Research Article

Effects of Plant Growth-Promoting Rhizobacteria and Seedling Density on Morphologic Characteristics and Nutrient Content of Corn Fodder

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Abstract | This research assesses the impact of plant growth-promoting rhizobacteria and seedling density on morphology and nutritional quality of fodder (Maize or Corn). The research was arranged according to a randomized, complete factorial design. Factor A was PGPR levels (0, 5, 10, and 15 ml/l) expressed as P, hereafter and Factor B was the density (0.26, 0.33, 0.40, and 0.47 g/cm^2) hereafter refers as D. Parameters observed in the research included germination percentage, plant height, fresh weight, dry weight, and proximate analysis. Results indicates that both level of P and D significantly influenced the measured parameters. Interaction of the factors P and D has a highly significant effects ($p<0.01$) on germination rate, plant height, fresh and dry weight. The combination of P at 15 ml/l with D 0.47 g/cm^2 showed the highest germination. Whereas, morphological traits like plant height, the optimal combination was associated to P1D2 with P 5 ml/l and D 0.33 g/cm^2. Similarly, fresh and dry weights indicated the best combination in P2D3, with PGPR at 10 ml/l and a seedling density of 0.40 g/cm^2. The optimal combination for proximate analysis was P2D2, with PGPR at 10 ml/l and a seedling density of 0.33 g/cm^2. Furthermore, a positive correlation ($p<0.001$) was observed between fresh and dry weights (Naturally because both are the same), as well as between neutral detergent fiber (NDF) and acid detergent fiber (ADF). In conclusion, the research highlights potential of integrating PGPR with strategic seedling density management to optimize corn as fodder growth and nutritional value, presenting a promising approach for future primary forage production.

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Introduction

Excessive utilization of chemical fertilizers can result in soil contamination. In contrast to chemical fertilizers, organic fertilizers have ability to enhance soil fertility without causing any lasting impact on the soil health. Additionally, they are more cost-effective (Baghdadi *et al*., 2018). The research area has agricultural land degradation due to excessive use of chemical fertilizers in corn as forage cultivation, which is a significant cause (Jisna *et al*., 2021). Long-term use of synthetic fertilizers in crops has been shown to decrease soil organic matter and disrupt soil microbial populations, leading to a decline in soil health and productivity (Tripathi *et al*., 2020). Therefore, it is preferable to utilize organic fertilizers that are both environmentally friendly and sustainable. Recently, an increasing focus has been on enhancing agricultural sustainability and productivity through innovative methods such as plant growth-promoting rhizobacteria (PGPR).

Plant growth-promoting rhizobacteria offers numerous advantages in agriculture (de Andrade *et al*., 2023; Ojuederie *et al*., 2019). These beneficial bacteria promote plant growth and productivity (Hayat *et al*., 2010). Directly, PGPR enhances nutrient availability by solubilizing phosphorus and fixing nitrogen, which is crucial for optimal growth (de Andrade *et al*., 2023; Singh, 2023). PGPR also enhances soil quality, facilitates bioremediation, and manage stress to promote ecologically conscious and sustainable agriculture growth. PGPR activity benefits plant growth, both directly and indirectly (Kumar *et al*., 2021). The suitable property of rhizobacteria lies in their capability to enhance plant growth by solubilizing phosphate, fixing N, or producing growth hormones (Hasan *et al*., 2024). PGPR enhances plant growth through bio-stimulant properties. Additionally, it improves availability of vital nutrients (Sun and Shahrajabian, 2023). Also, processes of PGPR facilitate nutrient solubilization for efficient plant absorption (Bhat *et al*., 2023). PGPR has demonstrated encouraging outcomes in promoting sustainable corn and maintaining as corn forage yield and quality. Corn fodder, categorized as a forage crop, possesses a nutrient-rich composition forage supporting livestock nutrition (Gurawal *et al*., 2022). While prior research has extensively investigated advantages of PGPR in grain production, studies specifically targeting corn fodder is still limited (Kumar *et al*., 2015). Existing research adequately covers the influence of PGPR on corn fodder's morphological characteristics, productivity, and forage quality.

This research addressed effectiveness of PGPR treatments in corn as forage crop like others (Sorghum and Bermuda grass). The research indicated that combinations of PGPR combined with reduced N inputs could sustain forage yield and quality comparable to conventional N application rates (Sullins *et al*., 2023). PGPR can contribute positively to developing sustainable fodder production and sustainable agriculture (Arora *et al*., 2024; Kumar *et al*., 2023). Nevertheless, this research aims to fill this gap by examining the impact of various seeding densities and different levels of PGPR on morphological traits and nutrient composition of corn as forage crop. Seeding density, defined as the number of seeds sown per unit area, directly affects crop growth parameters such as germination rate, plant height, biomass production, and overall yield and forage quality (Finch-Savage, 2020). Optimal seeding density ensures adequate utilization of resources, including light, nutrients, space and water, which are critical for maximizing growth potential of fodder crops. In contrast, improper seeding density can lead to overcrowding, which causes resource competition, underutilization of available space, adversely affecting the yield and forage quality. Seeding density is recognized to influence on plant growth and nutrient absorption. However, varying levels of PGPR are acknowledged in promoting plant growth and enhancing nutrient uptake. This research highlights the synergistic effects of PGPR application and seedling density management on fodder yield and quality. The research was aimed to identify optimal conditions to improve fodder's yield and nutrition quality by investigating various combinations of PGPR levels and seedling densities on corn as forage crop.

Materials and Methods

This research was conducted at the Feed Processing Unit of the Dairy Cow Laboratory, Faculty of Animal Science, Hasanuddin University, Makassar, from January to March 2024. The chemical composition of forage was analyzed at the Feed Chemistry Laboratory, Faculty of Animal Science at Hasanuddin University in Makassar.

Research implementation

This research was arranged according to a randomized complete factorial design, with Factor A representing levels of the PGPR (as P) application at 0, 5, 10, and 15 ml/l, and Factor B representing seedling density (as D) at the rate of 0.26, 0.33, 0.40, and 0.47 g/cm². The research involved 16 treatment combinations within five replicates, resulting total 80 experimental units. The specific treatment combination is shown in Table 1. Seeds underwent a soaking process in PGPR for 12 hours. Following to place in a 25 cm × 33 cm container designed for the seedling growth, adhering to a predetermined density treatment. The environmental conditions were carefully controlled, maintaining at a temperature ranged between 20–27ºC, relative humidity (RH) exceeding 60%, and exposure to a low light intensity for 16 hours on daily basis. Additionally, water and essential nutrients were readily available throughout the growth phase, with the fodders receiving regular watering intervals every 3 hours on daily basis (You should explain in depth the sample size and process?).

Table 1: *Layout of the treatments.*

Density	Level of plant growth promoting rhizobacteria			
	P0 (0 ml/L)	**P1 (5 ml/L)**	**P2 (10 ml/L)**	**P3 (15 ml/L)**
D1 (0.26 g/cm²)	P0D1	P1D1	P2D1	P3D1
D2 (0.33 g/cm²)	P0D2	P1D2	P2D2	P3D2
D3 (0.40 g/cm²)	P0D3	P1D3	P2D3	P3D3
D4 (0.47 g/cm²)	P0D4	P1D4	P2D4	P3D4

Observed parameters

The observed parameters in this research were:

The germination percentage, is determined using the following formula:

$$Germination\ rate\ (GR\%) = \frac{Final\ number\ of\ seedling\ emerged}{Total\ number\ of\ seeds\ sown} \times 100$$

Plant height (cm) was measured from the base of the stem to the tip of the highest leaf (Bódi *et al*., 2008) at 7 days and 14 days after planting. Fresh and dry-weigh of fodder were measured where the fresh weight (gram) of each tray unit, (whole plants-square meter area) was harvested 14 days after planting. Subsequently, 500 grams from each tray unit were retained as subsamples, oven-dried at 70˚C for 4 days to measure dry weight (gram) (Tarawali, 1995).

The composition and nutritional content of dry fodder was comprises for proximate parameters such as crude protein (CP), crude fat (CF), crude fibre (CFi), nitrogen-free extract (NFE) (AOAC, 2000), as well as acid detergent fibre (ADF) and neutral detergent fibre (NDF) according to procedure explained by Van Soest *et al*. (1984).

Data analysis

SPSS computer-based software was used to analyze data of growth characteristics one-way analysis of variance (ANOVA) was conducted, followed by the Duncan Multiple Range Test (DMRT) to identify any significant differences within parameters. Additionally, Pearson correlations were performed to examine the relationship between morphological and proximate analyses. A principal component analysis was carried out using the corrplot function of the corrplot package in RStudio version 4.2.3.

Results and Discussion

Effects of plant growth-promoting rhizobacteria on morphologic characteristics

Plant growth-promoting rhizobacteria have emerged as effective biofertilizers that enhance plant growth. The effects of PGPR on the morphologic characteristics of corn as fodder are presented in Table 2.

PGPR treatment in Table 2 significantly influences ($p<0.05$) germination and exhibits a highly significant effect ($p<0.01$) on plant height by day 14, as well as on fresh and dry weight. The most favorable germination rate of 80.80% was observed in the P3D4 treatment. Administering PGPR at a 15 ml/liter concentration enhances germination rates. PGPR plays a role in synthesizing indole-3-acetic acid, a type of auxin that promotes seed germination. Moreover, PGPR directly enhances plant growth and germination by regulating plant hormone levels (Garcia *et al*., 2023).

dos Santos *et al*. (2020) highlighted that sustaining increased maize productivity necessitates higher fertilizer inputs, leading to elevated production costs and greater environmental degradation. In contrast, Kuan *et al*. (2016) reported that plant growth-promoting bacteria offer a viable biological alternative by fixing atmospheric N2 and delaying nitrogen remobilization, which is directly linked to plant senescence. This approach not only reduces the need for nitrogen fertilizer but also enhances maize yields, increasing cob height by up to 30.9%. Additionally, PGPR accelerates metabolic processes, increasing phytochemical and phenolic compound production while mitigating oxidative stress, thereby expediting germination (Batool *et al*., 2020). Regarding plant height, statistically similar heights were observed across treatments by day 7, with non-PGPR treatments showing a slight tendency toward greater height. However, by day 14, significant differences in plant height were evident, with the highest heights observed in the PGPR-treated plants. This difference can be attributed to the depletion of seed nutrients by day 7, prompting plants to rely on external nutrient sources by day 14. PGPR application stimulates gibberellins' production, promoting overall plant growth and root development (Backer *et al*., 2018).

Table 2: *Effects of plant growth-promoting rhizobacteria on morphologic characteristics of corn fodder.*

Treat-ments	GR	7^{th} -PH	14^{th}-PH	FW	DW
P0D1	67.60^{b-e}	19.12	31.20^{a}	530.80^{a}	79.94^{a}
P0D2	65.20^{a-c}	18.60	31.72^{a}	762.40^{c}	111.91^{c-d}
P0D3	64.80^{a-b}	18.92	32.48^{a-b}	931.00^{e}	135.36^{f-g}
P0D4	58.40^{a}	18.24	33.76^{b-c}	880.20^{d-e}	132.56^{e-g}
P1D1	67.20^{a-d}	22.26	36.96^{c-d}	719.00^{b-c}	87.86^{a-b}
P1D2	64.00^{a-b}	21.62	37.36^{d}	885.00^{d-e}	135.93^{g}
P1D3	73.60^{c-f}	20.28	37.52^{d}	873.60^{d-e}	119.15^{d-f}
P1D4	76.00^{e-f}	19.85	36.36^{c-d}	1041.00^{f-g}	133.66^{e-g}
P2D1	63.20^{a-b}	14.00	37.00^{c-d}	630.00^{b}	83.91^{a}
P2D2	63.20^{a-b}	14.56	36.96^{c-d}	640.00^{b}	127.74^{d-g}
P2D3	67.20^{a-d}	14.89	36.64^{c-d}	1120.00^{g}	157.24^{h}
P2D4	60.80^{a-b}	14.16	36.60^{c-d}	960.00^{e-f}	157.05^{h}
P3D1	60.80^{a-b}	13.32	33.60^{b}	800.00^{c-d}	95.68^{ab}
P3D2	74.40^{d-f}	14.24	35.36^{c-d}	760.00^{c}	125.24^{d-g}
P3D3	74.40^{d-f}	15.98	37.36^{d}	790.00^{c-d}	101.43^{b-c}
P3D4	80.80^{f}	15.64	37.40^{d}	910.00^{e}	117.93^{d-e}
Mean	67.60	17.23	35.52	827.06	118.91
P	*	Ns	**	**	**
K	**	*	**	**	**
P*K	**	Ns	**	**	**

*GR: Germination rate (%); PH: Plant height (cm); FW: Fresh weight (g/cm²); DW: Dry weight (g g/cm²); Means with a similar superscript letter in the same column are not significantly different. Least Significant Difference (LSD) Test significance level = 1%. ** significant at p(α) 0.01; ns non-significant.*

According to Paulus and Tooy (2024), PGPR doses of 10 ml/l and 15 ml/l showed optimal plant growth. Moreover, previous research demonstrated that PGPR doses of 5 ml/liter and 10 ml/liter resulted in higher fresh and dry-weight due to increased nitrogen absorption facilitated by PGPR (Sun *et al*., 2024). Seedling density showed a significant effect ($P<0.05$) on plant height and a very significant effect ($p<0.01$) on germination, day 14 plant height, and fresh and dry weight. Germination is closely linked to moisture availability during germination, with denser seed populations retaining more moisture. Sinkkonen (2006) observed that higher seed density promotes quicker and more extensive germination up to a certain threshold, although overcrowding can lead to interactions that inhibit early growth. Plant height increases with higher seedling density, influenced by competition and shading effects. Djaman *et al*. (2022) reported that under adequate nutrition, increased density does not affect plant height uniformly; shaded plants may grow taller but with weaker stems.

Conversely, Ozer (2003) found that high seedling density can reduce overall plant height. Similarly, fresh and dry-weight production increased with seedling density, which aligns with Thapa and Katwal (2024), the higher density can also lead to stunted growth due to overcrowding.

The interaction between PGPR application and seedling density significantly influenced ($p<0.01$) germination, plant height on the 14^{th} day, and fresh and dry weight. The optimal combination for morphological traits, plant height, P1D2 with PGPR at 5 ml/l and a seedling density of 0.33 g/cm^2. Meanwhile, fresh and dry weight showed P2D3 as the best combination with PGPR at 10 ml/l and a 0.40 g/cm^2 seedling density. The treatment combinations P1D3, P1D4, P3D2, P3K3, and P3D4 exhibited the highest germination rates. Seed germination represents a critical early phase in plant development, influenced by both physiological and environmental factors. Maintaining optimal moisture levels during germination and ensuring nutrient availability are crucial factors that enhance seed germination efficiency (Fu *et al*., 2024). The treatment P1D3 demonstrated the tallest plant height, indicating a strong correlation between plant height and nutrient availability. Adequate nutrient availability likely optimized growth conditions in the P1D3 treatment, minimizing plant nutrient competition.

Similarly, treatments such as P2D3 showed the highest fresh and dry weight, suggesting optimal nutrient availability facilitated enhanced biomass accumulation. PGPR application enhances growth by promoting the synthesis of growth hormones and increasing nutrient availability for plants (Yolcu *et al*., 2012). Moreover, optimal seeding density with optimal PGPR can enhance corn fodder's crude protein content, ether extract, and ash yield, thereby significantly improving its nutritional value and quality for livestock consumption (de Oliveira *et al*., 2023; Kumar *et al*., 2017). The effects of PGPR on the nutrient content of corn fodder are presented in Table 3 in detail.

Table 3: *Effects of plant growth-promoting rhizobacteria on nutrient content of corn fodder.*

Treat-ments	CP	CF	Cfi	NFE	Ash	ADF	NDF
P0D1	17.24	6.53	11.93	61.05	3.24	18.22	39.79
P0D2	15.97	5.19	13.55	61.89	3.40	12.02	28.75
P0D3	18.52	4.89	13.94	59.31	3.34	13.21	36.78
P0D4	16.19	6.43	12.89	61.32	3.17	10.75	30.45
P1D1	18.19	5.09	14.57	58.38	3.77	16.33	39.38
P1D2	19.72	4.66	15.90	56.31	3.42	12.99	32.36
P1D3	17.78	6.19	15.01	57.44	3.57	17.50	41.30
P1D4	18.03	5.11	12.75	60.24	3.88	15.97	39.09
P2D1	16.72	6.12	12.15	60.24	3.88	17.99	38.14
P2D2	16.01	5.28	8.13	67.70	2.88	19.17	41.22
P2D3	16.61	5.72	10.62	62.62	4.42	20.13	42.30
P2D4	15.36	5.66	8.87	66.89	3.23	23.18	44.58
P3D1	16.05	5.90	12.94	60.52	4.59	17.99	46.03
P3D2	14.87	5.27	10.13	65.54	4.18	19.17	44.91
P3D3	16.82	5.75	14.29	55.32	7.83	20.13	36.31
P3D4	15.79	5.31	12.76	62.18	3.95	23.18	44.37
Mean	16.87	5.57	12.53	61.06	3.92	17.37	39.11
Min	14.87	4.66	8.13	55.32	2.88	10.75	28.75
Max	19.72	6.53	15.9	67.70	7.83	23.18	46.03

CP: crude protein; CF: crude fat; Cfi: crude fiber; NFE: Nitrogen free extract; ADF: Acid detergent fiber; NDF: Neutral detergent fiber.

Descriptively, proximate results show in Table 3 that, each parameter demonstrated optimal values: crude protein, crude fat, and crude fiber were highest with the combinations P1D2, P0D1, and P1D2, respectively. The P2D2 treatment also exhibited the best NFE value and ash content. Additionally, the lowest values for ADF and NDF were found in the combinations P0D2 and P0D4. Thus, descriptively, the optimal combination was P1D3, which involved PGPR at 5 ml/l and a seedling density of 0.40 g/cm^2. Corn fodder's crude protein (CP) content ranged from 14.87% to 19.72%. It demonstrates that the application of PGPR descriptively enhances the CP content of corn fodder based on seedling density and the level of PGPR applied. This finding revealed a higher CP content than the 11.135% reported by Widiastuti *et al*. (2022) in corn fodder. Proximate analysis studies have revealed that corn fodder comprises approximately 11.135% CP, 4.950% crude fat (CF), 2.340% ash, 15.210% crude fiber (Cfi), and other essential components (Widiastuti *et al*., 2022). These findings indicate that corn fodder presents a promising alternative for ruminant feed due to its favorable nutritional profile. The highest CP value observed in the P1D2 treatment suggests that applying 0.5 ml of PGPR at a planting density of 0.33 results in superior CP yield. This increase in protein content due to PGPR application can be attributed to enhanced nitrogen availability, which facilitates protein synthesis in plants (Chattha *et al*., 2017). The CFi content of corn fodder, influenced by planting density and the level of PGPR application, resulted in the lowest CF content of 4.66%. The average CF content in all treatments ranged from 8.13% to 15.90%. These findings indicate the beneficial impact of PGPR on enhancing the CF content in corn. PGPR influences the fiber content of corn through various mechanisms. Research indicates that PGPR inoculation can stimulate the production of enzymes that modify plant fiber and cell wall components, such as lignin, in corn (Jha, 2018).

Additionally, PGPR inoculation is linked to increased plant biomass and alterations in plant growth parameters, resulting in changes in the fiber content of corn (Lin *et al*., 2019; Sadaghiani *et al*., 2011). Corn fodder's nitrogen-free extract (NFE) content ranged from a high of 67.70% to a low of 55.32%. NFE content of corn feed can vary depending on different cultivation methods and nutrient supplementation. Research on hydroponically grown corn fodder demonstrated that the NFE content peaked at 66.72% on the seventh day of growth (Naik *et al*., 2012).

Ash content of corn fodder in different treatments ranged from 2.88% to 7.83%. PGPR have been investigated for their effects on forage crops like corn and sorghum (Chattha *et al*., 2017). Studies indicate that integrating PGPR with nutrient management practices can notably decrease the ash content in corn fodder (Kumar *et al*., 2022). Overall, the combined findings suggest that PGPR application can play an important role in reducing the ash content of corn fodder, thereby improving its quality and suitability for ruminant consumption. The ADF content of corn feed ranged from 12.02% to 23.18%, and the NDF content varied from 28.75% to 46.03%. These findings clearly indicate that using PGPR leads to increased production of plant fiber. Despite the increasing ADF and NDF levels with higher PGPR applications, the fiber concentrations remain within safe limits for ruminant consumption. Generally, forages characterized by lower levels of NDF and ADF indicate improved digestibility and nutrient accessibility for livestock. Decreased NDF content signifies a reduction in structural carbohydrates, enhancing the ease of consumption and digestion by breaking down fiber. Conversely, elevated NDF levels can restrict dry matter intake and energy availability (Xu *et al*., 2023). It is crucial to maintain a balance because while reducing NDF can heighten dietary energy content, sufficient fiber is essential for optimal digestive function in ruminants.

Correlation analysis between all characteristics

The Pearson correlation matrix offers crucial insights into the relationships among the morphological traits in our research population of corn fodder treated with PGPR and seedling density. By analyzing the correlation coefficients, we understand how various morphological and nutrient characteristics interact and influence each other. The matrix shows correlations between each pair of traits, indicating the strength and direction of their linear relationships. Correlation analysis between all characteristics is presented in Figures 1 and 2.

Figure 1 illustrates a strong positive correlation ($p<0.001$) between fresh and dry-weight corn fodder. This relationship indicates that as the fresh weight of the corn fodder increases, the dry weight also tends to increase. This positive correlation suggests that factors contributing to higher fresh weight, such as better water and nutrient uptake, also enhance dry matter accumulation. Consequently, improvements in fresh weight are likely to be mirrored by corresponding gains in dry weight, reinforcing the overall quality and productivity of the corn fodder. The positive correlation between fresh and dry-weight in corn fodder suggests that factors enhancing fresh weight, like water and nutrient uptake, also boost dry matter accumulation, improving overall quality and productivity (de Oliveira *et al*., 2023; Subrahmanya *et al*., 2019).

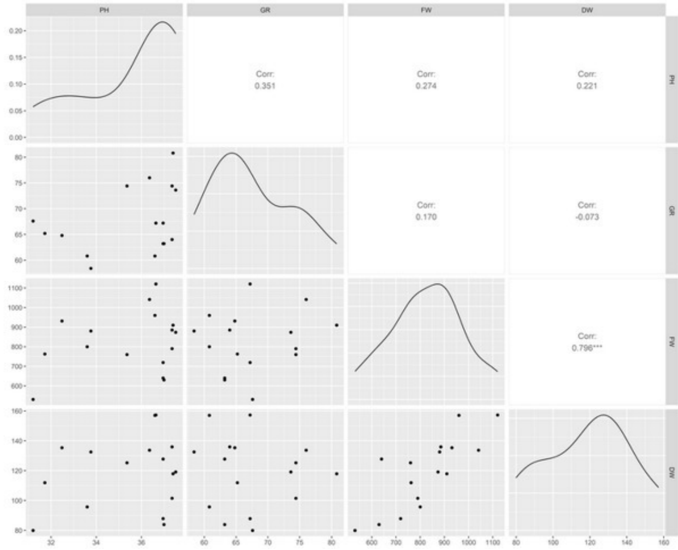

Figure 1: *Pearson correlation matrix of morphological traits. * p-value < 0.001.*

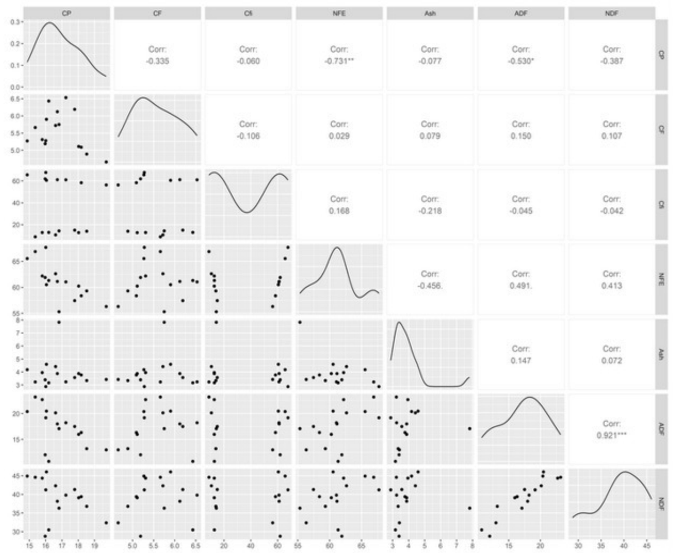

Figure 2: *Pearson correlation matrix of proximate analysis * p-value < 0.001. CP: Crude protein; CF: Crude fat; Cfi: Crude fiber; NFE: Nitrogen Free Extract; ADF: Neutral Detergent Fiber (NDF) and (ADF).*

Figure 1 also shows that the plant height parameter positively affects germination and fresh and dry-weight corn fodder. While germination positively correlates with fresh weight, it negatively correlates with plant dry weight. Plant height shows a positive correlation with germination rates. This relationship indicates that factors contributing to increased plant height, such as better nutrient uptake and efficient water use facilitated by PGPR, also enhance the initial stages of plant development. In line with previous research, PGPR enhances corn growth parameters like plant height and biomass, potentially reducing the need for nitrogen fertilization without compromising nutrient content (Kuan *et al*., 2016; Lin *et al*., 2019). In addition to the variety, certain agronomic practices implemented during crop production, particularly nutrient management strategies, significantly impact the accumulation of fodder yield (Rundan *et al*., 2023).

Plant growth-promoting rhizobacteria have been widely studied for their positive impact on crop growth and nutrient uptake (Lin *et al*., 2019). Research indicates that PGPR inoculants can substantially improve corn fodder's morphological traits and nutrient content (Song *et al*., 2022). Specifically, studies have shown that PGPR application enhances plant height, stem diameter, leaf area, and root morphology in corn, resulting in increased biomass production and nitrogen content. Moreover, the use of rhizosphere bacteria has been associated with improved plant biomass.

Figure 2 illustrates that an overall positive correlation exists among the observed parameters. Specifically, ADF and NDF parameters demonstrate a notably strong positive correlation ($p<0.001$). The data analysis reveals a very strong positive correlation between ADF and NDF. This relationship is considered as both parameters are metrics of fiber content, with ADF consisting of cellulose and lignin, while NDF includes the ADF component with hemicellulose. ADF and NDF are both crucial metrics for assessing fiber content. ADF comprises cellulose and lignin, whereas NDF includes the ADF component and hemicellulose. Research has demonstrated a strong positive correlation between ADF and NDF levels in various studies, particularly forage grasses and legumes (Arora *et al*., 2024; Elgersma and Søegaard, 2018). There is a strong correlation between fiber measurements and their relevance in evaluating corn fodder. Furthermore, the analysis demonstrates a weaker positive correlation between crude protein and both NDF and ADF. According to Mahanta *et al*. (2023), CP positively correlates with NDF and ADF in corn. In contrast, Almeida *et al*. (2020) reported that quality parameters in corn feed are interrelated, with CP showing an inverse relationship with NDF and ADF. This finding suggests that there is a slight increase in CP levels with rising fiber content. This relationship may be attributed to the presence of specific feedstuffs that contribute both protein and fiber.

Additionally, the figure illustrates a weaker positive correlation between CF and Nitrogen Free Extract (NFE). This indicates that higher CF content is associated with a marginal increase in NFE. The most plausible explanation for this relationship is the presence of non-fiber carbohydrates within high-fiber ingredients. NFE also exhibits a weaker positive correlation with both NDF and ADF. This observation suggests a complex interaction where an increase in fiber content slightly affects the amount of non-fiber carbohydrates. This highlights the relationship between fiber and carbohydrate components in feedstuffs. The crude fiber content of forage corn significantly influences its nutrient profile, especially the NFE content. Research on hydroponic corn feed has demonstrated that increased CF content tends to decrease NFE content. Moreover, research on the digestibility of CF in corn forage-based complete diets emphasized the critical role of CF digestibility in enhancing overall nutrient utilization (Aling *et al*., 2020; Gebremedhin, 2015). A negative correlation is also observed between CP and other measured parameters. A similar negative correlation exists between CF and CFi, with crude fiber also correlating with NDF, ADF, and ash content.

To comprehensively capture the variability across both categories of variables, the correlations may proceed in Principal Component Analysis (PCA). The resulting components are then interpreted to discern predominant patterns or relationships among morphological traits and proximate analysis parameters. Figure 3 depicts the PCA outcomes for morphological traits and proximate analysis.

Based on Figure 3, the resulting PCA Biplot effectively illustrates the relationships between various morphological and proximate analysis traits of corn fodder within a two-dimensional framework. The first principal component (Dim 1) accounts for 36.9% of the total variance in the dataset, while the second principal component (Dim 2) accounts for 31.1% of the total variance. Collectively, Dim 1 and Dim 2 explain the majority of the variability in the data. Variables with longer vectors and warmer colors, such as fresh-weight, dry-weight, and CP, are identified as significant contributors to the variability captured by the principal components. This PCA biplot provides a comprehensive visualization of the interaction among different morphological and nutritional traits of corn fodder, emphasizing the key variables that drive the observed variability within the dataset.

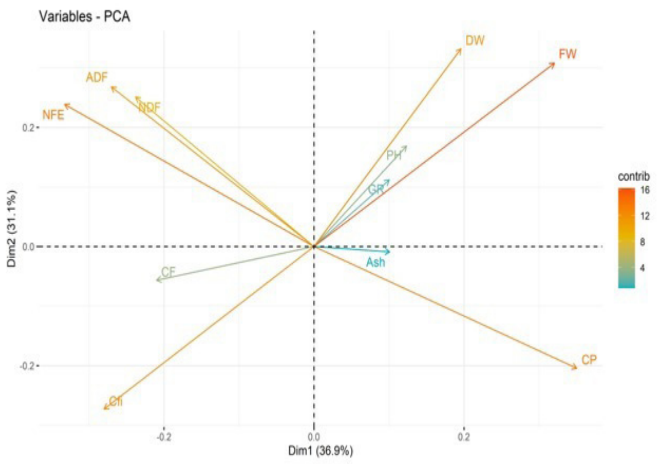

Figure 3: *Principal component analysis (PCA) on morphological traits and proximate analysis.*

Conclusion and Recommendations

The germination rate of corn fodder is positively influenced by higher levels of PGPR and seedling density, along with other factors such as plant height, fresh weight, and dry weight. A proximate analysis determined that the optimal combination was P2D2, with a PGPR concentration of 10 ml/l and a seedling density of 0.33 g/cm^2. Furthermore, a strong positive correlation ($p<0.001$) was found between parameters such as fresh and dry weight and between NDF and ADF. This research serves as a preliminary investigation into the effects of plant growth-promoting agents on corn fodder. However, further evaluation is required to assess the productivity and quality outcomes of post-plant growth-promoting applications in field conditions and their potential implications as primary forage for dairy cow production.

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Novelty Statement

In previous research, the application of plant growth-promoting rhizobacteria (PGPR) has primarily focused on enhancing the growth of food crops and plantations, including such as rice, corn, and soybeans. However, there remains limited notable literature regarding using PGPR in forages, particularly in fodder crops. This underexplored area presents an exciting opportunity to assess the potential benefits of PGPR in improving fodder's nutritional quality and growth performance, which could have significant implications for sustainable forage.

Author's Contribution

Ambo Ako, Renny Fatmyah Utamy, Syahdar Baba, Zulkharnaim Zulkharnaim: Conceived and designed the experiments, performed the field experiments, analyzed data, and wrote the paper.
Sri Gustina, Laode Alhamd, Indrawirawan and dan Aulia Uswa Noor Khasanah: Conceived and designed the experiments, dan performed the field experiments.
Andi Arif Rahman, Sitti Annisa Sukri and Rara Mufliha: Performed field experiment and data tabulation. **Zyahrul Ramadan**: Performed the data tabulation, analyzed data and wrote the paper.
Purnama Isti Khaerani: Analyzed data and wrote the paper.

Conflict of interest
The authors have declared no conflict of interest.

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