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Comparative effects of endemic plant growth-promoting rhizobacteria on morphological and nutritional characteristics of Hordeum vulgare and Avena sativa forages

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Received: 13/03/2026 Accepted: 29/09/2026 Published: 22/10/2026

Abstract

Plant–microbe interactions play a fundamental role in regulating plant growth and nutrient accumulation, thereby influencing forage yield and chemical composition. This study evaluated the effects of three PGPR strains on the growth and nutritional composition of two forage cereals, Hordeum vulgare and Avena sativa, cultivated under non-controlled field conditions. Morphological characteristics, including plant height and the leaf to stem and spike ratio, along with chemical composition, including dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (ADL), were measured at the early heading stage. In Barley, inoculation with BT1 considerably increased DM (25.34%) and OM (91.33%), whereas in oats, BT2 improved crude protein and crude fat by 9.42% and 12.4%, respectively. Fibre fractions exhibited species- and strain-dependent responses, with NDF, ADF, and ADL increasing in barley following BT2 inoculation, while the highest ADF and ADL values in oat were recorded in the control treatment. Plant height in barley increased by 82.15% under BT3, whereas the leaf-to-stem and spike ratios were generally unaffected by PGPR inoculation. In conclusion, PGPR biofertilizers can selectively improve forage productivity and nutritional value, providing a sustainable strategy for cereal forage production.

Keywords: Plant growth-promoting rhizobacteria (PGPR);, Sustainable agriculture, Barley; Oat, Nutritional composition

1. Introduction

Forage crops form the foundation of primary livestock production systems, providing the nutrients that support animal health, growth, and productivity. With the rising global demand for meat and dairy products, improving the quality and yield of forage crops has become increasingly important. Among these forages, barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) stand out for their adaptability to diverse agro-climatic conditions, high biomass potential, and valuable nutritional profiles (Forgács et al., 2025; Gul et al., 2023). To sustain high yields and improve forage quality, conventional agricultural systems have relied heavily on chemical fertilizers. However, their excessive and prolonged use has raised serious concerns about soil degradation, environmental pollution, and declining soil fertility (Carroll et al., 2023; Hossain et al., 2022). As a result, there is growing interest in sustainable and eco-friendly alternatives, among which plant growth-promoting rhizobacteria (PGPR) have emerged as promising candidates (wang et al., 2024). Plant growth-promoting rhizobacteria are a diverse group of beneficial soil bacteria that colonize the rhizosphere and enhance plant growth and development through a range of direct and indirect mechanisms (Sun et al., 2025). These mechanisms include biological nitrogen fixation, phosphate solubilisation, production or modulation of phytohormones such as indole-3-acetic acid (IAA), siderophore production, and enhancement of antioxidant defence systems (Etesami et al., 2023; Lee et al., 2023; Miljaković et al., 2020; Sabater et al., 2025). Consequently, PGPR inoculation has been associated with improvements in plant growth, biomass accumulation, nutrient acquisition, and stress tolerance in several agricultural species (Jamily, 2019; Sagar et al., 2024). Studies on maize (*Zea mays* L.) have shown that PGPR inoculation improves drought tolerance and overall plant growth (Azeem et al., 2022). In *Festuca arundinacea*, PGPR strains have been shown to improve phytoremediation efficiency in contaminated soil (Eskandary et al., 2017). In contrast, in soybeans, inoculation has been shown to enhance plant growth and nutrient accumulation (Yoshida & Teaumroong, 2026). Similar benefits have been observed in other species, including alfalfa, clover

and tomato, where PGPR provided adequate protection against pathogens (Lee et al., 2023; Rashid et al., 2021; Rosier et al., 2023). Despite the growing body of research on PGPR, their effects on forage cereals remain comparatively less characterised, particularly when responses are evaluated at specific developmental stages. Plant biomass accumulation and chemical composition change throughout development as carbon and nutrients are redistributed among plant organs (Stirling et al., 2022). Consequently, the developmental stage at which plant responses are assessed can influence the observed effects of microbial inoculation on both biomass production and forage quality. The early heading stage is of particular interest in forage cereals because it coincides with the onset of reproductive development and changes in assimilate allocation and tissue composition. Evaluating plant responses at this stage can therefore provide information on whether PGPR inoculation is associated with changes in biomass accumulation and nutritional characteristics during an important phase of forage development. Accordingly, the present study investigates the effects of PGPR inoculation on the growth and nutritional composition of barley and oat, with particular emphasis on the early heading stage. The study aims to characterise the responses of these two forage cereals to microbial inoculation and to evaluate the potential contribution of PGPR to forage productivity and nutritional quality within more sustainable crop production systems.

2. Materials and Methods

Plant growth-promoting rhizobacteria treatments

Three endemic PGPR isolates from the collection of Pr. Moumene S. (Laboratory of Aromatic and Medicinal Plants, University of Blida 1) developed under project D00L05UN09012210001, was used. Bacterial suspensions were prepared following a modified protocol described by Todorova & Kozhuharova (2010). Isolates were grown for 12–18 h at 30 °C, then diluted and adjusted to an optical density of 1×10^{8} cells·ml^{-1} for seed inoculation and irrigation.

2.1 Experimental Design and Plant Material

The field experiment was conducted at the University of Saad Dahleb's experimental station in Blida, Algeria, from 3 December 2023 to 1 April 2024. The experimental site is located in the bioclimatic sub-humid zone, with the following geographical coordinates: latitude 36°30'35" N, Longitude 2°52'26" E, and an altitude of 178 m.

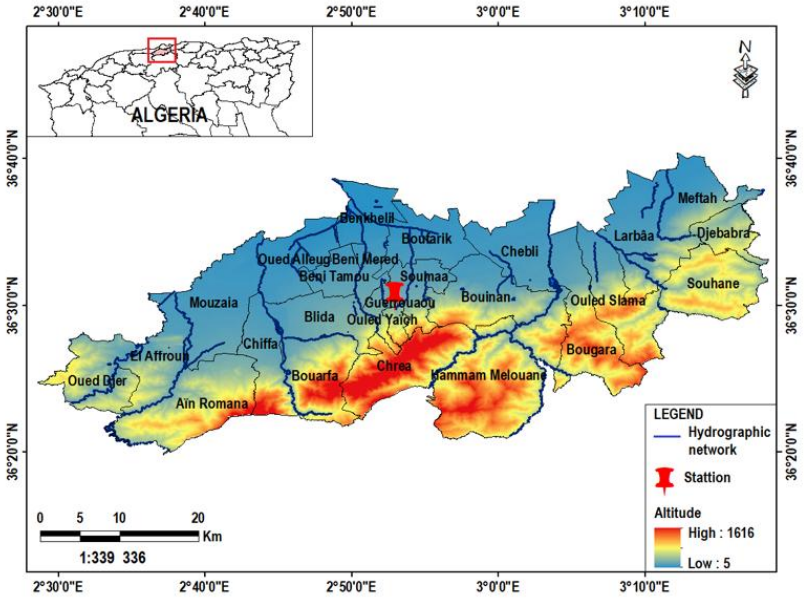


Figure 1. Geographical location of the experimental site in Blida, Algeria,

The experimental soil at the study site was classified as clay loam with a neutral pH (7.16). Its nutrient content, mineral composition, and physicochemical properties are summarized in Table 1.

Table 1. Physical and chemical properties of the experimental soil.

OM (%)	N (%)	P (ppm)	K (ppm)	Cl^{-} (meq·100g^{-1})	SO_4^{2-} (meq·100g^{-1})	Ca^{2+} (meq·100g^{-1})	CEC (meq·100g^{-1})	EC (µS/cm)	Total limestone (%)
0.64	1.3	50.68	203.5	0.56	1.72	1.2	25.45	84	6.5

Barley (*Hordeum vulgare* L var. Saida) and the Oat (*Avena sativa* L var. Gharbi) seeds underwent a surface disinfection process using 70% ethanol for 2 minutes, then sterilized with 5% sodium hypochlorite solution for 6 minutes, followed by multiple rinses with sterile distilled water to remove any residual sterilizing agent (Khenaka et al., 2019). The sterilized seeds

were primed for 30 minutes with the PGPR treatments. After the treatment, the seeds were sown in bags filled with 20 kg of non-sterilized soil and placed in an open field condition. Post-sowing, each seedling bag received 20 ml of one of the PGPR treatments (BT1, BT2, and BT3) at a concentration of 10^8 cells·ml^{-1}, whereas the control treatment received no PGPR inoculation. Weeding was done by hand when necessary, and no pesticides or herbicides were applied. The plants were irrigated during periods without precipitation. The experiment was conducted using a completely randomized design (CRD) with sixteen replicates.

2.2 Measurement of Growth and Quality Parameters

Growth parameters

The measurements were taken to assess the impact of PGPR treatments on crop performance, focusing on plant height and the leaf-to-stem and spike ratio. The leaf-to-stem and spike ratios were calculated by separating and weighing 100 g of fresh biomass for each treatment, distinguishing between leaves, stems, and spikes, and estimating their proportions. The height of the plants was measured in centimeters from the base to the tip of the farthest leaf using a measuring tape.

Evaluation of Nutritive Value

Fresh plant samples at the early heading stage were first oven-dried at 60 °C for 36 hours to improve preservation. After drying, the samples were finely ground (1 mm) and then stored hermetically to determine the chemical composition. The nutritional value of both *H. vulgare* L. and *A. sativa* L. was determined using the AOAC (1990) method. To determine the dry matter (DM) content, a portion of the fresh samples was chopped and dried at 105 °C for 24 hours (2 g for each sample). The organic matter (OM) was assessed by ashing the dried samples first at 200 °C for 1 h and 30 min, followed by combustion at 500 °C for 2 h and 30 min, as depicted in equation 1. Crude protein (CP) was determined by the Kjeldahl method. In contrast, the ether extract (EE) was determined using the Soxhlet extraction method. Fiber fractions, including neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), were determined using the fiber bag technique according to the Ankom technology method, following the methods described by Van Soest et al. (1991) and Robertson and Van Soest (1981), respectively. Heat-stable amylase and sodium sulfite were used in the NDF procedure. For ADL determination, the ADF residues were treated with 72% sulfuric acid for 3 h, then thoroughly rinsed with hot water, dried at 105 °C, and finally ashed at 600°C for 2 h to quantify lignin content. The nutritional components of the samples were determined in triplicate.

(1) Organic matter (% DM) =100 – Ash content (% DM).

Statistical Analysis

All statistical analyses were performed using R software. Analysis of variance (ANOVA) was applied with species and treatment as fixed factors, followed by Tukey's (HSD) test at $p < 0.05$. Principal Component Analysis (PCA) was conducted on the standardized dataset. Hierarchical Cluster Analysis (HCA) was performed to classify samples based on their nutritional characteristics and evaluate similarities among treatments and species.

3. Results and Discussion

Understanding plant responses to biofertilizer applications, particularly those involving plant growth-promoting rhizobacteria, requires a comprehensive evaluation of morphological and nutritional parameters. Plant Growth Promoting-rhizobacteria are well known for enhancing plant growth through mechanisms such as nutrient solubilization, biological nitrogen fixation, production of phytohormones such as indole-3-acetic acid (IAA) and gibberellins, synthesis of siderophores that improve iron uptake, induction of systemic resistance against pathogens, and modulation of stress-related metabolic pathways to improve tolerance to abiotic stresses (Sabater et al., 2025; Saxena et al., 2020). In our study, PGPR inoculation positively influenced plant height, with distinct patterns observed across the two forage species and the different plant growth-promoting rhizobacteria treatments, as shown in Figure 2. Both the type of treatment and the plant species influenced height, but the manner in which treatments affected one species did not depend on the other ($p > 0.05$). *Avena sativa* showed a clear response to the microbial treatments, especially under BT2, where the average height reached 72.32±1.85 cm, followed by BT1 (69.94±4.07 cm) and BT3 (69.76±5.58 cm), compared to 60.83±2.84 cm in the control. In contrast, *H. vulgare* displayed relatively stable heights across all treatments, with slight variation. The mean plant height was 77.23±8.10 cm in the control, 79.40±4.24 cm under BT1,78.70±4.71 cm under BT2, and 82.15±5.12 cm under BT3. These closely related values suggest that microbial treatments had a limited impact on *H. vulgare* height, which maintained a relatively consistent growth pattern across treatments. This growth-promoting effect may be linked to the modulation of plant hormone signal transduction pathways, as previously demonstrated by Yuan et al. (2025), where inoculation with PGPR strain altered the expression of key genes involved in hormonal signaling pathways, such as indole-3-acetic acid (IAA) and cytokinin, with upregulation of *AUX/IAA* genes suggesting enhanced auxin activity and increased plant height. The observed increase in *H. vulgare* height, particularly with BT3, reflects the well-documented role of these beneficial microorganisms.

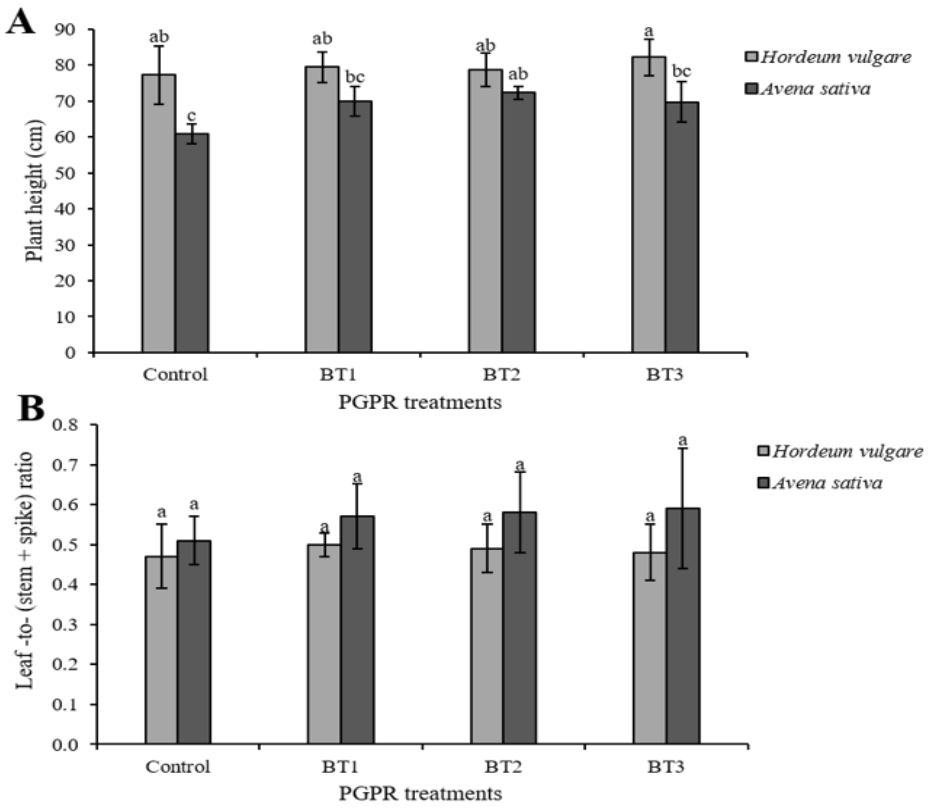


Figure 2. Effects of different Plant growth-promoting rhizobacteria on *Hordeum vulgare* and *Avena sativa* plants (A) Plant height, (B) Leaf-to-stem and spike ratio. The graph represents mean values, with error bars indicating standard deviation. Tukey's test indicates that values followed by the same letter are not significantly different ($p < 0.05$)

For instance, Çağlar et al. (2023) demonstrated that inoculation with PGPR significantly increased the height of *H. vulgare* compared to uninoculated controls. Likewise, Gupta et al. (2024) observed increased shoot length in *A. sativa* inoculated with PGPR under drought stress. As for the leaf-to-stem and spike ratio, the differences were mainly driven by species rather than by the treatments. *Avena sativa* consistently showed higher values across all treatments, ranging from 0.51±0.06 (control) to 0.57±0.08 (BT1) and 0.58±0.10 (BT2), peaking at 0.59±0.15 under BT3, highlighting its greater allocation to foliar biomass. Meanwhile, *H. vulgare* maintained lower and more uniform ratios, 0.47±0.08 (control), 0.48±0.07 (BT3), 0.49±0.06 (BT2), and 0.5±0.03 (BT1). These trends suggest that the observed variation is primarily due to the two species' inherent morphological traits rather than the microbial treatments. The leaf-to-stem and spike ratio provided additional insight into biomass distribution. Oat plants generally exhibited a higher ratio, indicating a greater allocation of biomass to leaf tissue relative to structural and reproductive parts. In contrast, barley showed a lower ratio, possibly because it tends to prioritize the development of spikes and thicker stems. Although inoculation-induced variations in the leaf-to-stem and spike ratio, these differences did not amount to a distinct treatment effect. Comparable findings have been reported in other crops, such as the inoculation of *Mentha arvensis* with *Brevibacterium halotolerans* and the application of PGPR in combination with recommended fertilizer doses in maize (Rundan et al., 2021; Singh et al., 2019). Both showed no detectable influence on the leaf-to-stem ratio, despite improvements being observed in other growth parameters.

3.1 Nutritional value

In addition to enhancements in morphological parameters, PGPR treatments also improved nutritional quality, as reflected by increased dry and organic matter contents, higher crude protein and ether extract levels, and reduced cell wall concentrations. Plant growth-promoting rhizobacteria affected dry matter concentrations differently across treatments and plant species ($p<0.001$ for both factors) as presented in Table 2. In *Hordeum vulgare*, BT1 application yielded the highest dry matter content (25.33%), exceeding that of all other treatments. By contrast, the lowest value was observed in BT2 (19.94%). For *Avena sativa*, dry matter also peaked under BT1 (25.49%), but remained close to the control level. Values under BT3 and BT2 were lower, but the decline was less pronounced than in barley. These observations indicate that while both species benefited most from BT1, *H. vulgare* exhibited a wider range of responses across treatments, whereas *A. sativa* maintained more stable dry matter levels. These trends suggest that oats responded more favorably overall to PGPR inoculation than barley. The presence of a treatment × plant interaction implies that the overall pattern of responses was consistent among species. The enhancement in dry matter, particularly under BT1, indicates improved assimilate accumulation and resource-use efficiency. While dry matter is often used as a proxy for total feed mass, its biological significance lies in its reflection of carbon fixation and partitioning dynamics. Several studies have reported that inoculation with a specific PGPR strain increases dry matter production, supporting the growth-promoting potential of specific microbial metabolites under both field and controlled conditions (Ferioun et al., 2024; Kálmán et al., 2024; Khurshid et al., 2025). However, in our study, not all the

treatments were able to achieve higher dry matter, which may be linked to suboptimal compatibility between the microbial inoculant and the plant host, as reported in a previous study by Huang et al. (2018), where they found that applied *Bacillus mycoides* strain produced growth-promoting volatiles such as 2,3-butanediol and 3-hydroxy-2-butanone, which were absent in other strains of the same species. This supports the idea that intraspecific variability in the biosynthesis of bioactive compounds can significantly affect plant quality, likely through interactions with phytohormonal signaling or by modulating carbon allocation strategies (Kai et al., 2020). Therefore, the superior performance of BT1 on dry matter in barley and oats could reflect its unique metabolite profile. Plant growth-promoting rhizobacteria can enhance root system architecture and water and nutrient uptake, thereby promoting photosynthetic activity and increasing carbon fixation, leading to enhanced production of dry and organic matter (Radhakrishnan et al., 2017; Samaniego-Gámez et al., 2016). In this study, the organic matter content was influenced by plant growth-promoting rhizobacteria treatment, with a statistically confirmed interaction between the two factors, indicating that the response to microbial inoculation varied depending on the plant. In barley, the highest organic matter concentration was observed under BT1 (91.33% DM), followed by BT2 (90.82% DM) and the control (90.75% DM). while the lowest organic matter was recorded under BT3 (90.39% DM), suggesting a higher ash content accumulation. *Avena sativa*, on the other hand, showed a different trend. The highest organic matter value was observed under BT3 (91.01% DM), exceeding that of the control group. This divergent response underscores the species-specific nature of PGPR effects on forage quality. Organic matter in plant tissues, which reflects the sum of energy-yielding compounds such as carbohydrates, was enhanced under PGPR treatments for both species. Plant organic matter directly contributes to ruminal fermentation, microbial growth, and the production of volatile fatty acids (Dijkstra, 1994). Higher organic matter indicates improved metabolic richness of the forage, which supports energy supply for maintenance and production in ruminants. Hett et al. (2022) observed similar organic matter enrichment in PGPR-treated wheat, noting that the ash content was higher in the control group, which they attributed to synergistic microbial effects on both root function and shoot composition. Consistent with our findings, Pyati et al. (2025) observed that PGPR application increased the organic matter content of oats. This compositional gain is functionally integrated with the observed increases in crude protein, which were most prominent under BT2 in *Avena sativa* and BT1 in *Hordeum vulgare*. Among the evaluated parameters, crude protein content showed a clear and consistent increase in response to the treatment, regardless of plant species. The treatment effect was statistically supported (p=0.001), indicating that the biofertilizers enhanced nitrogen assimilation and protein biosynthesis in both forage crops. A species and interaction effect was also observed (p=0.007; p=0.03), suggesting no subtle differences in how each plant responded to the microbial inputs. For barley, all treatments increased crude protein content relative to the control. The most notable increase was observed under BT1(12.96% DM), followed by BT3 (12.65% DM) and BT2 (12.44% DM). Moreover, the highest crude protein content in oat was recorded in BT2 (13.48% DM), which outperformed the other treatments, whereas the control group showed the lowest value (12.32% DM). Although the range of increase was more modest than in barley, the data point to a stronger response to BT2 in oat, implying that the efficacy of PGPR formulations may vary with species-specific physiological pathways.

Table 2. Effect of PGPR treatments on major chemical components (% DM) in cereal plants.

Treatments	Plant	DM	OM	CP	EE
Control	*Hordeum vulgare*	21.73±0.65^{ab}	90.75±0.29^{a}	11.26±0.41^{b}	3.4±0.16^{b}
	Avena sativa	25.15±0.93^{a}	90.17±0.63^{a}	12.32±0.24^{a}	4.02±0.35^{a}
BT1	*Hordeum vulgare*	25.34±0.70^{a}	91.33±0.27^{b}	12.96±0.52^{a}	3.55±0.50^{b}
	Avena sativa	25.49±0.21^{a}	90.71±0.24^{a}	12.78±0.29^{a}	4.25±0.10^{a}
BT2	*Hordeum vulgare*	19.94±0.14^{b}	90.82±0.34^{a}	12.44±0.88^{a}	3.98±0.46^{b}
	Avena sativa	22.01±0.42^{ab}	90.22±0.37^{a}	13.48±0.15^{a}	4.52±0.13^{a}
BT3	*Hordeum vulgare*	20.15±3.54^{b}	90.3±0.14^{a}	12.65±0.53^{a}	3.98±0.27^{b}
	Avena sativa	24.31±0.58^{a}	91.01±0.13^{a}	13.02±0.12^{a}	4.13±0.1^{a}
p value					
T		<0.001	0.04	0.001	0.04
P		<0.001	0.06	0.007	<0.001
T*P		0.01	0.02	0.03	0.03

Means ±SD are shown in the table; each value is the mean of three replicates. Tukey's test indicates that values followed by the same letter are not significantly different ($p < 0.05$). DM: dry matter, OM: organic matter, CP: crude protein, EE: Ether extract. T: PGPR treatments, P: plant, T×P: interaction of PGPR treatments and plants.

Protein content is a critical determinant of forage quality, affecting not only rumen microbial growth but also the supply of amino acids to the host animal. Plant growth-promoting rhizobacteria contribute to nitrogen enrichment in plants via nitrogen fixation, increased nitrate reductase activity, and enhanced assimilation pathways (Singh et al., 2021). Furthermore, some PGPR can fix nitrogen and produce pigments, such as melanin, which protect nitrogenase enzymes from oxygen, allowing continuous nitrogen fixation even under aerobic soil conditions (Aasfar et al., 2021). Pandey et al. (2018) reported that inoculation with selected PGPR strains positively influenced crude protein accumulation in the grains of *Amaranthus hypochondriacus*. Similarly, subsequent research demonstrated that PGPR inoculation increased nitrogen content in chickpea plants, accompanied by a corresponding increase in crude protein concentration (Khan et al., 2021). Collectively, these findings indicate that PGPR may enhance nitrogen acquisition and assimilation, thereby contributing to improved protein accumulation and, consequently, the nutritional quality of plant tissues. Beyond that, ether extract also contributes significantly to the nutritional value of forage, particularly in terms of energy supply for ruminants. Although cereals such as *H. vulgare* and *A. sativa* are not primarily lipid-rich, microbially induced increases in fat content may be particularly relevant for ruminant nutrition, given the role of dietary lipids in improving overall feed efficiency and animal performance (Palmonari et al., 2024). Lipids are metabolically indispensable as structural components of membranes, precursors of signaling molecules, and energy reserves (Suh et al., 2022). In the present study, PGPR treatments augmented EE content in both barley and oats plants. This rise is likely mediated by microbial regulation of key enzymes in the acetyl-CoA-dependent fatty acid biosynthesis pathway, particularly in plastids, where acetyl-CoA carboxylase catalyzes the committed step of converting acetyl-CoA to malonyl-CoA (Ye et al., 2020). Ameliorated nutrient acquisition and auxin secretion by PGPR may stimulate photosynthetic efficiency and carbon fixation, leading to greater availability of triose phosphates and, subsequently, glyceraldehyde-3-phosphate, a critical precursor for both glycolysis and lipid biosynthesis (Fierli et al., 2022; Roudier et al., 2010). A study by Bai et al. (2024) on peanut inoculated with the *Tsukamurella tyrosinosolvens* P9 strain demonstrated significant upregulation of lipid metabolism-related genes and phospholipid biosynthesis, highlighting the role of PGPR in modulating fatty acid biosynthetic and signaling pathways. Furthermore, improved nitrogen availability, facilitated by rhizospheric bacteria in the treatments, can shift the carbon-nitrogen balance, favoring carbon partitioning towards lipid biosynthesis when the protein demands are relatively met (Winichayakul & Roberts, 2025). This concept is supported by studies on high-lipid crops, in which optimized N allocation enables greater carbon to be diverted to lipid storage without compromising photosynthetic efficiency or growth (Beechey-Gradwell et al., 2019; Mengesha, 2021). The accumulation of higher lipid content in A*vena sativa* under BT2 (4.52% DM) compared to control (4.02% DM) represents a 12.44% increase, whereas in *Hordeum vulgare*, BT2 and BT3 treatments both resulted in 3.98% DM, corresponding to a greater increase of 17.06% over the control and suggesting convergence in lipid biosynthetic capacity upon microbial stimulation. These interspecific differences likely reflect species-specific metabolic plasticity and responsiveness to microbial metabolites such as volatiles, which are known to enhance fatty acid synthesis via redox signaling (Baslam et al., 2021). Supporting this hypothesis, several studies have reported that inoculation with beneficial bacteria can enhance EE accumulation in cereals. For example, *Rhizobium* increased EE accumulation in *A. sativa* (Baidalin et al., 2024), while He and Zhang (2022) reported a similar effect in black barley following PGPR application. Consistent with these findings, our results indicate that PGPR treatments induced noticeable variations in the cell wall components of *H. vulgare* and *A. sativa*, as reflected by the changes in NDF, ADF, and ADL. Overall, significant effects were observed for the factors treatment (T) and plant species (P), as well as their interaction ($p < 0.05$). These results highlight that the response of fiber fractions to bacterial inoculation is not only treatment-dependent but also species-specific.

Table 3. Effect of PGPR treatments on cell wall components (% DM) in cereal plants

Treatments	Plant	NDF	ADF	ADL
Control	*Hordeum vulgare*	59.75±1.25^{a}	32.77±0.65^{ab}	2.12±0.13^{b}
	Avena sativa	58.4±1.4^{a}	34.88±0.96^{a}	3.96±0.3^{a}
BT1	*Hordeum vulgare*	58.72±0.78^{a}	31.53±0.58^{b}	2.04 ±0.22^{b}
	Avena sativa	58.73±1.00^{a}	34.64±0.97^{a}	3.42±0.02^{a}
BT2	*Hordeum vulgare*	60.62±0.06^{a}	33.95±0.35^{ab}	2.77±0.13^{c}
	Avena sativa	59.73±0.48^{a}	34.81±0.59^{a}	3.83±0.26^{a}
BT3	*Hordeum vulgare*	59.42±0.86^{a}	32.31±0.16^{b}	2.12±0.1^{b}
	Avena sativa	56.17±0.74^{b}	32.34±0.6^{b}	3.34±0.2^{ac}
p value				
T		0.003	<0.001	<0.001

P		0.002	<0.001	< 0.001
T*P		0.04	0.007	0.003

Means ±SD are shown in the table; each value is the mean of three replicates. Tukey's test indicates that values followed by the same letter are not significantly different ($p < 0.05$). NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, ADL: Acid Detergent Lignin, CF: crude fiber. T: PGPR treatments, P: plant, T×P: interaction of PGPR treatments and plants

For NDF, values ranged from 56.17% in *Avena* sativa under BT3 to 60.62% in *Hordeum vulgare* under BT2. These increases suggest greater accumulation of structural carbohydrates, such as cellulose, hemicellulose, and lignin, which constitute the main components of the plant cell wall. This observation is consistent with that of Utamy et al. (2025), who reported that inoculation with plant growth-promoting rhizobacteria can enhance plant cell wall biosynthesis in corn fodder. However, a higher NDF content, while associated with stronger plant tissues and increased resistance to stress, may also reduce forage digestibility due to a higher proportion of indigestible components (Xu et al., 2023). Acid detergent fiber content also varied significantly among treatments. The lowest ADF value (31.53% DM) was obtained in *H. vulgare* under BT1, while the highest (34.88% DM) occurred in the control of *A. sativa*. The general reduction of ADF content in inoculated plants compared with their respective controls suggests that PGPR inoculation may improve fiber digestibility by reducing the proportion of less digestible cell wall components, such as cellulose and lignin-associated polysaccharides. According to Singh et al. (2022), beneficial rhizobacteria can modulate plant enzymatic activities involved in cell wall deposition, leading to more flexible tissues with lower lignocellulosic density. In contrast, the moderate increases observed in barley BT2-treated plants may result from enhanced lignification, potentially linked to bacterial induction of defense-related pathways, such as phenylpropanoid metabolism, which contribute to lignin precursor formation (Yadav et al., 2023). Comparable findings have been reported in studies where PGPR inoculation combined with organic nutrient amendments produced variable effects on ADF content across different forage species, including berseem, cowpea, maize, and oats, with some outcomes depending on the plant species and specific plant microbe interactions (Onte et al., 2025; Pyati et al., 2025). Regarding ADL, *Avena sativa* generally exhibited higher values (3.96% in the control and 3.83% in BT2) than *Hordeum vulgare* (ranging from 2.04% to 2.77%). This reflects species-specific differences in lignin biosynthesis and deposition. Lignin is a key determinant of forage digestibility, and its accumulation is often inversely related to nutrient availability and degradability (Stypinski et al., 2024). The higher ADL in *Hordeum vulgare* treated with BT2 could indicate that treatment modulates lignification, potentially by influencing the expression of genes involved in monolignol biosynthesis. Similar findings were reported by Pyati et al. (2025), who observed that PGPR inoculation increased ADL content in *Avena sativa*, suggesting a potential microbial role in regulating lignin metabolism. The higher ADL levels observed in some treated plants may be associated with enhanced lignification and secondary cell-wall development, consistent with the observations reported by Divyanshu et al. (2022). In contrast, the lower ADF and ADL concentrations observed under other PGPR treatments were associated with a more favourable fibre profile, suggesting a potential improvement in forage quality. Overall, these findings indicate that the effects of PGPR on forage fibre characteristics were treatment-dependent, with some inoculants producing more favourable changes in structural fibre fractions than others.

3.2 Principal Component Analysis

The principal component analysis showed that the first two axes explained 75.4% of the total variance, with Dim1 accounting for 45.7% and Dim2 for 29.7%, indicating that the factorial plane provides a reliable and meaningful representation of the relationships among variables and samples.

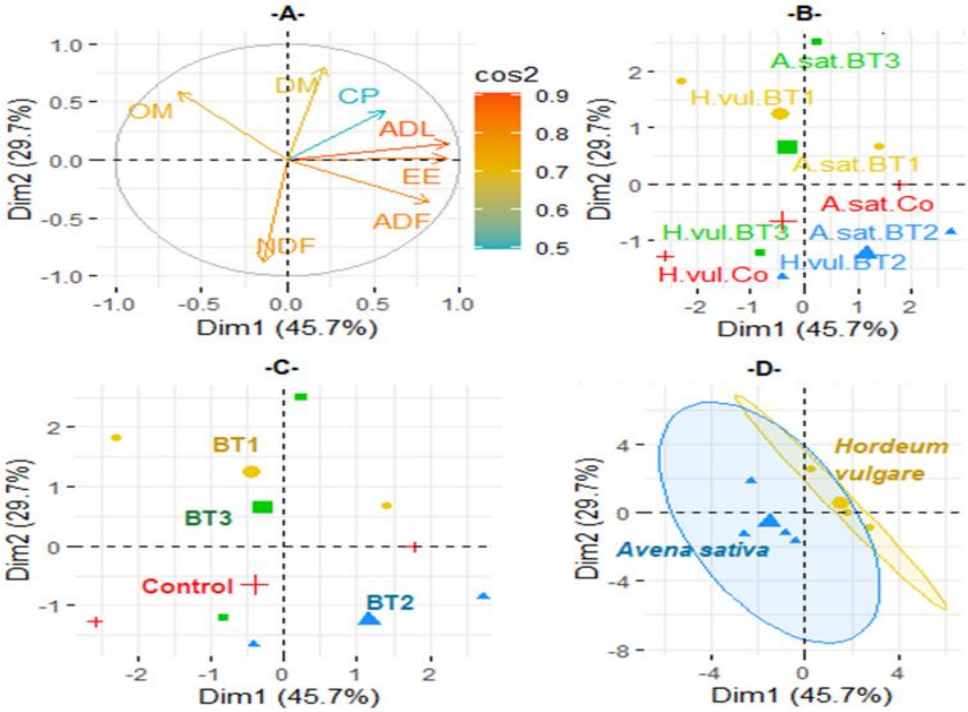


Figure 3. Principal component analysis (PCA) of the standardized dataset: (A) Correlation circle of variables; (B) Factorial map (Dim1 vs Dim2) showing the distribution of samples according to treatment and species; (C) Factorial map (Dim1 vs Dim2) illustrating treatment effects; (D) Biplot (Dim1 vs Dim2) highlighting species differentiation.

The correlation circle (Fig.3. A) highlights the structure of variable relationships. Dim1 is positively associated with CP (r= 0.56; cos^2= 0.31), EE (r= 0.93; cos2= 0.86), ADL (r= 0.94; cos^2= 0.88), and ADF (r= 0.83; cos2= 0.68), indicating that this axis represents a gradient of biochemical richness and structural fiber components. In contrast, OM is negatively correlated with Dim1 (r = −0.63; cos^2 = 0.40), suggesting an inverse relationship between organic matter content and the accumulation of lignocellulosic and lipid compounds. This opposition reflects differences in biomass composition and possibly plants maturity or metabolic allocation. The second axis (Dim2) is primarily defined by dry matter (DM), which shows a strong positive correlation (r = 0.80; cos^2 = 0.64), along with a moderate contribution from OM (r = 0.58; cos^2 = 0.34). In contrast, NDF is strongly negatively correlated with Dim2 (r = −0.87; cos^2 = 0.77), indicating a clear opposition between dry matter accumulation and cell wall components. This suggests that higher DM is associated with improved biomass consolidation and reduced structural constraints, which may enhance forage digestibility.

The projection of samples (Fig.3. B and C) reveals a clear separation according to treatments. Samples associated with BT2 are located in the positive region of Dim1. They are strongly linked to CP, EE, ADL, and ADF, indicating that this treatment promotes the accumulation of protein and structural compounds. Conversely, control samples are positioned on the negative side of Dim1 and are associated with OM, reflecting a distinct metabolic profile characterized by lower structural complexity. Along Dim2, treatments BT1 and BT3 are mainly distributed in the positive region, showing a stronger association with DM and OM, which indicates improved biomass production and organic matter accumulation under these treatments. In contrast, control samples are more closely associated with NDF, suggesting a higher proportion of fibrous cell wall components and potentially lower digestibility.

The species discrimination (Fig.3. D) is particularly evident. *Hordeum vulgare* samples cluster in the positive regions of both Dim1 and Dim2, reflecting higher levels of CP, EE, ADL, ADF, and DM, which indicates a profile characterized by both nutritional richness and structural development. In contrast, *Avena sativa* samples are mainly distributed in the negative regions of both axes. They are associated with higher OM and NDF, suggesting a composition richer in fibrous components and less metabolically concentrated tissues. The separation ellipses further confirm a clear distinction between species, indicating that species identity is a major source of variability, likely due to inherent physiological and metabolic differences affecting nutrient partitioning and cell wall composition.

3.3 Hierarchical Cluster Analysis

The distribution of samples in the factorial space highlights the formation of three distinct clusters, reflecting both species effect and treatment influence (Figure 4). The first cluster (green ellipse) groups *Avena sativa* samples (*A. sativa* Co, *A. sativa* BT1, and *A. sativa* BT2), all located on the positive side of Dim1. The proximity of these points indicates a high degree of intra-species homogeneity, suggesting that the nutritional composition of *Avena sativa* is relatively stable across treatments. This clustering implies that, for this species, treatment effects are less discriminant than species-specific traits and that its biochemical profile remains consistent regardless of PGPR application. In contrast, the second cluster (black ellipse)

includes *Hordeum vulgare* samples (*H. vulgare* Co, *H. vulgare* BT2, and *H. vulgare* BT3), positioned in the negative regions of both Dim1 and Dim2.

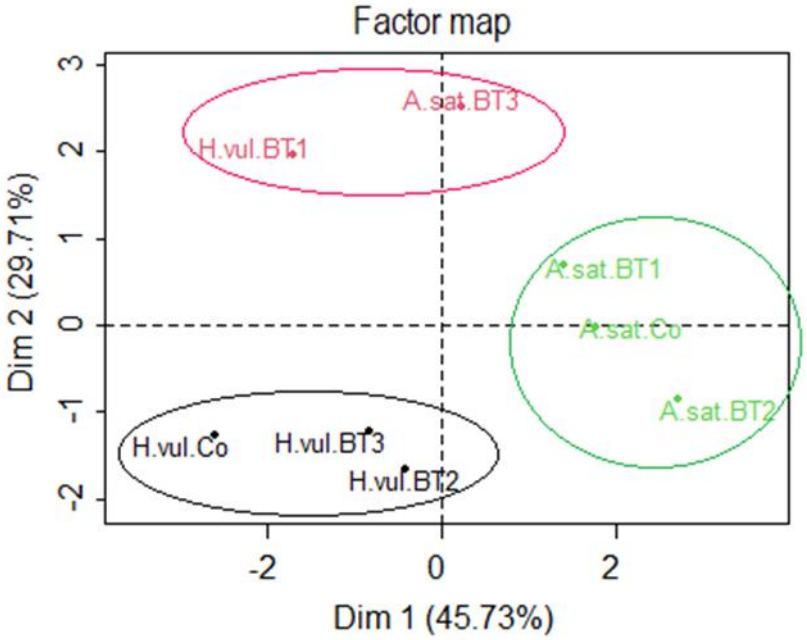


Figure 4. Factorial map derived from hierarchical cluster analysis (HCA) of treatments applied to *Avena sativa* and *Hordeum vulgare* based on nutritional parameters.

This separation from *Avena sativa* confirms a strong species effect, indicating that *Hordeum vulgare* possesses a distinct nutritional profile, likely related to differences in fiber composition, organic matter distribution, and metabolic allocation. The third cluster (red ellipse) is particularly noteworthy, as it groups *H. vulgare* BT1 and *A. sativa* BT3, which are separated from their respective species clusters and positioned in the positive region of Dim2. This indicates that these treatments exhibit specific nutritional characteristics that override the species effect, leading to a convergence in their biochemical profiles. Their position along Dim2 suggests a strong association with variables such as dry matter (DM) and organic matter (OM), as previously identified in the PCA.

4. Conclusion

This study demonstrated that PGPR inoculation influenced the growth and nutritional characteristics of barley and oat at the early heading stage. Bacterial treatments generally increased plant height and significantly modified several forage chemical parameters, including dry matter, organic matter, crude protein, ether extract, neutral detergent fibre, acid detergent fibre, and acid detergent lignin. However, the magnitude and direction of these responses differed between barley and oat and among the bacterial strains, indicating that PGPR effects were dependent on both the plant species and the inoculant applied. The observed responses at the early heading stage further demonstrate that PGPR can influence plant performance and forage composition during this important developmental phase. Overall, the findings support the potential of selected PGPR strains as biological inputs for improving forage production and nutritional quality, while highlighting the need for appropriate strain–plant combinations. Further studies should evaluate these interactions across different developmental stages, growing seasons, and environmental conditions to determine the consistency and agronomic applicability of PGPR-based approaches in forage production.

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