability through biological nitrogen fixation, which promotes chlorophyll synthesis, enhances photosynthetic efficiency, and subsequently contributes to improved overall plant growth and development (17).

Table 5. Effect of faba bean cultivars and *Rhizobium* inoculation on chlorophyll content (mg g^{-1} FW)

Varieties	With Pollination	Without Pollination
Local	2.41	1.82
Italian	2.12	1.65
Turkish	1.96	1.51
LSD (0.05)	0.18	

Genetic Analysis of the Studied Traits

The genetic analysis revealed considerable variation among the evaluated faba bean cultivars for all studied traits. The values of genotypic variance were higher than those of environmental variance, indicating that a large proportion of the observed variation was attributed to genetic differences among cultivars rather than environmental effects.

Furthermore, the studied traits exhibited high broad-sense heritability estimates, particularly the number of root nodules (81.4%) and seed yield per plant (76.9%). These high heritability values indicate that these traits are predominantly controlled by genetic factors and can be effectively improved through direct selection in breeding programs.

The high heritability estimates observed for these traits suggest the presence of substantial genetic variability and a good potential for genetic

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improvement, particularly for traits associated with nodulation efficiency and productivity. Therefore, selecting superior genotypes based on these characteristics may contribute to the development of faba bean cultivars with enhanced performance under gypsiferous soil conditions.

Table 6. Genetic parameters of the studied traits

Genetic Improvement(%)	Heritability (%)	Environmental Variation	Genetic Variation	Trait
18.6	72.3	12.4	32.5	Plant height
25.3	81.4	20.1	85.2	Number of nodes
21.7	76.9	15.8	45.7	Plant yield

Conclusion

The present study demonstrated that *Rhizobium* inoculation had a significant and beneficial effect on the agronomic and physiological performance of faba bean cultivated under gypsiferous soil conditions at the Research Station of the Department of Soil Science and Water Resources, College of Agriculture, Tikrit University, during the 2025–2026 growing season.

All evaluated cultivars (Local, Italian, and Turkish) showed a positive response to bacterial inoculation, which resulted in significant improvements in vegetative growth traits, particularly plant height, as well as root-related traits represented by the number of root nodules. These results confirm the successful establishment of the symbiotic relationship between faba bean plants and *Rhizobium* bacteria under the stress conditions imposed by gypsiferous soils.

The improvement in growth performance was reflected in yield-related traits, as inoculated plants exhibited higher seed yield per plant compared with non-inoculated plants. This finding highlights the important role of *Rhizobium* inoculation in enhancing nutrient-use efficiency, particularly nitrogen availability through biological nitrogen fixation. In addition, physiological traits, especially chlorophyll content, showed considerable improvement in inoculated plants, indicating enhanced photosynthetic activity as a result of improved plant nutritional status.

Genetic analysis revealed significant variation among cultivars in their response to *Rhizobium* inoculation. The local cultivar outperformed the Italian and Turkish cultivars for most of the studied traits, indicating its superior genetic efficiency in adaptation to local environmental conditions and in establishing an effective association with *Rhizobium* bacteria.

Overall, the findings of this study confirm that biological inoculation with *Rhizobium* represents an effective and sustainable agricultural approach for improving faba bean productivity under gypsiferous soil conditions. Furthermore, the selection of genetically efficient cultivars with superior responsiveness to inoculation

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is essential for achieving optimum crop performance and sustainable production.

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