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Assessment of Adaptability and Genetic Variability among Lucerne (*Medicago sativa* L.) Accessions for Green Fodder Yield and Associated Traits

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ABSTRACT

Lucerne (*Medicago sativa* L.) is a perennial forage crop valued for its high biomass production, nutritive value and repeated harvesting potential. Identification of local germplasm is essential for lucerne improvement and breeding. This study assessed the potential of 12 lucerne accessions for green fodder yield and associated morphological traits under the agro-ecological conditions of Farooqabad, Punjab, Pakistan during 2025–2026. The experiment was conducted in a randomized complete block design with three replications and green fodder yield was recorded from three successive cuts. Analysis of variance revealed significant differences among genotypes for plant height, number of tillers, number of leaves, leaf area and green fodder yield whereas culm thickness showed a non-significant genotypic effect. Green fodder yield ranged from 60.37 to 79.63 t ha⁻¹ with G2 (79.63 t ha⁻¹) and G5 (78.89 t ha⁻¹) recording the highest yields while G12 showed the lowest yield (60.37 t ha⁻¹). Correlation analysis indicated a positive association of green fodder yield with number of tillers ($r = 0.66$) whereas negative associations were observed with plant height ($r = -0.27$), number of leaves ($r = -0.27$) and culm thickness ($r = -0.51$). The first two principal components accounted for 68.46% of the total variation. Green fodder yield and the number of tillers contributed to the differentiation of genotypes in this study. The results demonstrated considerable phenotypic variation among the evaluated accessions and identified G2 and G5 as promising materials for further evaluation. These accessions together with other high-performing genotypes may provide useful material for subsequent multi-environment testing and lucerne improvement programs under Pakistani conditions.

Introduction

Lucerne (*Medicago sativa* L.) is well known as alfalfa and is an important forage crop worldwide (Zhang and Wang, 2025). Besides being rich in nutrients and having high palatability, it has the advantage of fixing atmospheric nitrogen which can improve the productivity of subsequent crops and capacity to provide repeated harvests (Patra and Paul, 2022). Lucerne is an autotetraploid ($2n = 4x = 32$) outcrossing specie. Therefore, there are many genes for each trait and there is variation between individual plants and between different plant populations (Ghazzal et al., 2026). Breeding lucerne for improved characteristics is complicated due to presence of numerous genes for traits of economic importance. Lucerne breeding programs have primarily focused on improvement of forage yield, forage quality and tolerance to biotic and abiotic stresses. The combination of high forage yield and nutritional makes alfalfa very valuable in livestock production. It is important to assess and maintain different alfalfa germplasm to choose the best parents in a breeding program (Zafar et al., 2026).

Lucerne similar to other forage species has a comparatively high genetic variability for various morphological and yield-associated characteristics. Traits such as plant height, number of tillers, branching, leaf development, stem thickness, fresh forage yield and dry matter yield contribute directly or indirectly to overall biomass production (Ghazzal et al., 2026).

Multivariate statistics allow us to interpret intricate patterns relating to numerous characteristics. Correlation analysis can identify magnitude and strength of relationships various traits have with forage yield, one can better decipher traits to be utilized for indirect selection for forage yield (Michelucci, 2025). By reducing correlated variables into a smaller number of principle components and identifying the qualities that contribute most to the observed variance among accessions, principal component analysis (PCA) offers a multivariate method for summarizing complex trait interactions. Thus, PCA allows the determination of traits that account for maximum variability in accessions. Given these attributes, PCA can be utilized to recognize various phenotypic groups contained within germplasm and in relation to other plants recognize predominant lines of variability. In conjunction with correlation analysis, recent work has illustrated the effectiveness of PCA in analysis of alfalfa forage yield (Matthew, 2025).

It is necessary to evaluate several accessions in a given ecological region to better understand the genetic and phenotypic variability of genotypes. Quantitative traits including those affecting forage and biomass yield are affected by both genetics and the environment. Of interest in those evaluations is the identification of accessions that are well adapted to the region and have the best combination of yield and plant attributes (Dominguez et al., 2025). Baxevanos, (2024) reported that not all traits associated with biomass are stable and genotype x environment interactions affect the stable expression of yield. Selection of accessions for further evaluation should be based on the integration of the expression of various traits and the variability of the population.

The objective of this study is to determine the genetic variability and adaptability of lucerne accessions for fodder yield and associated morphological traits. The study allow identification of accessions best adapted to the farooqabad location and define the relationships between the various morphological traits and fodder yield.

Materials and Methods

Experimental Site and Genetic Material

The field trial was conducted at the Experimental Seed Production Unit (ESPU), Farooqabad, Sheikhpura, Punjab, Pakistan during the 2025–2026 cropping season. Assessments were carried out on 12 alfalfa (*Medicago sativa* L.) genotypes within the framework of Adaptability / Punjab Uniform Fodder Yield Trial.

Field Management and Experimental Design

Sowing was done on October 28, 2025. The field experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each experimental plot was $1.8\text{m} \times 5\text{m}$ (9.0 m^2) in size and contained 4 rows spaced 45cm apart. Standard agronomic practices and weed control operations were performed on all experimental units in order to facilitate establishment of the experimental crop. Forage harvesting was done by taking three successive cuts, the first being done on February 10, 2026 and subsequent cuts being done 30–40 days thereafter based on the growth stage of the crop and its regrowth.

Yield and Morphological Measurements

Green fodder yield (kg/plot) was recorded at all three cuts consecutively and measured the fresh biomass harvested from the entire plot and converted to tons per hectare. Morphological parameters were evaluated exclusively during the first harvest cut (February 10, 2026). Within each plot, three randomly plants were selected for data recording. The recorded parameters are; Plant Height (From the ground to the main plant apex), Tillers per Plant (Total tillers mean counted from the selected plants in

each plot), Leaves per Tiller (The mean number of leaves counted from the selected main tillers), Culm Thickness (Measured by means of Digital calipers), Leaf Area (Quantified on representative trifoliate leaf samples by measuring leaf length (L) and leaf width (W) using the formula:

$$\text{Leaf Area} = L \times W \times 3 \times 0.75$$

where 0.75 represents the shape correction coefficient for the individual leaflets, multiplied by 3 to represent all leaflets of the trifoliate leaf.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the randomized complete block design (RCBD) using repeated measurements to estimate the sources of variation and associated F -ratios. Tukey's method initially called 'Honestly significant difference (HSD)' was adopted to estimate pairwise mean comparison of all studied traits (Steel et al., 1997). Pearson correlation coefficients (r) were calculated between the morphological traits and total green fodder yield using the statistical software. The principal component analysis was performed to measure variability and to understand relationship among variables.

Results and Discussion

Analysis of Variance for Green Fodder Yield and Associated Traits

Analysis of variance showed significant differences between 12 genotypes of Lucerne for most of the studied morphological traits and combined green fodder yield. Genotypic effects were significant for plant height and highly significant for number of tillers, number of leaves, leaf area and green fodder yield (Table 1). In contrast, culm thickness showed a non-significant genotypic effect. The coefficient of variation for green fodder yield was lowest at 3.05%, indicating high experimental precision.

Table 1. Combined analysis of variance for morphological traits and green fodder yield of lucerne genotypes

Source of variation	df	Plant height	Number of Tillers	Number of Leaves	Culm thickness	Leaf area	Green fodder yield
Replications	2	-	-	-	-	-	-
Genotypes	11	2.87*	5.98**	16.61**	0.76 ^{ns}	4.12**	28.44**
Error	22	-	-	-	-	-	-
Total	35	-	-	-	-	-	-

ns = non-significant; * = significant at $P < 0.05$; ** = highly significant at $P < 0.01$

Genotypic performance and Tukey's HSD test

Tukey's HSD test further compare the genotypes on the basis of mean for the traits showing significant ANOVA effects. For green fodder yield, genotype means ranged from 60.37 to 79.63 t ha⁻¹ (Figure 1). G2 recorded the highest yield (79.63 t ha⁻¹), followed by G5 (78.89 t ha⁻¹) and both belonged to group A. G6 (76.30 t ha⁻¹) and G7 (76.67 t ha⁻¹) were statistically comparable with the highest-yielding genotypes as indicated by their AB grouping. G12 produced the lowest yield (60.37 t ha⁻¹). The remaining genotypes showed intermediate performance while G9, G10 and G11 were classified in the lower yielding.

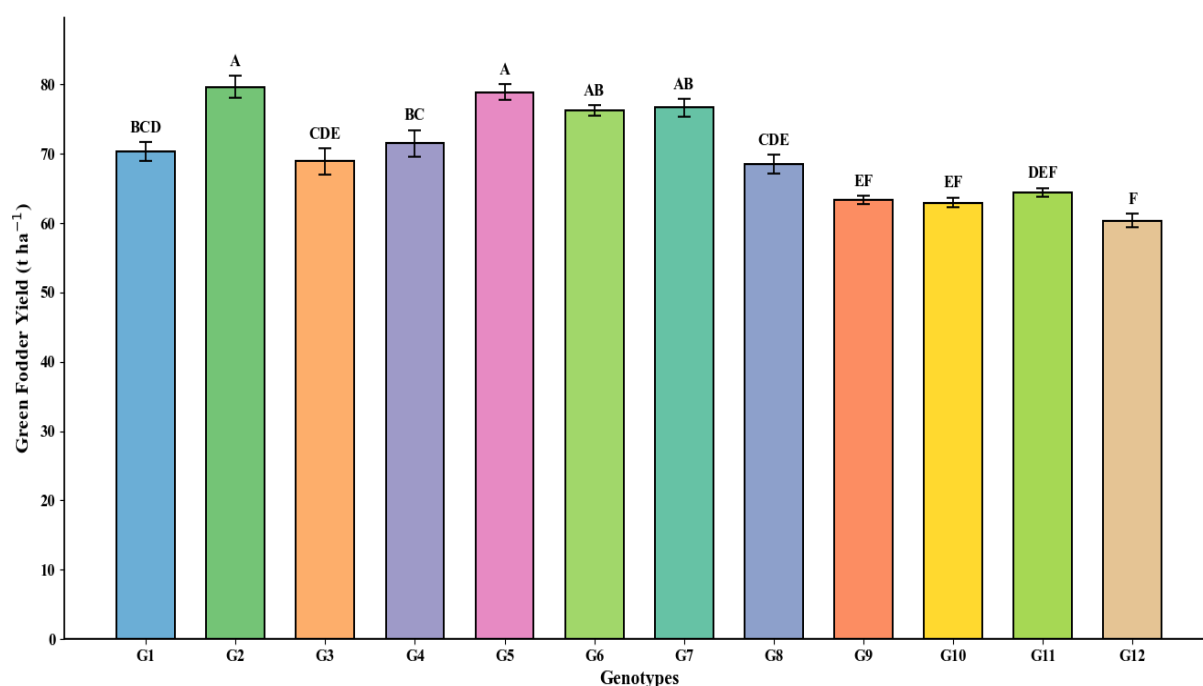


Figure 1. Genotypic variation combined with green fodder yield of lucerne accessions. Bars represent means $\pm$ SE. Different letters indicate significant differences among genotypes according to Tukey's HSD test at $P \leq 0.05$.

Variation was observed among the genotypes for morphological characters. For the morphological traits, G11 showed the greatest plant height (81.67 cm), G1 the highest number of tillers (6.67), G6 the highest number of leaves (39.67) and G7 the largest leaf area (1223.0 mm²). Culm thickness did not show significant differences among genotypes with all means belonging to the same Tukey group. Overall, the results demonstrate considerable phenotypic variation among the tested lucerne accessions particularly for green fodder yield, number of leaves, number of tillers and leaf area providing a basis for further assessment of trait associations and multivariate diversity through correlation and PCA.

Table 2. Mean performance of lucerne genotypes for morphological traits and green fodder yield with Tukey's HSD comparisons

Genotypes	Plant height (cm)	No. of tiller (NOL)	No. of leave (NOL)	Culm Thickness (mm)	Leaf Area (m ²)	Green Fodder Yield (t/ha ⁻¹)
G1	69.67AB	6.67A	21CD	3.67	920.9AB	70.37BCD
G2	70.67AB	6AB	17D	3.18	844AB	79.63A
G3	72.33AB	4.67ABCD	30ABC	3.23	846AB	68.89CDE
G4	66B	4.67ABCD	14.33D	3.2	1199.7A	71.48BC
G5	68.33AB	6.33A	22CD	3.18	1198.1A	78.89A
G6	76AB	5.67ABC	39.67	3.08	954.4AB	76.30AB
G7	76.33AB	5ABCD	29BC	3.42	1223A	76.67AB
G8	68AB	3.33CD	18.33D	2.95	733.3B	68.52CDE
G9	68.67AB	2.67D	19.67CD	3.43	944.2AB	63.33EF
G10	76.67AB	5.33ABC	34.33AB	3.47	1027.2AB	62.96EF
G11	81.67A	4.33ABCD	29.33ABC	3.28	1186.1A	64.44DEF
G12	75.67AB	3.67BCD	36.33AB	3.47	1121.2AB	60.37F

HSD @ 0.05	14.316	2.58	10.46	NS	422.85	6.36
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Correlation

Correlation analysis showed that the evaluated alfalfa genotypes differed significantly in the relationships among morphological traits and green fodder yield. Correlation of morphological traits with green fodder yield (GFY) of lucerne is shown in Figure (1). Number of leaves (NOL) was positively correlated with plant height ($r = 0.78$) and leaf area ($r = 0.30$), and thickness of the culm ($r = 0.39$) indicating that taller plants having more no. of leaves. Tiller number was negatively correlated with plant height ($r = -0.02$). Number of tillers were positively correlated to green fodder yield ($r = 0.66$) indicating a plant having more no. of tillers tended to have more biomass yield. Taller and having more tillering varieties of lucerne had the highest GFY. According to previous research, higher tiller numbers are associated with higher green forage yield in alfalfa (Strbanovic *et al.*, 2017). However, in the present study, GFY exhibited negative correlation with plant height ($r = -0.27$), along with leaf number ($r = -0.27$) and a moderate negative association with culm thickness ($r = -0.51$), while its association with leaf area is negligible ($r = 0.02$). Previous studies have shown that across different lucerne (*Medicago sativa* L.) varieties and under different environmental conditions, the number of tillers per plant, plant height and forage yield are often interrelated. However, the strength and magnitude of these associations can vary. Takawale *et al.*, (2019) reported positive phenotypic associations of 0.94 between plant height and tiller number of alfalfa, and 0.72 between plant height and green fodder yield across 31 alfalfa accessions. Thicker culms, higher number of leaves, and larger leaf area, are expected to result in higher biomass yield. Differences between the present study and the previously cited alfalfa studies may be attributed to a combination of factors including genetics of the alfalfa germplasm, growth conditions, cutting schedule, relative contribution of yield components and other factors. Overall, the correlation pattern suggests that tiller production was more closely related to GFY than to plant height, leaf number, leaf area or culm thickness in the present lucerne study. This conclusion is in accordance with previous alfalfa studies where tiller/stem number was identified as an important component linked to forage productivity (Farshadfar *et al.*, 2023).

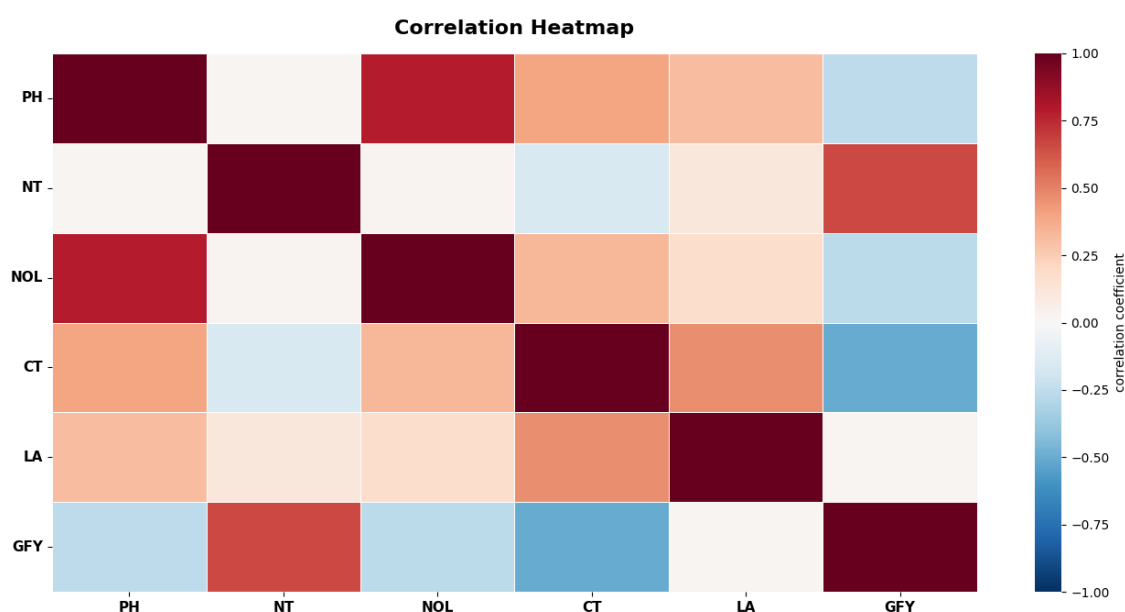


Figure 2. Correlation analysis between different attributes of alfalfa genotypes

Principal Component Analysis

Principal Component Analysis (PCA) was carried out on twelve lucerne genotypes for traits like plant height, number of tillers and leaves, culm thickness, leaf area and green fodder yield (Fig. 2). The first two principal components PC1 and PC2 accounted for 41.79% and 26.67% respectively and collectively accounted for 68.46% of the total variance. Results of the analysis showed substantial phenotypic variation for the assessed traits along with distribution of genotypes in all the four quadrants. PCA has been found to be effective in evaluation of diversity of alfalfa germplasm, based on forage yield and yield components. The first component PC1 was aligned to the major form of variation. Plant height and number of leaves and leaf area were aligned to positive side of PC1. Genotypes having high green fodder yield and number of tillers were located on the negative side of PC1. These vectors are, for the most part, consistent with previous correlation results. As shown in previous analyses, GFY has a

positive correlation with tiller number, and a negative correlation with other morphological traits. The prominent position of GFY on biplot indicates that forage yield has an effect on the differentiation of the genotypes along PC1. PC2 accounts for 26.67% of the total variation and shows positive projections of GFY and number of tillers. Culm thickness had a close position to the origin of the biplot. In general, GFY and no. of tillers explains a large part of variation among genotypes in the study. PCA has typically shown that first principal components are attributed to overall forage yield and yield components such as no. of leaves and plant height.

Genotypes in the biplot were generally widely distributed. G1, G2 and G5 were positioned in the negative region of PC1 and aligned toward the GFY-associated vector. While G10 and G11 were positioned in the positive region of PC1 near to PH, NOL and CT vectors. G7 was placed in the positive region of PC1 with association to LA vector. G8, G9, and G12 placed far from the origin indicating these have more distinct trait values for principal components. In contrast, G3 and G6 were positioned closer to central region, suggesting intermediate in multivariate analysis. This diversity in positions shows the presence of variation within evaluated germplasm.

The orientation of GFY opposite to morphological vectors shows that the evaluated genotypes differed in the combination of vegetative attributes that influenced the development of various forage yield components. From the results of this study, it concluded that traits contributed to forage yield are complex. Similarly, previous studies have documented variation in plant height and forage yield among lucerne germplasm, emphasizing the importance of evaluating multiple characteristics during selection.

According to the results of this study, genotypes with multivariate profiles can be used in alfalfa breeding programs. Analysis showed that genotypes located farther away from the center of the biplot are less genetically related, and are therefore more dissimilar. The biplot should be considered alongside genotype means, analysis of variance, correlation coefficients and genetic variability parameters when identifying potential parental material. Previous studies also used multivariate analysis to assess germplasm adaptability and identified useful diversity for breeding (Inostroza *et al.*, 2021; Sayed *et al.*, 2022)

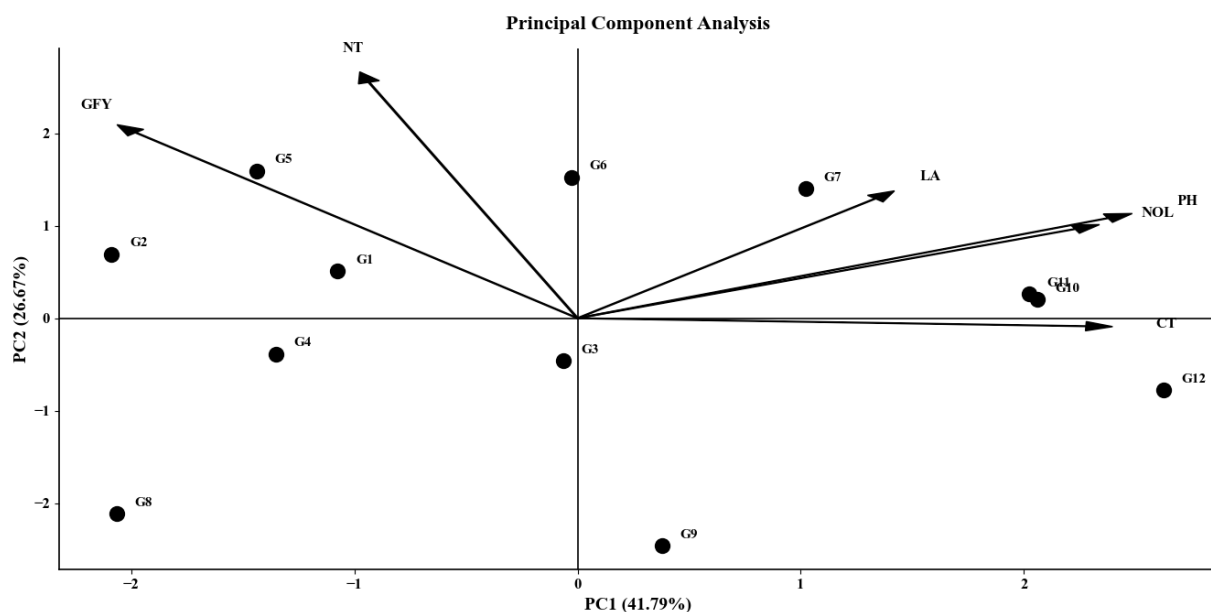


Figure 3. Distribution of Studied Morphological and Yield contributing Traits and genotypes Across Two Principal Component Axes on Biplot

Conclusion

The present study evaluated phenotypic expressions of 12 accessions of lucerne under the agro-ecological conditions of Farooqabad, Punjab. Variation was significant for plant height, number of tillers, number of leaves, leaf area and green fodder yield while culm thickness showed limited variation. Green fodder yield varied considerably among accessions with G2 and G5 recording the highest yields of 79.63 and 78.89 t ha⁻¹ respectively while G12 produced the lowest yield. Correlation analysis indicated that number of tillers had positive association with green fodder yield suggesting its potential usefulness as a selection trait although the relationships of yield with other morphological characteristics were variable. Principal component analysis

of the various traits of the lucerne accessions showed that first two components accounted for 68.46% of the total variation, and highlighting the importance of green fodder yield and tiller number in differentiating the evaluated material.

Overall, G2 and G5 emerged as promising accessions for green fodder production under the conditions of the present study. However, their performance should be validated across multiple locations and seasons before making conclusions regarding broad adaptability. The observed variation among accessions provides useful phenotypic resources for further selection, multi-environment testing and incorporation of promising material into lucerne breeding programs aimed at improving fodder productivity under Pakistani agro-ecological conditions.

Author Contributions

Swaiba Rani conducted the field investigations and performed the experimental work. Neelam Chaudhary and Muhammad Faisal Shafiq provide critical guidance on experimental design and statistical analysis. Sikander Hayat, Aamir Shehzad and Muhammad Jabbar Hussain contributed in developing initial draft of the paper. Syed Ali Murtaza Shah, Maham Sajid and Fariha Amjad prepared the final manuscript. All authors subsequently critically reviewed and revised the manuscript, contributed to its intellectual refinement, verified the data and analyses and approved the final version for submission.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

Policy Addressed

Forage security, livestock development and climate-smart agricultural policies should promote the evaluation and dissemination of high-yielding, locally adapted lucerne germplasm supported by efficient water and nutrient management and region-specific selection strategies to enhance sustainable fodder production, livestock productivity and resilience under changing agro-ecological conditions in Pakistan.

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