

Adaptive potential of legume-rhizobia symbiosis under saline soil conditions: Comparative evaluation of native salt-tolerant rhizobial inoculants

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ABSTRACT

Soil salinization is a major global constraint to sustainable agriculture because it severely limits legume productivity by disrupting both plant growth and biological N fixation. This study aimed to evaluate the symbiotic efficiency and agronomic potential of native salt-tolerant rhizobial strains isolated from naturally saline soils of the Ararat Valley, Armenia, for improving legume performance under saline conditions. A two-layer soil-sand system was developed to simulate progressive root-zone salinity under field-relevant conditions, using a non-saline upper layer for seed establishment and a saline lower horizon (electrical conductivity 4.0-6.2 dS m⁻¹) for stress exposure. Selected strains were tested on soybean (*Glycine max* (L.) Merr.), peanut (*Arachis hypogaea* L.), chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.), fenugreek (*Trigonella foenum-graecum* L.), and common bean (*Phaseolus vulgaris* L.) by assessing nodulation, plant growth, biomass accumulation, seed productivity, and seed protein content. In soybean, inoculation with selected *Bradyrhizobium japonicum* strains increased yield by 37%-45% relative to the non-inoculated control ($P < 0.05$), with strain MDC 5789 showing the highest symbiotic efficiency. In peanut, strain MDC 5812 increased productivity by 125% ($P < 0.05$). Across all tested legumes, inoculation significantly improved nodulation and plant growth under salinity, although the magnitude of response was strongly host dependent. In common bean, strain MDC 5700 maintained effective nodulation under 6.2 dS m⁻¹ and increased shoot biomass and seed protein content by 11.1% ($P < 0.05$). These results demonstrate that indigenous salt-tolerant rhizobia are promising candidates for development of region-specific biofertilizers and represent an effective biological strategy for improving legume productivity and sustainability in salt-affected agroecosystems.

Key words: Common bean, chickpea, fenugreek, legume-rhizobium symbiosis, lentil, microbial inoculants, pea, peanut, salt-tolerant rhizobia, soil salinity, soybean.

INTRODUCTION

Soil salinization is one of the most severe forms of land degradation and a major global threat to agricultural sustainability, food security, and ecosystem stability. According to recent global assessments, more than one billion hectares of land worldwide are affected by salinity, including approximately 20%-30% of irrigated agricultural areas, and this proportion is expected to increase further due to climate change, increasing evapotranspiration, freshwater scarcity, sea-level rise, and unsustainable irrigation practices (Butcher et al., 2016; FAO, 2021; Shokri et al., 2024).

Salinity constrains plant growth through osmotic stress, ionic toxicity, nutrient imbalance, and oxidative damage. Elevated concentrations of soluble salts reduce soil water potential, limiting root water uptake and

inducing physiological drought, while excessive accumulation of Na^+ and Cl^- disrupts ion homeostasis, damages cellular membranes, and impairs photosynthesis and C metabolism, ultimately reducing biomass and yield (Shrivastava and Kumar, 2015; Navarro-Torre et al., 2023).

Legume crops are particularly vulnerable because their productivity depends not only on plant stress tolerance but also on the functionality of the highly sensitive rhizobium-legume symbiosis. Salinity negatively affects nodulation, nodule metabolism, and biological N fixation (BNF), often before visible symptoms of plant stress appear (Egamberdieva et al., 2019; Balasubramaniam et al., 2023). Since legumes contribute substantially to sustainable agriculture through natural N input and restoration of soil fertility, maintaining their productivity under saline conditions has become an important agronomic priority. The use of salt-tolerant rhizobia has emerged as a promising biological strategy for improving legume productivity in salt-affected soils. Certain rhizobial strains exhibit remarkable physiological plasticity, enabling survival and maintenance of symbiotic efficiency under elevated salinity through mechanisms such as osmotic adjustment, ion homeostasis, exopolysaccharide production, antioxidant defense, and metabolic flexibility (Lindström and Mousavi, 2020). These traits have gained increasing relevance in the context of microbial biostimulants and climate-smart agriculture (Backer et al., 2018; Rouphael and Colla, 2020).

Among adaptive traits, metabolic flexibility is particularly important because utilization of diverse C substrates determines rhizosphere competitiveness and long-term symbiotic performance. Organic acids such as succinate and fumarate are major host-derived C sources during N fixation, whereas polyols contribute directly to osmoprotection under stress (Poole et al., 2018). Therefore, phenotypic metabolic profiling remains an important complementary tool for selecting effective biofertilizer candidates, especially when integrated with molecular characterization.

A major limitation of many salinity studies is the poor ecological realism of experimental systems. Most laboratory assays use homogeneous NaCl application, whereas field salinity is typically heterogeneous, with salts accumulating in soil deeper horizons. A two-layer soil-sand system can provide a practical approach for evaluating plant-microbe interactions under progressive root-zone salinity. In such a system, a low-salinity upper layer provides favorable conditions for seed germination and early seedling establishment, whereas subsequent root growth into the underlying saline soil results in progressive exposure to salt stress (Machado and Serralheiro, 2017; Shahid et al., 2018). The effectiveness of rhizobial inoculation also depends strongly on host genotype and host-strain compatibility. Increasing evidence indicates that native co-adapted plant-microbe combinations often outperform non-native commercial inoculants under environmental stress, supporting the concept of regionally adapted microbial inoculants (Goginyan et al., 2025). These issues are particularly relevant in the Ararat Valley, the principal irrigated agricultural region of Armenia, where secondary salinization has become a serious agricultural challenge due to intensive irrigation, shallow groundwater, poor drainage, and high evapotranspiration. More than one-third of irrigated soils in the region are affected by salinity, making it an important natural model for testing biologically based solutions (Ghazaryan et al., 2020).

To our knowledge, this is the first comparative study to evaluate multiple legume-rhizobium systems with native Armenian salt-tolerant isolates under progressive root exposure to naturally saline soil. The objective of the present study was to evaluate native salt-tolerant rhizobial strains isolated from saline soils of the Ararat Valley using a two-layer soil-sand system designed to simulate natural field conditions. By comparing their effects across several economically important legume crops and assessing physiological, symbiotic, and agronomic responses, this study aimed to identify promising regionally adapted microbial inoculants for sustainable agriculture in Armenia and other salt-affected dryland regions.

MATERIALS AND METHODS

Study site and experimental design

A controlled pot experiment was conducted to evaluate the effectiveness of selected salt-tolerant rhizobial strains in maintaining symbiotic performance and improving legume productivity under saline conditions. A two-layer soil-sand system was established to provide favorable conditions for seed germination and early seedling establishment, followed by progressive root exposure to naturally saline soil. Approximately 300 kg naturally saline soil was collected from the Arazap (40.0417° N, 44.1473° E) and Ranchpar (40°01'40" N, 44°22'10" E) sites in the Ararat Valley (approximately 40.0° N, 44.3° E), Ararat Province, Armenia. The saline soil was placed as the lower layer in experimental containers and covered with a 10 cm layer of washed river sand,

creating a two-layer soil-sand profile in which seeds germinated in the upper low-salinity layer and roots progressively penetrated the underlying saline horizon during plant development. All experiments were conducted under controlled greenhouse conditions (Munns and Tester, 2008). A schematic representation of the experimental design is presented in Figure 1.

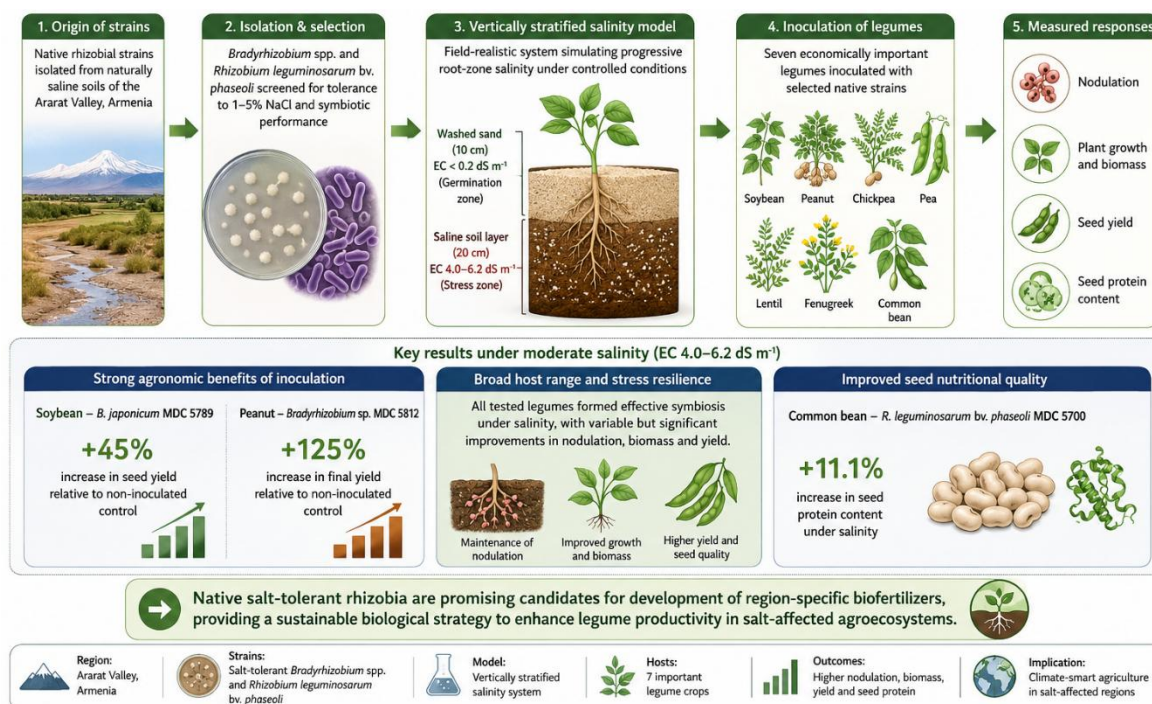


Figure 1. Schematic representation of the experimental design used to evaluate legume-rhizobium symbioses under the two-layer soil-sand system conditions.

Soil physicochemical characterization

Prior to use in the experiment, the collected saline soils were subjected to physicochemical characterization following standard analytical procedures (Page et al., 1982; Rhoades, 1996). Soil pH and electrical conductivity (EC) were measured in a 1:5 soil-to-distilled water extract using a calibrated pH/EC meter (Multi 3620 IDS, WTW/Xylem Analytics, Weilheim, Germany). The Arazap soil was classified as moderately saline and slightly alkaline, with pH values ranging from 7.9 to 8.1 and EC of 6.2 dS m⁻¹. Total dissolved salts were 0.8%, consisting primarily of chloride (0.45%) and sulfate (0.51%), with lower concentrations of bicarbonates (0.33%) and carbonates (0.20%). The Ranchpar soil exhibited pH values of 7.4–7.9 and EC of 4.0 dS m⁻¹, also corresponding to moderate salinity.

Estimation of salinity load in the experimental system

To characterize soil sodicity, the sodium adsorption ratio (SAR) was calculated according to Richards (1954). The experimental system functioned as a two-layer soil-sand system in which the upper sand layer maintained negligible salinity (EC < 0.2 dS m⁻¹), while the lower saline horizon retained the original EC values of the collected soils (4.0–6.2 dS m⁻¹), thereby enabling progressive root-zone salt exposure during plant development.

Rhizobial strains and inoculum preparation

Salt-tolerant rhizobial strains belonging to *Bradyrhizobium japonicum*, *Bradyrhizobium* spp., *Mesorhizobium ciceri*, *Rhizobium leguminosarum*, *R. leguminosarum* bv. *lens*, *R. leguminosarum* bv. *phaseoli*, and *Sinorhizobium meliloti* were obtained from the culture collection of the Scientific and Production Center “Armbiotechnology”, Yerevan, Armenia. The rhizobial strains and their corresponding legume hosts used in the inoculation experiments are summarized in Table 1. The strains had previously been isolated from saline soils and root nodules of the Ararat Valley and selected based on their ability to grow on yeast extract mannitol agar (YMA) supplemented with 1%-5% NaCl. Cultures were maintained on YMA (Vincent, 1970) and incubated at 28 ± 1 °C for 3-5 d. For inoculum preparation, single colonies were transferred to yeast extract mannitol broth (YMB) and grown at 28 °C with shaking (150 rpm) to late exponential phase. Cell density was standardized to 10^8 - 10^9 CFU mL⁻¹ (OD₆₀₀ = 0.8-1.0) before inoculation (Somasegaran and Hoben, 1994; Zahran, 1999; Atlas, 2010).

Table 1. Rhizobial strains used in the study and their corresponding legume host plants.

Rhizobial species	Strain (MDC No.)	Host plant used in this study
<i>Bradyrhizobium japonicum</i>	MDC 5783	Soybean (<i>Glycine max</i> (L.) Merr.)
	MDC 5784	
	MDC 5787	
	MDC 5789	
	MDC 5798	
<i>Bradyrhizobium</i> spp.	MDC 5804	Peanut (<i>Arachis hypogaea</i> L.)
	MDC 5805	
	MDC 5807	
	MDC 5808	
	MDC 5809	
	MDC 5812	
<i>Mesorhizobium ciceri</i>	MDC 6048	Chickpea (<i>Cicer arietinum</i> L.)
<i>Rhizobium leguminosarum</i>	MDC 5609	Pea (<i>Pisum sativum</i> L.)
<i>R. leguminosarum</i> bv. <i>lens</i>	MDC 6006	Lentil (<i>Lens culinaris</i> Medik.)
<i>R. leguminosarum</i> bv. <i>phaseoli</i>	MDC 5700	Common bean (<i>Phaseolus vulgaris</i> L.)
<i>Sinorhizobium meliloti</i>	MDC 5545	Fenugreek (<i>Trigonella foenum-graecum</i> L.)

Carbon utilization analysis using API system

Comparative physiological profiling of C utilization was performed for the soybean (*Glycine max* (L.) Merr.)-associated *Bradyrhizobium japonicum* strains (MDC 5783, MDC 5784, MDC 5787, MDC 5789, and MDC 5798) and peanut (*Arachis hypogaea* L.)-associated *Bradyrhizobium* spp. (MDC 5804, MDC 5805, MDC 5807, MDC 5808, MDC 5809, and MDC 5812) using the API 50 CH system (bioMérieux, Craponne, France) according to the manufacturer’s instructions. These two host-associated groups were selected for metabolic profiling because they comprised multiple strains, enabling comparative assessment of within-group metabolic diversity. The rhizobial strains used for chickpea (*Cicer arietinum* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.), fenugreek (*Trigonella foenum-graecum* L.), and common bean (*Phaseolus vulgaris* L.) were each represented by a single selected strain and were therefore not included in this comparative metabolic profiling. Fresh bacterial biomass from 48 h YMA cultures was suspended in sterile 0.85% NaCl to a turbidity equivalent to McFarland 2.0 and used to inoculate the test strips. Plates were incubated aerobically at 28 ± 1 °C for 48-72 h, and reactions were scored as positive (+) or negative (–). Each strain was tested in triplicate.

Plant material and seed preparation

The tested crops were soybean, peanut, chickpea, pea, lentil, common bean, and fenugreek. Seeds were surface-sterilized using 70% ethanol (30-60 s), followed by 2%-3% sodium hypochlorite (3-5 min), thoroughly rinsed with sterile distilled water, and pre-germinated on moist sterile filter paper at 25 ± 2 °C for 48-72 h. Uniform seedlings were inoculated immediately before planting with 1 mL bacterial suspension per seed (10^8 - 10^9 CFU mL⁻¹). Each treatment consisted of three independent biological replicates, with 10 plants per replicate. Plants were grown in the greenhouse under controlled conditions (16:8 h light/dark, 24-26 °C day/18-20 °C

night; relative humidity 60%-70%). At flowering and/or maturity, plants were evaluated for shoot length, shoot and root dry biomass, nodule number, nodule dry weight, pod/seed production, and final yield (Hunt, 1990). Seed protein content was determined using the Kjeldahl method, and total N was converted to crude protein using the standard conversion factor of 6.25.

Statistical analysis

All experiments were conducted using three independent biological replicates ($n = 3$), with 10 plants included in each biological replicate. Data are presented as mean $\pm$ standard error (SE). Statistical significance was assessed by one-way ANOVA. When more than two treatments were compared, Tukey's honestly significant difference (HSD) test was used for post hoc multiple comparisons. Differences were considered significant at $p \leq 0.05$. Because the number of treatment groups differed among experiments, the corresponding residual degrees of freedom also varied. The residual degrees of freedom were 12 for the soybean experiment (six treatment groups), 14 for the peanut experiment (seven treatment groups), 4 for each two-treatment comparison in the comparative legume experiment, and 6 for the common bean experiment (three treatment groups). The relatively small number of independent biological replicates represents a limitation of the study and may reduce statistical power, particularly in experiments involving only two or three treatment groups.

RESULTS AND DISCUSSION

Physiological and metabolic characteristics of soybean- and peanut-associated *Bradyrhizobium* strains

Comparative analysis of C source utilization revealed substantial physiological and metabolic diversity among the studied strains of *Bradyrhizobium japonicum* and *Bradyrhizobium* spp. despite their shared taxonomic affiliation (Figure 2). Such variability reflects differences in catabolic flexibility and ecological specialization that may influence rhizosphere competence, persistence, host colonization, and symbiotic performance under saline conditions, where metabolic plasticity is increasingly recognized as a key determinant of rhizobial adaptation (Lindström and Mousavi, 2020).

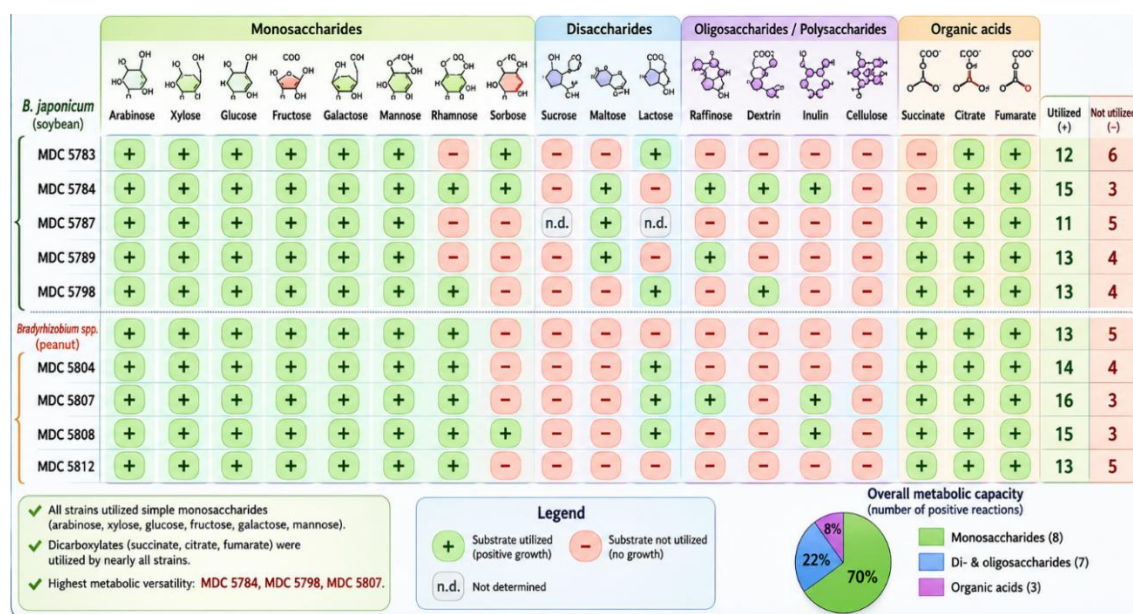


Figure 2. Carbon source utilization profiles of soybean-associated *Bradyrhizobium japonicum* and peanut-associated *Bradyrhizobium* spp. strains determined using the API 50 CH system (bioMérieux, Craponne, France).

All strains efficiently utilized simple monosaccharides (arabinose, xylose, glucose, fructose, galactose, mannose), indicating conserved carbohydrate transport systems and central metabolic pathways essential for rhizosphere colonization and early symbiotic establishment (Geddes and Oresnik, 2014; Poole et al., 2018; Liu et al., 2024). In contrast, marked differences were observed in the utilization of secondary carbohydrates (disaccharides, oligosaccharides, polyols), suggesting intraspecific metabolic diversification associated with ecological adaptation and host-driven niche specialization (Andrews and Andrews, 2017).

Among soybean-associated *B. japonicum*, strain MDC 5784 showed the broadest substrate spectrum, followed by MDC 5798 and MDC 5789, indicating greater metabolic versatility and adaptive potential, traits often linked to enhanced environmental resilience (Remigi et al., 2016; Kumawat et al., 2023). In contrast, peanut-associated isolates displayed a narrower but more conserved metabolic profile, consistent with stronger host specialization and co-adaptation.

Sorbose utilization, detected only in strains MDC 5783, MDC 5784, and MDC 5808, may represent an adaptive trait relevant to osmotic tolerance, since polyol metabolism contributes to osmotic adjustment and membrane stabilization under salinity stress. Nearly all strains efficiently utilized succinate and fumarate, confirming a conserved capacity for dicarboxylate metabolism, a key determinant of bacteroid energy supply and N fixation efficiency (Poole et al., 2018; Mendoza-Suárez et al., 2020). By contrast, cellulose was not utilized by any strain, indicating dependence on readily available low-molecular-weight C sources.

Overall, soybean-associated strains demonstrated greater metabolic heterogeneity than peanut-associated isolates, suggesting higher ecological plasticity under progressive salinization. From an applied perspective, strains MDC 5784, MDC 5798, and MDC 5807 appear particularly promising for development of regionally adapted inoculants for saline agroecosystems (Rouphael and Colla, 2020). These findings indicate that intraspecific metabolic diversity is an important functional determinant of stress tolerance and long-term inoculant performance.

Salt tolerance and symbiotic efficiency under saline conditions

A two-layer soil-sand system developed in this study provided a field-relevant platform for evaluating legume-rhizobium interactions under progressive saline stress. Unlike conventional assays based on homogeneous NaCl application, this model reproduced the spatial heterogeneity typical of naturally saline agricultural soils. A 10 cm upper layer of washed river sand ($EC < 0.2 \text{ dS m}^{-1}$) ensured favorable conditions for seed germination and early growth, whereas the underlying saline horizon, composed of naturally salt-affected soils from Arazap and Ranchpar in the Araks River floodplain, maintained moderate salinity ($4.0\text{--}6.2 \text{ dS m}^{-1}$) representative of the Ararat Valley agroecosystem.

This design created progressive root-zone salt exposure, closely simulating field conditions in irrigated arid and semi-arid environments, where salts accumulate in deeper soil layers due to evapotranspiration, insufficient leaching, and shallow groundwater (Machado and Serralheiro, 2017; Shahid et al., 2018). As a result, it enabled a more realistic assessment of rhizobial functionality than conventional homogeneous systems, particularly regarding maintenance of nodulation and biological N fixation during later developmental stages.

Clear differences among inoculated treatments demonstrated substantial variation in functional salt tolerance among the tested strains. Because germination occurred under non-saline conditions, subsequent differences primarily reflected tolerance to sustained root-zone salinity rather than variation in seed emergence, an important distinction since salinity often impairs nodulation and nitrogenase activity before visible biomass reductions occur (Egamberdieva et al., 2019; Balasubramaniam et al., 2023). The persistence of active nodulation confirmed that selected strains retained infectivity, competitiveness, and metabolic activity under elevated salinity, consistent with known adaptive mechanisms including osmotic adjustment, ion homeostasis, exopolysaccharide production, antioxidant defense, and biofilm formation. A major advantage of this stratified model was its ability to distinguish simple bacterial survival from true functional symbiotic tolerance. Many strains classified as salt-tolerant *in vitro* fail under greenhouse or field conditions because survival alone does not ensure successful root colonization or N fixation (Lindström and Mousavi, 2020; Trivedi et al., 2022). By enabling simultaneous evaluation of these processes under realistic conditions, the model substantially improved the predictive value of strain selection.

The results confirm that a two-layer soil-sand system is an effective tool for identifying rhizobial strains with genuine functional salt tolerance. The maintenance of symbiotic efficiency under progressive root-zone salinity

supports the use of native salt-tolerant rhizobia as a promising strategy to improve legume productivity and strengthen climate-resilient agriculture in salt-affected dryland ecosystems.

Soybean-*Bradyrhizobium japonicum* symbiosis under salinity

Inoculation of soybean seeds with selected salt-tolerant strains of *B. japonicum* significantly improved nodulation, seed formation, and overall productivity compared with the non-inoculated control under identical saline conditions (Table 2), confirming their capacity to establish and maintain effective symbiosis under moderate root-zone salinity (4.0-6.2 dS m⁻¹).

The absence of nodules in the non-inoculated control indicated that indigenous soybean-compatible rhizobia were either absent or physiologically inactive in the saline soil. Consequently, plants relied solely on native soil N and showed the lowest productivity (58.0 ± 1.6 g seed mass per 10 plants; 100-seed weight 15.4 ± 0.4 g), consistent with previous reports that salinity suppresses native rhizobial populations and limits spontaneous nodulation in soybean (Egamberdieva et al., 2019; Lindström and Mousavi, 2020; Balasubramaniam et al., 2023). All inoculated treatments formed abundant nodules (230.0 ± 11.5 to 560.5 ± 27.0 per 10 plants), confirming that the selected strains retained infectivity and nodulation capacity under salinity. This agrees with earlier studies showing that salt-tolerant *B. japonicum* maintains symbiotic function through osmotic adjustment, membrane stabilization, exopolysaccharide production, and antioxidant defense. A major outcome was the substantial increase in soybean productivity, with relative yields ranging from 137.6% to 144.8% of the control, corresponding to a 38%-45% increase in seed production under salinity. This confirms the strong agronomic value of rhizobial inoculation as a strategy to mitigate salt-induced yield losses (Balasubramaniam et al., 2023).

Table 2. Nodulation and productivity of soybean inoculated with *Bradyrhizobium japonicum* under salinity (Ranchpar salt marsh). Means followed by different letters differ significantly according to Tukey's HSD test ($p \leq 0.05$). All inoculated treatments significantly increased soybean yield compared with the non-inoculated control, whereas nonsignificant differences were detected among the inoculated treatments.

Treatment	Nodules	Nodule dry weight	Seeds	Seed weight	100-seed weight	Yield
	Nr 10 plants ⁻¹	g	Nr 10 plants ⁻¹	g	g	%
Control	—	—	360.0 ± 9.8	58.0 ± 1.6	15.4 ± 0.4	100.0 ± 2.5 ^b
Inoculated, strain MDC N°						
5783	381.5 ± 18.2	1.0 ± 0.05	565.9 ± 24.0	97.0 ± 3.8	22.1 ± 0.7	143.5 ± 4.2 ^a
5784	400.6 ± 19.0	1.2 ± 0.06	610.4 ± 26.5	110.3 ± 4.1	21.6 ± 0.6	140.3 ± 3.9 ^a
5787	230.0 ± 11.5	2.0 ± 0.10	550.0 ± 22.8	96.2 ± 3.5	21.2 ± 0.6	137.6 ± 3.7 ^a
5789	560.5 ± 27.0	1.3 ± 0.07	535.9 ± 23.5	103.5 ± 4.0	22.3 ± 0.7	144.8 ± 4.5 ^a
5798	482.9 ± 23.0	0.8 ± 0.04	650.0 ± 28.0	119.5 ± 4.6	21.5 ± 0.6	139.6 ± 4.0 ^a

Among the tested strains, MDC 5789 showed the highest overall efficiency, combining the greatest nodule number, highest relative yield, and largest seed size, indicating superior N fixation efficiency and host compatibility (Lindström and Mousavi, 2020). Strain MDC 5783 also performed strongly despite producing fewer nodules, suggesting that nodule functionality may be more important than nodule abundance alone. Strain MDC 5798 formed numerous but smaller nodules, whereas MDC 5787 produced fewer but larger nodules without superior yield, confirming that nodule morphology alone is a poor predictor of symbiotic effectiveness (Ferguson et al., 2019).

Despite these physiological differences, yield did not differ significantly among inoculated treatments, indicating that all tested strains provided similarly strong agronomic benefits. Overall, the results demonstrate that native salt-tolerant *B. japonicum* can effectively alleviate salinity stress in soybean and support their use as promising bioinoculants for saline agroecosystems such as the Ararat Valley and other salt-affected dryland regions.

Peanut-*Bradyrhizobium* sp. symbiosis under salinity

A similarly strong positive response to inoculation was observed in peanut inoculated with selected *Bradyrhizobium* spp. under a two-layer soil-sand system (Table 3). As in soybean, all inoculated treatments significantly outperformed the non-inoculated control, confirming that rhizobial inoculation effectively mitigated the negative effects of moderate salinity on plant growth and productivity.

Table 3. Efficiency of symbiosis between peanut nodulating bacteria (*Bradyrhizobium* spp.) and host plants under saline conditions (Ranchpar salt marsh). Inoculation with *Bradyrhizobium* spp. strains significantly improved peanut productivity under saline soil conditions (ANOVA, $p < 0.001$). The highest yield was obtained with strain 5812 (90.0 g m^{-2}), followed by strains 5804 and 5807, which formed the same statistical group according to Tukey's HSD test. Control plants showed the lowest productivity. Means followed by different letters differ significantly according to Tukey's HSD test ($p \leq 0.05$).

Treatment	Shoot length	Shoot dry weight	Number of nodules	Dry weight of nodules	Number of pods	Seed weight	100-seed weight	Yield
	cm	g		g		g	g	g m^{-2}
Control	25.0 ± 0.8	18.3 ± 0.6	–	–	20.0 ± 0.7	16.0 ± 0.5	24.0 ± 0.7	40.0 ± 1.2^d
Inoculated, strain MDC N°								
5804	32.0 ± 1.0	35.8 ± 1.2	200.0 ± 8.5	0.310 ± 0.015	47.0 ± 1.8	32.4 ± 1.1	32.0 ± 0.9	81.0 ± 3.4^{ab}
5805	28.0 ± 0.9	33.3 ± 1.1	181.6 ± 7.8	0.280 ± 0.014	36.2 ± 1.5	27.4 ± 0.9	28.0 ± 0.8	68.5 ± 2.8^b
5807	31.5 ± 1.0	30.0 ± 1.0	143.0 ± 6.2	0.230 ± 0.011	38.0 ± 1.5	29.6 ± 1.0	29.6 ± 0.8	74.0 ± 3.0^{ab}
5808	24.8 ± 0.8	24.4 ± 0.8	134.3 ± 5.8	0.200 ± 0.010	26.0 ± 1.0	21.6 ± 0.7	28.4 ± 0.8	54.0 ± 2.1^c
5809	25.0 ± 0.8	24.0 ± 0.8	180.6 ± 7.5	0.420 ± 0.020	30.8 ± 1.2	20.2 ± 0.7	29.2 ± 0.8	50.5 ± 2.0^c
5812	28.0 ± 0.9	32.2 ± 1.1	210.7 ± 9.0	0.400 ± 0.019	44.4 ± 1.7	36.0 ± 1.2	33.1 ± 0.9	90.0 ± 3.6^a

Under non-inoculated conditions, peanut plants showed clear salt-induced growth suppression, with reduced shoot length ($25.0 \pm 0.8 \text{ cm}$), low shoot dry biomass ($18.3 \pm 0.6 \text{ g}$), and poor final productivity ($40.0 \pm 1.2 \text{ g m}^{-2}$), consistent with previous reports describing osmotic stress, impaired photosynthesis, and nutrient imbalance under salinity (Isayenkov and Maathuis, 2019). Inoculation markedly improved vegetative and reproductive performance, enhancing shoot growth, biomass accumulation, nodulation, and pod production, indicating that the introduced strains remained physiologically active and symbiotically competent under saline conditions (Egamberdieva et al., 2019). Among the tested isolates, strain MDC 5812 showed the highest symbiotic efficiency, producing the greatest nodule number (210.7 ± 9.0 per plant) and final yield ($90.0 \pm 3.6 \text{ g m}^{-2}$), representing a 125% increase relative to the control. This suggests highly efficient N fixation and strong adaptation to saline stress (Tittabutr et al., 2006). Strain MDC 5804 also performed strongly, producing 200.0 ± 8.5 nodules and the highest shoot biomass ($35.8 \pm 1.2 \text{ g}$), indicating pronounced stimulation of vegetative growth. Intermediate but significant improvements were observed with strains MDC 5805 and MDC 5807, whereas MDC 5808 and MDC 5809 showed weaker responses, confirming that symbiotic effectiveness under salinity is strongly strain-dependent and cannot be predicted solely from taxonomic identity (Lindström and Mousavi, 2020; Trivedi et al., 2022). The observed variation also corresponded with the metabolic diversity described in Figure 2, supporting a direct link between physiological plasticity and stress resilience.

The peanut results demonstrate that native salt-tolerant *Bradyrhizobium* strains substantially alleviated salinity-induced growth and yield losses, highlighting their strong potential as bioinoculants for saline agroecosystems such as the Ararat Valley and other salt-affected dryland regions.

Comparative response of legume crops under saline conditions

To evaluate the broader applicability of the selected salt-tolerant rhizobial strains, additional experiments were conducted with several economically important legume crops using the same two-layer soil-sand system. The saline substrate collected from Arazap (6.2 dS m^{-1}) represented moderately saline soil, while the overlying sand layer enabled early seedling establishment under low salinity followed by progressive root exposure to osmotic and ionic stress.

All tested legumes established effective symbiosis under saline conditions; however, the magnitude and physiological expression of the response varied markedly among host species, indicating strong host-dependent differences in intrinsic salt tolerance and symbiotic adaptability (Tables 4 and 5). These differences likely reflect variation in root architecture, ion exclusion, osmotic adjustment, and nodule sensitivity (Isayenkov and Maathuis, 2019; Balasubramaniam et al., 2023). Among the tested crops, chickpea showed relatively strong adaptation, with only moderate reductions in nodulation and shoot growth, indicating that vegetative development was more sensitive than symbiosis. Pea demonstrated exceptional stability, maintaining nodulation and even increasing shoot biomass under salinity, consistent with hormetic responses reported under moderate stress (van Zelm et al., 2020). Lentil showed moderate sensitivity, with slight reductions in nodulation and biomass but unchanged plant height. Fenugreek exhibited the highest overall stability, with virtually unchanged nodulation and biomass under salinity, confirming its strong salt resilience (Banakar et al., 2022; Khachatryan et al., 2023; Kumawat et al., 2023). In contrast, soybean showed a more complex adaptive response: Stable nodulation accompanied by reduced shoot length but increased shoot and root biomass, indicating stress-induced biomass redistribution rather than direct growth inhibition.

Table 4. Comparative evaluation of salt-tolerant rhizobial strains based on biometric parameters of legumes under saline and non-saline conditions (Arazap, flowering stage). Comparative analysis showed that salinity had the strongest negative effect on chickpea, where all nodulation and growth parameters significantly decreased under saline conditions ($p < 0.05$). In contrast, fenugreek and soybean maintained relatively stable symbiotic performance, indicating higher tolerance to salt stress. Means followed by different letters differ significantly according to Tukey's HSD test ($p \leq 0.05$).

Crop	Strain MDC N°	Treatment	Nodules	Nodule dry weight	Shoot dry weight	Shoot length	Root dry weight
			nr plant ⁻¹	g plant ⁻¹	g plant ⁻¹	cm plant ⁻¹	g plant ⁻¹
Chickpea	6048	Salt marsh + sand	7.07 ± 0.32 ^b	0.20 ± 0.01 ^b	4.40 ± 0.15 ^b	30.0 ± 1.0 ^b	0.95 ± 0.04 ^a
		Soil + sand	10.00 ± 0.40 ^a	0.37 ± 0.02 ^a	4.80 ± 0.17 ^a	43.2 ± 1.4 ^a	0.80 ± 0.03 ^b
Pea	5609	Salt marsh + sand	15.00 ± 0.60 ^a	0.10 ± 0.01 ^a	3.90 ± 0.14 ^a	30.7 ± 1.0 ^a	0.10 ± 0.01 ^a
		Soil + sand	15.80 ± 0.63 ^a	0.20 ± 0.01 ^a	2.40 ± 0.09 ^a	34.4 ± 1.1 ^a	0.10 ± 0.01 ^a
Lentil	6006	Salt marsh + sand	8.67 ± 0.35 ^a	0.10 ± 0.01 ^a	1.47 ± 0.05 ^a	32.0 ± 1.0 ^a	0.10 ± 0.01 ^a
		Soil + sand	10.50 ± 0.42 ^a	0.10 ± 0.01 ^a	1.72 ± 0.06 ^a	32.0 ± 1.0 ^a	0.13 ± 0.01 ^a
Fenugreek	5545	Salt marsh + sand	9.00 ± 0.36 ^a	0.09 ± 0.01 ^a	1.20 ± 0.04 ^a	27.0 ± 0.9 ^a	0.09 ± 0.01 ^a
		Soil + sand	9.10 ± 0.36 ^a	0.09 ± 0.01 ^a	1.06 ± 0.04 ^a	29.6 ± 1.0 ^a	0.10 ± 0.01 ^a
Soybean	5789	Salt marsh + sand	9.02 ± 0.36 ^a	0.05 ± 0.01 ^a	2.40 ± 0.08 ^a	18.0 ± 0.6 ^a	0.30 ± 0.01 ^a
		Soil + sand	9.62 ± 0.38 ^a	0.09 ± 0.01 ^a	1.75 ± 0.06 ^a	15.0 ± 0.5 ^a	0.20 ± 0.01 ^a

Table 5. Effect of the salt-tolerant strain MDC 5700 (*Rhizobium leguminosarum* bv. *phaseoli*) on common bean under saline and non-saline conditions (Arazap, flowering stage). Inoculation with the salt-tolerant strain MDC 5700 significantly improved growth and nodulation of common bean under both saline and non-saline conditions. Plants grown in non-saline soil showed the highest shoot growth and nodulation ($p < 0.05$), whereas saline conditions reduced but did not eliminate the positive symbiotic effect. Protein content also increased in inoculated treatments. Means followed by different letters differ significantly according to Tukey's HSD test ($p \leq 0.05$).

Parameter	Control (non-inoculated)	Salt marsh (EC 6.2 dS·m ⁻¹)	Soil (EC 2.5 dS·m ⁻¹)
Shoot length, cm	35.9 ± 1.2 ^c	42.4 ± 1.4 ^b	58.9 ± 1.9 ^a
Shoot dry weight, g plant ⁻¹	28.0 ± 0.9 ^c	43.5 ± 1.5 ^b	56.7 ± 1.8 ^a
Nodules, nr plant ⁻¹	—	13.7 ± 0.6 ^b	25.8 ± 1.0 ^a
Protein content, %	12.6 ± 0.4 ^b	14.0 ± 0.5 ^{ab}	14.9 ± 0.5 ^a

Overall, nodulation was generally less sensitive to salinity than vegetative growth, suggesting that biological N fixation remained functional even when host development was partially constrained (Lindström and Mousavi, 2020). Based on overall performance, apparent salinity resilience ranked approximately as follows: Fenugreek $\approx$ pea > soybean $\approx$ chickpea > lentil. These findings demonstrate that native salt-tolerant rhizobia can sustain effective symbiosis across multiple legume hosts, but agronomic success depends strongly on host physiology, emphasizing the need for host-specific inoculant selection for saline agroecosystems such as the Ararat Valley.

Response of common bean to inoculation under salinity

The response of common bean inoculated with strain MDC 5700 (*R. leguminosarum* bv. *phaseoli*) was evaluated separately (Table 5) because the experimental design directly compared saline (EC 6.2 dS m⁻¹) and non-saline (EC 2.5 dS m⁻¹) conditions, allowing direct assessment of how salinity modified the benefits of inoculation.

Under saline conditions, inoculated plants formed 13.7 ± 0.6 nodules per plant, whereas the non-inoculated control remained completely non-nodulated, confirming that strain MDC 5700 retained infectivity and symbiotic competence despite elevated salinity. This is particularly relevant because common beans are among the more salt-sensitive grain legumes, and its rhizobial symbiosis is highly vulnerable to osmotic and ionic stress. Inoculation significantly improved vegetative growth under both conditions (Xu et al., 2026). Shoot length increased from 35.9 ± 1.2 cm in the control to 42.4 ± 1.4 cm under salinity and 58.9 ± 1.9 cm under non-saline conditions, while shoot dry biomass increased from 28.0 ± 0.9 to 43.5 ± 1.5 and 56.7 ± 1.8 g plant⁻¹, respectively. Although growth remained partially constrained by salinity, these results demonstrate substantial mitigation of salt-induced growth suppression (Xu et al., 2026).

A notable outcome was the improvement in seed protein content, which increased from $12.6 \pm 0.4\%$ in control to $14.0 \pm 0.5\%$ under salinity and $14.9 \pm 0.5\%$ under non-saline conditions, indicating that inoculation enhanced not only growth but also grain nutritional quality through improved nitrogen fixation and assimilation (Rouphael and Colla, 2020).

Overall, although salinity reduced the magnitude of the inoculation effect, it did not eliminate it, confirming that strain MDC 5700 possesses sufficient functional salt tolerance and represents a promising candidate for common bean cultivation in salt-affected soils.

Comparative interpretation across host species

Taken together, the comparative experiments demonstrate that inoculation with selected salt-tolerant rhizobial strains effectively maintained nodulation, plant growth, and physiological performance across multiple legume species under moderate salinity (EC 4.0-6.2 dS m⁻¹). All tested symbioses remained functional, confirming the broad agronomic potential of native rhizobia isolated from long-term saline environments. However, the magnitude and expression of the response were strongly host dependent. Soybean and peanuts showed the greatest productivity gains, whereas chickpea and pea maintained the highest symbiotic stability. Lentil displayed moderate sensitivity, while fenugreek exhibited exceptional tolerance with minimal physiological disturbance. Common beans retained significant benefits, although their response improved under reduced salinity. These differences confirm that salinity tolerance in legume-rhizobium systems depend not only on bacterial stress tolerance but also on host-specific traits, including developmental plasticity, ion regulation, root architecture, and host-strain compatibility (Lindström and Mousavi, 2020; Balasubramaniam et al., 2023).

Successful development of microbial inoculants for saline agroecosystems should rely on host-strain-specific selection rather than bacterial salt tolerance alone. This principle is particularly relevant for salt-affected regions such as the Ararat Valley, where regionally adapted biofertilizers may provide a sustainable tool for improving legume productivity and maintaining biological nitrogen fixation under increasing soil salinization.

CONCLUSIONS

The present study demonstrated that native salt-tolerant rhizobial strains isolated from naturally saline soils of the Ranchpar and Arazap (Ararat Valley, Armenia) possess strong adaptive potential and can maintain effective symbiosis with multiple economically important legume crops under moderate salinity stress. Across all experimental systems, inoculation significantly improved nodulation, plant growth, biomass accumulation, and productivity, confirming the importance of host-compatible, stress-adapted rhizobia for sustaining legume performance in salt-affected soils.

Clear strain-dependent differences in symbiotic efficiency were observed. In Soybean, *Bradyrhizobium japonicum* strain MDC 5789 produced the strongest yield response (37%-45%), while in peanut strain MDC 5812 showed the highest symbiotic effectiveness. Comparative evaluation across additional legumes confirmed pronounced host-dependent responses, highlighting the importance of host-strain compatibility in determining performance under salinity.

A major methodological contribution of this study was the use of a two-layer soil-sand system, which realistically simulated progressive root-zone salt exposure and enabled more reliable discrimination of rhizobial functional tolerance than conventional homogeneous salinity assays.

Overall, the results confirm that targeted selection of indigenous salt-tolerant rhizobia represents a promising strategy for developing region-specific microbial inoculants. The identified strains are strong candidates for biofertilizer development aimed at improving biological N fixation, reducing dependence on synthetic N fertilizers, and supporting climate-smart agriculture in the Ararat Valley and other salt-affected dryland agroecosystems.

Author contribution

Conceptualization: S.H., T.S., V.H. Methodology: S.H., T.S. Validation: V.G., V.H. Formal analysis: G.K., R.H., A.Z. Resources: V.G. Data curation: S.H., T.S., V.G. Writing-original draft: V.G. Writing-review & editing: S.H. Supervision: S.H., V.G. Project administration: V.G. Funding acquisition: V.G. All co-authors reviewed the final version and approved the manuscript before submission.

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