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Isolation and functional analysis of indigenous *Rhizobium* spp. from common bean root nodules

Tehmina Zafar[1], Nasir Rahim[1], Asma Imran[2], Muawya A. Alasasfa[3], Omar Mahmoud Al Zoubi[4], Majid Mahmood Tahir[1], Afshan Majeed[1], Aqeel Ahmad[5], Raees Ahmed[6*], Nilay Kayin[7]

[1] Department of Soil and Environmental Sciences, Faculty of Agriculture, University of Poonch Rawalakot, Rawalakot 12350, Pakistan
[2] Department of Soil and Environmental Biotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad 38040, Pakistan
[3] Department of Plant Production, Faculty of Agriculture, Mutah University, Karak, Jordan
[4] Department of Biology, College of Science in Yanbu, Taibah University, Yanbu Governorate, Saudi Arabia
[5] Department of Agronomy, Faculty of Agriculture, University of Poonch Rawalakot, Rawalakot 12350, Pakistan
[6] Department of Plant Pathology, Faculty of Agriculture, University of Poonch Rawalakot, Rawalakot 12350, Pakistan
[7] Graduate Education Institute, Bilecik Şeyh Edebali University, Türkiye
* Corresponding author's e-mail: raees@upr.edu.pk

ABSTRACT

In the Himalayan region, the identification and isolation of native rhizobia strains for promoting symbiotic nitrogen (N) fixation in common beans have remained unexplored owing to the rough terrain of the region. To bridge the research gaps, this research was designed to determine physico-chemical characteristics of soil samples collected from 27 sites situated at different altitudes (707–2134 m), grow common bean plants in pots filled with the collected soils, and subsequently isolate and characterize indigenous Rhizobium strains from common bean nodules. The bacterial populations were assessed using the most probable number (MPN) technique, which ranged from 1.0 × 10^6–8.0 × 10^5 cells per gram of soil. Out of 27 isolates tested for biochemical traits, twelve isolates exhibited N fixation potential, 24 isolates were positive for indole-3-acetic acid, and 8 isolates displayed zinc mobilization potential. Moreover, 23 and 21 isolates were positive for combined carbon medium source (CCM) and acid production, respectively, while 6 bacterial isolates were found capable of producing alkali. Interestingly, catalase activity was exhibited by 22 isolates, and only 4 isolates effectively solubilized the insoluble phosphate, indicating the significant need to inoculate common beans for boosting grain yield and nutritional value. Thereafter, common bean plants sown in collected soils under a controlled environment revealed that the shoot length varied from 28.6 cm to 48.5 cm, while root length ranged from 6.85 to 22.4 cm. Similarly, the weight of fresh and dry shoots ranged between 4.59–11.5 g and 1.45–4.69 g $plant^{-1}$, respectively, while the fresh and dry weight of roots varied between 0.57–1.60 g and 0.27–0.93 g per plant. For developing biofertilizer, isolates (BR3, BR10, BR18, BR20, BR25, BR30, and BR31) may be recommended for high salt tolerance, while BR1, BR8, BR10, BR17, and BR20 stand out in terms of tolerance to varying pH levels owing to superior genetics and better physiological and ecological adaptations.

Keywords: *Phaseolus vulgaris* L., rhizobium isolation, nodulation, nitrogen fixation, biofertilizer.

INTRODUCTION

Globally, common beans (*Phaseolus vulgaris* L.) hold the greatest economic value among legumes by contributing 85% to the total global production of beans. It has become a major cultivated pulse crop across the sub-Himalayan region of Azad Jammu and Kashmir (AJK), Pakistan, where it holds dietary significance in the local cuisines of millions of people (Ali et al., 2020). Recently, it has gained popularity, and its demand is on the rise for high nutritional value

(protein, fiber, vitamins, and essential minerals (Ali et al., 2020; Guan and Bai, 2024). Moreover, several functional properties (for lowering cholesterol, blood glucose levels, and anti-aging effects) of common beans have led to a pronounced increment in consumer demand (Shandily and Sharma 2017). Although it is widely cultivated in the region, local demand could not be fulfilled owing to significantly lower yield (0.5–5 tons per hectare) (Ali et al., 2020). Particularly, suboptimal yields have been recorded in the districts of Neelum, Bagh, Poonch, etc., which have seriously compromised the food and nutritional security (Ali et al., 2020). One of the prime reasons for the lower productivity of common beans has been identified as the deficiency of nitrogen (N) in mountainous soils due to extensive leaching with torrential rainfall spells (Marschner and Petra, 2012; Ali et al., 2020).

Although mineral N fertilizers are being applied to supply N, promoting biological nitrogen fixation (BNF) in leguminous crops offers an economical, sustainable, and eco-friendlier management practice (Abbas et al., 2024; Iqbal et al., 2024). Additionally, the use of chemical fertilizers has been linked to environmental pollution in the form of severe soil degradation, eutrophication, and emission of greenhouse gases (GHGs) (Sarfraz et al., 2023). Moreover, loss of N fertilizers as nitrate occurs in the soil through leaching, which poses serious ecological risks (Kizilgeci et al., 2021; Yildirim et al., 2022). To increase common bean yield in the sub-Himalayan region, the development of biofertilizers, specifically Rhizobium inoculants, can potentially be achieved through a sustainable increment in bioavailable N build-up in the soils (Akdeniz et al., 2019; Ali et al., 2019). Inoculating legumes with native Rhizobium has remained effective in boosting the growth and grain yield of several field crops (Iqbal et al., 2017). It has been inferred that Rhizobia tend to develop symbiotic associations with the roots of legumes through nodulation, leading to N fixation through the BNF process (Meena, 2023). The quantity of N fixed through the BNF process depends on symbiotic efficiency, which is determined by the genetic diversity of the rhizobia population and their N-fixing capability, rhizobia-host compatibility, and their pedo-environmental adaptability (Chen et al., 2024). However, these findings are limited in scope owing to remarkably different pedo-climatic conditions of the sub-Himalayan region, and the absence of common beans-specific research warrants conducting site-specific studies in this region.

Besides N fixation potential, Rhizobium strains offer many other benefits, such as plant growth-promoting attributes including biosynthesis of indole-3-acetic acid (IAA), siderophore secretion, solubilization of phosphate, and mobilization of zinc (Martens and Grosser, 2018). Additionally, these can metabolize a wide range of carbon and N sources and produce enzymes like catalase, urease, and protease, aiding in nutrient cycling and biotic and abiotic stress mitigation (Dincă et al., 2020). Likewise, their ability to tolerate extreme pH, salinity, and temperature, along with resistance to heavy metals, enhances their survival in diverse environments and potential use in bioremediation in the rough terrain of the Himalayas (Nawaz et al., 2020). Moreover, it has been reported that Rhizobium contributes 50–70% of global BNF, accounting for over 100–180 million metric tons annually. Furthermore, legume-Rhizobium symbiosis tends to boost the fertility of soil but also reduces dependence on synthetic fertilizers, thereby mitigating environmental depletion (Burris and Roberts, 2016). Therefore, it has become vital to study, identify, and screen native rhizobia strains for promoting BNF and to compensate for N losses caused by soil erosion, nutrient depletion, and severe run-off due to steep topography (Shah, 2021).

Thus, there is a pressing need to identify and utilize region-specific native rhizobia that can function effectively in soils having varying pH and salinity levels as biofertilizers under local agro-meteorological conditions. Encouraging smallholder farmers to use Rhizobium inoculants for common beans has remained neglected owing to the absence of region-specific studies in the sub-Himalayas. Thus, it was hypothesized that soil physico-chemical traits vary with altitude along with rhizobia population, whereas growing legumes like common beans may lead to the recognition of local rhizobia strains capable of performing the biological nitrogen fixation process owing to their tolerance to different saline levels and pH values. Therefore, the current study was performed to evaluate the potential of native Rhizobium species from unexplored soils of the Himalayan region (Poonch Division of AJK), aiming to isolate and characterize rhizobia strains from bean root nodules for anticipated use as biofertilizers.

MATERIALS AND METHODS

Site characteristics, soil sampling, and physico-chemical analyses

The research was carried out in the Poonch region of AJK, Pakistan. The AJK lies in the north of Pakistan and spans a geographical area of 13,297 sq km, located between 33–34° N latitude and 73–74° E longitude (Figure 1). The region is predominantly mountainous, with significant climatic variability. The annual rainfall varies between 1000 mm and 2000 mm, average maximum temperatures range from 20–32 °C, and minimal temperatures vary from 4–7 °C (Akbar, 2017). Soil samples were collected from 27 cultivated fields from the study locality (across four districts of Poonch division, including Poonch, Bagh, Haveli, and Sudhnoti). Site selection was based on recorded meteorological data using a portable weather station (Kestrel 5500, USA). At each site, composite soil samples were taken from a 0–15 cm depth of soil and stored in sterile polythene bags. Therafter, samples were shifted to the Laboratory and refrigerated at 4 °C until further analysis for determining the physicochemical properties, microbial enumeration, and bacterial isolation. The collected soil samples from each location were analyzed to determine their texture, organic matter content, pH, EC, total nitrogen, and rhizobial population using the most probable number (MPN), by the following standard method. Soil texture was determined by the Bouyoucos hydrometer method. Soil pH was examined by the method described by McLean et al. (1982), while electrical conductivity EC was assessed by saturated paste extract 1:2 (w/v) (Bohn et al., 1985), and organic matter content was measured through the Walkly and Black method (Nelson and Sommers, 1981). Moreover, total nitrogen was estimated by following the Kjeldahl method (Bremner and Mulvaney, 1982), and the bacterial population in the soil samples was estimated using the serial dilution technique (Somasegaran and Hoben, 1994).

Isolation and purification of bacterial isolates

Plastic pots (5 kg capacity) were filled with the collected soils, and common bean seeds were sown. At the flowering stage, root nodules were harvested, surface sterilized with 2.5% sodium hypochlorite for 2–3 minutes, followed by 70% ethanol, and rinsed with sterile distilled water. Sterilized nodules were crushed in the presence of a few drops of sterile water, and the suspension was streaked onto Yeast Extract Mannitol Agar (YEMA) plates containing Congo red (Nelson and Child, 1981). Samples were incubated at 28 ± 2 °C for 3–5 days. Distinct colonies were selected and sub-cultured to obtain pure isolates, which were preserved for further analysis (Vincent, 1970). During the screening process of collected rhizobia strains through morphological and biochemical means, a small number of isolates exhibited characteristics

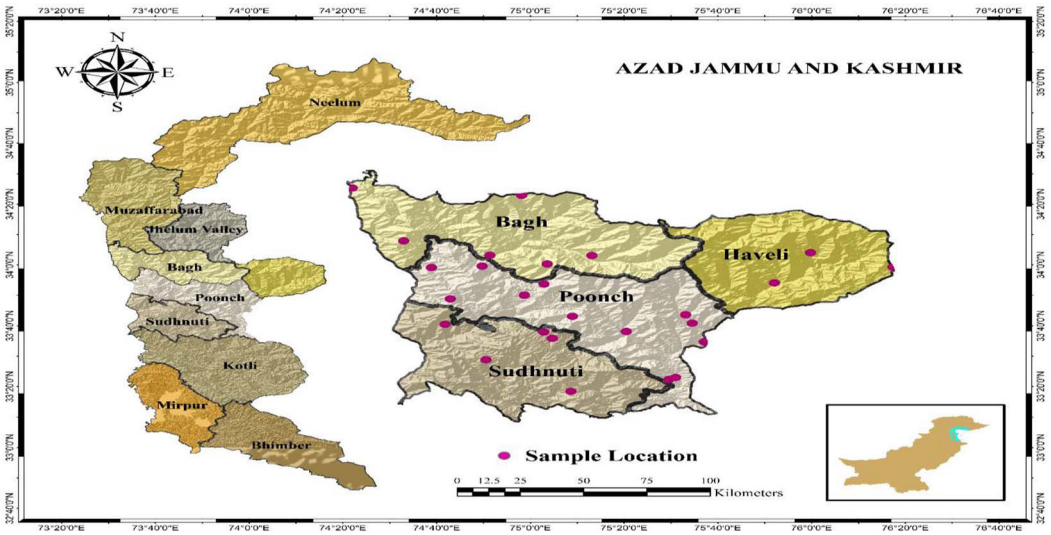

Figure 1. The geographical coordinates of the study locality (Himalayan regions of Azad Jammu and Kashmir, Pakistan), and sampling sites are represented by pink points on the right side

inconsistent with Rhizobium spp, which were excluded from further analysis, because the focus of the study was only Rhizobium isolates from common bean nodules.

Biochemical characterization, phosphate solubilization, and indole-3-acetic acid production

Colony morphology (color, shape, size, margin, and elevation) was observed visually and recorded. Cell morphology and Gram's reaction were examined by the Gram staining procedure using a light microscope under 100X magnification (Somasegaran and Hoben, 1985). Pikovskaya's agar containing tricalcium phosphate (TCP) was spot inoculated with a single colony and incubated at 28 ± 2 °C for 10 days. Halo zones were recorded, and the solubilization index (SI) was calculated (Edi-premono et al., 1996; Pikovskaia, 1948). For quantitative analysis, isolates were grown in Pikovskaya's broth at 28 ± 2 °C for 10 days. Cultures were centrifuged (12,000 rpm, 10 min), and the supernatant was analyzed for soluble P using the phosphomolybdate blue method at 882 nm (Halder et al., 1990). Bacterial isolates were grown in Okon's malate medium for IAA detection and quantification (Okon et al., 1977). Tryptophan (100 mg/L) was supplemented as the precursor of IAA. After one week of incubation, a qualitative assessment of IAA was carried out using the spot test method. Quantitative estimation of IAA was performed using the ethyl acetate oxidation method, followed by HPLC analysis with Turbochrom software (Bric, 1991).

Zinc mobilization acid/alkali, catalase production, and growth of the combined carbon medium

To assess zinc solubilization efficiency, 20 µL of bacterial suspensions (104 CFU ml^{-1}) were spotted on the center of carbon-free mineral salts medium (MSM) containing insoluble ZnO and incubated at 28±2 °C. Colony sizes and the diameters of the halo zones around the bacteria were recorded in three replicates, and the zinc solubilizing index (ZSI) was calculated by following the procedure suggested by Saravanan et al. (2007). Isolates were streaked on YEMA plates with 1.5 mL/100 mL bromothymol blue (BTB). Color change after 24–48 hours at 28 ± 2 °C to determine acid (yellow) or alkali (blue) production (Somasegaran and Hoben, 1994). Likewise, a bacterial smear on a slide was treated with a drop of 3% hydrogen peroxide (H_2O_2). Bubble formation was used as confirmation of catalase activity (Prince, 2009). Moreover, isolates were streaked on combined carbon-source medium (CCM) and incubated at 28 ± 2 °C for 3–5 days. Growth was recorded to assess utilization of combined carbon sources (Madigan et al., 2018; Rodriguez Caceres, 1982).

Nitrogen fixation activity, pH, thermal, and salt tolerance

Bacterial isolates (20 µL) were introduced into semisolid nitrogen-free malate (NFM) medium in Eppendorf tubes. The color changed from green to blue, and pellicle formation within 5-10 days indicated nitrogen fixation (Amin and Latif, 2013). Likewise, growth of isolates was assessed on YEMA plates adjusted to pH levels ranging from 4.0 to 12.0 described by Patel et al. (2020). Plates were kept at 28 ± 2 °C, and growth was observed for estimating pH tolerance. Moreover, thermal adaptability was tested by incubating isolates on YEMA at different temperatures (37 °C, 45 °C, 50 °C, and 55 °C) by using the method of Patel et al. (2020), and growth response was recorded. To assess halotolerance, isolates were streaked on YEMA plates supplemented with NaCl at concentrations of 2%, 3%, 4%, and 6% (w/v), thereafter, growth was observed after incubation (Cardoso et al., 2017).

Assessment of plant infectivity

To evaluate host specificity and the symbiotic effectiveness of the isolates, a pot experiment was performed in a greenhouse environment. Common bean seeds were surface sterilized using 2.5% sodium hypochlorite for 2–3 minutes and were grown in plastic pots. No fertilizer was applied in pots. After germination, 1 mL of inoculum of each bacterial isolate was inoculated into the plant roots. An N-free Hoagland solution was given to each pot. The non-inoculant treatment was not kept because the aim of the trial was to comparatively evaluate the performance of isolates employed to inoculate common bean seeds. Plants were harvested after 45 days of germination, and data were recorded for nodulation and growth parameters such as

nodule numbers, fresh and dry weight of nodules, shoot length, fresh and dry weight of shoot, root length, fresh and dry weight of root (Winaro and Lie, 1979). The trial had three replications of each treatment, and it was repeated twice to validate the recorded findings.

Statistical analysis

The data collected from glasshouse trials (three replications and the trial repeated twice) were subjected to analysis of variance (one-way ANOVA) using a completely randomized design (CRD) to estimate the overall significance among treatments. Thereafter, mean values of treatments were compared using the honest significant difference (HSD) test at a 5% probability level.

RESULTS

Site characteristics, soil physico-chemical properties, and rhizobia population

Samples of soil were taken from different locations across the sub-Himalayan regions of Poonch Division of Azad Jammu and Kashmir, Pakistan, situated at different altitudes, with a minimum of 707 m at Azad Pattan and a maximum of 2134 m at Sudhan Gali. At the time of sampling, soil temperatures ranged from 15 °C to 30 °C, and relative humidity varied from 34.2% (Sudhan Gali) to 83% (Azad Pattan). These variations reflect the diverse agroecological conditions of the region and highlight the importance of site-specific microbial characterization. The physicochemical analysis of soil samples revealed substantial variation across the 27 sampling sites (Table 1). The soils were classified into various textural classes, including loam, silt loam, silty clay loam, and sandy loam, with loam being the most prevalent, occurring at 12 of the sites. Soil pH ranged from 6.32 (slightly acidic) to 8.31 (slightly alkaline), with most values falling within the neutral range of 6.5 to 7.5. Organic matter (OM) content varied considerably, with the highest range recorded in Banjosa (2.38%) and the lowest in Azad Pattan (0.58%). Total nitrogen content remained generally low across the sites. The maximum N was found at Sudhan Gali (0.19%), while the minimum was observed at Azad Pattan (0.01%), indicating a potential nitrogen limitation for crop production, especially in low-N sites. Electrical conductivity (EC) values across all locations were low, indicating non-saline conditions. The highest EC value was recorded at Arja (0.093 dS/m) and the lowest at Harighal (0.012 dS/m) (Table 1). Moreover, the recorded findings revealed that rhizobia population varied significantly between sites, ranging from 5.4×10^6 to 8.0×10^5 CFU g^{-1}, while the lowest count was observed at Abbaspur (1.0×10^6 CFU g^{-1}) (Table 1).

Colony and cell morphology of rhizobia isolates

The bacterial isolates obtained from the different sampling sites showed considerable variations in their colony (colony size, shape, color, margin, motility, and Gram reaction) and cell morphology (Table 2). White-color colonies were dominant across 12 isolates, while pink and yellowish color colonies appeared in five and two bacterial isolates, respectively, while red and creamy white colonies were recorded for 2 isolates each (Table 3). Most of the bacterial isolates (18) exhibited regular colony margins, while 6 isolates showed irregular margins. The bacterial isolates (27 in total) exhibited varying degrees of motility in terms of highly motile (12), moderately motile (5), and less motile (9). The presence of both Gram-positive (23) and Gram-negative (4) bacteria across sampling sites was confirmed through Gram staining.

Indole-3-acetic acid (IAA) secretion by bacterial isolates

Out of all 27 collected isolates, 24 were tested positive for IAA production in the qualitative spot test (Table 4). A qualitative analysis of indole-3-acetic acid production by bacterial isolates enriched with L-tryptophan is presented. Quantitatively, 10 isolates produced indole-acetic acid (IAA) in concentrations ranging from 51.6-89.6 µg/mL in tryptophan-amended media. Overall, the results revealed that isolates BR1, BR3, BR10, BR18, BR30, and BR31 were the most efficient producers.

Nitrogen fixing potential, growth in combined carbon medium, acid and alkali production

The N fixation analysis showed that among the 27 bacterial isolates, 12 isolates were capable of fixing N, while the remaining isolates were negative in terms of their N-fixing potential (Table 3).

Table 1. The physicochemical characteristics of soil and rhizobia population in soil samples collected from 27 sites in the sub-Himalayan region

Sr. No	Sampling sites	Soil textural class	pH	S.0. M. (%)	Total N (%)	Soil Ece	MPN/ CFU g^{-1} soil
1	Palandri	Silt loam	7. 50	1.16	0.02	0.087	5.0×10^4
2	Gora	Silty clay loam	7.21	0.70	0.15	0.081	4.0×10^6
3	Trarkhel	Loam	7.41	1.41	0.05	0.041	4.0×10^5
4	Bloach	Loam	7.75	0.90	0.04	0.075	5.0×10^5
5	Azad pattan	Silt loam	7.78	0.58	0.02	0.016	2.0×10^5
7	Abbaspur	Sandy loam	6.91	0.87	0.07	0.036	1.0×10^6
8	Barigali	Loam	6.93	1.46	0.03	0.054	7.0×10^6
9	Chattra	Loam	7.53	1.14	0.04	0.023	5.1×10^6
10	Thandikasi	Silty clay loam	7.31	1.13	0.08	0.048	7.0×10^3
11	Hajira city	Silt loam	7.11	0.77	0.07	0.081	2.0×10^3
12	Mandole	Loam	6.81	1.24	0.14	0.027	3.0×10^6
14	Tetrinote	Loam	6.96	1.11	0.02	0.057	6.0×10^6
15	Rawalakot	Loam	7.28	2.12	0.11	0.063	7.0×10^3
16	Paniola	Silt loam	7.83	1.54	0.06	0.046	5.0×10^6
17	Tain	Silt loam	8.02	0.98	0.09	0.033	6.0×10^4
18	Thorar	Loam	7.93	1.17	0.03	0.018	3.0×10^4
19	Banjosa	Sandy loam	6.51	2.38	0.17	0.029	5.0×10^5
20	Khurshdabad	Loam	6.86	1.10	0.05	0.081	5.2×10^5
21	Kohalla	Loam	7.57	1.39	0.04	0.032	5.4×10^6
22	Dirkot	Silty clay loam	7.22	0.94	0.07	0.064	4.0×10^3
23	Arja	Silt loam	7.66	1.23	0.18	0.093	3.0×10^4
25	Harighal	Sandy loam	7.49	1.13	0.10	0.012	5.0×10^6
26	Bagh	Silt loam	7.69	0.99	0.16	0.029	5.0×10^3
27	Sudhan Gali	Silt loam	7.12	2.21	0.19	0.084	4.3×10^5
29	Upper Gagrar	Loam	6.94	0.89	0.05	0.019	5.0×10^2
30	Kalamola	Silt loam	6.79	1.60	0.16	0.071	8.0×10^5
31	Kahuta	Loam	7.30	1.49	0.08	0.021	7.0×10^6

Note: ECe= electrical conductivity of saturation extract; SOM= soil organic matter; CFU; colony formation unit; N%=total nitrogen.

Likewise, bacterial isolates were assessed for their ability to utilize different carbon sources based on their growth in CCM medium. As per recorded findings, twenty-three isolates showed growth, suggesting robust metabolic flexibility and high adaptability in carbon-rich environments such as the rhizosphere. Acid and alkali production was determined using BTB-supplemented YEM agar. Twenty-one isolates produced acids, leading to a color change indicative of pH reduction, while 6 isolates produced alkaline compounds (Table 3).

Phosphate and zinc solubilization, and catalase activity

The biochemical characterization of 27 rhizobia isolates collected from various locations across Poonch Division, Azad Jammu and Kashmir, revealed considerable functional diversity in terms of phosphorus (P) and zinc (Zn) solubilization. The P solubilization was evaluated using both qualitative and quantitative methods. In the qualitative analysis, only 4 isolates formed distinct halo zones on Pokrovsky's agar, indicating the ability to solubilize inorganic phosphate. Among these, BR15 and BR20 showed the highest phosphorus solubility index (SI) (Table 3). In the quantitative assay, phosphate solubilization ranged from 1.12-21.5 µg/mL, with BR15 (21.5 µg/mL) and BR20 (19.2 µg/mL) again showing the highest solubilizing potential (Table 3). Moreover, twelve of the 27 isolates demonstrated the ability to mobilize zinc (Zn) from insoluble ZnO (Table 3), as evidenced by halo zone formation

Table 2. Morphological characterization of rhizobia isolates collected from soil samples in the sub-Himalayan region of Azad jammu and Kashmir, Pakistan

Colony morphology							Cell morphology	
Sampling Sites	Isolates	Size	Shape	Colony color	Texture	Growth	Margin	Motality
Palandri	BR1	Large	Rod	White	Gummy	Fast	Irregular	Motile
Gora	BR2	Large	Rod	Milky white	Gummy	Fast	Regular	Highly motile
Trarkhel	BR3	Mesdium	Rod	White	Gummy	Fast	Regular	Motile
Bloach	BR4	Small	Round	Off-white	Gummy	Fast	Regular	Highly motile
Azad pattan	BR5	Large	Rod	White	Gummy	Fast	Irregular	Less motile
Abbaspur	BR7	Medium	Rod	Redish	Gummy	Slow	Regular	Highly Motile
Barigali	BR8	Small	Rod	Pinkish	Gummy	Fast	Irregular	Highly Motile
Chattra	BR9	Small	Round	White	Gummy	Fast	Regular	Motile
Thandikasi	BR10	Large	Rod	White	Gummy	Fast	Regular	Highly motile
Hajira city	BR11	Large	Round	Yellowish	Gummy	Slow	Irregular	Motile
Mandole	BR12	Large	Rod	Pinkish	Gummy	Fast	Regular	Less motile
Tetrinote	BR14	Medium	Rod	Off-white	Gummy	Fast	Regular	Less motile
Rawalakot	BR15	Small	Rod	Creamy	Gummy	Fast	Regular	Motile
Paniola	BR16	Large	Round	White	Gummy	Fast	Regular	Motile
Tain	BR17	Large	Rod	Pinkish	Gummy	Fast	Regular	Less motile
Thorar	BR18	Large	Rod	White	Gummy	Fast	Regular	Less motile
Banjosa	BR19	Small	Rod	Yellowish	Gummy	Fast	Regular	Highly motile
Khurshdabad	BR20	Large	Rod	Off-white	Gummy	Slow	Irregular	Highly motile
Kohalla	BR21	Large	Rod	Redish	Gummy	Fast	Regular	Highly motile
Dirkot	BR22	Medium	Round	Creamy	Gummy	Fast	Regular	Motile
Arja	BR23	Large	Rod	Pinkish	Gummy	Fast	Irregular	Less motile
Harighal	BR25	Medium	Rod	White	Gummy	Fast	Regular	Highly motile
Bagh	BR26	Medium	Round	Off-White	Gummy	Fast	Regular	Motile
Sudhan Gali	BR27	Large	Rod	White	Gummy	Fast	Regular	Motile
Upper Gagral	BR29	Medium	Round	Pinkish	Gummy	Fast	Regular	Motile
Kalamola	BR30	Large	Rod	Milky-white	Gummy	Fast	Irregular	Highly motile
Kahuta	BR31	Medium	Rod	White	Gummy	Fast	Regular	Highly motile

on MSM agar. The highest Zn Solubilizing Index (ZSI) was recorded for five isolates, suggesting that these strains possess efficient mechanisms for Zn mobilization. Table 3 also depicts the presence of catalase activity, indicating oxidative stress tolerance, that was observed in 22 isolates, and the other five isolates were negative as far as catalase production was concerned.

pH and salt tolerance

The results of this study revealed significant variability among the isolates in terms of pH, temperature, and salt tolerance (Tables 4 and 5). Among all, isolate BR7 demonstrated the widest pH tolerance (pH 4.0-7.0), indicating its strong adaptability to acidic to neutral soils. Other isolates, such as BR1, BR2, BR3, BR5, BR10, BR18, and BR31, exhibited good growth within pH 5.0–7.0, suggesting their potential for slightly acidic to neutral conditions. Isolates including BR4, BR8, BR12, BR17, and BR20 showed tolerance in slightly alkaline ranges (pH 6.0–8.0). A few isolates, such as BR10, BR14, BR17, and BR20, exhibited broader pH adaptability (pH 5.0–8.0), making them suitable for a wider range of soil types. Moderately tolerant isolates like BR22, BR23, and BR25 performed well in the pH range of 5.0–8.0. Moreover, the salt tolerance of the isolates was evaluated at increasing NaCl concentrations of 2%, 3%, 4%, and 6%, to assess their resilience in saline soils (Table 6). All isolates demonstrated growth at 2% NaCl, indicating broad adaptability to moderate salinity levels. However, tolerance decreased with rising salinity. At 4% NaCl, only seven isolates,

Table 3. Biochemical characterization of rhizobia isolates separated from soil samples collected from study localities situated at different altitudes in the sub-Himalayan region

Isolates	P- solubility index (cm)	Zn-solubility index (cm)	IAA (ug/mL)	NFM analysis	CCM analysis	Acid/Alkali production on BTB	Catalase activity	P-quantification (ug/mL)	IAA- quantification (ug/mL)
BR1	–	0.7	++	++	+	+	–	1.53	74.3
BR2	–	2.4	+	–	+	+	+	1.97	51.6
BR3	–	1.4	++++	+	+	+	–	1.83	88.2
BR4	–	–	+	+	+	+	+	2.25	27.0
BR5	–	1.1	+++	–	+	+	+	1.33	76.0
BR7	0.3	–	–	–	+	+	+	1.65	11.2
BR8	–	–	++	++	+	–	+	2.23	66.7
BR9	–	–	++	–	+	+	+	1.77	29.3
BR10	–	2.0	+++	+	+	+	+	1.30	71.7
BR11	0.7	–	++	–	–	–	+	16.7	31.0
BR12	–	–	+	+	+	+	+	2.31	20.4
BR14	–	–	++	+	+	–	+	1.15	29.8
BR15	2.0	2.3	–	–	–	+	+	19.2	10.1
BR16	–	–	+	–	+	+	+	3.12	33.0
BR17	–	–	+	–	+	–	–	1.36	39.1
BR18	–	–	++++	–	+	+	+	1.67	89.6
BR19	–	–	++	–	+	+	+	1.71	43.5
BR20	2.4	2.6	+	+	–	+	+	21.5	38.6
BR21	–	–	+	–	+	+	+	2.20	15.3
BR22	–	–	++	–	+	–	+	1.90	57.3
BR23	–	–	+	–	–	+	+	2.62	22.6
BR25	–	–	+	+	+	+	+	1.43	28.0
BR26	–	–	–	–	+	+	–	1.34	10.6
BR27	–	–	++	+	+	+	+	1.58	47.6
BR29	–	0.9	+	–	+	–	–	2.61	24.2
BR30	–	–	+++	+	+	+	+++	1.24	68.3
BR31	–	–	++++	+	+	+	++	2.53	73.4

Note: The symbol, +, represents the positive reaction or the presence of the traits, while the symbol, -, shows the negative reaction or absence of traits.

BR3, BR10, BR18, BR20, BR25, BR30, and BR31, retained robust growth.

Nodulation efficiency and plant growth characteristics

In the current study, a significant difference was recorded in nodule number and the fresh and dry weights of root nodules across different sites (Tables 6 and 7). The nodules per plant varied from a minimum (13) at the Harighal site to a maximum (36) at the Khurshdabad site. Among 27 sites obtained from various locations of Poonch division, Azad Jammu and Kashmir, 17 soils showed several nodules higher than 20, 6 soils had 13–20 nodules, while 3 soils showed the absence of nodulation. The observed variability in nodulation parameters across different sites indicates the important role of environmental and microbial factors in shaping the symbiotic association between common bean (Phaseolus vulgaris L.) and native rhizobia in the study locality. Moreover, variation in the growth performance, like root growth of common bean, concerning location. Growth traits exhibited significant differences across the various locations. The shoot length varied from 28.6 cm to 48.5 cm, while root length ranged from 6.85 to 22.4 cm. Similarly, the weight of fresh and dry shoots ranged between 4.59–11.5 g and 1.45–4.69 g plant-1, respectively. Fresh and dry weight of roots varied between 0.57–1.60 g and 0.27–0.93 g per plant.

Table 4. Response of rhizobia isolates to varying pH levels

Isolates	4.0	4.5	5.0	6.0	7.0	8.0	Isolates	4.0	4.5	5.0	6.0	7.0	8.0
BR1	–	–	+	+	+	+	BR16	–	–	–	+	+	–
BR2	–	–	+	+	+	–	BR17	–	–	+	+	+	+
BR3	–	–	+	+	+	–	BR18	–	–	+	+	+	–
BR4	–	–	–	+	+	+	BR19	–	+	+	–	+	–
BR5	–	–	+	+	+	–	BR20	–	+	+	+	+	+
BR7	+	+	+	+	+	–	BR21	–	–	+	+	–	–
BR8	–	–	+	+	+	+	BR22	–	–	+	+	–	–
BR9	–	–	–	–	+	+	BR23	–	+	+	–	+	–
BR10	–	–	+	+	+	+	BR25	–	–	–	+	+	–
BR11	–	+	+	–	–	–	BR26	–	–	–	+	+	+
BR12	–	–	–	+	+	+	BR27	–	–	–	+	+	+
BR14	–	+	+	–	–	+	BR29	+	+	+	–	+	–
BR15	–	+	+	–	+	–	BR30	–	–	–	+	+	–
----------							BR31	–	–	+	+	+	–

Note: *All the isolates were also checked for growth, pH up to 12, but no growth was found from pH 9–12. Positive reactions are marked with a + sign, and negative reactions with a – sign.

Table 5. Response of rhizobia isolates to varying salt concentrations

Isolates	Salt Tolerance at (2% NaCl)	Salt Tolerance at (3% NaCl)	Salt tolerance at (4% NaCl)	Salt tolerance at (6% NaCl)	Isolates	Salt Tolerance at (2% NaC)	Salt Tolerance at (3% NaCl)	Salt Tolerance at (4% NaCl)	Salt tolerance at (6% NaCl)
BR1	+	–	–	–	BR17	+	–	–	–
BR2	+	–	–	–	BR18	+	+	+	–
BR3	+	+	+	–	BR19	+	–	–	–
BR4	+	–	–	–	BR20	+	+	+	–
BR5	+	+	–	–	BR21	+	–	–	–
BR7	+	–	–	–	BR22	+	+	–	–
BR8	+	–	–	–	BR23	+	–	–	–
BR9	+	+	–	–	BR25	+	–	+	–
BR10	+	+	+	–	BR26	_	–	–	–
BR11	+	–	–	–	BR27	+	+	–	–
BR12	+	–	–	–	BR29	+	–	–	–
BR14	+	+	–	–	BR30	+	+	+	–
BR15	+	+	–	–	BR31	+	+	+	–
BR16	+	–	–	–	----------				

Note: *All the isolates were also checked for growth at 2-6% NaCl concentrations, but no growth was found 6% salt concentration. Positive reactions are marked with +, and negative reactions with a – symbol.

DISCUSSION

The present research was conducted to examine the potential of native *Rhizobium* species from unexplored soils of the sub-Himalayan region (Poonch Division, AJK, Pakistan), aiming to isolate and characterize rhizobacterial strains from common bean root nodules for their anticipated utilization as biofertilizers. The physicochemical analysis of soil samples showed significant variation across the sampling sites. From the recorded findings of the soil pH (6.5–7.5), it might be inferred that the soil pH has been favorable for the activity of microorganisms and plant growth. In addition, the stability of pH values across most of the sites indicates well-buffered soils, which are

Table 6. Assessment of nodulation potential and growth traits (nodule number, fresh and dry weight, shoot fresh and dry weight) of common bean grown under greenhouse conditions using soils collected from different locations in the sub-Himalayan region

Sampling sites	Nodulation	Number of nodules plant^{-1}	Fresh weight of Nodules (mg plant^{-1})	Dry weight of nodules (mg plant^{-1})	Shoot length (cm)	Fresh weight of shoot (g plant^{-1})
Palandri	+	25.7cd	223.0g	99.2c	38.5cd	6.24c
Gora	+	20.6e	211.7h	78.6de	30.7de	8.67b
Trarkhel	+	29.4b	327.5d	113.2a	35.4d	8.33b
Bloach	+	21.5e	218.0g	76.3e	28.6e	5.80c
Azad-pattan	+	18.9f	206.1i	70.4f	33.8de	6.70c
Abbaspur	–	–	–	–	31.0e	4.96d
Barigali	+	23.1d	219.8g	94.1cd	40.3c	9. 20b
Chattra	+	17.2f	202.1i	70.8f	46.1ab	11.2a
Thandikasi	+	24.1d	221.6g	98.0c	40.9c	7.30c
Hajira	–	–	–	–	35.0d	6.89c
Mandole	+	15.9g	189.0j	70.1f	30.2de	7.18c
Tetrinote	+	27.3c	315.6e	108.5b	45.9ab	9.48b
Rawalakot	+	29.9b	331.2d	103.9bc	38.0cd	5.66cd
Paniola	+	21.8e	217.5g	73.3ef	33.2d	7.55c
Tain	+	13.8h	180.4jk	93.6cd	43.6b	10.3ab
Thorar	+	26.0c	230.7f	73.0ef	28.9e	4.99d
Banjosa	+	31.7b	340.1c	103.1bc	40.5c	9.87b
Khurshdabad	+	36.0a	347.5b	112.0a	29.8de	5.10d
Kohalla	+	26.8c	310.6ef	103.2bc	46.5ab	11.5a
Dirkot	+	17.5g	212.8h	83.4d	34.4d	6.89c
Arja	+	20.9e	209.4h	79.8de	39.7c	8.33c
Harighal	+	13.0h	175.2k	80.9de	44.9b	9.50b
Bagh	–	–	–	–	–	6.68c
Sudhan gali	+	18.4f	203.8i	83.0d	29.3de	4.59d
Uppergagral	+	30.6b	324.0d	100.3c	48.5a	10.7ab
Kalamola	+	35.0ab	351.4a	110.3ab	44.0b	8.26b
Kahuta	+	29.8b	318.2e	99.6c	47.3a	5.67cd
CV values		6.861	4.209	9.301	8.886	5.658

advantageous for maintaining consistent growing conditions over time (Fageria et al., 2011; Strawn et al., 2015). These findings underline the necessity of selecting rhizobia isolates based on soil pH to optimize symbiotic effectiveness and ensure successful legume-rhizobium interactions in diverse agroecological zones (Brady and Weil, 2017). Likewise, OM content varied considerably (0.58–2.38%) in the soil samples collected from study sites situated at different altitudes, and these findings are in concurrence with those of Havlin et al. (2014), who inferred that soils richer in OM had better structure, higher water-holding capacity, and enhanced nutrient availability. Contrastingly, soils with low OM restricted microbial proliferation and reduced crop productivity. In addition, in our study, the lowest EC levels suggest that the soils are well-suited for a wide range of crops, including legumes like common beans, as high salinity can hinder plant nutrient uptake (Rengasamy, 2010). Interestingly, the total N content across the sites remained generally low, and it may be attributed to one of the primary limiting factors for plant growth in the region. Low levels of N necessitate inoculation of legume seeds with native rhizobia strains to promote biological N fixation, which can assist crop plants in achieving a greater part of their N requirement.

In this study, the microbial population varied greatly among all sites. These results indicate a

Table 7. Assessment of nodulation potential and growth traits (shoot dry weight, root length, root fresh and dry weight) of common bean grown under greenhouse conditions using soils collected from different locations in the sub-Himalayan region

Sampling sites	Nodulation	Dry weight of shoot (g plant-1)	Root length (cm)	Fresh weight of root (g plant-1)	Dry weight of root (g plant-1)
Palandri	+	0	7.48g	1.27bc	0.72d
Gora	+	3.11b	10.1ef	0.89e	0.56g
Trarkhel	+	4.30ab	13.4de	1.17cd	0.80cd
Bloach	+	2.16d	9.22f	1.30bc	0.78cd
Azad-pattan	+	3.13b	15.8d	1.43b	0.50fg
Abbaspur	–	1.87d	6.85h	1.35b	0.63e
Barigali	+	4.10ab	8.62fg	1.16c	0.84c
Chattra	+	4.29ab	7.90g	0.78f	0.27j
Thandikasi	+	3.28b	16.2c	1.22c	0.90a
Hajira	–	2.64c	13.9de	1.14d	0.58f
Mandole	+	2.33cd	17.3bc	1.39b	0.49g
Tetrinote	+	4.36ab	15.0d	0.88fg	0.68de
Rawalakot	+	1.86d	12.5e	0.96e	0.71d
Paniola	+	2.14d	16.9c	1.11d	0.53fg
Tain	+	2.77c	9.29f	0.70g	0.39h
Thorar	+	1.58d	14.1de	1.37b	0.69d
Banjosa	+	3.48b	21.3ab	1.40b	0.51fg
Khurshdabad	+	1.45e	18.6b	1.14d	0.93a
Kohalla	+	4.19ab	12.0e	0.69g	0.65e
Dirkot	+	2.84c	9.60f	0.57h	0.31i
Arja	+	2.39cd	16.5c	1.42b	0.82c
Harighal	+	3.38b	22.4a	1.60a	0.62e
Bagh	–	1.99d	17.0bc	1.33b	0.80cd
Sudhan gali	+	1.49de	10.8f	0.91e	0.48g
Uppergagral	+	4.69a	20.8b	1.49ab	0.70d
Kalamola	+	3.18b	23.0a	1.19d	0.87b
Kahuta	+	1.73d	17.6bc	0.99e	0.55f
CV values		7.627	9.254	6.248	5.025

substantial presence of native *Rhizobia* spp. in the mountainous soils of the sub-Himalayan region. It may be inferred that variability in microbial population was probably owing to differences in soil organic matter content, soil moisture, and pH. These factors have been positively correlated with microbial population and diversity in prior studies (Zhang, 2017). Moreover, it has been reported that soils with higher microbial populations tend to be more biologically active and better able to support nutrient cycling, which benefits soil fertility and plant productivity (Smith et al., 2024). In the current study, out of 27 isolates, 23 were negative in Gram's reaction. These findings agreed with several studies that classified most rhizobium, such as *Sinorhizobium*, *Bradyrhizobium*, and *Mesorhizobium*, as Gram-negative bacteria (Lado et al., 2019). The study revealed that rhizobia identification and biochemical characterization effectively assist in differentiating among the bacterial isolates. Additionally, the evaluation of these biochemical properties identifies rhizobia's potential as PGPR and for their use in the development of biofertilizers (Smith et al., 2024). Overall, these results underscore rhizobia's role in improving phosphorus bioavailability in soils, particularly within the pH range of 6.0–7.0, where phosphorus solubility is generally optimal (Fageria et al., 2011). The highest zinc solubilizing index (ZSI) was recorded for five bacterial isolates. Isolates with low ZSI values may be less effective in facilitating zinc uptake by plants (Saravanan

et al., 2007; Iqbal et al., 2023). Moreover, in this study, isolates including BR1, BR3, BR10, BR18, BR30, and BR31 were the most efficient in IAA production. This variation in IAA production may be due to genetic differences in IAA biosynthesis pathways, particularly in genes such as ipdC and amiE, which influence tryptophan metabolism (Labrazi, 2020), and high-IAA-producing strains are valuable for promoting root elongation and enhancing nutrient uptake. Likewise, the variability in N-fixing potential may be due to differences in the presence or expression of nitrogenase (nif) genes, which are responsible for atmospheric nitrogen reduction (Lindstorm and Mousavi, 2020).

It was recorded in our study that out of 27 rhizobia isolates, 23 had high adaptability in carbon-rich environments. Efficient carbon metabolism supports multiple PGPR traits like N fixation, exopolysaccharide production, and colonization ability (Sugawara and Sadowsky, 2014). On the other hand, 21 isolates were acid-producing. Acid production is often linked to organic acid exudation, which facilitates nutrient solubilization, especially phosphate (Sundarajan and Reddy, 2016). Furthermore, most isolates showed catalase activity in this study. Previously, it has been inferred that catalase played a key role in detoxifying hydrogen peroxide, allowing bacteria to survive under stressful environmental conditions (Imlay, 2013), and this trait enhances rhizobia persistence in diverse and sometimes hostile soil ecosystems. Temperature and salt tolerance are crucial traits for rhizobia intended for use in variable climatic conditions. It was noted that at 4% NaCl, only seven isolates (BR3, BR10, BR18, BR20, BR25, BR30, and BR31) outperformed, while BR1, BR8, BR10, BR17, and BR20 stand out in terms of tolerance to varying pH levels. It may be interpreted that these isolates had significantly higher levels of physiological adaptability (by virtue of synthesizing compatible solutes such as proline, trehalose, etc.), genetic superiority (osm genes, ion transporter genes), and outstanding symbiotic adaptations (stronger cell membrane owing to fatty acid deposition) that allowed them to perform their functions effectively under salt stress and varying pH levels, by avoiding ion toxicity, osmotic stress, membrane damage, thus these specific isolates may be the potential candidates for seed inoculation in salt-affected soils, where they can maintain symbiotic performance and support plant growth in a saline environment (Zou et al., 2021). Moreover, these strains could contribute significantly to sustainable agriculture by ensuring crop production on marginal lands through vigorous germination and robust plant growth in sub-optimal conditions (Chauhan et al., 2014).

In the current study, a significant difference was recorded in nodule number and the fresh and dry weights of root nodules of common bean grown in soils collected from different sites. The significant differences in nodule number and fresh and dry weights of nodules could be due to differences in soil texture, nutrient status, pH, organic matter, and native microbial diversity, which substantially influence nodulation efficiency (Sadiq et al., 2023). The cultivation of common beans is a cost-effective technique that substantially increases the income of farmers in the Himalayan region of Pakistan. It was observed that common bean coated with different isolates of rhizobium revealed significant variations in the root, shoot, nodulation, and overall biomass. Our results indicate that inoculation of rhizobia strains before sowing dramatically improves initial seedling growth and enhances the yield of grains. The significant variation recorded in shoot length across different sites emphasizes the influence of environmental heterogeneity, such as nutrient status, soil moisture, and genotype, and environment interactions on the growth performance of beans (Castiano et al., 2023).

CONCLUSIONS

The recorded findings were in line with the research hypothesis, as significant variation in soil physico-chemical traits was recorded with increasing altitude of the site, whereas the rhizobia population also pronouncedly differed among soil samples. This foundational study provides valuable insight into the native rhizobia diversity present in the unexplored mountainous agroecosystems of the sub-Himalayan region. A range of bacterial isolates was identified with multiple traits, including N2-fixing, P-solubilizing, IAA, Acid/Alkali production, and catalase activity. These findings establish a strong basis for further exploration. However, detailed molecular and functional characterization of the promising strains is recommended to confirm their taxonomic identity and functional genes. For developing biofertilizer, isolates (BR3, BR10, BR18, BR20, BR25, BR30, and BR31) may be recommended for high salt tolerance, while BR1, BR8, BR10, BR17, and BR20 stand out in terms of tolerance to varying pH levels. It is

suggested that these isolates demonstrated significantly higher physiological adaptability, genetic superiority, and outstanding symbiotic adaptations that assisted them in surviving under salt stress and varying pH levels by mitigating the deleterious effects of ion toxicity, osmotic stress, and membrane damage. Moreover, although phenotypic and biochemical characterization have provided valuable preliminary identification of rhizobia strains in this study, to reveal a definitive taxonomic classification of collected rhizobia strains, it is suggested that the use of molecular approaches such as 16S rRNA sequencing or multilocus sequence analysis must be performed as an important direction for future work. Furthermore, future research works must assess the effectiveness of rhizobia isolates as biofertilizers for sustainable legume production in mountainous areas of the Himalaya under abiotic stress-prone environments. It is recommended that these selected native *Rhizobia* spp. be considered for biofertilizer development, which can increase legume (common bean, soybean, cowpea, cluster bean, field peas, etc.) productivity, reduce dependence on expensive synthetic fertilizers, reduce the risk of ecological disruptions, and impart climate resilience to modern legume-based cropping systems in the Himalayas.

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REFERENCES

1. Abbas R.N., Iftikhar A., Iqbal A., Erden Z., Alqahtani M.D., Almutairi K.F., Iqbal M.A. (2024). Counteracting heat, salinity, and osmotic stresses by reconciling seed size and sowing depth for bolstering germination and seedling growth of cluster bean (*Cyamposis tetragonoloba* L. Taub.). *Polish Journal of Environmental Studies*. https://doi.org/10.15244/pjoes/195278
2. Akbar K.F. (2017). Potential impacts of climate change on plant diversity of hilly areas of Azad Kashmir and their mitigation. *Journal of Mountain Area Research, 2*, 37–44.
3. Akdeniz H., Bozkurt M.A., Hosaflioglu I., Islam M.S., Hossain A., El Sabagh M., Iqbal M.A., El Sabagh A. (2019). Yield and nutritive value of Italian ryegrass (*Lolium italicum* L.) is influenced by different levels of nitrogenous fertilizer. *Fresenius Environmental Bulletin, 28*(12), 8986–8992.
4. Ali A., Khan S.A., Ali S., Ali N., Khan S.M., Hussain I. (2020). Morphological and biochemical characterization of locally available kidney beans. *Pure and Applied Biology, 9*, 528–537.
5. Ali M., Rauf S. (2020). Soil erosion and fertility loss in the mountainous regions of Azad Jammu and Kashmir: Causes and remedial strategies. *Pakistan Journal of Agricultural Sciences, 57*, 1031–1039.
6. Ali S., Fatima A., Bukhari Q.A., Iqbal M.A., Rana M.A., Afzaal S. (2019). Effect of nitrogen fertilization on polyphenols in *Trachyspermum ammi* and *Foeniculum vulgare* under various lights interception. *Z Arznei-Gewurzpfla, 23*(2), 67–71.
7. Amin A., Latif Z. (2013). Detoxification of mercury pollutant by immobilized yeast strain Candida xylopsoci. *Pakistan Journal of Botany, 45*, 1437–1442.
8. Bohn H.L., Neal B.L.M., O'Connor G.A. (1985). *Soil Chemistry, 2nd ed.* John Wiley & Sons, New York, pp. 22169–22175.
9. Brady N.C., Weil R.R. (2017). *The Nature and Properties of Soils, 15th ed*. Pearson Education, New York.
10. Bremner J.M., Mulvaney C.S. (1982). *Nitrogen total*. In: Methods of Soil Analysis. Agronomy No. 9, Part 2: Chemical and Microbiological Properties, 2nd ed. Page A.L. (ed.). American Society of Agronomy, Madison, pp. 595–624.
11. Bric J.M., Bostock R.M., Silverstone S.E. (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. *Applied and Environmental Microbiology, 57*, 535–538.
12. Burris R.H., Roberts G.P. (2016). Biological nitrogen fixation. *Annual Review of Nutrition, 13*, 317–335.
13. Cardoso A.A., Andraus M.P., Borba T.C.O., Martin-Didonet C.C.G., Ferreira E.P.B. (2017). Characterization of rhizobia isolates obtained from nodules of wild genotypes of common bean. *Brazilian Journal of Microbiology, 48*, 43–50.
14. Castiano W., Schifino Wittmann M.T., Polyakov A., Grüdtner L. (2023). Combining ability of common bean (*Phaseolus vulgaris* L.) genotypes for root traits across diverse environments. *Plant Breeding, 142*, 368–379.
15. Chauhan P.S., Singh R., Malla M.A. (2014). Salt tolerance mechanisms of rhizobia: Impact on the legume–rhizobia symbiosis under saline conditions. *Soil Biology and Biochemistry, 76*, 112–120.
16. Chen W., Liu Y., Wang X., Zhang Y. (2024). Molecular mechanisms governing rhizobium–legume symbiosis: Signaling, infection, and nitrogen fixation. *Molecular Plant-Microbe Interactions, 37*, 120–134.
17. Clemens S. (2018). Zn and Fe biofortification: The right chemical environment for human bioavailability. *Plant Science, 225*, 52–57.

18. Dincă M., Şumălan R., Manole A. (2020). Biochemical characterization of some rhizobacterial strains with plant growth-promoting potential. *Research Journal of Agricultural Science, 52*, 16–22.
19. Edi-Premono M., Maowad A., Vleck P.L.G. (1996). Effect of phosphate solubilizing Pseudomonas purida on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science, 11*, 13–23.
20. Fageria N.K., Baligar V.C., Jones C.A. (2011). *Mineral Nutrition of Field Crops, 3rd ed*. CRC Press, Boca Raton.
21. Guan X., Bai Y. (2024). The role of common beans (*Phaseolus vulgaris* L.) in combating malnutrition and enhancing global nutrition. *Frontiers in Nutrition, 11*, 1385232.
22. Halder A.K., Mishra K.A., Bhattacharyya P., Chakrabartty K.P. (1990). Solubilization of rock phosphate by Rhizobium and Bradyrhizobium. *Journal of General and Applied Microbiology, 36*, 81–92.
23. Havlin J.L., Beaton J.D., Tisdale S.L., Nelson W.L. (2014). *Soil Fertility and Fertilizers: An Introduction to Nutrient Management*. Pearson, Upper Saddle River.
24. Imlay J.A. (2013). The molecular mechanisms and physiological consequences of oxidative stress: Lessons from a model bacterium. *Nature Reviews Microbiology, 11*, 443–454.
25. Iqbal, S., Iqbal, M.A., Li, C., Iqbal, A. and Abbas, R.N. (2023). Overviewing drought and heat stress amelioration – From plant responses to microbe-mediated mitigation. *Sustainability, 15*, 1671.
26. Iqbal A., Abbas R.N., Al Zoubi O.M., Alasasfa M.A., Rahim N., Tarikuzzaman M., Aydemir S.K., Iqbal M.A. (2024). Harnessing the mineral fertilization regimes for bolstering biomass productivity and nutritional quality of cowpea [*Vigna unguiculata* (L.) Walp]. *Journal of Ecological Engineering, 25*(7), 340–351.
27. Iqbal A., Iqbal M.A., Iqbal A., Aslam Z., Maqsood M., Ahmad Z., Farooq M.U., Abbas G., Faisal M. (2017). Boosting forage yield and quality of maize (*Zea mays* L.) with multi-species bacterial inoculation in Pakistan. *Phyton International Journal of Experimental Botany, 86*, 84–88.
28. Kizilgeci F., Yildirim M., Islam M.S., Ratnasekera D., Iqbal M.A., Sabagh A.E. (2021). Normalized difference vegetation index and chlorophyll content for precision nitrogen management in durum wheat cultivars under semi-arid conditions. *Sustainability, 13*, 3725. https://doi.org/10.3390/su13073725
29. Lado J., Puy I., Solans M., Fabra A. (2019). Plant growth-promoting potential of Bacillus strains isolated from legume nodules. *Symbiosis, 77*, 191–200.
30. Lebrazi H. 2020. Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. *Journal of Genetic Engineering and Biotechnology, 18*, 71.
31. Lindström K., Mousavi S.A. (2020). Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology, 13*, 1314–1335.
32. Madigan M.T., Bender K.S., Buckley D.H., Sattley W.M., Stahl D.A. (2018). *Brock Biology of Microorganisms, 14th ed*. Pearson, Boston, pp. 71–74.
33. Marschner H., Petra M. (2012). Marschner's mineral nutrition of higher plants. *Soil Biology and Biochemistry, 30*, 1275–1280.
34. Martens M., Grosser S. (2018). Rhizobium diversity and symbiotic effectiveness in different legume species. *Applied and Environmental Microbiology, 84*, 14–17.
35. McLean E.O. (1982). *Soil pH and lime requirement*. In: Methods of Soil Analysis: Part 2 – Chemical and Microbiological Properties, 2nd ed. Page A.L. (ed.). American Society of Agronomy and Soil Science Society of America, Madison, pp. 199–224.
36. Meena, V.K. (2023). Diverse rhizobial strains as biofertilizers for sustainable legume cultivation. *Environmental Sustainability, 6*, 987–1001.
37. Mohamed, M.H. and Abdel-Lateif, K.S. (2021). Salt tolerance mechanisms of rhizobial isolates and their potential role in enhancing legume productivity under saline conditions. *Rhizosphere, 19*, 100369.
38. Nasar, S., Shaheen, H., Murtaza, G., Tinghong, T., Arfan, M. and Idrees, M. (2023). Socioeconomic evaluation of common bean (*Phaseolus vulgaris* L.) cultivation in providing sustainable livelihood to the mountain populations of Kashmir Himalayas. *Plants, 12*, 213.
39. Nawaz, I., Muhammad, F., Ali, S. and Ali, G.M. (2020). Primary evaluation of seed characteristics of common bean landraces collected from Himalaya region of Pakistan. *Sarhad Journal of Agriculture, 36*, 33–41.
40. Nelson, D.W. and Sommers, L.E. (1982). *Total carbon, organic carbon, and organic matter*. In: Methods of Soil Analysis: Part 2 – Chemical and Microbiological Properties, 2nd ed. Page, A.L. (Ed.). American Society of Agronomy and Soil Science Society of America, Madison, pp. 539–579.
41. Nelson, L.M. and Child, J.J. (1981). Nitrogen fixation and hydrogen metabolism by Rhizobium leguminosarum isolates in pea root nodules. *Canadian Journal of Microbiology, 62*, 2279–2285.
42. Okon, Y., Albercht, S.L. and Burris, R.H. (1977). Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. *Applied and Environmental Microbiology, 22*, 85–88.

43. Patel, A.K., Banjerae, U., Kumara, A., Singh, R.K. and Pandey, K.D. (2020). Temperature and pH tolerant Rhizobium leguminosarum bv. viciae strains with plant growth promotion traits. *Journal of Pure and Applied Microbiology, 14*, 2603–2609.
44. Pikovskaia, R.I. (1948). Metabolism of phosphorus in soil connection with vital activity of some microbial species. *Mikrobiologiya, 17*, 362–370.
45. Prince, C.P. (2009). *Catalase test*. In: Practical Manual of Medical Microbiology. Jaypee Brothers Medical Publishers, New Delhi, pp. 112–113.
46. Rengasamy, P. (2010). Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology, 37*, 613–620.
47. Rodriguez Caceres, E.A. (1982). Improved medium for isolation of *Azospirillum* spp. *Applied and Environmental Microbiology, 44*, 990–991.
48. Sadiq, M., Rahim, N., Iqbal, M.A., Alqahtani, M.D., Tahir, M.M., Majeed, A. and Ahmed, R. (2023). Rhizobia inoculation supplemented with nitrogen fertilization enhances root nodulation, productivity, and nitrogen dynamics in soil and black gram (*Vigna mungo* (L.) Hepper). *Land, 12*, 1434.
49. Saravanan, V.S., Madhaiyan, M. and Thangaraju, M. (2007). Solubilization of zinc compounds by different bacterial species. *Applied Soil Ecology, 36*, 49–56.
50. Sarfraz M., Khaliq A., Tahir M.M., Iqbal M.A., Rehman Z., Shan Q. (2023). Saffron spice quality in response to integrated nutrient management under lesser Himalayan conditions of Rawalakot Azad Jammu & Kashmir. *Journal of Plant Nutrition*, https://doi.org/10.1080/01904167.2023.2254322
51. Shah, S.M. (2021). Nutrient status of soils in the hilly regions of Azad Jammu and Kashmir: A case study from Rawalakot. *Soil and Environment, 40*, 116–125.
52. Shandilya, U.K. and Sharma, A. (2017). Functional foods and their benefits. *Journal of Nutrition, Health and Food Engineering, 7*, 2–5.
53. Smith, A., Johnson, B. and Patel, R. (2024). Soil microorganisms: Their role in enhancing crop nutrition and health. *Sustainability, 16*, 345–359.
54. Somasegaran, P. and Hoben, H.J. (1994). *Handbook for Rhizobia*. Springer-Verlag, New York, p. 380.
55. Somasegaran, P. and Hoben, H.J. (1985). *Methods in Legume–Rhizobium Technology*. NifTAL Project and MIRCEN, University of Hawaii, Paia.
56. Strawn, D.G., Bohn, H.L. and O'Connor, G.A. (2015). *Soil Chemistry, 4th ed.* John Wiley & Sons, Hoboken.
57. Sugawara, M. and Sadowsky, M.J. (2014). The role of *Sinorhizobium meliloti* transport systems in carbon utilization and competitiveness. *Frontiers in Microbiology, 5*, 72.
58. Sundararajan, M. and Reddy, B. (2016). Acid production by rhizobia and its effects on nutrient uptake in legume crops. *Agricultural Microbiology, 35*, 45–52.
59. Vincent, J.M. (1970). *Manual for Practical Study of Root Nodule Bacteria*. IBP Handbook 15. Blackwell, Oxford.
60. Winaro, R. and Lie, T.A. (1979). Competition between Rhizobium strains in nodule formation, interaction between nodulating and non-nodulating strains. *Journal of Plant, 51*, 135–142.
61. Yildirim M., Kizilgeci F., Albayrak O., Iqbal M.A., Akinci C. (2022). Grain yield and nitrogen use efficiency in spring wheat (*Triticum aestivum* L.) hybrids under different nitrogen fertilization regimes. *Journal of Elemetology, 27*(3), 627–644. https://doi.org/10.5601/jelem.2022.27.3.2241
62. Zhang, H., Jiang, N., Zhang, S., Zhu, X., Wang, H., Xiu, W., Zhao, J., Liu, H., Zhang, H. and Yang, D. (2024). Soil bacterial community composition is altered more by soil nutrient availability than pH following long-term nutrient addition in a temperate steppe. *Frontiers in Microbiology, 15*, 68–74.
63. Zhang, L. (2017). Nitrogen and phosphorus pollution in the world's rivers and lakes. *Nature Communications, 8*, 1–9.
64. Zou, J., Liang, Y., Qin, L. and Zhu, T. (2021). Role of salt-tolerant rhizobia in improving the growth and nitrogen fixation of legumes under salinity stress. *Frontiers in Plant Science, 12*, 658908.