STUDYING AND UTILIZATION
OF PLANT GENETIC RESOURCES

Original article
UDC 635.657:631.53:631.8
DOI: 10.30901/2227-8834-2025-4-20-29

The effect of bacterial seed inoculation on agronomic characteristics of chickpea

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Background. The use of biofertilizers can be an eco-friendly method and, in addition to reducing chemical inputs, can be considered a climate-smart agricultural option in semiarid regions.
Materials and methods. A field trial was aimed to investigate the effect of biofertilizers (F_1: control, F_2: Nitroxin, F_3: *Mesorhizobium*, and F_4: PhosphoBARVAR) on the growth of chickpea genotypes (G_1: ILC-482, G_2: 'Pirouz', and G_3: 'Jam') in the Meshginshahr area, Iran.
Results. The highest longitudinal growth was recorded with the use of different biofertilizers (F) in G_1. The lateral growth and number of secondary branches were higher in $G_2 + F_2$ than in the others. The highest aboveground biomass was obtained in $G_1 + F_1$ and $G_1 + F_4$. Inoculation with *Mesorhizobium* resulted in the highest number of root nodules in G_2 and G_3. The application of *Mesorhizobium* also increased the number of pods. The highest grain yield was obtained in G_1+F_3 (1.43 t ha^{-1}), and $G_1 + F_2$ (1.35 t ha^{-1}).
Conclusion. The response of genotypes to bacterial inoculations was different. The weakest growth performance and grain yield production was recorded in G_1 without bacterial inoculation. The results showed that the ILC-482 line along with inoculation with *Mesorhizobium* produced economically acceptable grain yield.

Keywords: aboveground biomass, grain yield, *Mesorhizobium*, Nitroxin, root nodules, semiarid regions

Acknowledgements: this research was supported by the Ministry of Science, Research and Technology (MSRT). We are grateful to the local farm workers who provided technical assistance, expertise, or access to resources.
The authors thank the reviewers for their contribution to the peer review of this work.

For citation: Janmohammadi M., Ebadi-Segherloo A., Sabaghnia N., Mohebodini M. The effect of bacterial seed inoculation on agronomic characteristics of chickpea. *Proceedings on Applied Botany, Genetics and Breeding*. 2025;186(4):20-29. (In Russ.). DOI: 10.30901/2227-8834-2025-4-20-29

ИЗУЧЕНИЕ И ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ РАСТЕНИЙ

Научная статья
DOI: 10.30901/2227-8834-2025-4-20-29

Влияние бактериальной инокуляции семян на агрономические показатели нута

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Актуальность. Использование биоудобрений может быть экологически чистым методом повышения урожайности нута и, помимо сокращения использования химических веществ, может считаться климатически оптимизированным вариантом ведения сельского хозяйства в полузасушливых регионах.
Материалы и методы. Полевое испытание было направлено на изучение влияния биоудобрений (F_1: контроль; F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR) на рост генотипов нута (G_1: ILC-482, G_2: 'Pirouz', G_3: 'Jam') в районе Мешгиншехр, Иран.
Результаты. Самый высокий продольный рост зафиксирован при использовании различных биоудобрений у G_1. Боковой рост и количество вторичных ветвей были выше у G_2 + F_2, чем в других вариантах. Наибольшая надземная биомасса получена у G_1 + BF_1 и G_1 + F_4. Инокуляция *Mesorhizobium* привела к максимальному количеству корневых клубеньков у G_2 и G_3. Применение *Mesorhizobium* также увеличило количество бобов. Самую высокую урожайность зерна получили в вариантах G_1 + F_3 (1,43 т/га) и G_1 + F_2 (1,35 т/га).
Заключение. Реакция генотипов на бактериальные инокуляции была различной. Слабейшие показатели роста и урожайности зерна зафиксированы у G_1 без бактериальной инокуляции. Результаты показали, что линия ILC-482 вместе с инокуляцией *Mesorhizobium* даст экономически приемлемый урожай зерна.

Ключевые слова: надземная биомасса, урожайность зерна, *Mesorhizobium*, Nitroxin, корневые клубеньки, полузасушливые регионы

Благодарности: исследование проведено при поддержке Министерства науки, исследований и технологий (MSRT). Благодарим местных фермеров, оказавших техническую и экспертную помощь или предоставивших доступ к ресурсам.
Авторы благодарят рецензентов за их вклад в экспертную оценку этой работы.

Для цитирования: Джанмохаммади М., Эбади-Сегерлу А., Сабагния Н., Мохебодини М. Влияние бактериальной инокуляции семян на агрономические показатели нута. *Труды по прикладной ботанике, генетике и селекции*. 2025;186(4):20-29. DOI: 10.30901/2227-8834-2025-4-20-29

Introduction

Chickpea, or garbanzo bean (*Cicer arietinum* L.), is an important legume for human consumption or animal feed. Chickpea ranks third in terms of production among leguminous crops, after common bean and pea (Madurapperumage et al., 2021). This food legume is classified as a cool-season grain legume and can grow well through its vegetative stage to the flowering stage under relatively cool temperatures (Mukherjee et al., 2022). Chickpea is relatively drought-tolerant, and this feature has made *C. arietinum* particularly attractive to researchers and farmers, making chickpea a reasonable option for crop rotation in cereal-based systems (Spalevic et al., 2025). Chickpea production in 2023 is estimated at 16.5 million tons (https://www.fao.org/faostat/en/#data/QCL). India is the main producer, accounting for 75% of the total production (12.3×10^6 tons), followed by Australia (0.94×10^6 tons), Türkiye (0.58×10^6 tons), Russia (0.53×10^6 tons), and Ethiopia (0.45×10^6 tons). Chickpea contains 60% of carbohydrates, 22% of protein, 2% of fat, and 3% of ash. The nutritional quality of chickpea is associated with acceptable amounts of nutritionally essential amino acids, such as arginine and lysine, and sulfur-containing amino acids, vitamins, and trace elements, such as iron, some vitamins, and fiber (Landi et al., 2021). Relatively high levels of proteins in chickpea have made this food legume a low-cost alternative to animal protein products. The area under chickpea cultivation in Iran during 2023 was 423,000 ha and the grain production was 175,000 t (https://www.fao.org/faostat/en/#data/QCL).

Although chickpea has a mutualistic relationship with nitrogen-fixing bacteria, it appears that the lack of suitable bacterial strains in the soil of semiarid regions significantly reduces the supply of biological nitrogen and makes this plant dependent on chemical inputs (Abd-Alla et al., 2023). Therefore, the importance of using environmentally friendly nutritional methods to achieve acceptable performance and raise production is increasingly emphasized. Some rhizobium bacteria can provide biological nitrogen fixation through root nodule formation and also improve the availability of nutrients, such as phosphate and micronutrients, through the production of phytosiderophores, ultimately increasing plant growth and yield. These beneficial bacteria may have positive impact on environmental cleanliness (Khoso et al., 2024).

In recent decades, with the advancement of various aspects of microbiology, resistant and efficient strains of beneficial bacteria have been identified or produced. Such strains are relatively well adapted to the conditions of semiarid regions and can tolerate harsh climatic and edaphic conditions. By forming a bacterial consortium containing selected beneficial bacteria, they have been offered as various biofertilizers, whose efficiency can vary depending on the biological and physicochemical conditions of the soil, climatic conditions, soil biome, soil nutritional management, crop rotation, and crop type (Figiel et al., 2025). Inoculation of crop seeds with the provided beneficial bacterial consortia causes the bacteria to attack the roots after the germination and root emergence stage, and after infection, symbiotic relationships with beneficial bacteria are quickly formed (Brambilla et al., 2022). These relationships improve the availability of nutrients to the developing seedling.

Numerous soil microbes are normally applied as biofertilizers, such as nitrogen-fixing soil bacteria and cyanobacteria, phosphate-solubilizing bacteria. They can be used along with the combination of molds and fungi. Consistently, phytohormone producing bacteria are also used in biofertilizer formulation. Although, in general, any type of microorganisms that stimulates the growth and yield increase in crops is considered a biofertilizer, hitherto the focus in the formation of beneficial bacterial consortia has been on rhizobia and phosphate-solubilizing bacteria. Rhizobia are aerobic and autotrophic bacteria that can perform symbiotic nitrogen fixation after infection of the roots of crops and root nodule formation (Nosheen et al., 2021). *Azotobacter* is also used in the application of these fertilizers. This bacterium is nonmutualistic, nonphotosynthetic, and diazotrophic, with the ability to convert atmospheric nitrogen to usable forms. In addition to providing nitrogen through biosynthesis and the release of phytosiderophores and phytohormones, *Azotobacter* can establish crosstalk with the roots of crop plants and induce its positive effects (Sumbul et al., 2020). *Azospirillum* bacteria are equipped with the enzyme nitrogenase that can utilize atmospheric nitrogen by converting them to NH_3, and after some biochemical changes it can be available for the plant roots. *Azospirillum* also has a significant impact on phosphate solubilizing and stimulating the biosynthesis of phytohormones. These bacteria are also resistant to some biological stresses, such as salinity (Fukami et al., 2018). If the appropriate strain is present and favorable soil conditions are available, chickpea roots are inherently capable of establishing a symbiotic relationship with *Mesorhizobium ciceri* and benefiting from ammonium produced from biological nitrogen fixation. In addition, this bacterium also contributes significantly to the absorption of potassium and phosphorus in the plant (El-Saadony et al., 2024). However, there are some genetic differences among cultivars in their response to bacterial inoculation. Besides, there is still no comprehensive information on the application of different biofertilizers to chickpea genotypes. The intention of this investigation was to assess the effects of chickpea seed inoculation with *Mesorhizobium*, Nitroxin, and PhosphoBARVAR biofertilizers on growth performance in a temperate climate zone in northwest Iran.

Material and methods

Site description

A field trail was designed on a private farm in the Meshginshahr area (37.41°N, 38.33°E, 1562 m above sea level) during the 2022 growing season. This research is part of a MSc project and the obtained results will be subsequently confirmed by observations over 2–3 years. The site is located on the slopes of Mount Sabalan and, according to the Köppen–Geiger climate classification, the climate of the area is cold semiarid, with 324 mm long-term average of precipitation and an average annual temperature above 8°C. Some of the meteorological elements of the studied area are shown in Table 1. Combined soil samples were prepared from a depth of 0 to 30 cm before primary soil tillage and sent to the Water and Soil Laboratory for chemical analysis. The soil texture was silty loam. Its characteristics were as follows: pH 7.24 (1 : 1 soil to H_2O), total nitrogen content 0.19%, $CaCO_3$ content 7.24%, EC 0.98 ds m^{-1}, organic C 0.49%, P 7.21 ppm, and K 247 ppm.

On-farm trial implementation

In this experiment, the effect of seed inoculation with different bacterial consortia in three chickpea genotypes was studied. Biofertilizers in four levels were applied, including F_1: control (intact seed); F_2: Nitroxin (*Azotobacter chroococcum* + *Azospirillum lipoferum*); F_3: *Mesorhizobium* (*Mesorhizobium cicer*); F_4: PhosphoBARVAR (*Pantoea agglomerans* + *Pseudomonas putida*). The used chickpea (*Cicer arietinum*)

Table 1. Various meteorological elements recorded at Meshginshahr Synoptic Station during the 2022 cropping season

Таблица 1. Метеорологические характеристики, зарегистрированные на метеорологической станции Мешгиншехр в течение вегетационного сезона 2022 г.

	Apr.	May	Jun.	Jul.
Precipitation (mm)	41.5	57.1	73.6	24.2
Mean temperature (ºC)	9	13	18.1	19.8
Dew Point (ºC)	3	8	11	12
Wind Speed (m s^{-1})	6.3	5.9	9.4	3.2
Humidity %	69	72	72	71

genotypes were: G_1: ILC-482 (Kabuli type), G_2: 'Pirouz' (Desi type), and G_3: 'Jam' (Kabuli type), obtained from the Dryland Agricultural Research Institute, Iran. Bacterial populations with a density of 10^8 colony-forming units per mL were obtained from the Soil and Water Research Institute, Karaj, Iran. The ILC-482 line (Kabuli type) has relatively high drought tolerance and performs better under water deficit stress conditions compared to other chickpea genotypes. It was earlier revealed that the degree of drought resistance depended on the size of seeds (Mafakheri et al., 2010). ILC-482 has larger seeds so may be more sensitive to drought conditions. Cv. 'Pirouz' (Desi type) was selected and improved from local populations in western Iran over the past three decades. This cultivar is sensitive to salinity stress, but its tolerance to drought stress is relatively favorable. However, it has acceptable performance compared to newer cultivars. Cv. 'Jam' (Kabuli type) is a relatively high-yielding chickpea cultivar with large seeds, high protein content, and resistance to the *Ascochyta* blight disease. This cultivar can be grown in cold or subtropical regions as a winter crop and produce acceptable yields in these areas due to its high cold tolerance. The previous crop grown in the farm was winter wheat, and the studied site remained as fallow (unplanted) during the fall and winter seasons. In December 2021, the soil was initially plowed with a moldboard plow and then 15 t ha^{-1} of farmyard manure was applied to the soil surface and mixed with the soil to a depth of 15 cm via a mini rotavator. In mid-March 2022, secondary tillage was carried out using the disc and spring tooth to make a fine seedbed. Then, the soil surface was shaped into ridges and furrows using a hiller-furrower. The distance between ridges was 25 cm and the seeds were sown on top of the ridges at a depth of 5 cm after bacterial inoculation. Seed sowing was done manually on April 5. Seedling emergence lasted until April 28, and harvesting was carried out on July 24. Bacterial inoculation was performed by applying a liquid slurry of bacteria, along with bacterial gum and some nutrients required by the bacteria, to the seed surface at atmospheric pressure. Three seeds were sown as clump planting at the same spot. After the seedlings were fully established, excess seedlings were removed along with the first weeding at the 4-leaf stage, and a distance of 8 cm was maintained between plants. The final plant density per area unit (1 m^2) was 50 plants. Each experimental plot consisted of 12 planting rows, 4 m long. Drip irrigation (tape-type) was used for watering the field. Irrigation was carried out during periods without rainfall and when the soil moisture content fell to less than 21% (up to 60% of field capacity, FC). Weed control was carried out by hand weeding in three stages during vegetative growth and flowering. Diazinon, a nonsystemic insecticide and acaricide from the organophosphate group, was used in the early podding stage.

Agronomic characters

The relative water content (RWC) was calculated at the early flowering stage according the methods described by D. Soltys-Kalina et al. (2016), RWC = [(FW–DW) / (TW–DW)] × 100. Leaf samples were prepared from the upper leaves and their fresh weight obtained by the sensitive digital scale (FW), then immediately they were placed in distilled water for 8 h and their turgid weight were measured (TW). The turgid leaves were dried in an electric oven at 65°C and their dry weight (DW) was calculated. To assess the number of root nodules (NN) at the seed-setting stage, the roots of the plants were carefully pulled out of the soil by digging a 30 cm deep hole adjacent to the planting rows, and the NN was counted on roots of 15 plants, randomly. Plants were harvested at the stage of full maturity (browning of pods), through quadrat sampling and by randomly placing a square frame (1 m^2), in the middle parts of the plots. The plants inside the square frame were evaluated for the number of pods, the number of grains per plant, and the number of secondary branches. The plants inside the frame were cut from the ground surface and, after drying in an oven at 60°C for 72 hours, their biomass was measured. Also, after threshing and separating the straw, the grain yield per area unit was measured. Through a grain counter, three groups of 100 grains each were separated and their 100-grain weight was measured. The harvest index was calculated through the ratio of biomass to grain yield.

Statistical interpretation

Two-way ANOVA was used for the analysis of variance. ANOVA of the gathered data was performed with the SAS software (v. 9.1). The experiment was conducted as a factorial (3 × 4) based on a randomized complete block design with three replications. Factor A included 4 bacterial inoculation levels and factor B included three chickpea genotypes. The means comparison was executed using the Least Significant Difference (LSD) test. PCA was accomplished using the Minitab software (v. 16). Figures and box plots were drawn with the Excel and SPSS software (v. 25).

Results and discussion

Plant height evaluation showed that the interaction effects of genotype (G) and biofertilizer (F) on this indicator were significant ($p < 0.01$). The tallest plant was recorded in cv. 'Jam' with the application of biological fertilizers (Table 2). The lowest plant height (20.97 cm) was observed in cv. 'Pir-

Table 2. Evaluation of agronomic characteristics in different chickpea genotypes under different bacterial inoculation conditions at the time of planting time in the Meshginshahr area, Ardabil, Iran

Таблица 2. Оценка агрономических характеристик разных генотипов нута при различных условиях бактериальной инокуляции во время посева в районе Мешгиншехр (Ардебиль, Иран)

Genotypes		PH	NSB	RWC	PN	GN	HGW	HI
ILC-482		27.18^{b}	2.42^{c}	73.77^{c}	20.94^{b}	33.47^{b}	32.20^{a}	19.46^{c}
‘Pirouz’		22.62^{c}	2.97^{a}	75.29^{b}	23.09^{a}	36.34^{a}	21.43^{c}	20.74^{b}
‘Jam’		31.90^{a}	2.82^{b}	77.19^{a}	17.57^{c}	27.84^{c}	31.82^{b}	21.71^{a}
Biofertilizers								
F_1		24.40^{c}	2.56^{b}	73.81^{c}	18.56^{c}	27.81^{c}	27.97^{b}	20.13^{b}
F_2		27.65^{b}	2.86^{a}	74.73^{bc}	21.22^{ab}	35.55^{a}	2.58^{a}	20.96^{a}
F_3		28.81^{a}	2.66^{b}	75.41^{b}	21.85^{a}	34.87^{a}	28.65^{a}	21.34^{a}
F_4		28.08^{ab}	2.83^{a}	77.72^{a}	20.51^{b}	31.98^{b}	28.74^{a}	20.13^{a}
ILC-482	F_1	24.96^{c}	2.20^{j}	72.43^{d}	18.57^{e}	27.97^{f}	31.10^{f}	21.56^{b}
	F_2	27.26^{b}	2.32^{ij}	73.73^{cd}	21.83^{bcd}	36.29^{bc}	32.40^{b}	21.63^{b}
	F_3	28.53^{b}	2.70^{efg}	73.86^{cd}	22.43^{bcd}	35.80^{bc}	32.36^{b}	22.96^{a}
	F_4	27.96^{b}	2.48^{hi}	75.06^{bcd}	20.96^{d}	33.83^{cd}	32.96^{a}	20.68^{bcd}
Pirouz	F_1	20.97^{e}	2.99^{bc}	73.00^{d}	21.41^{cd}	32.07^{de}	21.26^{h}	19.27^{de}
	F_2	22.36^{de}	3.43^{a}	75.83^{bc}	23.56^{ab}	41.60^{a}	21.60^{g}	20.76^{bc}
	F_3	23.80^{cd}	2.50^{ghi}	75.70^{bc}	24.53^{a}	37.96^{b}	21.46^{gh}	21.72^{ab}
	F_4	23.36^{d}	2.96^{bcd}	76.63^{b}	22.86^{abc}	33.73^{cd}	21.40^{gh}	21.22^{b}
Jam	F_1	27.26^{b}	2.59^{fgh}	76.00^{bc}	15.70^{f}	23.39^{g}	31.56^{e}	19.35^{cde}
	F_2	33.33^{a}	2.58^{cde}	74.63^{bcd}	18.28^{e}	28.75^{f}	31.76^{de}	20.50^{bcd}
	F_3	34.10^{a}	2.78^{efd}	76.66^{b}	18.60^{e}	30.85^{def}	32.11^{bc}	19.32^{de}
	F_4	32.93^{a}	3.07^{b}	81.46^{a}	17.27^{e}	28.40^{f}	31.86^{cd}	18.48e
		***P* value**						
G		< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F		< 0.0001	0.0002	0.0002	< 0.0001	< 0.0001	< 0.0001	0.0039
G×F		0.0027	< 0.0001	0.0569	0.9570	0.0922	< 0.0001	0.0058

Note: F_1: control (no seed inoculation); F_2: Nitroxin; F_3: Mesorhizobium; F_4: PhosphoBARVAR; PH: plant height, cm; NSB: number of secondary branches; RWC: relative water content, %; PN: pod number; GN: grain number; HGW: 100-grain weight, g; HI: harvest index, %. *P* values less than 0.01 are significant at the 1% statistical level

Примечание: F_1: контроль (без инокуляции семян); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR; PH: высота растения, см; NSB: количество вторичных ветвей; RWC: относительное содержание воды, %; PN: число бобов; GN: число зерен; HGW: масса 100 зерен, g; HI: индекс урожая, %. Значения *P* менее 0,01 статистически значимы на уровне 1%

ouz’ without the use of biological fertilizers. In the ILC-482 line, although the use of biological fertilizers increased plant height by 11% compared to the control (F1), there was no difference between the biological fertilizers in terms of plant height.

The interaction effect of F × G was also significant ($p < 0.01$) for the number of secondary branches (NSB). The lowest number of NSB was recorded in line ILC-482 without bacterial inoculation or with the use of Nitroxin. The highest NSB (3.43) was obtained in ‘Pirouz’ with the use of Nitroxin. In cv. ‘Jam’, the use of PhosphoBARVAR (F4) significantly increased the NSB. The results of ANOVA for RWC showed that the effects of F and G on this parameter were significant ($p < 0.01$). Among the genotypes, ‘Jam’ showed the highest RWC value (77.19%), the lowest value of this indicator was observed in line ILC-482 (73.77%). The highest effect of biofertilizers on RWC was achieved with the use of F4.

The evaluation of the number of root nodules on a plant (NN) showed that among the genotypes, the highest NN was observed in 'Jam' (12.55), and the lowest recorded for the ILC-482 line (10.18) and cv. 'Pirouz' (11.08) without significant difference. Comparison of the mean for the effect of the F×G feature on the NN values showed that the highest NN was obtained in 'Jam' with *Mesorhizobium* inoculation (18.10) and the lowest NN in 'Pirouz' without bacterial inoculation. The difference between the genotypes in terms of NN was evident; however, among the biofertilizers, the use of *Mesorhizobium* had the highest effect on NN (16.14) and the use of PhosphoBARVAR (11.05) was the second. The use of Nitroxin had a negligible effect on NN compared to the control (Fig. 1).

Grain number (GN) evaluation also indicated a similar trend. Application of Nitroxin, *Mesorhizobium* and PhosphoBARVAR increased GN by 27%, 25% and 14%, respectively, compared to the control. The interaction effect of F × G on 100-grain weight was also significant at the 1% level.

The main effect of biofertilizer application (F) and the genotype (G) on aboveground biomass was significant ($p < 0.01$). PhosphoBARVAR application produced the highest biomass (6.24 t ha^{-1}), followed by *Mesorhizobium* (6.09 t ha^{-1}) and Nitroxin (5.97 t ha^{-1}), and the biomass was the lowest under the F_1 conditions (5.83 t ha^{-1}). Among the genotypes, the highest biomass was recorded in ILC-482 (6.25 t ha^{-1}), while the biomass in 'Pirouz' was 5.93 t ha^{-1}, and in 'Jam' 5.90 t ha^{-1} (Fig. 2). The highest biomass was recorded for ILC-482 with the application of *Mesorhizobium* (6.41 t ha^{-1}) and PhosphoBARVAR (6.29 t ha^{-1}). The lowest biomass was recorded in 'Jam' without bacterial inoculation (5.69 t ha^{-1}).

The main effects of F and G on pod number per plant (PN) were significant at the 1% level. Cv. 'Pirouz' had the highest PN (23.09) and 'Jam' had the lowest PN (17.57). Comparing the mean for each component among the biofertilizer levels, the highest PN was recorded with the application of *Mesorhizobium* (21.85) and Nitroxin (21.22), which increased the PN by 7% and 14% compared to the control (see Table 2). Grain number (GN) evaluation also indicated a similar trend. The application of Nitroxin, *Mesorhizobium* and PhosphoBARVAR increased GN by 27%, 25% and 14%, respectively, over the control. The effect of the F × G feature on 100-grain weight was also significant at the 1% level. The heaviest seeds were obtained in the ILC-482 line with the application of PhosphoBARVAR (32.96 g). The lowest 100-seed weight was obtained in 'Pirouz' (21.26 g) without the application of biofertilizer. For 'Pirouz', the application of Nitroxin had the greatest effect. For 'Jam', the application of *Mesorhizobium* had the greatest effect on seed weight. The effect of the F × G feature on seed yield was significant, and the highest yield was obtained in the ILC-482 line with the application of *Mesorhizobium* (1.43 t ha^{-1}). The lowest grain yield was obtained in cv. 'Pirouz' without bacterial inoculation (1.09 t ha^{-1}). Under the conditions without application of biofertilizers, the highest yield was recorded for ILC-482 (1.3 t ha^{-1}). This trend was also observed under the conditions of Nitroxin and *Mesorhizobium* application. The yield response of 'Jam' to the application of biological fertilizers was not significant, and the lowest yield was recorded under bacterial inoculation conditions for this cultivar. However, the lowest yield was shown by cv. 'Pirouz' under the conditions without bacterial inoculation (Fig. 3). Interestingly, only the application of *Mesorhizobium* had a minor effect on the grain yield of 'Jam'.

Comparisons of the harvest indices (HI) among the genotypes showed that 'Jam' had the highest HI and ILC-482 showed the lowest value. Inoculation with different types of bacteria significantly increased HI compared to the control. Comparisons of the genotype × inoculation interaction effects showed that the highest HI was obtained in ILC-482 and also in 'Pirouz' with *Mesorhizobium* inoculation.

The trait vector monoplot is shown in Figure 4. In this figure, the evaluated agronomic characters that have sharp angles demonstrate a positive and significant correlation (Cos 0°= 1; correlation coefficient). The results indicated that

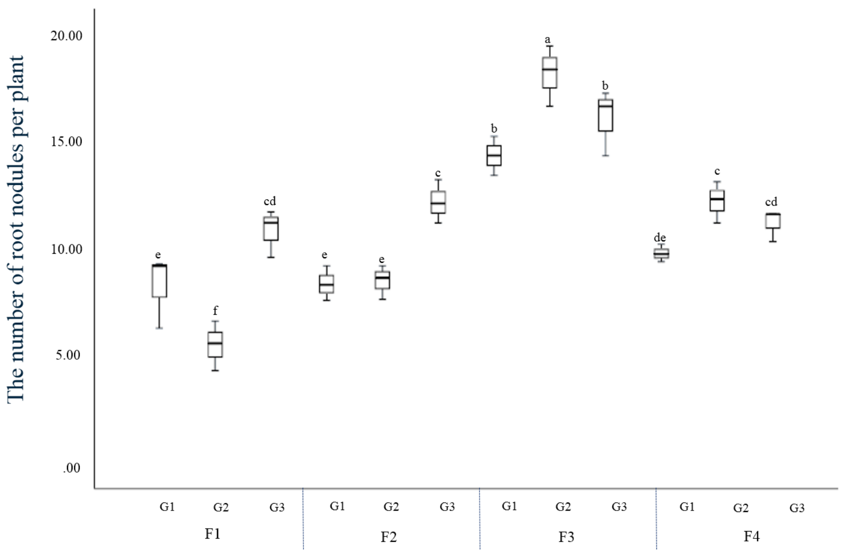

Fig. 1. Number of nodules on the chickpea root affected by the application of different biofertilizers in the Meshginshahr area, Ardabil, Iran. G_1: line ILC-482; G_2: 'Pirouz'; G_3: 'Jam'. F_1: control (no seed inoculation); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR

Рис. 1. Количество клубеньков на корне нута, образовавшихся в результате применения различных биоудобрений в районе Мешгиншехр (Ардебиль, Иран). G_1: линия ILC-482; G_2: сорт 'Pirouz'; G_3: сорт 'Jam'. F_1: контроль (без инокуляции семян); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR

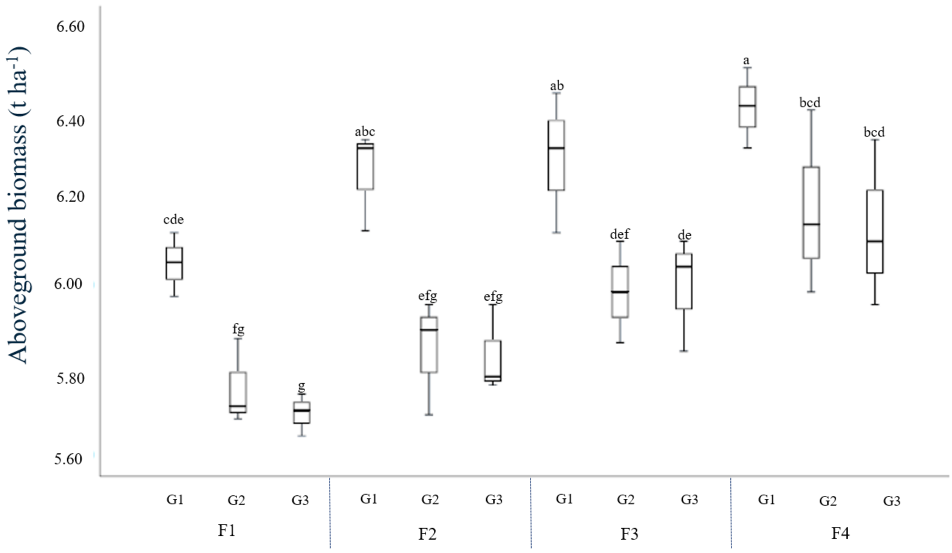

Fig. 2. **The effect of bacterial seed inoculation at the time of planting on the aboveground biomass of chickpea genotypes cultivated in the Meshginshahr area, Ardabil, Iran. G_1: line ILC-482; G_2: 'Pirouz'; G_3: 'Jam'. F_1: control (no seed inoculation); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR**
(columns with the same letters do not contain statistically significant differences)

Рис. 2. **Влияние бактериальной инокуляции семян во время посева на надземную биомассу генотипов нута, выращиваемых в районе Мешгиншехр (Ардебиль, Иран). G_1: линия ILC-482; G_2: сорт 'Pirouz'; G_3: сорт 'Jam'. F_1: контроль (без инокуляции семян); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR**
(столбцы с одинаковыми буквами не имеют статистически значимых различий)

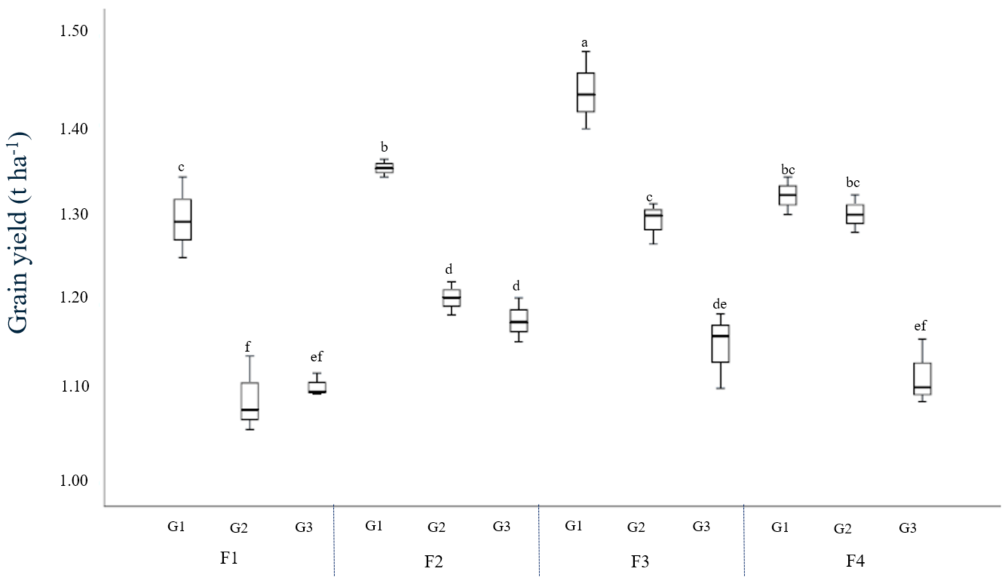

Fig. 3. **Grain yield evaluation in different chickpea genotypes under the influence of different biofertilizers in the Meshginshahr area, Ardabil, Iran. G_1: line ILC-482; G_2: 'Pirouz'; G_3: 'Jam'. F_1: control (no seed inoculation); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR**

Рис. 3. **Оценка урожайности зерна различных генотипов нута под влиянием разных биоудобрений в регионе Мешгиншехр (Ардебиль, Иран). G_1: линия ILC-482; G_2: сорт 'Pirouz'; G_3: сорт 'Jam'. F_1: контроль (без инокуляции семян); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR**

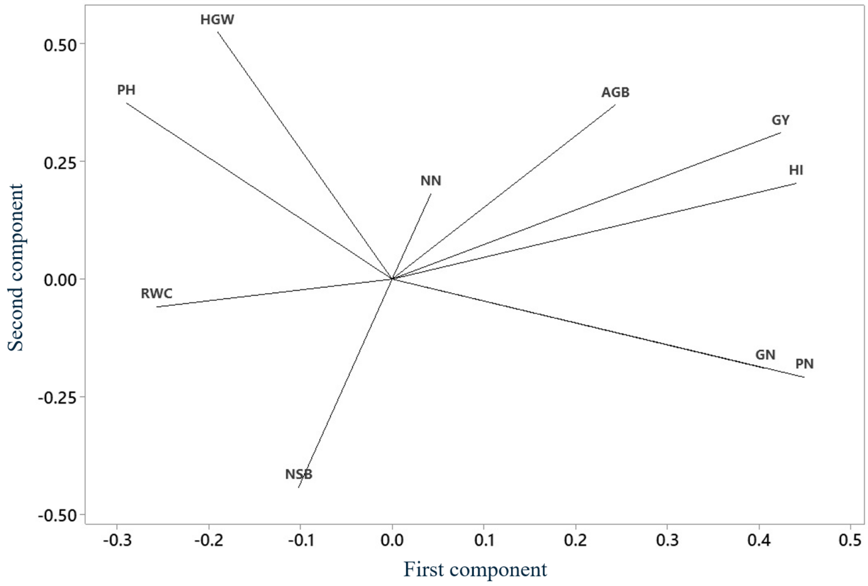

Fig. 4. **Linear dimensionality reduction on the monoplot of principal component analysis to describe the correlation between different agronomic characters of chickpea genotypes under different biofertilizer application conditions in northwest Iran. NN: nodule number per root; GY: grain yield; ABG: aboveground biomass; PH: plant height; NSB: number of secondary branches; RWC: relative water content; PN: pod number; GN: grain number; HGW: 100-grain weight; HI: harvest index**

Рис. 4. **Сокращение линейной размерности с помощью монографического анализа главных компонент для описания корреляции между различными хозяйственно значимыми признаками нута при различных условиях применения биоудобрений на северо-западе Ирана. NN: количество клубеньков на корне; GY: урожайность зерна; ABG: надземная биомасса; PH: высота растения; NSB: количество вторичных ветвей; RWC: относительное содержание воды; PN: число бобов; GN: число зерен; HGW: масса 100 зерен; HI: индекс урожая**

such indicators as seed number, pod number, biomass, and nodule number per root showed a positive correlation with seed yield. Also, 100-seed weight was positively correlated with plant height and RWC (see Fig. 4). The number of lateral branches was negatively and significantly correlated with nodule number per root (Cos 180°= –1; correlation coefficient).

Principal Component Analysis (PCA) to describe the spatial location and similarity between the combined treatments (score plot) is shown in Figure 5. In the figure, the first component separated G_3 (‘Jam’) from G_1 (ILC-482) and G_2 (‘Pirouz’), and the second separated G_2 (‘Pirouz’) from G_3 (‘Jam’) and G_1 (ILC-482). The close location of F_1 and F_4 indicated a slight effect of PhosphoBARVAR compared to the control in the ILC-482 line. In cv. ‘Pirouz’, the effects of inoculation with *Mesorhizobium* were somewhat similar to PhosphoBARVAR. The best performance under the conditions of *Mesorhizobium* application was observed in the ILC-482 line. For ‘Jam’, the effects of *Mesorhizobium* and Nitroxin on the studied indicators were largely similar.

The results indicated that the ‘Jam’ genotype had greater longitudinal growth and that this genotype exhibited good potential for breeding improvement for mechanized harvesting with a combine. In the aforementioned genotype, bacterial inoculation was also able to increase plant height. Greater height in chickpea plants is considered a desirable trait for mechanized harvesting, so cultivars with increased height have a greater advantage. Genotypes whose plants are 30–40% taller than those of the existing cultivars and have the semierect to erect type of growth habit will make the chickpea cultivars suitable for mechanized harvesting. The average chickpea yield in the semiarid regions of Iran is lower than the global average due to a large number of environmental constraints. One of the reasons for this is the lack of recognition of the diversity among cultivars for agro-climatic zones (Rahimi-Moghaddam et al., 2023). Utilization of greater chickpea diversity and examining a greater number of different genotypes can facilitate the selection of the best genotype compatible with the environment and also show the effectiveness of biological fertilizers more accurately. Cv. ‘Pirouz’, one of the old and native populations selected in western Iran, had the lowest plant height, and inoculation with *Mesorhizobium* and PhosphoBARVAR improved the height in this genotype.

A brief comparison of the Kabuli and Desi varietal types showed that the longitudinal growth of the stem in a Desi genotype is less than that in the Kabuli one, and it seems that this component is largely controlled by genetics. The highest number of lateral branches in ‘Pirouz’ was obtained by inoculation with *Azotobacter chroococcum* + *Azospirillum lipoferum*. The most important factors affecting the number of lateral branches in chickpea are genetic predisposition, plant density, and environmental factors such as access to nutrients and water (Koul et al., 2022). The effectiveness of *Azotobacter* + *Azospirillum* inoculation depends to some extent on a crosstalk between them and the host plant, and it seems that the genetic background played a more crucial role in the formation of symbiotic relationships and its effects on lateral growth (Sun et al., 2025). A brief comparison of the Desi type and Kabuli genotypes indicated that cv. ‘Pirouz’ produced more pods, but due to its low 100-grain weight, the final grain yield was lower compared to the Kabuli genotypes. While some researchers have reported poor performance of Desi-type chickpeas, ‘Pirouz’ is likely to have been well adapted to the regional conditions. Allocation of photoassimilate resources to reproductive and grain yield is one of the key factors affecting yield improvement (Patrick, Colyvas, 2014), and

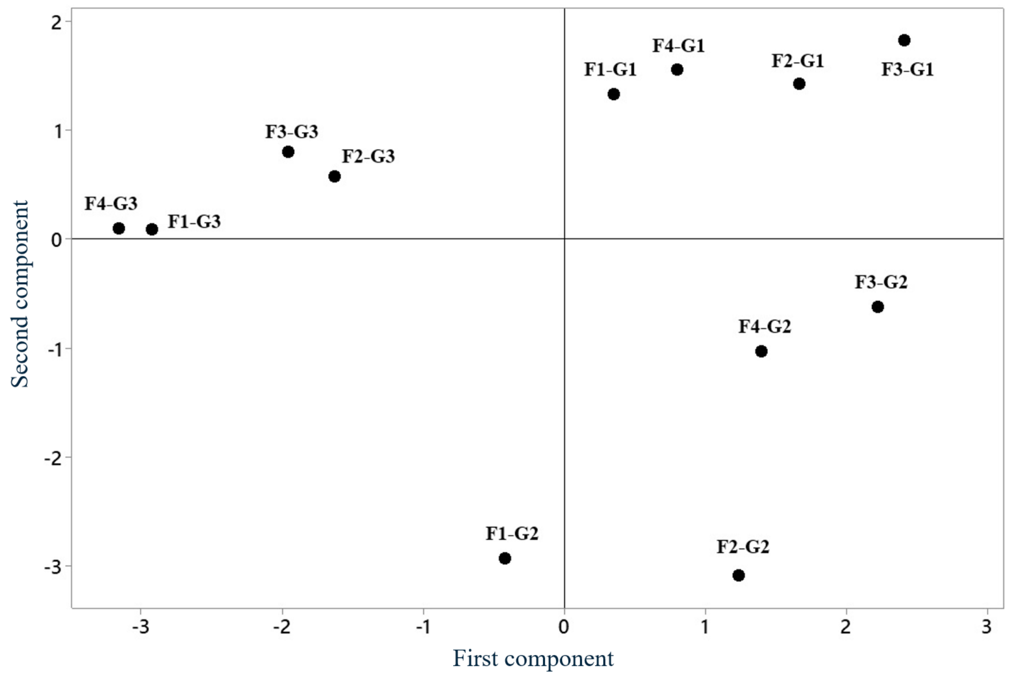

Fig. 5. Information content summarized via PCA to describe the spatial arrangement and similarity of combined impacts (genotype and biofertilizers) and their effect on the evaluated agronomic indicators. G_1: ILC-482; G_2: 'Pirouz'; G_3: 'Jam'. F_1: control (no seed inoculation); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR

Рис. 5. Содержание информации, обобщенное с помощью PCA для описания пространственного расположения и сходства комбинированных воздействий (генотипа и биоудобрения) и их влияния на оцениваемые хозяйственно значимые признаки. G_1: линия ILC-482; G_2: сорт 'Pirouz'; G_3: сорт 'Jam'. F_1: контроль (без обработки семян); F_2: Nitroxin; F_3: *Mesorhizobium*; F_4: PhosphoBARVAR

in the Desi-type chickpea, poor allocation can be one of the reasons for reduced grain yield. The response of Desi-type chickpeas to inoculation with *Pantoea agglomerans* and *Pseudomonas putida* was more pronounced. Seed inoculation with phosphate-solubilizing bacteria may increase the conversion of insoluble forms of phosphorus in the soil into soluble forms and improve plant growth and nutrient uptake (Wu et al., 2019). Furthermore, these bacteria can improve seed germination, seedling establishment, and root extension. Also, *P. agglomerans* is recognized for N assimilation and P solubilization, while *P. putida* can encourage root system development and nutrient adsorption (Vasseur-Coronado et al., 2021).

Bacterial inoculation, especially with *Azotobacter chroococcum* + *Azospirillum lipoferum* and *Mesorhizobium cicer*, increased the number of nodules per plant, especially in Kabuli genotypes. These results showed that bacterial seed inoculation and providing the necessary symbiotic relationships further increased the yield of the Kabuli genotypes, while the Desi-type chickpea was likely capable of higher performance under environmental constraints. Bacterial inoculation with phosphate-solubilizing bacteria in cv. 'Pirouz' increased the number of root nodules more than with other biofertilizers. The relative water content in cv. 'Jam' with phosphorus fertilizer inoculation was higher than under other conditions. This could be due to water saving through reduced transpiration or increased water absorption in the soil. Comparison of yield components in the Desi-type and Kabuli genotypes showed that despite the high grain yield in Kabuli genotypes, increasing yield components, such as the number of pods and the number of seeds per plant, can still be a breeding goal in these genotypes. Assessing the impacts of bacterial inoculation on various plant characters showed that overall, the inoculation with *Mesorhizobium cicer* had a significant improving effect in both Desi-type and Kabuli chickpeas compared to the control. Although the main role of *Mesorhizobium cicer* is in providing nitrogen to nitrogen-consuming processes in plants, such as the biosynthesis of enzymes, chlorophyll and structural proteins (Lindström, Mousavi, 2020). Given the low amount of available phosphorus in the soil, the use of phosphate-solubilizing bacteria can have a significant impact. Perhaps the application of animal manure in the present study had partially reduced the expected effect of bacterial inoculation with *Pantoea agglomerans* and *Pseudomonas putida*. The results showed that bacterial inoculation of the ICL-482 line with *Mesorhizobium cicer* could be a beneficial agronomic option in improving crop performance in the studied area.

Conclusion

Among the evaluated agronomic indicators, ABG, NN, HI, seed number, and PN showed the highest correlation with grain yield and are considered appropriate selection indices for evaluating the performance of chickpea genotypes. The response of growth and grain yield components in genotypes to biofertilizers was variable. Although all biofertilizers improved growth, the improving effect of *Mesorhizobium* inoculation was greater than in the case with other biofertilizers. The lowest growth and grain yield were recorded for cv. 'Jam' without bacterial inoculation. Despite its high lateral growth, cv. 'Pirouz' cannot be a suitable option for mechanized harvesting due to its low plant height. The grains produced in the ICL-482 line were heavier under the application of biofertilizers than under other conditions. The results showed that the application of biofertilizers, especially *Mesorhizobium*, and planting the ICL-482 line under the agro-climatic conditions of the studied area resulted in more acceptable performance.

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Contribution of the authors: the authors contributed equally to this article.
Вклад авторов: все авторы сделали эквивалентный вклад в подготовку публикации.

Conflict of interests: the authors declare no conflicts of interests.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

The article was submitted on 02.07.2025; approved after reviewing on 15.10.2025; accepted for publication on 30.10.2025.
Статья поступила в редакцию 02.07.2025; одобрена после рецензирования 15.10.2025; принята к публикации 30.10.2025.