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ORIGINAL PAPER

Harnessing the Efficacy of a *Rhizobium* Strain in Individual and Consortium Mode to Promote Sustainable Lentil Production and Biofortification under Diverse Soil Conditions

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Abstract

Purpose Supplementing chemical fertilizers with bio-fertilizers is vital for sustaining agricultural productivity while reducing the adverse environmental effects of traditional fertilizers. Present study aimed to assess the effectiveness of *Rhizobium* inoculation (individual and consortium modes) compared to chemical fertilizers in enhancing lentil growth, yield, and nutrient fortification in lentil grains across different soil systems. **Methods***Rhizobium* and rhizobacteria were isolated from Bihar's Tal soil and screened for plant growth promoting activities, nodulation, and nutrients solubilization. A pot experiment involving thirteen treatments, each replicated three times, was conducted for evaluation. Out of 28 isolates, four strains- DPR2 (*Rhizobium leguminosarum* bv. *viciae*), DPB4 (*Bacillus cabrialesii*), DPP4 (*Pseudomonas synxantha*) and DPA1 (*Azotobacter salinestris*) strains were selected based on their plant growth promoting activities, nutrient solubilization capabilities and harmoniously coexist. **Results** In a pot experiment, the consortium of DPR2 + DPB4 + DPP4 + DPA1 exhibited the shortest germination time and significantly improved nodule formation, plant growth, yield and yield attributes across all soil types. Moreover, this consortium significantly increased biofortification of macronutrients (N, P and K) and micronutrients (Fe and Zn) in lentil grains compared to other treatments. Irrespective to treatments, the number of root nodules, plant growth, yield and yield attributes, nutrient accumulation in lentil grains were consistently highest in Tal soil and lowest in calcareous soil. **Conclusions** Consortium of DPR2 + DPB4 + DPP4 + DPA1 can enhance lentil yield and fortification of nutrients in grains. Thus, consortium may hold promise as a bio-fertilizer supplement to chemical fertilizers for sustainable lentil production in diverse soil systems.

Keywords Biofortification · Microbial consortia · Nutrient solubilization · Fe/Zn · *Rhizobium* · Yield

1 Introduction

Food security in sustainable agriculture production systems relies on the integration of various nutrient resources for crops. Despite this, many farmers, both resource-rich and resource-poor, heavily rely on chemical fertilizers due to their immediate visible effects and quick grain yield. However, this reliance leads to significant environmental and health issues. Excessive chemical fertilizer use leads to runoff and leaching, contaminating water with nitrogen, phosphorus, and other compounds. This harms aquatic ecosystems, causing algal blooms, fish kills, and water quality degradation (Bashir et al. 2020). Moreover, chemical fertilizers disrupt soil composition, leading to soil acidification, nutrient depletion, and decreased microbial activity. This degradation results in reduced soil fertility and productivity over time (Montemurro et al. 2015). Additionally, chemical fertilizers pose risks to human health through various pathways, including direct exposure during application, consumption of contaminated food and water (Naidu et al. 2021). Furthermore, they disrupt natural ecosystems by altering soil microbial communities and reducing biodiversity. These disruptions have cascading effects on ecosystem functions and services (Mishra et al. 2023a). Consequently,

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the intrinsic capacity of soil for optimal productivity is compromised, highlighting the urgent need for sustainable agricultural practices.

To combat these challenges and promote sustainable farming practices, a progressive approach integrating soil microbes into agricultural production systems is imperative. Soil microbes are vital for soil fertility and ecosystem health, driving processes like nutrient recycling, organic matter decomposition, and plant growth enhancement (Mishra et al. 2023b). Plant growth-promoting rhizobacteria (PGPR) directly improve plant growth by producing hormones and fixing nitrogen, while also suppressing pathogens and inducing plant resistance (Barbaccia et al. 2022). Biological nitrogen fixation contributes significantly to global agriculture (Shirvani and Yahaghi 2022). Various bacteria, including *Pseudomonas, Bacillus*, and *Azotobacter*, enhance plant growth and yield. Harnessing these microbes through biofertilization promotes agricultural sustainability (Kumar et al. 2022).

Bihar, an unexplored state in terms of microbial diversity, offers the potential for forming a consortium to develop a universal strain of bio-fertilizer. The state comprises diverse soil conditions including Tal, Diara, Chaur, and calcareous soil. Tal region, such as Mokama, is exempted from chemical fertilizer use and faces challenges in crop production. The soil in this region has a hard texture with wide cracks during summer and remains submerged for 3–4 months during the rainy season. Diara land, prone to flooding due to river meandering, exhibits varying soil properties with increasing distance from the riverbank Chaur areas, comprising lowlands, experience prolonged waterlogging. Calcareous soil, found in both arid and humid regions, contains calcium carbonate, leading to elevated pH levels and reduced availability of phosphorus and potassium (Diwakar and Singh 1992; Choudhary et al. 2019; Kumar et al. 2013).

Tal land can be selected for isolation of microbes, because of its challenged ecosystem and production of crop without any use of chemical fertilizer. There is natural farming system carried in Tal region mainly with legumes (mostly covered with lentil) (Singh 2018). Legume crops could play an important role in this context by delivering multiple services in line with sustainability principles (Stagnari et al. 2017). Among the legumes, lentil (*Lens culinaris*), known as the "poor man's meat," is a highly nutritious legume with high protein content, minerals, carbohydrates, fats, and lipids. It is one of the oldest cultivated food crops, originating in the Fertile Crescent of the Near East. Lentil have a 70% higher crop yield compared to cereals, making them economically beneficial (Preissel et al. 2015). They are easily digestible, have a short cooking time, and can tolerate a wide range of temperatures, including both high heat and frost conditions (Dhull et al. 2023). Lentil also thrives in poor soil conditions unsuitable for other crops. With a protein content of 20–26% (compared to cereals' 8–12%), lentil contribute significantly to meeting the world's protein demand. The economic and ecological importance of legumes, including lentil, is highlighted by their extensive cultivation and commercialization, as well as their ability to obtain nitrogen through symbiotic associations with soil bacteria called rhizobia (Clúa et al. 2018).

Researchers have observed that *Rhizobium* bacteria effectively fix nitrogen (Kebede 2021; Alemneh et al. 2020). However, they may not contribute significantly to fulfilling other nutrient requirements of the plant beyond nitrogen. To address the limitations of individual *Rhizobium* strains, the use of microbial consortia comprising multiple strains has gained attention. Such consortia offer benefits like improved survival, multinutrient supply, and growth regulation (Fairhurst 2013). Researchers have explored various combinations of *Rhizobium* with other soil bacteria (Pathania et al. 2020; Kumar & Chandra 2008; Iqbal et al. 2012) to enhance lentil growth and yield. Despite, there is still a lack of comprehensive research on the efficacy of microbial consortia, particularly in diverse soil systems like those found in Bihar, India. While individual strains of *Rhizobium* have been studied, their effectiveness in meeting lentil's nutrient requirements beyond nitrogen fixation remains uncertain. Moreover, the potential of microbial consortia, combining *Rhizobium* with other beneficial bacteria, to address these limitations and enhance lentil yield and nutrient fortification in grains has not been thoroughly explored.

We hypothesize that the consortium of *Rhizobium* and other plant growth-promoting rhizobacteria, isolated from Bihar's Tal soil, will exhibit superior performance in enhancing lentil growth, yield, and nutrient fortification compared to both chemical fertilizers and individual strains of *Rhizobium*. Specifically, we expect that this consortium will promote faster germination, increased nodule formation, improved plant growth, and higher yield across different soil types. Therefore, the current investigation was aimed to isolate effective *Rhizobium* strains and other plant growth-promoting rhizobacteria and evaluate their performance in different soil systems in terms of plant growth, yield, and nutrient acquisition in grains.

2 Materials and Methods

2.1 Isolation of *Rhizobium* and Rhizobacteria

Lentil plants and rhizospheric soils were collected from the Tal region of Mokama, Bihar. This region lies between latitude 25°10'N to 25°35'N and longitude 85°5'E to 86°8'E in the districts of Patna, Nalanda, and Munger in central Bihar.

The pinkish red coloured nodules were carefully separated from the roots and washed to remove soil contaminants. To isolate *Rhizobium*, 1 gram of nodules was washed with a 0.1% mercuric chloride solution for 3–4 min, followed by rinse with 70% ethanol for 1 min and finally washed with double-distilled sterile water for at least 1 min (Rudolph et al. 2015). The washed nodules were then crushed in 1 mL of double-distilled sterile water using a mortar and pestle. Serial dilutions ranging from 10^{-3} to 10^{-7} were prepared from the crushed nodules. These dilutions were spread-plated onto Congo red yeast extract mannitol agar. The plates were then incubated at 32 °C for 24 h. To isolate rhizobacteria, one gram of rhizospheric soil was taken and serially diluted up to 10^{-7} using double-distilled sterile water. The diluted soil samples were spread-plated on nutrient agar media, Jensen's media, and King's B media. Three replications were performed for each dilution, and the plates were incubated at 30 °C for 24 h. Selection of different isolates was based on the colony's morphotypes. The single isolated colony from streaked plates was taken out to prepare slants and stored in refrigerator at 4^0C for further use.

2.2 Screening of *Rhizobium* and Rhizobacteria

Bacterial isolates were screened for viz. nitrogen fixation, phosphorus solubilization, potassium solubilization, zinc solubilization and production of siderophore, indole acetic acid (IAA), HCN and ammonia. Nitrogenase activity of the selected isolates was determined using Acetylene reduction assay (ARA) (Lee and Yoshida 1997) expressed in terms of nanomoles of ethylene per milligram of protein per hour. For phosphorus solubilization screening, isolates were spotted on Pikovskaya's Agar media (Pikovskaya 1948). For potassium solubilization, isolates were spotted on modified Aleksandrove agar media plates (Rajawat et al. 2016). For zinc solubilization, isolates were tested on tris-minimal salt media supplemented with 1% ZnO (Fasim et al. 2002). Incubation at 30 °C allowed for the observation of clear zones indicating solubilization abilities. Siderophore production ability of isolates was analyzed by chrome azurol S (CAS) dye solution method (Milagres et al. 1999). IAA production ability of isolates was examined using Patten and Glick (2002) method. Castric's method (Castric 1975) and Dye's method (Dye 1962) were used for detection of HCN and ammonia production ability of isolates, respectively.

2.3 Nodulation Test

To check the potentiality of proper strain to infect their specific host an experiment was taken on the basis of what the potential strain can be selected efficiently. In this experiment, an assembly of bottle was made which had two parts. The upper part of assembly contained sterilized vermiculite and sand in 1:1 ratio (Leonard 1943), whereas the lower part of it contained the nitrogen free plant nutrient media. Here, the nutrient media provided all the nutrients except nitrogen which can be made available by Rhizobial inoculation. After that, the lentil seeds were soaked in the broth culture of *Rhizobium* and added charcoal powder. After 24 h of incubation, the inoculated seeds were sown in the upper part of the Leonard Jar.

2.4 Survival Competence for *Rhizobium*

Rhizobium isolates were evaluated using various types of soil extract agar media plates. To prepare the soil extract, each soil type (mesh size, 2 mm) was soaked in a water solution (1:1 ratio) in a beaker for 24 h, followed by centrifugation at 5000 rpm for 20 min at 20 °C (Aagot et al. 2001). The resulting supernatant was collected in a separate beaker and mixed with glucose (1 g/L) and agar powder (18 g/L), adjusted according to the volume of the extract, and sterilized in an autoclave at 15 lbs pressure (121 °C) for 15 min (Subba Rao 1977). Subsequently, soil extract agar plates were prepared and inoculated with each isolate. The inoculated plates were then incubated for 2 days to observe full growth. The isolate exhibiting maximum growth on each type of soil extract agar medium was selected, representing the optimal performance under the respective soil nutrient conditions.

2.5 Identification of Potential *Rhizobium* and Rhizobacterial Isolates

Four bacterial isolates (DPB4, DPP4, DPA1, and DPR2), selected based on their plant growth promoting activities, underwent further study. Genomic DNA extraction was performed using the ZR Fungal/Bacterial DNA Mini PrepTM kit (ZYMO Research Corporation, USA). The amplified 16 S rRNA gene fragments were obtained using universal primers PA (5′-AGAGTTTGATCCTGGCTCAG-3′) and PH (5′-AAGGAGGTGATCCAGCCGCA-3′) (Edwards et al. 1989) and purified using a Qiagen purification kit. Subsequently, sequencing of the purified 16 S rRNA gene fragments was conducted by Sci-Genome Pvt. Ltd., Bangalore. The resulting sequences were analyzed for maximum homogeneity with available 16 S rRNA gene sequences in the NCBI database using BLASTn. Sequence alignment was performed using the CLUSTAL W program, and a phylogenetic tree was constructed using Mega-X software (Kumar et al. 2018) employing the bootstrap method. Ancestral states were inferred using the Maximum Likelihood method (Nei and Kumar 2000) with 1000 bootstrap replications and the Kimura 2-parameter model (Kimura

1980). These sequences have been submitted to NCBI, with the accession numbers- OR136499, OR137932, OR136354 and OR131586 for the promising isolates- DPR2, DPB4, DPP4 and DPA1, respectively.

2.6 In vitro Compatibility of Selected Isolates

To ensure the seamless integration of DPB4, DPP4, DPA1 and DPR2 isolates, a compatibility test was carried out. All the isolates were cross streaked on nutrient agar plates and incubated in BOD at 30 °C for 48 h.

2.7 Soil Collection for Pot Experiment

Collection of soil samples was done from top layer to 15 to 20 cm deep. Soil was collected in such a way that the whole field should cover while taking soil in zig-zag fashion. For each type, about 5 quintals of soil was collected from Mokama Tal, Begusarai, Darbhanga and Pusa campus, thus total 20 quintals of soil was collected. Each type soil was mixed separately by breaking clogs and making a homogeneous mixture.

2.8 Chemical Properties of Soils

The active hydrogen ion concentration was determined by Digital pH meter (Elico) (Jackson 1973). Total soluble ion concentration of soil was determined by Conductivity Bridge (Elico) (Jackson 1973). Available nitrogen in soil was determined by alkaline potassium permanganate ($KMnO_4$) method (Subbiah and Asija 1956). For estimation of available phosphorus (P), soil was extracted with 0.5 M $NaHCO_3$ (Olsen et al. 1954) and determined on spectrophotometer (Tandon et al. 1993). Available potassium (K) was determined by extracting the soil with 1 N ammonium acetate (pH 7.0) and K content in the extract was measured by flame photometer (Jackson 1973). Organic carbon content was determined using Walkley and Black (1934) method. The micronutrient such as Zn and Fe content in soil was determined by the calculation of the transmitted wavelength of light in Atomic Absorption Spectrophotometer (Lindsay and Norvell 1978).

2.9 Pot Experiment

A pot experiment was conducted to evaluate the performance of lentil *Rhizobium* in individual and consortium mode in different types of soil systems in relation to days of germination, plant growth, nodulation, yield and yield attributes and acquisition of N, P, K Fe and Zn in lentil grains. The efficacy of the *Rhizobium* in individual and consortium mode along with different doses of NPK fertilizer was checked in 4 types of soil conditions and effect of individual *Rhizobium* and consortia of *Rhizobium* with rhizobacteria were compared with the chemical fertilizer application.

The indigenous (Desi) variety of lentil was used and grown in pots measuring 30 cm in diameter and 30 cm in height. The study included a total of four different soil types: Tal, Diara, Chaur, and calcareous. Each pot was filled with 10 kg of the respective soil type. Soil used in pot experiment was sterilized for 3 consecutive days at 121 °C, 15 psi for 40 min. The inocula for the selected bacterial isolates (DPB4, DPP4, DPA1 and DPR2) were prepared by inoculating them in their respective broths and incubated at 30 °C, 160 rpm for 24 h, to maintain 10^9 CFU mL^{-1}. The lentil seeds were treated with the inoculated broth mixture, while the control seeds were treated with a blank solution and charcoal. Ten seeds were sown in each pot and following germination thinned to five plants in each pot. The recommended doses of fertilizers (RDF) were applied to the experimental setup based on the specific ratios of N, P, K, Fe and Zn (20 kg N ha^{-1}, 40 kg P_2O_5 ha^{-1} and 20 kg K_2O ha^{-1}, 25 kg $FeSO_4$ ha^{-1} and 40 kg $ZnSO_4$ ha^{-1}) as described in the study by Roy et al. (2018). There were a total of 52 treatments, with 13 treatments for each type of soil. The experiment followed a Completely Randomized Design with three replications.

The treatments applied to the lentil plants in the pots were as follows: T1: Control (no additional treatment); T2: 25% of RDF; T3: 50% of RDF; T4: 75% of RDF; T5: 100% of RDF; T6: *Rhizobium leguminosarum* bv. *viciae* DPR2; T7: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Azotobacter salinestris* DPA1; T10: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13: *Rhizobium leguminosarum* bv. *viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1.

2.10 Analysis of Days of Germination, Plant Growth, Biomass and Yield

Lentil crop growth parameters were analyzed to evaluate the effect of selected isolates. Plants from replicated pots were uprooted and used for measuring the different crop parameters including days of germination, root length at 40 days after sowing (DAS), shoot length at 40 DAS, number of branches per plant at 40 DAS, number of nodules per plant

at 40 DAS, weight of 100 grains, number of pods per plant, number of seeds per pod and grain yield per pot.

2.11 Estimation of Nitrogen, Phosphorus and Potassium in Grains

For estimation of N, samples were digested by $H_2SO_4 + H_2O_2$ in presence of $CuSO_4$, K_2SO_4, and Se in 10:4:1 ratio. The digested plant samples were taken for nitrogen estimation by modified Kjeldahl method (Jackson 1973). For estimation of phosphorus, Plant samples were digested with tri-acid (nitric acid + perchloric acid + sulphuric acid in ratio of 10:3:1). Digested sample were determined by Vanado-molybdo phosphoric acid yellow colour method (Jackson 1973). Whereas potassium content was determined by using flame photometer (Jackson 1973).

2.12 Estimation Fe and Zn Content in Grains

Fully dried seeds were ground to fine powder. 0.5 g of powdered sample were taken and digested with 10 ml of tri acid mixture (nitric acid: perchloric acid: sulphuric acid in 10:3:1 ratio) on hot plate at 300ºC until it gets colorless. Digested mixture transferred to a volumetric flask of 50 ml, the flask was filled up to 50 ml with double distilled water. Analysis was done using atomic absorption spectrophotometer (Lindsay and Norvell 1978).

2.13 Statistical Analysis

All the Data obtained from experiments was used for analysis of mean values of three replications of each treatment and then statistically analyzed by using Minitab 17 statistical software using one way analysis of variance (ANOVA). Grouping information between mean values of obtained data of each experiment was carried out by Fisher LSD method and 95% confidence ($P \leq 0.05$).

3 Results

3.1 Isolation of *Rhizobium* and Rhizobacteria from Tal Lentil

A total of 10 distinct *Rhizobium* morphotypes including-DPR1, DPR2, DPR3, DPR4, DPR5, DPR6, DPR7, DPR8, DPR9 and DPR10) were isolated from lentil root nodules collected from Tal region of Mokama, Bihar. Likewise, 18 rhizobacteria morphotypes including-DPB1, DPB2, DPB3, DPB4, DPB5, DPP1, DPP2, DPP3, DPP4, DPP5, DPP6, DPA1, DPA2, DPA3, DPA4, DPA5, DPA6 and DPA7) were isolated from rhizosphere soil from the same area.

3.2 Screening of *Rhizobium* and Rhizobacteria

Among the isolates, DPR2, a *Rhizobium* isolate, exhibited the highest nitrogen fixation ability, with a rate of 45.62 nanomoles of ethylene per milligram of protein per hour. Regarding phosphate solubilization, out of the 28 isolates, rhizobacteria isolates-DPB4 and DPP4 displayed the maximum P-solubilization ability. In terms of IAA production, rhizobacteria isolate-DPB4 produced the highest amount of IAA (102.64 µg mg^{-1} protein). For zinc solubilization, rhizobacteria isolates-DPB4, DPP4, DPA1 and DPR2 displayed better Zn solubilization compared to the other isolates. Regarding K-solubilization activity, 9 isolates (DPB3, DPB4, DPP3, DPP4, DPA1, DPA4, DPR2, DPR5 and DPR9) demonstrated positive results. In terms of siderophore production, rhizobacteria isolates- DPB4 and DPP4 displayed remarkable results. Among the tested isolates, DPB4 exhibited the highest HCN production ability. Furthermore, *Rhizobium* isolate-DPR2 demonstrated the most significant result in ammonia production compared to other isolates (supplementary Table 1).

3.3 Nodulation Test

Lentil seeds inoculated with the potential *Rhizobium* sp. were sown in jars and allowed to grow for 25 days to promote proper nodule formation. Among the isolates, DPR2 demonstrated the best nodulation results (Supplementary Fig. 1).

3.4 Survival Competence of *Rhizobium*

All the isolates of *Rhizobium* were purified and incubated on soil extract agar derived from different soil types. The compatibility of each isolate was examined using various soil extracts and among them, isolates DPR2, DPR5, DPR7, and DPR8 exhibited notable growth on all types of soil. In the quantitative analysis conducted using colony forming units on different soil extract agar media; DPR2 displayed the most impressive performance (supplementary Table 2).

3.5 Identification of the Isolates

DPR2, DPB4, DPP4 and DPA1 exhibited superior plant growth promoting (PGP) activities and survival competence, leading to their selection for further study. Through 16 S rRNA gene sequencing and phylogenetic analysis using the NCBI database, the isolates DPR2, DPB4, DPP4, and DPA1 were identified as *Rhizobium leguminosarum* bv. *viciae* strain DPR2 (Accession No. OR136499), *Bacillus cabrialesii* strain DPB4 (Accession No. OR137932), *Pseudomonas synxantha* strain DPP4 (Accession No. OR136354)

and *Azotobacter salinestris* strain DPA1 (Accession No. OR131586), respectively (Fig. 1).

3.6 In Vitro Compatibility of Selected Isolates

Compatibility results observed in cross streaking methods indicate how well different microbial isolates could coexist without inhibiting each other's growth. Cross streaking results reveled that all the microbes were able to grow alongside without one suppressing the growth of the other. Therefore, excellent compatibility was observed among all selected isolates (Supplementary Fig. 2).

3.7 Chemical Properties of Soils

All the four types of soils were characterized for their chemical properties. Soil collected from different four places of Bihar had different appearance in colour and texture. Nitrogen content was the highest in Tal soil (212.23 kg ha^{-1}) followed by chaur soil (185 kg ha^{-1}) and Diara soil (175.62 kg ha^{-1}). Phosphorus content was maximum for Diara soil (20.15 kg ha^{-1}) followed by chaur soil (13.94 kg ha^{-1}) and Tal soil (13.35 kg ha^{-1}). Potassium content was maximum in Tal soil (390.85 kg ha^{-1}) followed by Chaur (344.60 kg ha^{-1}) and Diara (219.24 kg ha^{-1}). Iron content was maximum for Chaur soil (24 mg kg^{-1}) followed by Tal

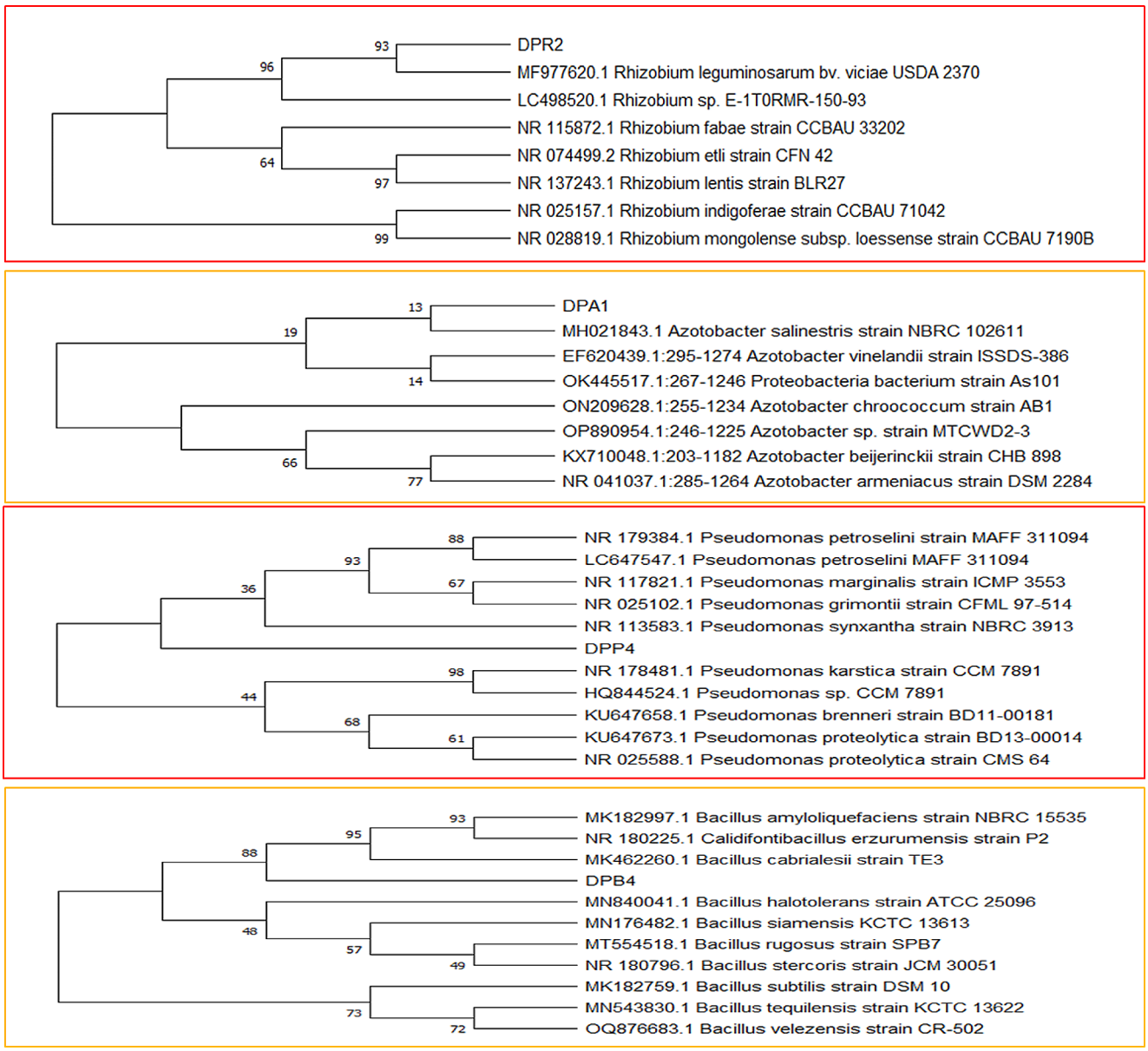


Fig. 1 Phylogenetic tree constructed using Maximum Likelihood method based on Nei and Kumar (2000) with 1000 Bootstrap replications for promising plant growth promoting isolates derived from Clustal W alignment of 16 S rDNA partial sequences

soil (21.07 mg kg^{-1}). Zinc content was maximum in Diara soil (0.92 mg kg^{-1}) followed by Chaur soil (0.88 mg kg^{-1}) and Tal soil (0.84 mg kg^{-1}). pH was maximum for calcarious soil (8.4 pH) followed by Diara soil (8.12 pH). EC was maximum for Diara soil (0.21 ds m^{-1}) followed by Tal soil (0.16 ds m^{-1}). Organic carbon was maximum for Chaur soil (0.70%) followed by Tal soil (0.64%) and Diara soil (0.51%) (Supplementary Table 3).

3.8 Effect of *Rhizobium* in Individual and Consortium mode on Germination

Consortia comprising *Rhizobium*, *Bacillus, Pseudomonas*, and *Azotobacter* demonstrated the shortest germination time, significantly surpassing both control and fertilizer treatments across all soil systems (Table 1). Likewise, irrespective to soils, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* showed the shortest germination time among the treatments and reduced the germination time by 22.30% and 21% over the 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 2a). Irrespective to treatments, Chaur soil exhibited the shortest germination time, followed by Diara soil (Supplementary Fig. 3). Analyzing the days of germination via ANOVA revealed a high significance for both soil types and treatments at 5% and 1% probability levels, respectively. However, their interaction did not yield significant results (supplementary Table 4).

3.9 Effect of *Rhizobium* in Individual and Consortium Mode on Root Nodulation

The number of root nodules per plant significantly varied among the treatments in different soil systems. Among the fertilizer treatments, 100% RDF treatment resulted significantly higher number of root nodules per plant across all soil systems (Table 1). Generally, inoculation of in individual and consortium mode significantly increased number of root nodules over the control and fertilizer treatments across all soil systems. Consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* had the highest number of root nodules among the treatments across all soil systems (Table 1). Irrespective to soils, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* increased the number of root nodules per plant by 90% and 64% over the 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 2a). Irrespective to treatments, the highest number of root nodules was recorded in Tal soil which was statistically similar to Chaur soil and Diara soil. The numbers of root nodules in Tal, Chaur and Diara soils were at par over the calcareous soil (Supplementary Fig. 3). The ANOVA for number of root nodules showed that soil types and treatments were highly significant at 1% probability. However, their interaction was not significant (supplementary Table 4).

Table 1 Effect of Rhizobium in individual and consortium mode on seed germination and number of root nodules in different soil system*

Treatments	Germination time (Days)				Number of root nodules $plant^{-1}$			
	Calcareous soil	Chaur soil	Diara soil	Tal soil	Calcareous soil	Chaur soil	Diara soil	Tal soil
T1	11.00 ± 1.00^{a}	10.00 ± 0.53^{a}	10.33 ± 0.58^{a}	10.00 ± 0.50^{a}	3.33 ± 1.15^{h}	7.33 ± 1.15^{d}	7.33 ± 0.58^{h}	10.00 ± 2.65^{c}
T2	10.67 ± 0.58^{ab}	10.00 ± 0.56^{a}	10.33 ± 0.58^{a}	10.00 ± 0.70^{a}	4.00 ± 2.00^{gh}	8.00 ± 2.00^{d}	8.33 ± 1.53^{gh}	12.67 ± 2.08^{c}
T3	10.33 ± 0.58^{abc}	9.67 ± 0.58^{ab}	10.00 ± 0.53^{ab}	$9.67 \pm 0.58a^{b}$	6.33 ± 2.52^{fg}	8.67 ± 1.15^{d}	10.33 ± 2.52^{fg}	12.33 ± 0.58^{c}
T4	10.00 ± 0.40^{abcd}	9.33 ± 0.58^{ab}	9.67 ± 0.58^{abc}	9.33 ± 0.58^{abc}	7.00 ± 3.00^{ef}	10.00 ± 0.00^{cd}	$12.00 \pm 3.46d^{ef}$	10.67 ± 1.53^{c}
T5	9.67 ± 0.58^{abcde}	9.00 ± 0.46^{abc}	9.33 ± 0.58^{abcd}	9.33 ± 0.58^{abc}	7.33 ± 2.08^{ef}	11.33 ± 1.15^{bcd}	11.00 ± 1.00^{efg}	13.33 ± 1.53^{bc}
T6	9.00 ± 0.30^{abcdef}	9.00 ± 0.60^{abc}	9.33 ± 0.58^{abcd}	9.33 ± 0.58^{abc}	9.33 ± 4.16^{bcde}	12.33 ± 2.52^{bcd}	14.00 ± 2.00^{cd}	14.3 ± 1.15^{bc}
T7	8.00 ± 0.50^{def}	7.33 ± 0.58^{cd}	8.00 ± 1.00^{cde}	8.00 ± 1.00^{bcd}	11.33 ± 4.16^{abc}	18.67 ± 2.52^{abc}	13.67 ± 2.89^{cde}	14.67 ± 2.52^{bc}
T8	9.33 ± 0.58^{abcde}	8.00 ± 1.00^{bcd}	8.33 ± 1.15^{bcde}	9.33 ± 0.58^{abc}	10.67 ± 5.03^{bcd}	18.33 ± 3.51^{abc}	13.33 ± 1.53^{cde}	14.33 ± 3.21^{bc}
T9	8.67 ± 0.58^{bcdef}	8.33 ± 0.58^{abcd}	8.33 ± 0.58^{bcde}	8.67 ± 0.58^{abcd}	8.00 ± 2.00^{def}	19.00 ± 2.00^{ab}	13.67 ± 1.53^{cde}	15.33 ± 2.52^{bc}
T10	8.00 ± 1.00^{def}	7.33 ± 0.58^{cd}	7.67 ± 0.58^{de}	7.67 ± 0.58^{cd}	10.67 ± 6.43^{bcd}	19.67 ± 3.06^{ab}	16.00 ± 4.00^{bc}	16.33 ± 4.16^{abc}
T11	8.33 ± 1.53^{cdef}	8.67 ± 1.53^{abcd}	8.33 ± 0.58^{bcde}	8.67 ± 0.58^{abcd}	8.67 ± 0.58^{cdef}	18.33 ± 2.52^{abc}	14.00 ± 2.00^{cd}	21.33 ± 5.51^{ab}
T12	7.67 ± 0.58^{ef}	7.00 ± 0.12^{d}	7.33 ± 0.58^{de}	7.33 ± 0.58^{d}	12.00 ± 1.00^{ab}	23.00 ± 3.61^{a}	18.67 ± 4.04^{ab}	16.67 ± 1.53^{abc}
T13	7.33 ± 0.58^{f}	7.00 ± 0.21^{d}	$7.33 \pm 0.58de$	7.33 ± 0.58^{d}	13.67 ± 3.21^{a}	24.67 ± 7.09^{a}	$19.67 \pm 1.53a$	23.67 ± 3.06^{a}

*Data are the average of three replicates ± SD; Grouping information between mean values of obtained data was carried out by Fisher LSD method and 95% Confidence ($P \leq 0.05$). Different letter point out significant differences in a column

Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

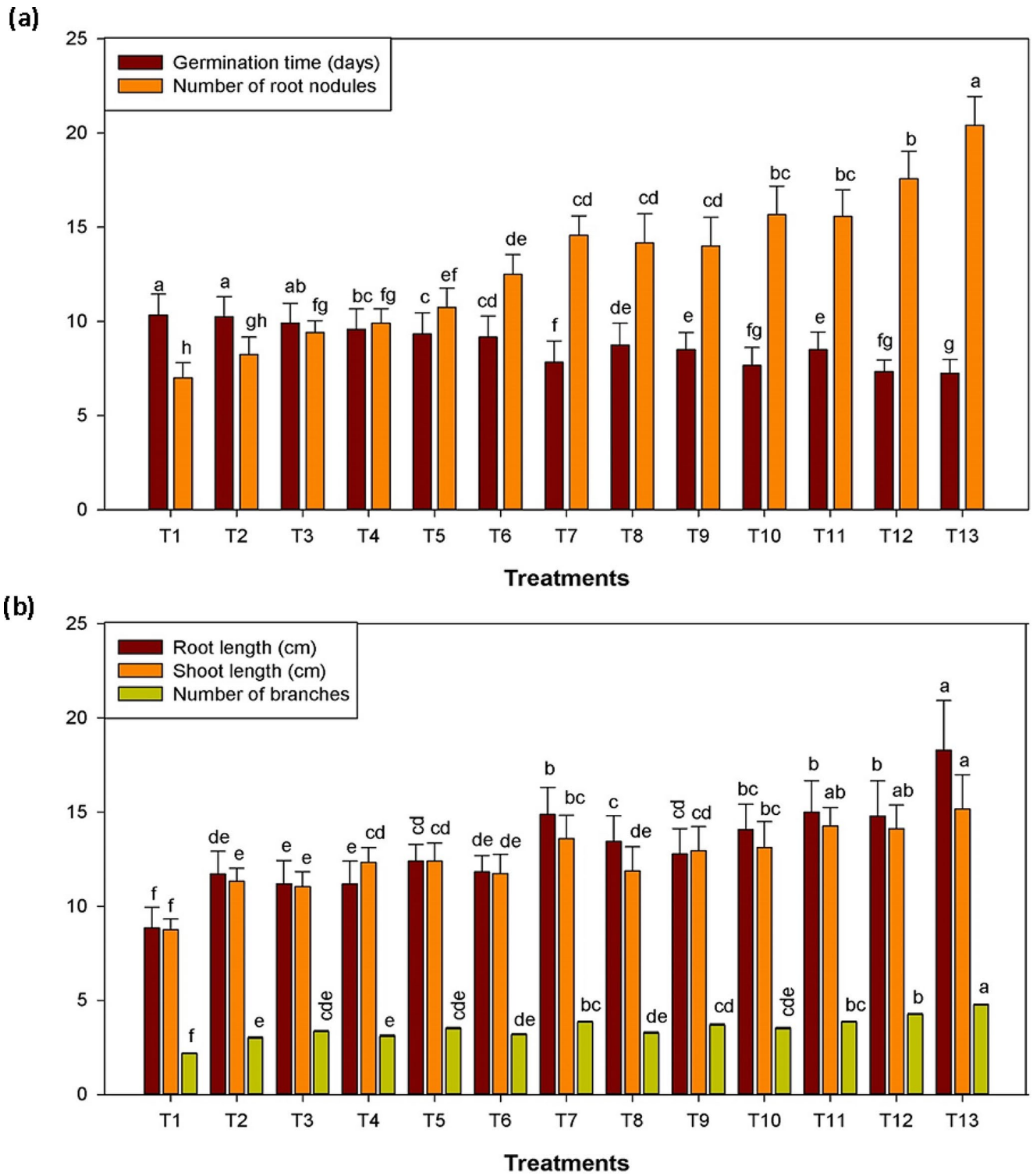


Fig. 2 Effect of *Rhizobium* in individual and consortium mode on seed germination, number of nodules and plant growth irrespective to soil. # **a:** germination, number of nodules; **b:** plant growth. *The data represent the average of three replicates, with error bars indicating the standard deviation (SD). Different letters denote significant differences within a column. Grouping was determined using the Fisher LSD method with a 95% confidence level ($P \leq 0.05$). Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

3.10 Effect of *Rhizobium* in Individual and Consortium Mode on Plant Growth

Across all soil systems, application of consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* significantly enhanced root length and shoot length over the individual *Rhizobium* and fertilizers treatments (supplementary Table 5). Irrespective to the soil, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* increased root length by 47.50% and 54.80% over the 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 2b). Whereas, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* increased shoot length by 22.27% and 29.26% over the 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 2b). Irrespective to treatments, Chaur soil resulted in the longest roots and shoots, followed by Tal soil (Supplementary Fig. 3). The ANOVA for root length and shoot length showed that soil types and treatments were highly significant at 1% probability. Soil type × treatment interaction was highly significant at 1% probability for root length (supplementary Table 4). Whereas, in terms of shoot length, soil type × treatment interaction was highly significant at 5% probability (supplementary Table 4).

The number of branches per plant varies across the treatments and soil systems. Consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* consistently showed the significantly higher number of branches per plant compared to control in all soil systems (Table 2). Irrespective to soil, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* increased number of branches by 35.71% and 49.84% over the 100% RDF and individual *Rhizobium* treatment, respectively. Irrespective to treatments, Tal soil exhibited the highest number of branches per plant followed by Chaur soil (Supplementary Fig. 3). The ANOVA for number of branches showed that soil types and treatments were highly significant at 1% probability. Besides, soil type × treatment interaction was not significant (supplementary Table 4).

3.11 Effect of *Rhizobium* in Individual and Consortium Mode on Yield Attributes of Lentil

Individual *Rhizobium* treatment did not show any significant effect on number of pods plant^{-1}, number of seeds pod^{-1} and weight of 100 seeds compared to fertilizer treatments (Tables 2 and 3). However, *Rhizobium* in combination with other rhizobacteria resulted in a significant increase in number of pods plant^{-1}, number of seeds pod^{-1} and weight of 100 seeds across all soil systems. Across all soil systems, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* exhibited significantly the highest number of pods plant^{-1}, number of seeds pod^{-1} and weight of 100 seeds across all soil systems (Tables 2 and 3). Irrespective to soil, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* enhanced number of pods plant^{-1} by 195%, 64% and 61.55% over the control, 100% RDF, and individual *Rhizobium* treatment, respectively (Fig. 3a). Consortia

Table 2 Effect of Rhizobium in individual and consortium mode on number of branches and number of pods plant^{-1} in different soil system*

Treatments	Number of branches plant^{-1}				Number of pods plant^{-1}			
	Calcareous soil	Chaur soil	Diara soil	Tal soil	Calcareous soil	Chaur soil	Diara soil	Tal soil
T1	1.00 ± 0.54^{b}	2.67 ± 0.58^{b}	2.33 ± 0.58^{c}	2.67 ± 0.58d	20.00 ± 2.00^{h}	33.00 ± 5.00^{f}	30.33 ± 5.51^{e}	29.33 ± 5.13^{h}
T2	1.67 ± 0.58^{ab}	3.33 ± 0.58^{ab}	3.33 ± 0.58^{abc}	3.67 ± 0.58^{bcd}	23.33 ± 1.53^{gh}	42.00 ± 3.00^{ef}	32.67 ± 2.52^{e}	39.00 ± 2.65^{gh}
T3	2.00 ± 0.06^{ab}	3.67 ± 0.58^{ab}	3.67 ± 0.58^{abc}	4.00 ± 1.00^{abcd}	28.33 ± 4.51^{fg}	45.00 ± 9.17^{def}	44.00 ± 2.00^{de}	43.00 ± 4.36^{fgh}
T4	1.67 ± 0.58^{ab}	3.67 ± 1.15^{ab}	3.67 ± 0.58^{abc}	3.33 ± 0.58^{cd}	37.00 ± 3.46^{de}	49.00 ± 8.72^{cdef}	47.33 ± 5.69^{d}	49.67 ± 2.08^{efg}
T5	1.67 ± 0.58^{ab}	4.00 ± 1.00^{ab}	4.00 ± 0.00^{abc}	4.33 ± 0.58^{abcd}	38.00 ± 2.00^{de}	53.00 ± 9.85^{cdef}	54.00 ± 2.65^{cd}	58.00 ± 3.61^{def}
T6	1.33 ± 0.58^{ab}	3.67 ± 0.58^{ab}	3.67 ± 0.58^{abc}	4.00 ± 0.20^{abcd}	35.33 ± 2.52^{e}	58.00 ± 6.08^{bcde}	64.33 ± 2.52^{bc}	48.67 ± 7.09^{efg}
T7	2.00 ± 0.10^{ab}	4.67 ± 0.58^{ab}	3.33 ± 0.58^{abc}	5.33 ± 0.58^{ab}	62.00 ± 5.29^{b}	66.33 ± 10.60^{abcde}	71.33 ± 2.08^{ab}	81.67 ± 5.69^{abc}
T8	2.00 ± 0.07^{ab}	3.33 ± 0.58^{ab}	3.33 ± 0.58^{abc}	4.33 ± 0.58^{abcd}	52.67 ± 3.21^{c}	66.33 ± 4.93^{abcd}	63.67 ± 5.51^{bc}	70.00 ± 5.00^{bcd}
T9	2.67 ± 0.58^{ab}	3.67 ± 0.58^{ab}	3.67 ± 0.58^{abc}	4.67 ± 0.58^{abc}	33.33 ± 17.62^{ef}	65.00 ± 9.17^{abcd}	58.33 ± 3.51^{bcd}	54.00 ± 11.53^{defg}
T10	2.67 ± 0.58^{ab}	4.33 ± 0.58^{ab}	3.00 ± 0.00^{bc}	5.00 ± 1.00^{abc}	42.33 ± 3.51^{d}	69.33 ± 7.23^{abc}	68.33 ± 6.51^{bc}	71.00 ± 3.61^{bcd}
T11	2.00 ± 0.04^{ab}	4.00 ± 1.00^{ab}	4.67 ± 0.58^{ab}	4.67 ± 0.58^{abc}	66.67 ± 4.51^{ab}	63.33 ± 4.73^{abcde}	64.00 ± 10.54^{bc}	63.67 ± 10.60^{cde}
T12	3.00 ± 0.06^{ab}	4.67 ± 0.58^{ab}	4.67 ± 0.58^{ab}	4.67 ± 0.58^{abc}	70.33 ± 4.51^{a}	79.00 ± 5.57^{ab}	83.33 ± 4.04^{a}	86.67 ± 5.51^{ab}
T13	3.33 ± 0.58^{a}	5.00 ± 1.00^{a}	5.00 ± 1.00^{a}	5.67 ± 0.58^{a}	72.33 ± 3.51^{a}	80.00 ± 6.08^{a}	85.67 ± 2.52^{a}	95.33 ± 7.02^{a}

*Data are the average of three replicates ± SD; Grouping information between mean values of obtained data was carried out by Fisher LSD method and 95% Confidence ($P \leq 0.05$). Different letter point out significant differences in a column

Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

Table 3 Effect of Rhizobium in individual and consortium mode on number of seeds plant-1 and weight of 100 seeds in different soil system*

Treatments	Number of seeds pod^{-1}				Weight of 100 seeds (g)			
	Calcareous soil	Chaur soil	Diara soil	Tal soil	Calcareous soil	Chaur soil	Diara soil	Tal soil
T1	1.28 ± 0.05^{f}	1.38 ± 0.04^{e}	1.34 ± 0.09^{f}	1.37 ± 0.04^{e}	2.16 ± 0.23^{f}	2.58 ± 0.03^{g}	2.51 ± 0.04^{e}	2.48 ± 0.09^{f}
T2	1.30 ± 0.07^{ef}	1.40 ± 0.08^{e}	1.42 ± 0.05^{ef}	1.42 ± 0.08^{de}	2.26 ± 0.09^{ef}	2.62 ± 0.03^{fg}	2.57 ± 0.06^{e}	2.57 ± 0.04^{f}
T3	1.35 ± 0.06^{def}	1.42 ± 0.04^{de}	1.40 ± 0.11^{ef}	1.40 ± 0.05^{de}	2.38 ± 0.03^{def}	2.68 ± 0.04^{efg}	2.77 ± 0.18^{e}	2.64 ± 0.03^{ef}
T4	1.41 ± 0.02^{cdef}	1.41 ± 0.04^{de}	1.49 ± 0.05^{def}	1.42 ± 0.04^{de}	2.40 ± 0.07^{cdef}	2.97 ± 0.13^{d}	2.73 ± 0.04^{e}	2.85 ± 0.05^{de}
T5	1.44 ± 0.09^{bcdef}	1.55 ± 0.07^{bcde}	1.59 ± 0.06^{bcde}	1.58 ± 0.06^{bcd}	2.48 ± 0.04^{bcdef}	3.33 ± 0.05^{bc}	2.77 ± 0.09^{e}	3.12 ± 0.03^{cd}
T6	1.41 ± 0.04^{cdef}	1.48 ± 0.09^{cde}	1.52 ± 0.05^{cdef}	1.55 ± 0.07^{cde}	2.40 ± 0.04^{cdef}	2.86 ± 0.07^{de}	2.65 ± 0.06^{e}	2.65 ± 0.06^{ef}
T7	1.55 ± 0.11^{bc}	1.64 ± 0.06^{abc}	1.67 ± 0.05^{abcd}	1.68 ± 0.04^{abc}	2.71 ± 0.14^{abcd}	3.35 ± 0.06^{bc}	3.35 ± 0.19^{a}	3.32 ± 0.03^{bc}
T8	1.50 ± 0.03^{bcd}	1.53 ± 0.06^{bcde}	1.57 ± 0.05^{bcde}	1.57 ± 0.04^{cd}	2.50 ± 0.02^{bcdef}	2.78 ± 0.03^{ef}	2.67 ± 0.15^{e}	2.70 ± 0.02^{ef}
T9	1.50 ± 0.03^{bcd}	1.53 ± 0.05^{bcde}	1.51 ± 0.05^{def}	1.57 ± 0.06^{cd}	2.42 ± 0.07^{cdef}	2.77 ± 0.06^{ef}	2.68 ± 0.10^{e}	2.50 ± 0.25^{f}
T10	1.58 ± 0.12^{abc}	1.70 ± 0.05^{ab}	1.71 ± 0.07^{abc}	1.72 ± 0.05^{abc}	2.76 ± 0.09^{abc}	3.38 ± 0.04^{bc}	3.27 ± 0.27^{ab}	3.42 ± 0.03^{ab}
T11	1.49 ± 0.07^{bcde}	1.61 ± 0.14^{abcd}	1.63 ± 0.05^{abcd}	1.62 ± 0.15^{bc}	2.62 ± 0.07^{bcde}	3.22 ± 0.10^{c}	2.88 ± 0.20^{bc}	3.01 ± 0.17^{d}
T12	1.64 ± 0.06^{ab}	1.75 ± 0.04^{a}	1.75 ± 0.09^{ab}	1.76 ± 0.02^{ab}	2.82 ± 0.04^{ab}	3.47 ± 0.07^{b}	3.48 ± 0.17^{a}	3.42 ± 0.04^{ab}
T13	1.77 ± 0.04^{a}	1.81 ± 0.06^{a}	1.81 ± 0.03^{a}	1.81 ± 0.04^{a}	3.06 ± 0.30^{a}	3.69 ± 0.03^{a}	3.52 ± 0.14^{a}	3.67 ± 0.04^{a}

*Data are the average of three replicates ± SD; Grouping information between mean values of obtained data was carried out by Fisher LSD method and 95% Confidence ($P \leq 0.05$). Different letter point out significant differences in a column

Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* enhanced number of seeds pod^{-1} by 34.32%, 16.88% and 20.80% over the control, 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 3b). Likewise, weight of 100 seeds were enhanced by 43%, 19.11% and 32.20% compared to control, 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 3b). Irrespective to treatments, the highest numbers of pods plant^{-1}, seeds pod^{-1} and weight of 100 seeds were significantly recorded higher in Tal soil followed by Chaur soil (Supplementary Fig. 4a and 4b).

3.12 Effect of *Rhizobium* in Individual and Consortium Mode on Lentil Grain Yield

In terms of grain yield per pot, there was considerable variation among the treatments across different soil systems. Among the fertilizer treatments, the 100% RDF treatment significantly increased grain yield compared to the control, but only in Chaur soil and Tal soil. The application of individual *Rhizobium* had a significant impact on lentil grain yield compared to the control, but this effect was observed solely in Tal soil (Table 4). In calcareous soil, Chaur soil, and Diara soil, both the 100% RDF and individual *Rhizobium* treatments showed statistically similar results. However, in Tal soil, the 100% RDF treatment showed significant superiority over the individual *Rhizobium* treatment (Table 4). Across all soil systems, when *Rhizobium* was combined with other rhizobacteria (T7-T13), there was a significant increase in grain yield compared to the individual *Rhizobium* treatments. The treatment with the consortium of *Rhizobium*, *Bacillus*, *Pseudomonas*, and *Azotobacter* consistently showed the highest grain yield across all soil systems (Table 4).

Irrespective to treatments, Tal soil consistently showed the highest grain yield across most of the treatments, followed by Chaur soil (Supplementary Fig. 4a). Irrespective to soils, fertilizer treatments significantly enhanced grain yield over the control and 100% RDF had the highest grain yield (Fig. 3a). Application of 100% RDF increased the grain yield by 10.33% over the control. Moreover, 100% RDF also showed significantly higher grain yield over the individual *Rhizobium* treatment. However, different consortium of *Rhizobium* with other rhizobacteria significantly enhanced grain yield over the other treatments. Consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* had the highest grain yield which enhanced grain yield by 24%, 12.33% and 16.54% over the control, 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 3a). The ANOVA for grain yield showed that soil types and treatments were highly significant at 1% probability. Besides, soil type × treatment interaction was not significant (supplementary Table 6).

3.13 Effect of *Rhizobium* in Individual and Consortium Mode on Biofortification of N, P and K

Fertilizer treatments were statistically significant over the control in relation to N accumulation in lentil grains. Among the fertilizer treatments, 100% RDF treatment (T5) was resulted significantly higher N content in lentil grains across all soil systems. Individual *Rhizobium* inoculation was statistically significant over the control across all soil systems in relation to N content in grains (Table 4). However, it was statistically similar to 100% RDF irrespective to soil (Fig. 4a). When *Rhizobium* was combined with other rhizobacteria, there was a further increase in N in grains compared to the individual *Rhizobium* treatments. The highest N content in lentil grains was observed with consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* across all soil systems (Table 4). Irrespective to soil, consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* increased N content in lentil grains by 36.82%, 23.31% and 23.15% over the control, 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 4a).

In terms of P and K accumulation in grains, there was considerable variation among the treatments in different soil system. Among fertilizer treatments, 100% RDF showed significant effect on P and K accumulation in grains over the control across all soil systems. Individual *Rhizobium* treatment was statistically similar with 100% RDF in relation to P and K accumulation in grains across all soil system (Table 5). Different combination of *Rhizobium* showed significant effect on P and K accumulation compared to individual *Rhizobium* treatment across all soil systems (Table 5). Consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* recorded significantly higher accumulation of P and K in lentil grains across all soil systems.

Irrespective to soil, all the fertilizer treatments were statistically similar to the control in relation to P content in lentil grains (Fig. 4a). Individual *Rhizobium* inoculation also did not show any significant effect on P accumulation in lentil grains. However, different consortia of *Rhizobium* with *Bacillus*, *Pseudomonas* and *Azotobacter* showed significantly higher accumulation of P in lentil grains compared to other treatments. Consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* enhanced P accumulation in lentil grains by 31.63%, 27.68% and 27.13% over the control, 100% RDF treatment and individual *Rhizobium* treatment, respectively. All treatments exhibited statistically similar potassium content in lentil seeds irrespective to soils (Fig. 4a). Irrespective to treatments, the highest amount of N, P and K in lentil grains were accumulated in Tal soil followed by Chaur soil. Whereas, the least amount of N, P and K in lentil grains were accumulated in calcareous soil (Supplementary Fig. 5a). The ANOVA for N, P and K content in lentil seeds showed that soil types and treatments were highly significant at 1% probability. Likewise, soil type × treatment interaction was also significant at 1% probability (supplementary Table 7).

3.14 Effect of *Rhizobium* in Individual and Consortium Mode on Biofortification of Fe and Zn in Lentil Grains

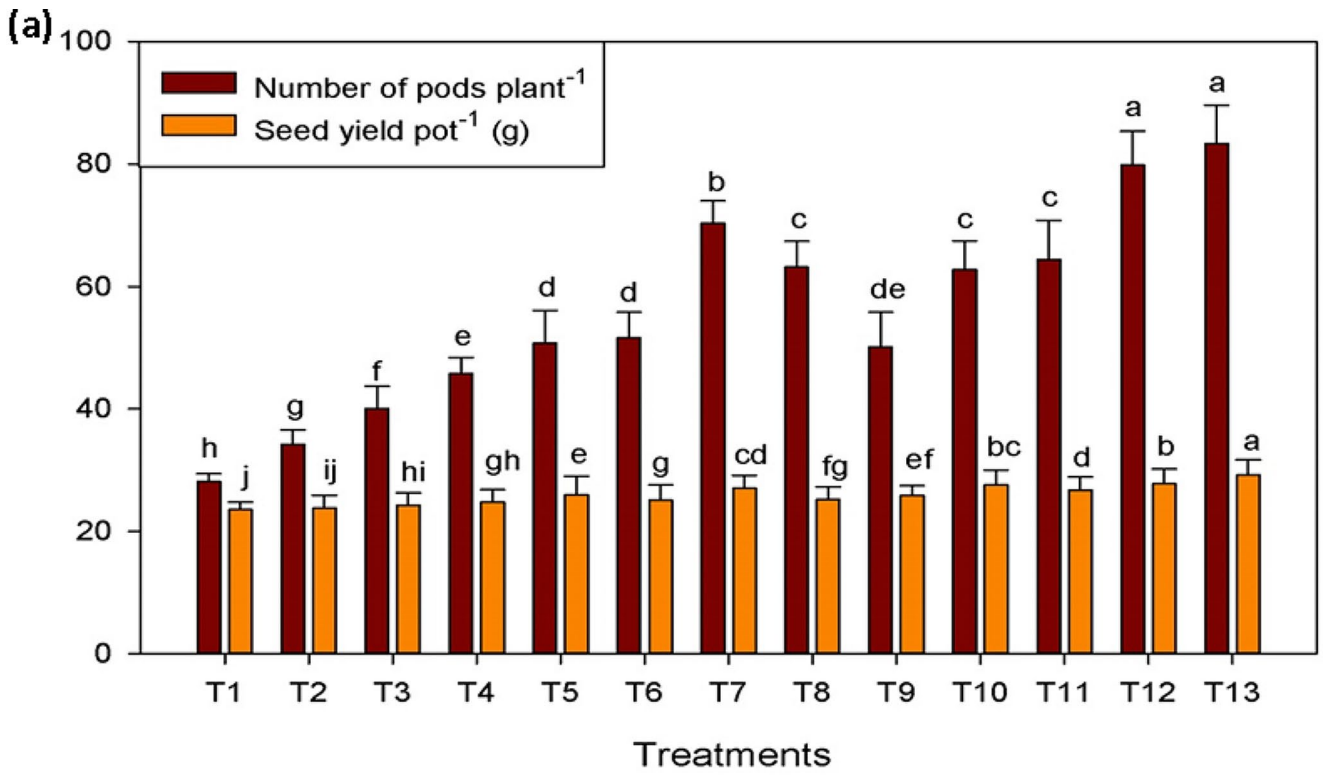
Fe and Zn content in lentil grains were significantly increased with the increase in RDF levels (T2-T5) over the control across all soil systems (Table 6). Individual *Rhizobium* inoculation did not show any significant effect on Fe and Zn content in lentil grains over the control across all soil systems (Table 6). However, as the *Rhizobium* was applied in combination with other microbial inoculants, Fe and Zn accumulation in lentil grains was significantly increased over the control. However, in most cases, the consortium mode of microbial inoculation outperforms the chemical fertilizer treatments in terms of Fe and Zn biofortification in all soil systems (Table 6). The highest Fe and Zn content in lentil grains was observed with four microbe consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* followed by three microbe consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* across all soil systems.

Irrespective to soil, application of 100% RDF increased Fe and Zn content by 44.60 and 55% in lentil grains over the control, respectively. Consortia of *Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter* enhanced Fe content by 82.54%, 26.24% and 80% over the control, 100% RDF and individual *Rhizobium* treatment, respectively. Likewise, Zn content was increased by 78.68%, 15.26% and 68% over the over the control, 100% RDF and individual *Rhizobium* treatment, respectively (Fig. 4b).

Irrespective to the treatments, Fe content in lentil grains was significantly higher in Tal soil and Diara soil. Fe content in lentil grains was non-significant in calcareous soil. Zn content in lentil grains was significantly higher in Chaur soil which was statistically similar to Tal and Diara soil and at par over the calcareous soil (Supplementary Fig. 5b). The ANOVA for Fe and Zn content in lentil grains showed that soil types and treatments were highly significant at 1% probability. Likewise, soil type × treatment interaction was also significant at 1% probability (Supplementary Table 7).

4 Discussion

Over time, the role of soil microbes in enhancing fertility and green energy production has gained attention. These microorganisms boost crop yield, aid in nitrogen fixation and nutrient cycling. Bio-fertilizers, relying on biological


(a)
Number of pods plant-1
Seed yield pot-1 (g)
100
80
60
40
20
0
h
j
g
ij
f
hi
e
gh
d
e
d
g
b
cd
c
fg
de
ef
c
bc
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b
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T7
T8
T9
T10
T11
T12
T13
Treatments

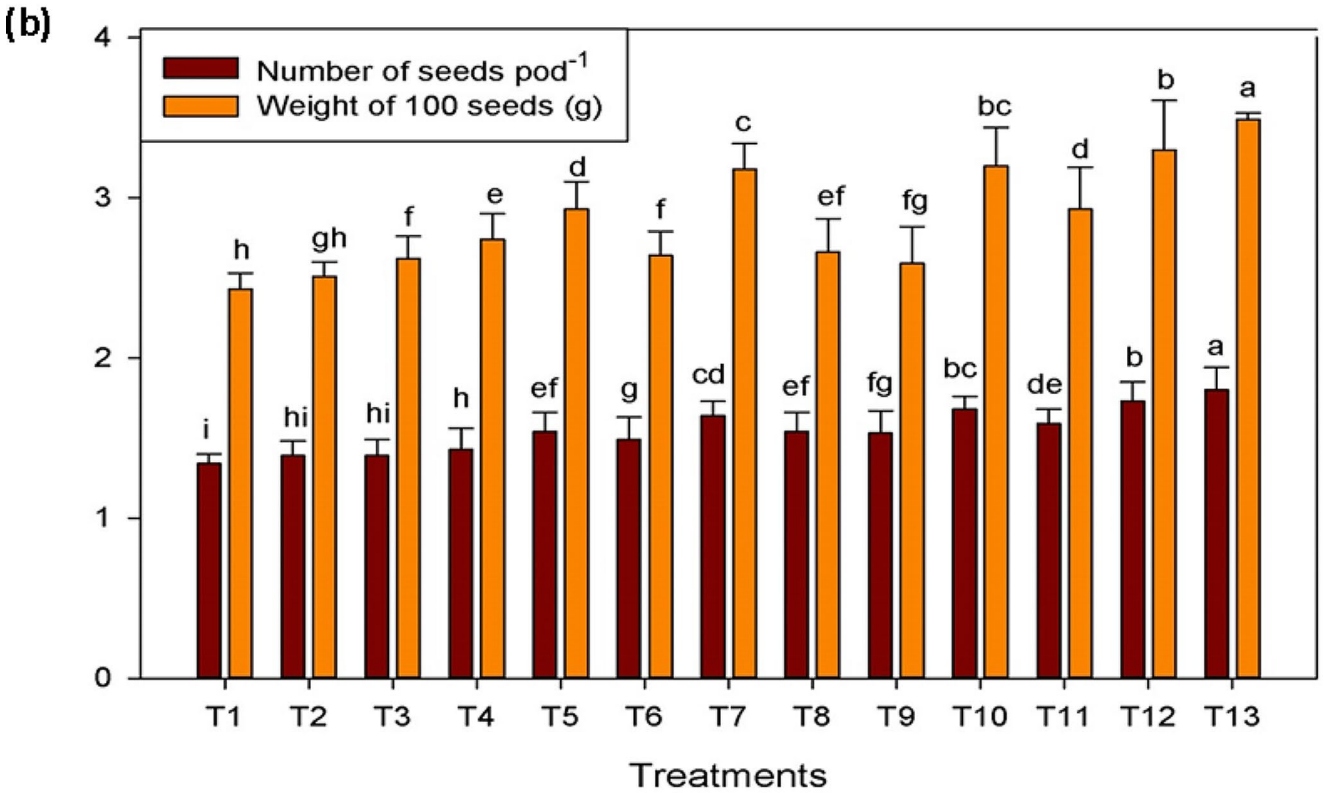
(b)
Number of seeds pod-1
Weight of 100 seeds (g)
4
3
2
1
0
i
h
hi
gh
hi
f
h
e
ef
d
g
f
cd
c
ef
ef
fg
fg
bc
bc
de
d
b
b
a
a
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10
T11
T12
T13
Treatments

Fig. 3 Effect of *Rhizobium* in individual and consortium mode on lentil yield and yield attributes irrespective to soil. # **a:** number of pods plant $^{-1}$, seed yield pot $^{-1}$ (g); **b:** number of seeds pod $^{-1}$, weight of 100 seeds (g). *The data represent the average of three replicates, with error bars indicating the standard deviation (SD). Different letters denote significant differences within a column. Grouping was determined using the Fisher LSD method with a 95% confidence level ($P \leq 0.05$). Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

processes, are crucial for sustainable agriculture (Itelima et al. 2018). However, their effectiveness varies by region. To address this, researchers aim to identify universal strains that can thrive across diverse soil conditions (Trivedi et al. 2021). In the present study, rhizobacteria and *Rhizobium* isolates from Tal region soil were evaluated in other soil conditions, including Diara, Chaur, and calcareous soil, alongside the Tal soil. The selection of Tal soil of Mokama was based on its status as an unexplored natural habitat for soil microbes. This region, spanning over 17,225 km^2, faces significant challenges, particularly during the monsoon season due to poor drainage facilities. As a result, farmers in this region primarily rely on Rabi crops (Choudhary et al. 2019). The farmers here cultivate indigenous crop varieties without the use of chemical fertilizers, weed control, or pest control chemicals. Therefore, the success of crop production in this region, particularly Rabi crops, depends heavily on the microbial population present in the soil. Lentil, among other pulses, cover a substantial area in the Tal region (Singh 2018). By studying the microbial communities in the Tal region and evaluating their performance in different soil types, this research aims to identify potential biofertilizer consortia that can adapt to various environmental conditions. Such findings would be valuable for promoting sustainable agriculture and increasing crop productivity in diverse regions.

Our research highlights diverse plant growth-promoting traits exhibited by various bacterial isolates, including P, K, and Zn solubilization, siderophore, IAA, HCN, and ammonia production. These findings deepen our understanding of how these microbes enhance plant growth and nutrient biofortification. Rhizospheric bacteria assist plants in accessing nutrients from the soil by hydrolyzing them into soluble forms, facilitating their uptake (Gupta et al. 2022; Bisht and Garg 2022; Singh et al. 2017a, b). Enhanced solubilization of P, K, and Zn by these bacteria holds promise for improving crop productivity and soil health, promoting sustainable agriculture (Iftikhar et al. 2024; Upadhayay et al. 2022). Siderophore-producing and zinc-solubilizing endophytes have been shown to enhance Fe and Zn accumulation in wheat plants, leading to a 100% increase in grain yield compared to control (Singh et al. 2017a, b, 2020).

The soil samples collected from different locations in Bihar exhibited variations in their chemical properties. The chemical properties of Diara and Tal soil in our study were consistent with the findings of Verma et al. (2011). Regarding calcareous soil, our study's results align with the findings of Chandan et al. (2019) for nitrogen (N), phosphorus (P), and potassium (K). Furthermore, our study's findings regarding Chaur soil from the Darbhanga district were consistent with the research conducted by Pandey et al. (2000), who examined the properties of soil collected from other Chaur regions in Bihar. The similarities in the chemical properties suggest a shared soil profile and composition within the Chaur regions.

In present study, the improved germination time observed in *Rhizobium* treatments suggests that the presence of *Rhizobium* and other microorganisms positively influenced seedling establishment and vigor. This may be attributed to the ability of PGPR to fix atmospheric nitrogen and enhance nutrient availability through various mechanisms such as phosphate solubilization, production of growth-promoting substances, and disease suppression. In our present study, Diara soil and calcareous soil had slightly longer germination times, indicating that they might have certain factors or conditions that delayed germination compared to the other soils. Tena et al. (2016) and Devi and Kumar (2020) likely investigated similar effect of *Rhizobium* on germination. Co-inoculation of the multi-traits soil bacteria with *Rhizobium* improve the agronomic traits in various crop was addressed by various researchers (Zaheer et at. 2019; Ibrahim and El-Sawah 2022).

The number of root nodules is an important parameter indicating the efficiency of nitrogen fixation in leguminous plants (Bekuzarova et al. 2020; Vasileva 2017). In the present study, the enhanced nodulation observed in *Rhizobium* treatments compared to chemical fertilizers can be attributed to the phenomenon whereby the application of chemical fertilizer may suppress the growth of native rhizobia in the soil, consequently leading to a negative impact on nodulation in plants (Ben-Laouane et al. 2020; Hinson and Adams 2020; Elgharably and Benes 2021). The presence of other microorganisms in the consortium may have facilitated nodulation by providing a conducive rhizosphere environment, stimulating the expression of nodulation genes, or promoting the proliferation of beneficial bacteria. Our findings are

Table 4 Effect of Rhizobium in individual and consortium mode on lentil seed yield and nitrogen content in seeds across different soil system*

Treatments	Seed yield pot^{-1} (g)				Nitrogen (g kg^{-1})			
	Calcareous soil	Chaur soil	Diara soil	Tal soil	Calcareous soil	Chaur soil	Diara soil	Tal soil
T1	20.78 ± 0.36^{c}	24.18 ± 0.62^{e}	24.61 ± 0.48^{d}	24.80 ± 1.2^{g}	20.74 ± 0.52^{f}	21.36 ± 0.09^{f}	20.68 ± 0.71^{g}	21.49 ± 1.05^{i}
T2	21.15 ± 0.24^{c}	24.54 ± 0.79^{cde}	24.74 ± 0.89^{cd}	24.83 ± 1.07^{g}	21.36 ± 0.74^{ef}	21.41 ± 0.04^{f}	21.56 ± 0.71^{fg}	21.50 ± 1.03^{i}
T3	21.39 ± 0.54^{c}	25.27 ± 0.38^{cde}	25.10 ± 1.18^{bcd}	25.39 ± 1.08^{fg}	22.39 ± 0.15^{def}	22.68 ± 0.04^{e}	21.94 ± 0.35^{fg}	22.62 ± 1.03^{h}
T4	22.33 ± 0.45^{bc}	25.54 ± 0.89^{cde}	25.57 ± 0.75^{bcd}	25.73 ± 1.12^{f}	22.38 ± 0.61^{def}	22.79 ± 0.04^{de}	23.50 ± 0.89^{de}	22.81 ± 1.04^{g}
T5	23.40 ± 1.15^{abc}	26.81 ± 0.29^{bc}	26.32 ± 0.38^{bcd}	27.54 ± 1.28^{d}	22.85 ± 0.23^{cde}	23.49 ± 0.04^{cd}	23.41 ± 0.48^{de}	23.78 ± 1.03^{f}
T6	22.29 ± 1.91^{bc}	25.54 ± 0.79^{cde}	25.79 ± 0.35^{bcd}	26.69 ± 1.12^{e}	22.83 ± 0.06^{cde}	23.51 ± 0.09^{cd}	22.77 ± 0.17^{ef}	24.54 ± 1.12^{e}
T7	25.39 ± 0.75^{ab}	26.84 ± 1.09^{bc}	27.02 ± 2.38^{abcd}	28.94 ± 1.24^{c}	24.19 ± 0.92^{bc}	25.71 ± 0.03^{ab}	25.61 ± 0.33^{bc}	26.62 ± 1.03^{b}
T8	22.46 ± 0.81^{bc}	25.67 ± 0.84^{cde}	26.06 ± 0.61^{bcd}	26.77 ± 1.18^{de}	22.66 ± 0.68^{cde}	25.01 ± 0.18^{b}	24.70 ± 0.42^{cd}	24.43 ± 1.03^{e}
T9	22.70 ± 0.29^{bc}	26.53 ± 0.71^{bcd}	26.97 ± 0.67^{abcd}	27.35 ± 1.05^{de}	23.27 ± 0.42^{cd}	24.18 ± 0.40^{c}	24.97 ± 0.20^{c}	24.72 ± 1.03^{d}
T10	25.56 ± 3.03^{ab}	28.07 ± 0.74^{ab}	27.68 ± 0.69^{ab}	29.27 ± 1.03^{bc}	23.89 ± 1.19^{cd}	26.21 ± 0.40^{a}	26.87 ± 0.40^{ab}	26.77 ± 1.08^{b}
T11	24.39 ± 1.57^{abc}	26.77 ± 0.90^{bcd}	27.48 ± 0.45^{abc}	28.53 ± 1.03^{c}	23.64 ± 0.18^{cd}	25.76 ± 0.66^{ab}	25.14 ± 0.22^{c}	25.68 ± 1.04^{c}
T12	25.56 ± 0.69^{ab}	28.07 ± 0.71^{ab}	27.65 ± 0.82^{ab}	30.00 ± 1.21^{b}	25.61 ± 0.15^{ab}	26.11 ± 0.16^{a}	27.39 ± 0.22^{a}	26.68 ± 1.03^{b}
T13	26.66 ± 0.85^{a}	29.41 ± 0.64^{a}	29.42 ± 0.79^{a}	31.44 ± 1.87^{a}	26.21 ± 0.30^{a}	25.84 ± 0.18^{a}	27.88 ± 0.25^{a}	27.37 ± 1.07^{a}

*Data are the average of three replicates ± SD; Grouping information between mean values of obtained data was carried out by Fisher LSD method and 95% Confidence ($P \leq 0.05$). Different letter point out significant differences in a column

Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6-*Rhizobium*; T7-*Rhizobium* + *Bacillus*; T8-*Rhizobium* + *Pseudomonas*; T9-*Rhizobium* + *Azotobacter*; T10-*Rhizobium* + *Bacillus* + *Pseudomonas*; T11-*Rhizobium* + *Pseudomonas* + *Azotobacter*; T12-*Rhizobium* + *Bacillus* + *Azotobacter*; T13-*Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter*

consistent with the study conducted by Debela et al. (2021), which reported similar results regarding the effects of co-inoculation of *Rhizobium* and PGPR strains on nodulation, and leghemoglobin content in lentil nodules.

In present study, the application of consortia inoculants, specially four microbe consortia, outperformed the sole application of chemical fertilizers or *Rhizobium* in all soil types. This suggests that the combined action of multiple beneficial microbes may enhance nutrient availability, stimulate root growth, and provide other growth-promoting factors, resulting in improved plant development (Araújo et al. 2009; Verma et al. 2012). In our study, *Bacillus, Pseudomonas* and *Azotobacter* demonstrated their abilities in phosphorus solubilization which may play a crucial role in increasing the availability of phosphorus in the rhizosphere. This availability of phosphorus has a positive impact on *Rhizobium* growth, nodulation efficiency, nitrogen fixation capacity, and overall nitrogen content in legume plant parts (Bashir et al. 2011). Biswas & Turner (2012) reported similar results, demonstrating that the use of consortia inoculants led to higher performance compared to the individual form application of *Rhizobium*.

These findings have practical implications for agricultural practices, particularly in areas with nutrient-deficient soils. The use of microbial consortia may be a promising approach for sustainable agriculture, as it can potentially reduce the reliance on synthetic fertilizers and enhance nutrient use efficiency. Irrespective to treatments, calcareous soil had shorter root and shoot lengths, suggesting that it may not provide as favorable conditions for root and shoot development. Calcareous soil, characterized by high pH and limited nutrient availability, showed the poorest performance in terms of plant growth parameters. Different soil types have varying nutrient profiles and physical characteristics, which significantly influence plant development (Neina 2019; Ma et al. 2020; Huang and Hartemink 2020; John et al. 2007).

In present study, the application of 100% RDF performed better than single *Rhizobium* strain with regards to yield and yield attributes. This finding is corroborated by Singh et al. (2022), who similarly observed that using *Rhizobium* alone had a lesser impact on both yield and yield attributes in lentil cultivation compared to the application of chemical fertilizers. However, application of *Rhizobium* in different combination of *Bacillus, Pseudomonas*, and *Azotobacter*, showed higher and more positive results compared to 100% RDF and individual *Rhizobium*. The positive effect of combined microbial inoculants on seed yield is evident in all soil systems, suggesting that these combinations can be beneficial for lentil cultivation regardless of soil type. These findings are in line with the research conducted by Tiwari et al. (2022) who found that consortia of *Rhizobium* + phosphate-solubilizing bacteria showed higher yield and yield attributes compared to chemical fertilizer or individual microbial inoculant. Kumar et al. (2021) also reported that inoculation of individual *R. leguminosarum* subsp. *viciae* RR1 increased yield by 19% and 36% compared to 100% RDF and 50% RDF levels, respectively. Whereas, co-inoculation of *R. leguminosarum* subsp. *viciae* RR1 with *Bacillus* sp. RB1 or *Pseudomonas* sp. RP1 increased yield by 25% over the 100% RDF level.

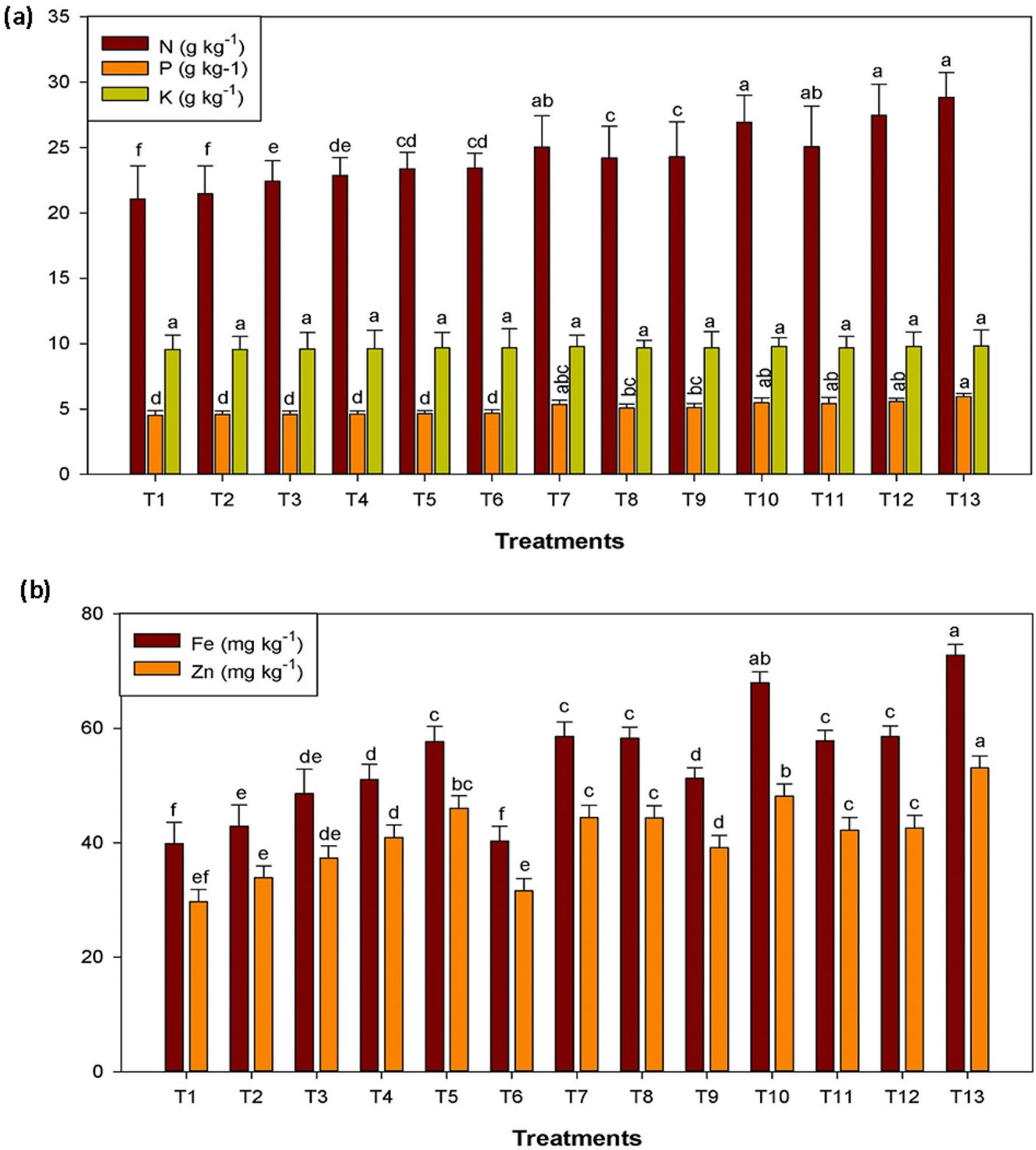

Fig. 4 Effect of *Rhizobium* in individual and consortium mode on biofortification of macronutrients and micronutrients in lentil seeds irrespective to soil. #**a:** macronutrients; **b:** micronutrients. *The data represent the average of three replicates, with error bars indicating the standard deviation (SD). Different letters denote significant differences within a column. Grouping was determined using the Fisher LSD method with a 95% confidence level ($P \leq 0.05$). Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

Table 5 Effect of Rhizobium in individual and consortium mode on phosphorus and potassium content in lentil seed in different soil system*

Treatments	Phosphorus (g kg^{-1})				Potassium (g kg^{-1})			
	Calcareous soil	Chaur soil	Diara soil	Tal soil	Calcareous soil	Chaur soil	Diara soil	Tal soil
T1	4.46 ± 0.03^{f}	4.33 ± 0.20^{i}	4.56 ± 0.12^{h}	4.54 ± 0.10^{j}	9.54 ± 0.16^{d}	9.55 ± 0.23^{i}	9.54 ± 0.51^{e}	9.55 ± 0.80^{g}
T2	4.53 ± 0.08^{ef}	4.47 ± 0.22^{h}	4.58 ± 0.20^{h}	4.59 ± 0.15^{i}	9.55 ± 0.22^{d}	9.56 ± 0.42^{i}	9.56 ± 0.61^{e}	9.58 ± 1.00^{g}
T3	4.53 ± 0.10^{ef}	4.48 ± 0.27^{h}	4.62 ± 0.10^{g}	4.60 ± 0.11^{i}	9.55 ± 0.32^{d}	9.63 ± 0.12^{h}	9.57 ± 0.51^{e}	9.63 ± 1.10^{f}
T4	4.51 ± 0.20^{ef}	4.65 ± 0.08^{g}	4.62 ± 0.34^{g}	4.65 ± 0.14^{h}	9.64 ± 0.21^{c}	9.66 ± 0.25^{g}	9.57 ± 0.42^{e}	9.67 ± 0.81^{e}
T5	4.57 ± 0.20^{de}	4.65 ± 0.09^{g}	4.74 ± 0.26^{f}	4.67 ± 0.16^{h}	9.65 ± 0.42^{c}	9.71 ± 0.42^{d}	9.64 ± 0.41^{d}	9.69 ± 0.73^{de}
T6	4.65 ± 0.01^{cd}	4.78 ± 0.24^{f}	4.75 ± 0.28^{f}	4.67 ± 0.20^{h}	9.65 ± 0.40^{c}	9.71 ± 0.31^{d}	9.64 ± 0.42^{d}	9.68 ± 0.90^{de}
T7	4.65 ± 0.03^{cd}	5.44 ± 0.23^{c}	5.57 ± 0.19^{d}	5.67 ± 0.12^{d}	9.74 ± 0.31^{ab}	9.79 ± 0.40^{b}	9.76 ± 0.33^{b}	9.78 ± 0.90^{b}
T8	4.65 ± 0.04^{cd}	4.97 ± 0.04^{e}	4.85 ± 0.30^{e}	5.21 ± 0.14^{g}	9.64 ± 0.10^{c}	9.67 ± 0.61^{g}	9.68 ± 0.40^{c}	9.68 ± 0.90^{de}
T9	4.73 ± 0.03^{bc}	5.15 ± 0.05^{d}	4.84 ± 0.30^{e}	5.52 ± 0.13^{f}	9.66 ± 0.10^{bc}	9.69 ± 0.40^{f}	9.66 ± 0.50^{cd}	9.70 ± 1.01^{d}
T10	4.76 ± 0.03^{b}	5.40 ± 0.07^{c}	5.65 ± 0.41^{c}	5.78 ± 0.11^{c}	9.76 ± 0.40^{a}	9.76 ± 0.50^{c}	9.78 ± 0.61^{b}	9.79 ± 0.71^{b}
T11	4.75 ± 0.03^{b}	5.40 ± 0.04^{c}	5.55 ± 0.41^{d}	5.60 ± 0.14^{e}	9.65 ± 0.20^{c}	$9.70 \pm 0.60e^{f}$	9.66 ± 0.70^{cd}	9.75 ± 0.91^{c}
T12	4.82 ± 0.01^{ab}	5.75 ± 0.05^{b}	5.85 ± 0.40^{b}	5.87 ± 0.10^{b}	9.76 ± 0.30^{a}	9.83 ± 0.56^{a}	9.79 ± 0.70^{ab}	9.81 ± 0.91^{b}
T13	4.85 ± 0.05^{a}	5.87 ± 0.06^{a}	5.96 ± 0.41^{a}	5.90 ± 0.22^{a}	9.75 ± 0.23^{a}	9.85 ± 0.49^{a}	9.82 ± 0.61^{a}	9.87 ± 1.2^{a}

*Data are the average of three replicates ± SD; Grouping information between mean values of obtained data was carried out by Fisher LSD method and 95% Confidence ($P \leq 0.05$). Different letter point out significant differences in a column

Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6-*Rhizobium*; T7-*Rhizobium* + *Bacillus*; T8-*Rhizobium* + *Pseudomonas*; T9-*Rhizobium* + *Azotobacter*; T10-*Rhizobium* + *Bacillus* + *Pseudomonas*; T11-*Rhizobium* + *Pseudomonas* + *Azotobacter*; T12-*Rhizobium* + *Bacillus* + *Azotobacter*; T13-*Rhizobium* + *Bacillus* + *Pseudomonas* + *Azotobacter*

Table 6 Effect of Rhizobium in individual and consortium mode on biofortification of Fe and Zn in Lentil seed in different soil system*

Treatments	Fe (mg kg^{-1})				Zn (mg kg^{-1})			
	Calcareous soil	Chaur soil	Diara soil	Tal soil	Calcareous soil	Chaur soil	Diara soil	Tal soil
T1	30.42 ± 1.33^{f}	45.21 ± 1.09^{f}	39.39 ± 1.09^{f}	44.45 ± 2.07^{f}	23.36 ± 0.29^{e}	31.51 ± 1.04^{g}	32.72 ± 1.22^{f}	31.24 ± 1.71^{f}
T2	32.48 ± 1.11^{e}	48.35 ± 2.21^{e}	43.50 ± 1.13^{e}	47.14 ± 2.07^{e}	27.55 ± 0.03^{d}	35.69 ± 1.02^{f}	36.88 ± 1.33^{e}	35.42 ± 1.40^{e}
T3	40.46 ± 1.15^{d}	53.82 ± 1.09^{de}	46.54 ± 1.19^{de}	53.52 ± 1.04^{de}	32.52 ± 0.20^{c}	38.70 ± 2.02^{e}	39.33 ± 1.02^{d}	38.72 ± 1.30^{de}
T4	43.56 ± 1.33^{cd}	56.79 ± 1.17^{d}	47.56 ± 1.06^{de}	56.33 ± 3.04^{d}	35.58 ± 0.26^{bc}	42.32 ± 1.03^{d}	43.40 ± 1.03^{cd}	42.23 ± 1.50^{d}
T5	45.93 ± 1.07^{c}	62.89 ± 1.39^{c}	55.85 ± 1.13^{b}	65.92 ± 5.04^{c}	37.79 ± 0.27^{b}	48.54 ± 1.08^{c}	48.48 ± 1.04^{c}	49.35 ± 1.30^{bc}
T6	30.42 ± 1.12^{f}	46.48 ± 1.07^{ef}	39.58 ± 1.07^{f}	44.82 ± 4.03^{f}	23.60 ± 0.10^{e}	33.26 ± 2.04^{fg}	34.36 ± 1.26^{ef}	35.14 ± 1.40^{e}
T7	46.50 ± 1.44^{bc}	62.35 ± 2.06^{c}	57.54 ± 1.06^{b}	67.86 ± 3.06^{c}	35.62 ± 0.25^{bc}	47.78 ± 1.03^{c}	46.73 ± 1.22^{c}	47.33 ± 1.13^{c}
T8	46.36 ± 1.13^{bc}	63.50 ± 1.36^{c}	56.75 ± 1.07^{b}	66.57 ± 3.05^{c}	36.16 ± 0.26^{b}	48.71 ± 1.03^{c}	46.14 ± 1.24^{c}	46.35 ± 1.70^{c}
T9	40.30 ± 1.65^{d}	56.75 ± 1.14^{d}	50.18 ± 1.05^{d}	57.75 ± 2.06^{d}	32.42 ± 0.25^{c}	42.78 ± 2.03^{d}	40.11 ± 1.32^{d}	41.25 ± 1.60^{d}
T10	49.21 ± 1.41^{b}	76.95 ± 1.13^{ab}	67.82 ± 1.03^{b}	77.91 ± 3.03^{ab}	37.41 ± 0.38^{b}	51.36 ± 1.05^{b}	52.18 ± 1.44^{b}	51.51 ± 1.32^{b}
T11	46.42 ± 1.18^{bc}	60.39 ± 2.13^{c}	57.42 ± 1.04^{c}	67.03 ± 2.16^{c}	31.52 ± 0.38^{c}	47.44 ± 1.06^{c}	44.32 ± 1.42^{c}	45.56 ± 1.16^{c}
T12	46.45 ± 1.27^{bc}	61.53 ± 1.07^{c}	58.02 ± 1.05^{c}	68.30 ± 4.07^{c}	30.44 ± 0.20^{c}	47.36 ± 1.04^{c}	45.74 ± 1.24^{c}	46.76 ± 1.09^{c}
T13	54.77 ± 1.31^{a}	78.71 ± 2.21^{a}	78.61 ± 1.03^{a}	79.06 ± 3.31^{a}	43.66 ± 0.41^{a}	54.64 ± 1.04^{a}	56.79 ± 1.32^{a}	57.19 ± 1.56^{a}

*Data are the average of three replicates ± SD; Grouping information between mean values of obtained data was carried out by Fisher LSD method and 95% Confidence ($P \leq 0.05$). Different letter point out significant differences in a column

Where, T1-Control; T2-25% of RDF; T3-50% of RDF; T4-75% of RDF; T5-100% of RDF; T6- *Rhizobium leguminosarum* bv. *Viciae* DPR2; T7- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4; T8- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4; T9- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Azotobacter salinestris* DPA1; T10- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4; T11- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1; T12- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Azotobacter salinestris* DPA1; T13- *Rhizobium leguminosarum* bv. *Viciae* DPR2 + *Bacillus cabrialesii* DPB4 + *Pseudomonas synxantha* DPP4 + *Azotobacter salinestris* DPA1

Tal soil consistently shows the highest lentil seed yield and yield attributes across most treatments. This suggests that Tal soil may be more suitable for lentil cultivation, providing better conditions for growth and yield. Calcareous soil had the lowest seed yield. It is worth noting that the specific response to microbial treatments may vary depending on the soil system and its inherent properties. The differences observed in the performance of the treatments across different soil systems highlight the influence of soil characteristics on plant-microbe interactions and subsequent seed development.

Our present findings also highlight the potential of plant growth-promoting rhizobacteria (PGPR) in replenishing chemical fertilizers. The co-inoculation of PGPR

can effectively reduce the reliance on chemical fertilizers, offering economic and environmental benefits. By enhancing nutrient availability, promoting plant growth, and facilitating nutrient uptake, PGPR can serve as an eco-friendly alternative to chemical fertilizers (Basu et al. 2021; Verma et al. 2019). This reduction in chemical fertilizer usage not only contributes to cost savings for farmers but also helps mitigate the negative environmental impacts associated with excessive fertilizer application (Chandini et al. 2019; Good and Beatty 2011).

Our results indicated that the type of soil, fertilizer dose, and microbial inoculants significantly influence the biofortification of N, P, K, Fe and Zn in lentil seeds. In our present study, the control treatment consistently exhibits the lowest nitrogen, phosphorus and potassium content in lentil seeds, indicating the importance of fertilizer application or microbial inoculation for N, P and K uptake. Combined inoculation of *Rhizobium* with *Bacillus*, *Pseudomonas*, and/or *Azotobacter* showed more prominent results compared to individual *Rhizobium* or fertilizer treatments. These results suggest that combine inoculation can enhance nitrogen fixation, phosphorus and potassium solubilization and improve N, P and K accumulation in lentil plants, leading to higher N, P and K content in the seeds. Similar results were also found by Zafar et al. (2012) over application of bio-fertilizer on lentil plant.

In our present study, the different percentages of the recommended dose of fertilizer (25%, 50%, 75%, and 100% of RDF) also influenced the concentrations of Fe and Zn in lentil seeds. Generally, increasing the fertilizer dose resulted in higher concentrations of Fe and Zn. This suggests that appropriate fertilization can enhance the bioavailability and uptake of these micronutrients by lentil plants. In our present study, *Rhizobium* inoculation did not show any significant effect on Fe/Zn fortification. But, the presence of *Rhizobium*, in combination with *Bacillus, Pseudomonas*, and/or *Azotobacter*, had a significant impact on the biofortification of Fe and Zn in lentil seeds over the control. This suggests that the combined application of multiple microbial strains can have synergistic effects on nutrient biofortification. Kumar et al. (2021) reported that *Rhizobium* inoculation did not show any significant effect on Fe fortification. But co-inoculation of *R. leguminosarum* subsp. *viciae* RR1with *Bacillus* sp. RB1 and/or *Pseudomonas* sp. RP1 significantly enhanced the Fe content in lentil seeds over the control. Gopalakrishnan et al. (2016) also reported that co-inoculation of seven strains of PGPRs increased the Fe content by 18% and 12% in chickpea and pigeonpea over the control, respectively.

Variations in Fe and Zn content were observed across different soil types, with Tal soil exhibiting higher levels compared to calcareous soil, attributed to differences in nutrient composition and soil characteristics (Malusà et al. 2016). Soil richness in Tal, Chaur, and Diara, along with higher organic carbon content, favored bio-fertilizer effectiveness compared to calcareous soil. Indigenous strains perform best in their native soil due to soil microorganism adaptability, while non-native strains may face challenges, reducing efficacy (Malusà et al. 2016; Debnath et al. 2019). Karaköy et al. (2012) reported a large variability in the landraces of Turkish lentil, particularly for Fe and Zn content. These values were frequently observed in most of the landraces, which supports our findings for the control group in all four soil conditions regarding Fe and Zn.

5 Conclusions

The study introduced a novel consortium of rhizospheric bacteria with *Rhizobium*, demonstrating superior performance in promoting lentil growth and nutrient acquisition across diverse soil systems. Notable traits such as enhanced P-solubilization, siderophore production, and micronutrient solubilization abilities were observed in selected strains. Consortia involving *Rhizobium* with *Bacillus cabrialesii*, *Pseudomonas synxantha*, and *Azotobacter salinestris* significantly improved germination time, root nodulation, plant growth, yield, and nutrient content in lentil seeds compared to individual *Rhizobium* treatments and different fertilizer treatments across all soil systems. These findings suggest the potential of the identified consortium as a universal biofertilizer solution for sustainable agriculture.

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Data Availability Data will be provided by corresponding author as per request.

Declarations

Competing interests No potential conflict of interest was reported by the author(s).

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