

Genetic variation and trait selection in ryegrass (*Lolium multiflorum* Lam.) across dry and rainy seasons in Peru

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Abstract

Ryegrass (*Lolium multiflorum* Lam.) is an important forage species in Peruvian livestock micro-watersheds; however, increasing climatic variability underscores the need to identify promising germplasm for breeding programs. Twenty-three accessions, including three widely used checks in the area, were evaluated under an augmented block design across three livestock micro-watersheds in the Amazonian Andes of Peru, using mixed linear models with REML/BLUP for the dry and rainy seasons and a combined analysis. The study aimed to determine variance components, Cullis heritability, and correlations across fifteen selected traits, as well as to identify promising accessions through the Elston index and principal component analysis for plant height, basal coverage area, dry matter concentration, crude protein, neutral detergent fiber, and in vitro digestibility. The results revealed significant variation among accessions for most agronomic traits, with the exception of stem diameter. Heritability for in vitro digestibility was zero during the dry season. Plant height, basal coverage area, spike and spikelets number exhibited high heritability (>0.60) in both seasons, while crude protein showed moderate heritability (dry season = 0.54; rainy season = 0.51). Dry matter concentration displayed high heritability during the rainy season and moderate heritability during the dry season. Principal component analysis indicated greater phenotypic differentiation during the rainy season (73.6%) than during the dry season (58.1%). Furthermore, the Elston index identified LM20 as the most promising accession in the combined analysis and dry season, outperforming the checks. LM5 showed performance comparable to the check varieties, whereas LM18 exhibited the most balanced profile during the rainy season. These findings provide a quantitative foundation for future breeding programs targeting the conditions of the Amazonian Andes.

Keywords Forage traits · Genetic inheritance · Heritability · Index selection · Multivariate analysis · Seasonal variation

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Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) is an annual forage grass widely used in warm regions, with a natural and deliberate domestication process¹. It is an essential feed resource for livestock systems in Latin America and Asia, particularly in Peru, Brazil, South Korea, Japan, and China^{2,3,4}. It exhibits high dry matter production potential per unit area, rapid growth, and good nutritional quality⁵, along with a greater nitrogen uptake capacity than other species within the genus⁶.

Although *Lolium* is a genus introduced to the Peruvian Andes, it exhibits broad genetic diversity with the potential to respond to adverse environmental conditions, including frost and drought resistance⁷. For this reason, livestock producers and farmers have contributed to preserving forage genetic resources adapted to local needs; however, the management of these resources has received limited attention despite its potential for breeding programs^{8,9}. Compared with other *Lolium* spp., *Lolium multiflorum* Lam. thrives across a wide range of environments¹⁰, with an adaptive range between 465 and 3,740 m a.s.l.^{11,12}, although its agronomic and nutritional performance is strongly influenced by the agroecological conditions of cultivation^{13,14}.

On a global scale, climate change has substantially affected more than 70% of the Earth's land surface by altering temperature and precipitation patterns¹⁵, and in Peru's Andean region and Amazon, water scarcity increasingly threatens agricultural production and food security^{16,17}. In the Amazonas Region, extreme drought events recorded between 2000 and 2024 show an increasing trend, and their frequency and intensity are projected to rise¹⁸, with negative consequences for pasture availability and the risk of livestock mortality^{19,20}. Under these conditions, the selection of promising materials must integrate attributes linked to persistence and nutritional value, such as in vitro digestibility, crude protein, and basal cover^{21,22}. Furthermore, land-use changes over the past decade have affected approximately 16% of the Peruvian Amazon; of this area, nearly 70% shows degradation associated with low-productivity native grasses and previously improved but currently degraded pastures²³, underscoring the urgency of elite materials with superior agronomic and nutritional traits under variable environmental conditions.

The diversity of local ryegrass accessions in the Peruvian Andes²⁴ provides a foundation for developing improved varieties adapted to the target region. Current breeding programs focus on yield, quality, and persistence traits under successive cuts and across seasons²⁵, and improvements in dry matter production of *Lolium perenne* have already been reported in the United Kingdom²⁶. However, in the Peruvian Andes, the selection of promising accessions to support future breeding programs targeting traits such as basal cover, plant height, dry matter concentration, in vitro digestibility, neutral detergent fiber, and crude protein remains largely unexplored. Identifying superior accessions first requires quantifying what proportion of phenotypic variation is under genetic control, that is, estimating variance components and heritability, since only heritable variation responds to selection²⁷. Furthermore, given that breeding objectives integrate productive and nutritional traits that may exhibit negative genetic correlations, such as the negative association between dry matter yield and crude protein²⁸, multi-trait selection indices offer an integrated criterion that selection based on a single trait cannot achieve. Different multi-trait selection approaches have been developed over time, with varying data requirements and underlying assumptions²⁹. In the present study, the Elston multiplicative index³⁰ was used as a selection criterion, following its established use in breeding programs for the simultaneous selection of productive and quality traits^{31,32}.

In this context, the livestock micro-watersheds of the Amazonas department offer suitable environments for evaluating the agronomic performance of ryegrass accessions outside their high-Andean areas of origin. Information on variance components and heritability for agronomic and nutritional traits in local ryegrass accessions, however, remains scarce under Amazonian Andean conditions, which limits assessment of their selection potential and their use in breeding programs. The present study therefore evaluated accessions from the highlands of northern Peru across three livestock micro-watersheds in Amazonas, with the objectives of (i) estimating variance components and heritability for agronomic and nutritional traits; (ii) determining correlations between

production and nutritive value traits; and (iii) identifying superior accessions using Elston's multi-trait selection index together with multivariate analyses, separately for the dry and rainy seasons. We hypothesized that (i) agronomic and nutritional traits show significant genetic variation among accessions, with heritabilities large enough to support a response to selection; (ii) production and nutritive value traits are significantly associated; and (iii) accession rankings differ between seasons, justifying season-specific selection criteria. Because the best ryegrass genotypes in one season are not necessarily the best in the other³³, we adopted a two-level analytical strategy: a combined model integrating the four harvests to estimate mean annual genetic performance, and season-specific models to characterize differential responses between the dry and rainy seasons. Accessions identified as superior through this approach could provide valuable germplasm for developing new cultivars adapted to livestock systems in the Amazonian Andes, and support the design of forage systems that are more resilient to climate variability.

Materials and methods

Description of the study area

Field experiments were conducted between April and December 2025 in the Amazonas Region, Peru. The study area comprised the livestock micro-watersheds of Molinopampa (6°12'34.25" S, 77°39'46.55" W; 2,377 m a.s.l.), Pomacochas (5°49'29.96" S, 77°58'5.01" W; 2,238 m a.s.l.), and Leimebamba (6°41'51.96" S, 77°48'19.07" W; 2,518 m a.s.l.). Soil sampling and analysis were performed in each micro-watershed in accordance with Peru soil regulations (Supreme Decree No. 013-2010-AG)³⁴. Sampling was performed using a zigzag transect with 10 sampling points. At each point, the soil surface was cleared and a spade was driven to a depth of 30 cm. The collected subsamples were mixed and homogenized. Subsequently, by the quartering method, a composite sample of 1 kg per livestock micro-watershed was obtained³⁵. All samples were coded and transferred to the Laboratorio de Investigación de Suelos y Aguas (LABISAG) of the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, accredited by the Instituto Nacional de Calidad (INACAL) of the Ministerio de la Producción, in accordance with standard NTP-ISO/IEC 17025:2017³⁶. Physicochemical properties were determined following the methodology of Bazán Tapia³⁷ (Supplementary Table S1).

Monitoring of environmental conditions

During the experimental period (April–December 2025), key meteorological variables were recorded during the dry (April–August) and rainy (September–December) seasons using a Vantage Pro2 weather station (Davis Instruments Corp., Hayward, CA, USA) installed in each micro-watershed. During the dry season, mean temperatures in Leimebamba, Molinopampa, and Pomacochas were 14.46 ± 1.30 °C, 14.02 ± 0.72 °C, and 15.30 ± 1.03 °C, respectively, whereas during the rainy season they were 13.41 ± 1.03 °C, 13.05 ± 1.05 °C, and 14.18 ± 0.81 °C (Fig. 1a). Total precipitation during the dry season was 274.8 mm in Leimebamba, 455.8 mm in Molinopampa, and 568.9 mm in Pomacochas, whereas during the rainy season it was 357.2 mm, 600.7 mm, and 1,401.7 mm in Leimebamba, Molinopampa, and Pomacochas, respectively (Fig. 1b). Relative humidity during the dry season for Leimebamba, Molinopampa, and Pomacochas was $86.15 \pm 4.26\%$, $88.13 \pm 3.68\%$, and $88.07 \pm 4.65\%$, respectively, whereas during the rainy season it was $88.94 \pm 2.48\%$, $90.62 \pm 3.25\%$, and $91.77 \pm 3.23\%$ (Fig. 1c).

Plant material collection

Twenty accessions of ryegrass were acquired from the Programa Nacional de Investigación de Pastos y Forrajes of the Instituto Nacional de Innovación Agraria (INIA), belonging to the Estación Experimental Agraria Baños del Inca, located in the Cajamarca Region (7°09'56" S, 78°27'07" W; 2,667 m a.s.l.). These accessions were

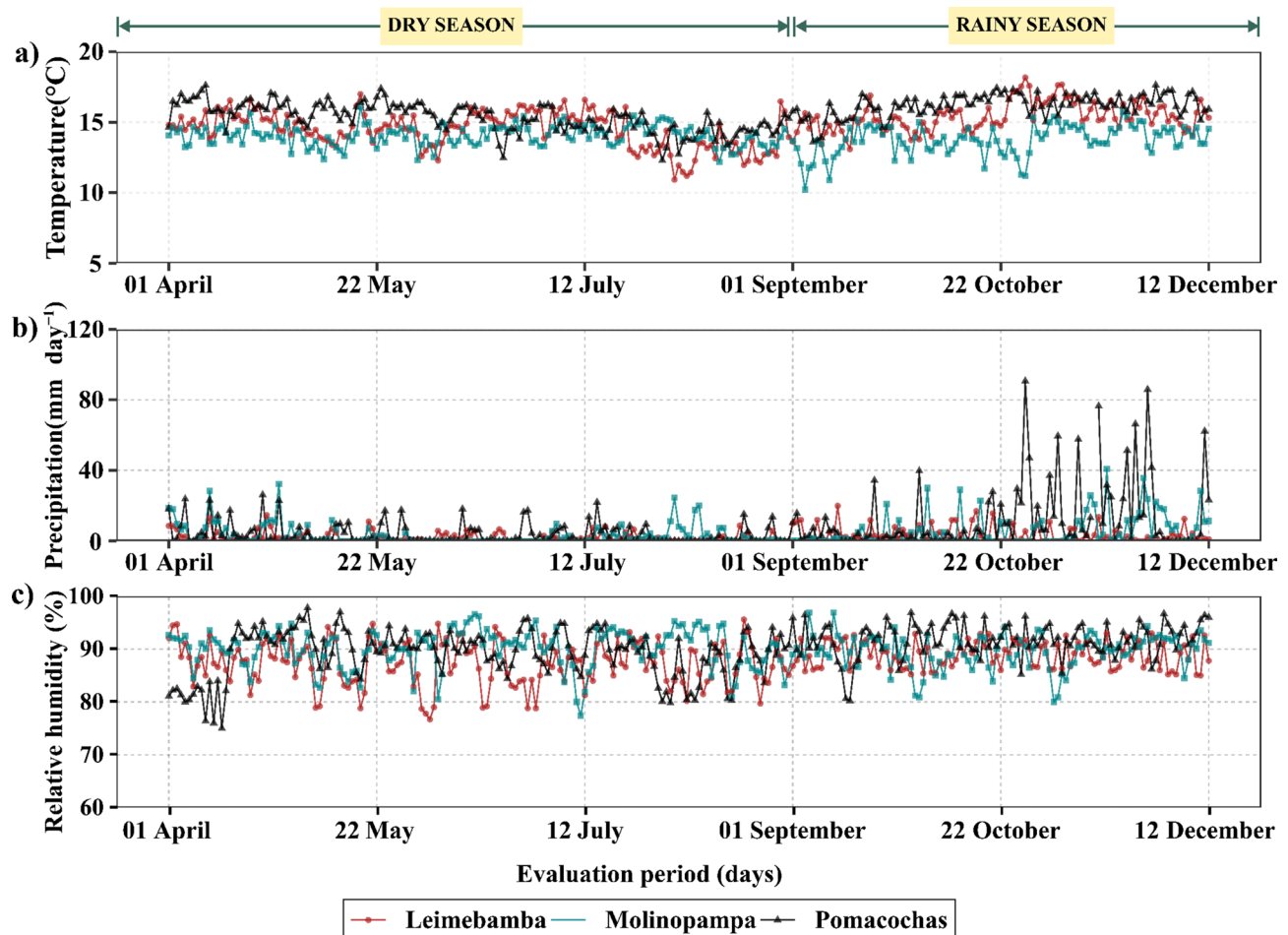


Fig. 1 Meteorological conditions across the three micro-watersheds. **(a)** Mean temperature, **(b)** relative humidity, and **(c)** precipitation recorded in Leimebamba, Molinopampa, and Pomacochas.

originally collected from different provinces of the Cajamarca Region and preserved in the genebank of the Instituto Nacional de Innovación Agraria (INIA) for future research; collection sites are detailed in Supplementary Table S2 and Supplementary Figure S1. In addition, three accessions frequently used by livestock farmers in the three micro-watersheds of the Amazonas region were included as controls: INIA 910–Kumymarca (LM21), the Cajamarquino ecotype (LM22), and PACCHA_EEAA (LM23) obtained from the INIA germplasm bank.

All accessions were vegetatively propagated from vigorous mother plants in excellent phytosanitary condition, in order to obtain genetic material identical to that of the mother plant. Planting was carried out by forming clumps (planting points) of eight tillers, each 12 cm long, extracted with a portion of soil adhering to the roots. The plant material was transported in kraft paper bags, with damp towels placed inside to prevent dehydration.

Experimental design

Field experiments were established independently in each of the three livestock micro-watersheds using an Augmented Block Design, a design commonly used for the primary evaluation of germplasm collections when plant propagation material is scarce³⁸. The spatial arrangement of experimental plots within each micro-watershed was generated using the RCBDAugmented function from the FieldHub package³⁹ in R version 4.5.0⁴⁰ within the RStudio environment⁴¹. The design consisted of five blocks arranged in horizontal rows (Fig. 2). Within each block, three control accessions or checks were included: INIA 910–Kumymarca (LM21), Ecotipo Cajamarquino

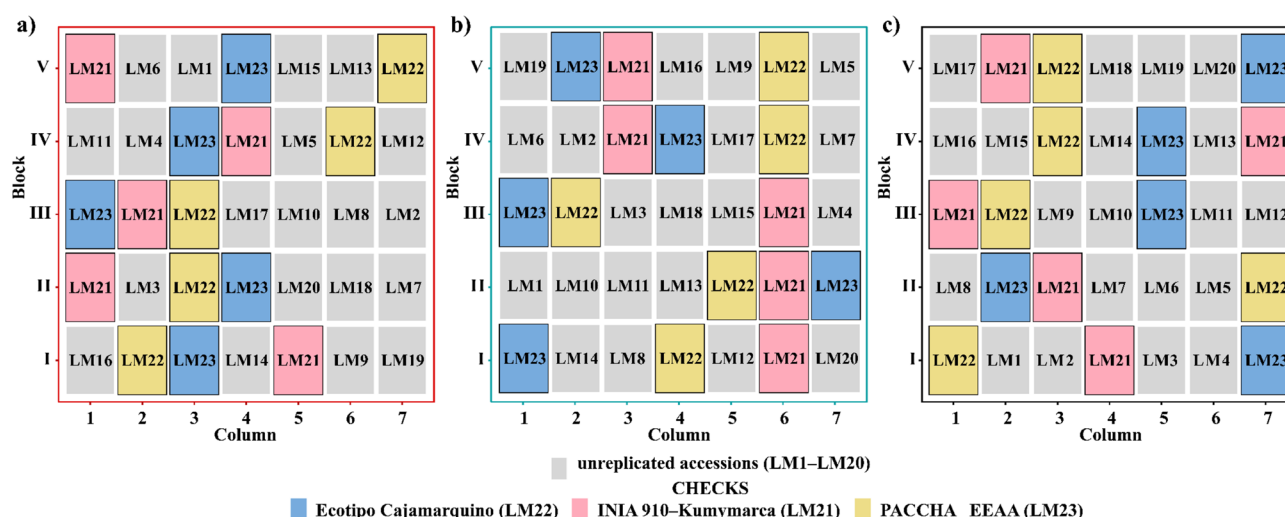


Fig. 2 Field layout of the augmented block design in the livestock micro-watersheds of Leimebamba (a), Molinopampa (b), and Pomacochas (c).

(LM22), and PACCHA_EEAA (LM23), placed in random positions and replicated across the five blocks, serving as internal references for the statistical adjustment of spatial heterogeneity. The remaining 20 experimental accessions (LM1–LM20) were not replicated, with each assigned to a single block. This design yielded a total of 35 plots per livestock micro-watershed (5 blocks $\times$ 7 columns), corresponding to Leimebamba (Fig. 2a), Molinopampa (Fig. 2b), and Pomacochas (Fig. 2c). Phenotypic evaluation was conducted over four successive cuttings: the first and second during the dry season, and the third and fourth during the rainy season.

Establishment of the experimental area

The experimental area was prepared by conventional tillage to a depth of 30 cm, followed by harrowing and surface leveling. Each experimental unit consisted of a 2 m $\times$ 3 m plot (6 m²), with 1 m separation between plots and between blocks. Establishment was carried out by manually transplanting ryegrass tillers, with eight clumps of eight tillers per plot, spaced 0.75 m apart between clumps and between rows. Two weeks before transplanting, poultry litter was applied as basal fertilization at a rate of 300 g m⁻², with N, P, and K concentrations of 26.7, 37.4, and 21.9 g kg⁻¹, respectively. This was the only fertilization throughout the entire experimental period.

During the two weeks following transplanting, sprinkler irrigation was applied (46 mm per application) to ensure establishment; thereafter, irrigation was suspended and the crop was maintained under natural rainfall. Weed control was performed manually every 10 days. Phytosanitary management was applied preventively at each cut, using the fungicide Protexin (carbendazim 500 g L⁻¹) and the insecticide Colam (thiamethoxam 141 g L⁻¹ + lambda-cyhalothrin 106 g L⁻¹), through uniform foliar spraying under the same protocol across the three micro-watersheds. Four successive cuts were performed at 5 cm above ground level: the first and second during the dry season at 60-day intervals, and the third and fourth during the rainy season at 45-day intervals.

Evaluation of agronomic traits

Plant height data were obtained by measuring from the base of the stem at ground level to the tip of the most extended leaf using a Bahco measuring tape (SNA Europe SAS, Éragry-sur-Oise, France). Values were expressed in cm. Basal cover area was determined using the ellipse area formula^{42,43} as shown in Eq. 1.

$$\text{Basal coverage area (cm}^2\text{)} = \pi \left(\frac{D1}{2} \right) \left(\frac{d2}{2} \right) \quad (1)$$

Where D1 is the major diameter and d2 is the minor diameter of each plant measured immediately after cutting at 5 cm above ground level.

The number of stems was recorded per clump in four clumps per experimental unit, and stem diameter was measured on eight stems using a digital caliper (Dasqua®, model 2000–2010, accuracy ± 0.02 mm) at 5 cm from the plant base⁴⁴. The number of leaves was quantified on eight tillers per clump, in four clumps per experimental unit. For leaf dimensions, eight fully expanded leaves were selected per clump from the middle third of the plant, excluding senescent or still-expanding leaves; leaf length was measured with the same digital caliper from the ligule to the apex, and width was measured at the midpoint of the blade, perpendicular to the midrib, both expressed in mm.

The number of spikes was counted at the end of each cutting cycle in four clumps per experimental unit, and five spikes randomly collected per clump were measured from the first node of the rachis to the spike apex⁴⁵, expressed in cm. The number of spikelets per spike was determined by counting the spikelets inserted on the rachis, on five randomly selected spikes per clump, in four clumps per experimental unit⁴⁶.

The number of tillers was assessed at each of the four cuts. In each experimental unit, four clumps were randomly selected, and within each clump, four tillers were marked with colored PVC rings (6 mm in diameter), placed loosely at the tiller base to avoid restricting growth, following the methodology of Laidlaw⁴⁷. At each successive cut, basal tillers that had emerged since the previous evaluation were identified and marked with a color-specific ring for each cycle, allowing the dynamics of new tiller emergence to be recorded throughout the experimental period.

For dry matter concentration, the clumps within plots were cut at a residual height of 5 cm, and the harvested forage was weighed in situ with an electronic balance (accuracy ± 5 g) to record fresh weight. Subsamples were oven-dried at 60 °C for 72 h until constant weight, and dry matter concentration (%) was calculated as dry weight divided by fresh weight $\times 100$.

Nutritive value

Samples for nutritional analysis were obtained from the same clumps used for agronomic measurements. Four clumps per experimental unit were harvested at a residual height of 5 cm, and the material from the four clumps was combined and homogenized into a single composite sample per experimental unit. The composite sample was cut with scissors to obtain 250 to 500 g of representative subsample, which was placed in labeled and tared kraft paper bags. The samples were transported to the Agrostology Laboratory at the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas, where they were dried at 60 °C for 72 h or until constant weight, and subsequently ground in a hammer mill to pass through a 2 mm sieve. Neutral detergent fiber (NDF) was determined using the ANKOM A200 procedure with neutral detergent solution in the presence of alpha-amylase and sodium sulfite, followed by acetone rinsing (5 min), evaporation at room temperature (~15 min), and final drying at 105 °C for 2 h.

In vitro dry matter digestibility (IVDMD) was determined using the ANKOM DAISY II system, following the standardized ANKOM Technology protocol for fibrous forages. Dried and ground samples were weighed at 0.50 g into ANKOM F57 filter bags prewashed with acetone (5 min) and air-dried. Buffer solutions A and B were prepared according to the manufacturer's specifications, preheated to 39 °C, and mixed in a 1:5 ratio (B:A) to reach pH 6.8, adding 1,600 mL per jar, which was placed in the incubator, allowing thermal stabilization for 30 min before adding the ruminal inoculum. The ruminal fluid was obtained from the ruminal contents of cattle slaughtered at an abattoir, collected immediately after slaughter in a thermos preheated to 39 °C, and transported to the laboratory within 30 min, where it was homogenized in a blender purged with CO₂ (30 s) and filtered through four layers of cheesecloth. Four hundred milliliters of ruminal inoculum were added per jar, which was

sealed and incubated at 39.5 ± 0.5 °C for 48 h with continuous agitation. After incubation, the bags were rinsed with cold distilled water and treated with neutral detergent solution to remove microbial residues, and IVDMD was calculated from the final NDF residue with blank-bag correction, applying the standard equations of the DAISY II method.

Crude protein concentration was determined by the Dumas dry combustion method⁴⁸. Samples were predried (40 ± 2 °C), ground in a knife mill and sieved to 150 µm (No. 100 mesh), and approximately 200 mg of sample were weighed into tared ceramic crucibles (± 0.1 mg). The analysis was performed on a LECO CN928 analyzer (LECO Corporation, St. Joseph, MI, USA), operated with the Cornerstone software, through combustion at 1,100 °C. Total released nitrogen was quantified with a thermal conductivity detector (TCD), and crude protein concentration was calculated by applying the conversion factor $N \times 6.25$. Quality control included sample blanks, certified reference materials, and duplicates in each analytical sequence.

Mixed linear models

To determine performance across cuts as a differential response between seasons, two analytical strategies were applied following the methodology of Matias et al.⁴⁹: a joint model that integrated the four cuts to estimate performance throughout the entire evaluation period, and a by-season model to independently assess the dry and rainy seasons with the aim of capturing the season-specific genetic control, since accessions with outstanding traits in one season are not necessarily the best in another³³, thereby complementing the selection capacity of the accessions⁵⁰.

Joint model for the four cuttings

Data were analyzed using mixed linear models, with accession treated as a random effect, allowing the estimation of best linear unbiased predictors (BLUPs) of genotypic values⁵¹, to accommodate for unbalanced datasets⁵². Variance components were estimated by restricted maximum likelihood (REML). For the joint analysis of the four cuts, Eq. 2 was fitted:

$$y_{ijklm} = \mu + L_i + G_j + C_k + (G \times C)_{jk} + (G \times L)_{ji} + B_{l(i)} + P_m + \epsilon_{ijklm} \quad (2)$$

where μ : intercept, L_i : fixed effect of the i -th livestock micro-watershed, G_j : random effect of the j -th accession, C_k : random effect of the k -th cut, $(G \times C)_{jk}$: random accession $\times$ cut interaction, $(G \times L)_{ji}$: random accession $\times$ livestock micro-watershed interaction, $B_{l(i)}$: random effect of block nested within livestock micro-watersheds, P_m : random effect of plot, ϵ_{ijklm} : residual error.

Model by season

The analyses by season (rainy and dry) were conducted independently, considering the interaction between livestock micro-watershed $\times$ cut as the environment, which generated six environments per season (3 livestock micro-watersheds $\times$ 2 cuts). Accession was established as a random effect to obtain the BLUPs of genotypic values. Variance components were estimated using restricted maximum likelihood (REML). Equation 3 was fitted:

$$y_{ijk} = \mu + G_i + E_j + (G \times E)_{ij} + P_k + \epsilon_{ijk} \quad (3)$$

where μ is the intercept, G_i is the random effect of the i -th accession, E_j is the random effect of the j -th environment, $(G \times E)_{ij}$ is the random accession $\times$ environment interaction, P_k is the random plot effect, and ϵ_{ijk} is the residual error.

Cullis heritability estimation

Heritability was estimated following the method of Cullis et al.⁵³, as shown in Eq. 4:

$$H^2_{\text{Cullis}} = 1 - \frac{V_{\Delta}^{BLUP}}{2\sigma_g^2} \quad (4)$$

where σ_g^2 is the genotypic variance estimated by REML y V_{Δ}^{BLUP} is the mean variance of pairwise differences between genotypic BLUPs.

Heritability was interpreted using the classification of Johnson et al.⁵⁴, defining three categories: low (<0.30), moderate (0.30–0.60), and high (>0.60).

Elston index

As a selection criterion, the Elston multiplicative index³⁰ was employed in this initial phase of the breeding program. The Elston index was chosen for the present study for the following reasons: (i) it has been previously used in breeding programs for the simultaneous selection of productive and quality traits; and (ii) its multiplicative nature penalizes low performance across traits, thereby favoring the selection of genotypes with balanced expression of the evaluated characteristics, which is a relevant feature for forages combining productive and nutritive-value attributes^{31,32}. In this study, the index integrated six agronomic and nutritive value traits: plant height, basal cover, dry matter concentration, crude protein, neutral detergent fiber (NDF), and in vitro digestibility, computed on standardized genotypic BLUPs (z-scores). For NDF, a reflection transformation was applied prior to index calculation, given that lower concentrations are associated with better nutritional value. This transformation reversed the ranking order while preserving the relative distances among accessions, ensuring that genotypes with lower NDF concentrations received higher index values, consistent with the desired selection direction. The Elston index multiplicatively penalized low trait values and, through the definition of minimum thresholds, reduced the possibility of compensation among traits, so that high performance in some traits could hardly compensate for failure to meet the minimum acceptable value in another, thereby favoring the selection of more balanced genotypes. The index was calculated using the following expression (Eq. 5):

$$E_i = \prod_{j=1}^J (s_{ij} - k_j), \quad k_j = \min(s_j) \quad (5)$$

where: E_i is the Elston index value for the i-th accession, $\prod_j =$ is the product across all j evaluated traits, s_{ij} is the standardized BLUP (z-score) of the j-th trait for the i-th accession, calculated as follows: k_j is the lower limit of the j-th trait, defined as the minimum z-score value observed across accessions for each trait, using the minimum z-score as the lower limit to ensure that all product terms remain non-negative (Eq. 6), where:

$$s_{ij} = \frac{(x_{ij} - \bar{x}_j)}{SD_j} \quad (6)$$

in which x_{ij} is the BLUP of the i-th genotype for the j-th trait, $\bar{x}_j$ is the mean y SD_j is the standard deviation of the j-th trait BLUPs across all accessions.

Data analysis

Data were analyzed in R version 4.5.0⁴⁰ using the RStudio environment⁴¹. Quantitative variables were analyzed using linear mixed models with the lmer() function from the lme4 package⁵⁵. The significance of random effects was assessed using the restricted likelihood ratio test implemented in the ranova() function from the lmerTest

package⁵⁶, with a significance level of $\alpha=0.05$. Heritability was estimated using the `h_cullis()` function from the `agriutilities` package⁵⁷.

Adjusted means per accession were extracted by changing the accession term from a random effect to a fixed effect, both in the joint model for the four cuts and in the by-season model. These values were subsequently used for principal component analysis (PCA) and phenotypic inter-trait correlation analyses. PCA was applied to agronomic and nutritional traits with direct implications for livestock productivity and forage quality: plant height, basal ground cover, dry matter concentration, crude protein, neutral detergent fiber, and in vitro digestibility. Prior to analysis, all variables were standardized using z-score transformation to ensure comparability across traits with different units. PCA was performed with the `prcomp()` function, and the results were visualized as a biplot generated with the `factoextra` package⁵⁸.

Associations among all evaluated phenotypic traits were estimated using Spearman correlation, with p-values computed via the `rcorr()` function from the `Hmisc` package⁵⁹. The strength of correlations was interpreted according to the correlation coefficient ranges proposed by Sudika et al.⁶⁰ and visualized as a correlation matrix constructed using the `corrplot` package⁶¹. Finally, Elston's multiplicative selection index was calculated using the `elston()` function from the `st4gi` package⁶².

Results

Estimation of variance components and heritability

The majority of agronomic and nutritional traits were affected by the accession effect, revealing genetic differences among accessions according to the likelihood ratio test (Table 1). However, stem diameter, in vitro digestibility, and neutral detergent fiber, showed no significant accession effect, indicating limited genetic variability in the evaluated population for these traits. The accession $\times$ cut interaction confirmed that accessions performed differently across cuts, except for leaf number and tiller number. The accession $\times$ livestock micro-watershed interaction influenced leaf number, stem diameter, basal cover area, dry matter concentration, in vitro digestibility, and

Table 1 Estimated variance components for accession (σ^2g), accession $\times$ cut interaction (σ^2gc), accession $\times$ livestock micro-watershed interaction (σ^2gl), cut (σ^2c), and block nested within livestock micro-watershed ($\sigma^2b_{(mw)}$); Cullis heritability (H^2_{Cullis}); and trait mean ($\bar{X}$) in ryegrass, based on a joint analysis across four cuttings.

Trait	σ^2g	σ^2gc	σ^2gl	σ^2c	$\sigma^2b_{(mw)}$	H^2_{Cullis}	$\bar{X}$
Plant height (cm)	105.64***	2.91*	2.05ns	33.45***	11.19*	0.84	84.52
Number of leaves	0.02*	0.00ns	0.01*	0.01*	0.00ns	0.51	3.91
Leaf length (cm)	8.77***	1.12***	1.26ns	12.80***	0.00ns	0.75	27.90
Leaf width (cm)	0.37***	0.06**	0.00ns	0.79***	0.00ns	0.72	7.68
Stem diameter (cm)	0.01ns	0.01**	0.01*	0.20***	0.00ns	0.30	2.50
Number of tillers	0.02*	0.00ns	0.00ns	0.14***	0.00ns	0.26	1.71
Number of stems	597.40*	199.30**	386.70*	3116.20***	199.60*	0.46	202.48
Basal coverage area (cm ²)	511.50**	160.20***	558.10***	2521.60***	0.00ns	0.61	132.17
Number of spikes	921.68***	373.22***	47.34ns	3678.85***	38.91ns	0.69	105.23
Spikes length (mm)	3.70**	1.05*	0.00ns	17.59***	2.01**	0.60	29.78
Number of spikelets	2.95***	0.43*	0.13ns	5.04***	0.06ns	0.74	21.75
Dry matter concentration (%)	1.87**	1.24**	1.69***	1.68***	0.00ns	0.54	21.88
In vitro digestibility (%)	0.00ns	1.12*	3.15**	3.97***	0.00ns	0.00	59.29
Neutral detergent fiber (%)	0.52ns	0.51**	2.23**	7.60***	0.00ns	0.25	51.68
Crude protein (%)	0.57*	1.05***	0.00ns	13.87***	0.00ns	0.41	15.60

Asterisks indicate the significance of the likelihood ratio test (LRT) for each random effect of the model, where: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant ($p > 0.05$).

neutral detergent fiber, indicating that accession performance varied by livestock micro-watershed for these traits. Cullis heritability estimates ranged from 0.00 (for in vitro digestibility) to 0.84 (for plant height), representing the minimum and maximum observed values. Overall, most traits exhibited moderate ($0.30 < H^2_{Cullis} < 0.60$) to high ($H^2_{Cullis} > 0.60$) heritabilities, while tiller number and neutral detergent fiber showed low values ($H^2_{Cullis} < 0.30$).

In each season, most traits were influenced by the accession effect, according to the likelihood ratio test (Table 2). For both the dry and rainy seasons, the accession effect was significant for nine traits: plant height, leaf width and length, basal cover area, number and length of spikes, number of spikelets, dry matter concentration, and crude protein. The number of leaves and the number of stems were affected by the accession effect only during the rainy season (Table 2); in the dry season, this significant effect disappeared, indicating limited genetic variability for these traits under that condition. In vitro digestibility showed no accession effect during the dry season, accompanied by a null variance component, whereas during the rainy season this effect was present. The environmental effect was significant for a greater number of traits during the rainy season than during the dry

Table 2 Estimated variance components for accession (σ^2_g), environments (σ^2_a), accession $\times$ environments interaction (σ^2_{ga}); Cullis heritability (H^2_{Cullis}); and trait mean ($\bar{X}$) in ryegrass, based on independent analyses for the dry and rainy seasons.

Dry season					
Trait	σ^2_g	σ^2_a	σ^2_{ga}	H^2_{Cullis}	$\bar{X}$
Plant height (cm)	92.93***	34.26**	31.83*	0.76	84.24
Number of leaves	0.01ns	0.00ns	0.04***	0.41	3.93
Leaf length (cm)	10.99***	3.21*	5.75***	0.75	30.63
Leaf width (cm)	0.49***	0.25*	0.08ns	0.71	8.43
Stem diameter (cm)	0.00ns	0.00ns	0.14***	0.07	2.86
Number of tillers	0.00ns	0.00ns	0.06***	0.00	1.97
Number of stems	319.40ns	840.50***	23.60ns	0.41	173.39
Basal coverage area (cm ²)	355.20***	0.00ns	776.80***	0.70	98.19
Number of spikes	313.20***	0.00ns	494.90***	0.65	64.42
Spikes length (mm)	5.72*	4.85ns	7.65**	0.53	33.15
Number of spikelets	2.86***	0.42ns	3.61***	0.70	23.49
Dry matter concentration (%)	2.52*	0.00ns	13.26***	0.48	21.93
In vitro digestibility	0.00ns	1.92*	6.47***	0.00	60.16
Neutral detergent fiber (%)	1.13*	1.53*	2.07***	0.45	49.97
Crude protein (%)	1.16**	0.00ns	1.02ns	0.54	17.85
Rainy season					
Trait	σ^2_g	σ^2_a	σ^2_{ga}	H^2_{Cullis}	$\bar{X}$
Plant height (cm)	136.9***	35.24***	0.00ns	0.85	84.74
Number of leaves	0.02*	0.01**	0.00ns	0.54	3.88
Leaf length (cm)	7.64***	3.06***	0.71ns	0.72	25.21
Leaf width (cm)	0.27***	0.27***	0.08ns	0.70	6.94
Stem diameter (cm)	0.00ns	0.07***	0.00ns	0.23	2.17
Number of tillers	0.00ns	0.01***	0.00ns	0.28	1.41
Number of stems	1286.00***	10755.00***	0.00ns	0.52	233.58
Basal coverage area (cm ²)	1309.51***	2705.97***	1549.23***	0.80	166.53
Number of spikes	2173.73***	2084.11***	78.37ns	0.72	145.69
Spikes length (mm)	4.36***	0.88*	0.40ns	0.64	26.37
Number of spikelets	3.14**	1.80***	0.47ns	0.63	20.05
Dry matter concentration (%)	3.66***	3.99***	4.75***	0.75	21.82
In vitro digestibility	3.60*	7.28***	17.12***	0.47	58.46
Neutral detergent fiber (%)	1.30ns	5.35***	0.00ns	0.42	53.34
Crude protein (%)	1.00*	1.36***	0.84*	0.51	13.37

Asterisks indicate the significance of the likelihood ratio test (LRT) for each random effect of the model, where: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant ($p > 0.05$).

season. The accession $\times$ environment interaction was less pronounced during the rainy season; only basal cover area, dry matter concentration, in vitro digestibility, and crude protein were significant.

In general, the rainy season showed higher Cullis heritability values (H^2_{Cullis}) than the dry season for plant height (0.85 vs. 0.76), number of leaves (0.54 vs. 0.41), stem diameter (0.23 vs. 0.07), number of tillers (0.28 vs. 0.00), number of stems (0.52 vs. 0.41), basal cover area (0.80 vs. 0.70), number of spikes (0.72 vs. 0.65), spike length (0.64 vs. 0.53), dry matter concentration (0.75 vs. 0.48), and in vitro digestibility (0.47 vs. 0.00). According to established thresholds, plant height, leaf width and length, basal cover area, number of spikes, and number of spikelets exhibited high heritability in both seasons ($H^2_{Cullis} > 0.60$). In contrast, dry matter concentration showed high heritability only during the rainy season, while neutral detergent fiber (NDF) and crude protein displayed moderate heritability in both seasons. In vitro digestibility exhibited zero heritability during the dry season and increased to moderate levels during the rainy season. Regarding trait means, the rainy season recorded higher values for number of stems (233.58 vs. 173.39), basal cover area (166.53 vs. 98.19 cm²), and number of spikes (145.69 vs. 64.42). In contrast, crude protein showed a higher mean during the dry season (17.85 vs. 13.37%). Taken together, these results indicate that the rainy season exhibited higher H^2_{Cullis} values and superior means for certain agronomic traits, whereas the dry season showed a higher mean crude protein content.

Trait correlations

Significant correlations among the evaluated phenotypic traits were observed in both the combined analysis (dry and rainy seasons) and each season separately (Fig. 3). Across all analyses (Fig. 3a and b, and 3c), plant height exhibited positive and significant correlations of moderate strength with leaf length ($r=0.55$ – 0.61), leaf width ($r=0.44$ – 0.46), and number of spikes ($r=0.62$ – 0.67), of moderate to strong strength with basal cover area ($r=0.44$ – 0.73), and strong correlations with spike length ($r=0.76$ – 0.82). Similarly, leaf length demonstrated a strong correlation with leaf width ($r=0.70$ – 0.76), as well as positive correlations of moderate strength with number of tillers ($r=0.57$ – 0.68) and basal cover area ($r=0.47$ – 0.60). Leaf width, in turn, revealed moderate correlations with stem diameter ($r=0.54$ – 0.69). Likewise, number of tillers was moderately correlated with number of stems ($r=0.51$ – 0.60) and displayed moderate to strong correlations with basal cover area ($r=0.67$ – 0.77), while number of stems exhibited strong correlations with basal cover area ($r=0.74$ – 0.96) and moderate to strong correlations with number of spikes ($r=0.45$ – 0.88).

Dry matter concentration and crude protein showed a significant negative correlation in both the combined analysis ($r=-0.63$) and the rainy season ($r=-0.63$) (Fig. 3a and c). On the other hand, in vitro digestibility was significantly and negatively correlated with neutral detergent fiber across all analyses, with strong correlations in the combined analysis ($r=-0.78$) (Fig. 3a) and the rainy season ($r=-0.82$) (Fig. 3c), while a moderate correlation was observed during the dry season ($r=-0.58$) (Fig. 3b). The number of spikelets was significantly and negatively correlated with neutral detergent fiber during the dry season ($r=-0.45$), and also negatively correlated with crude protein during the rainy season ($r=-0.42$).

Principal component analysis

Principal component analysis enabled the identification of multivariate patterns among accessions in the combined analysis (dry and rainy seasons) and in each season separately. In the combined analysis, the first two principal components explained 35.1% and 32.2% of the total variance, accounting for a cumulative 67.3% (Fig. 4a). In the dry season, PC1 and PC2 explained 33.9% and 24.2% of the variance, with a cumulative value of 58.1%, while in the rainy season both components explained 51.6% and 22.0%, accumulating 73.6% of the total variance (Fig. 4b and c).

In the combined analysis, crude protein showed an opposing orientation relative to the vectors of plant height, basal cover area, and dry matter concentration. Likewise, in vitro digestibility and dry matter concentration displayed vector orientations opposite to neutral detergent fiber. In the dry season, dry matter concentration and

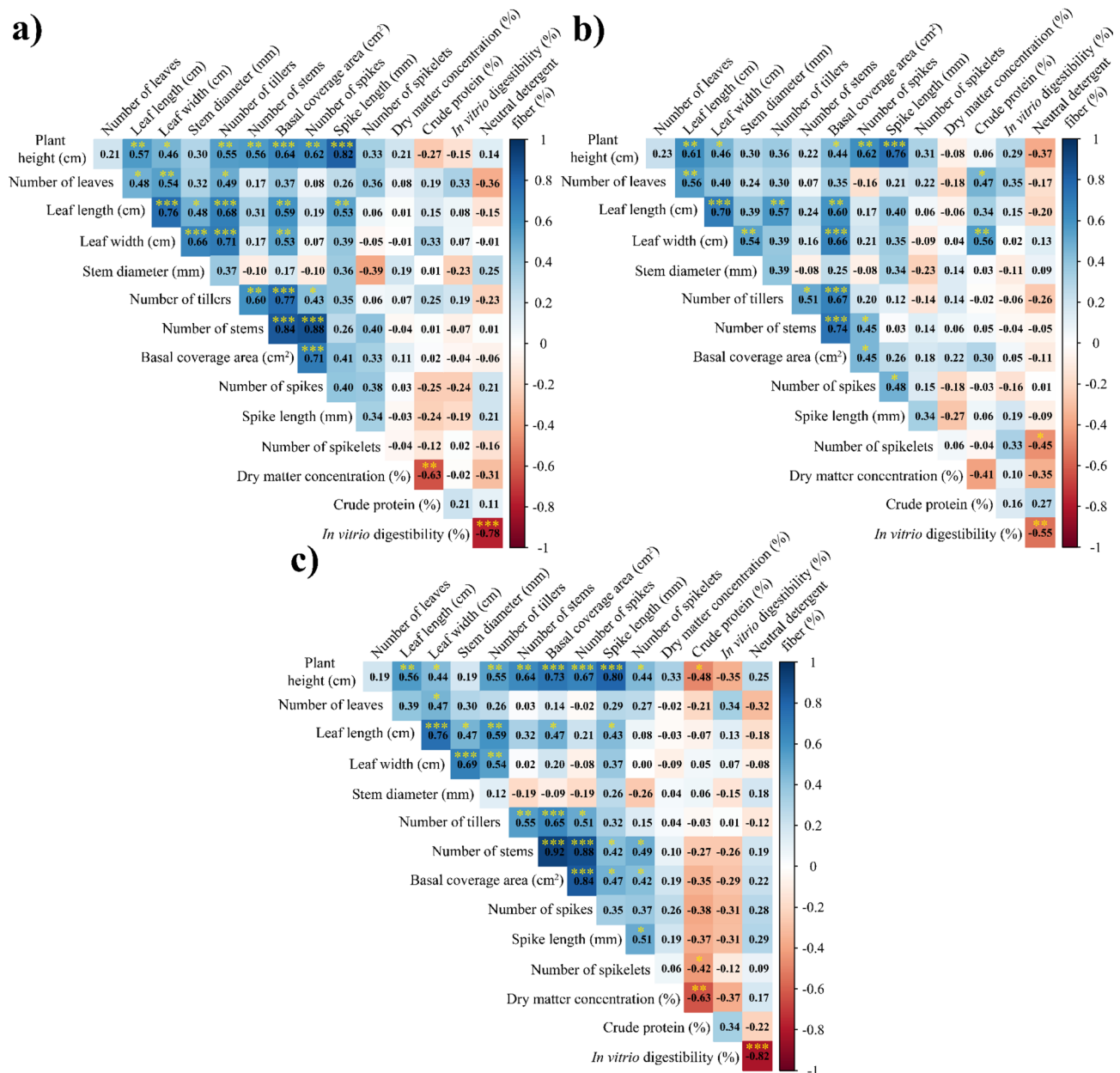


Fig. 3 Spearman correlations with adjusted means of phenotypic traits evaluated across both seasons combined (**a**), during the dry season (**b**), and during the rainy season (**c**).

in vitro digestibility showed similar vector orientations, while both were positioned in the opposite direction to neutral detergent fiber and crude protein. In the rainy season, dry matter concentration, plant height, and basal cover area exhibited similar orientations among themselves and opposite to crude protein. In contrast, *in vitro* digestibility showed an opposing direction to neutral detergent fiber, a pattern that remained consistent across all analyses. Accessions LM13 and LM4 were located in the quadrant associated with high crude protein and high *in vitro* digestibility in both the combined analysis and the rainy season. Accession LM10 showed a notable association with crude protein in the dry season. In contrast, LM20 was associated with basal cover area and plant height across all analyses, as shown in Fig. 4.

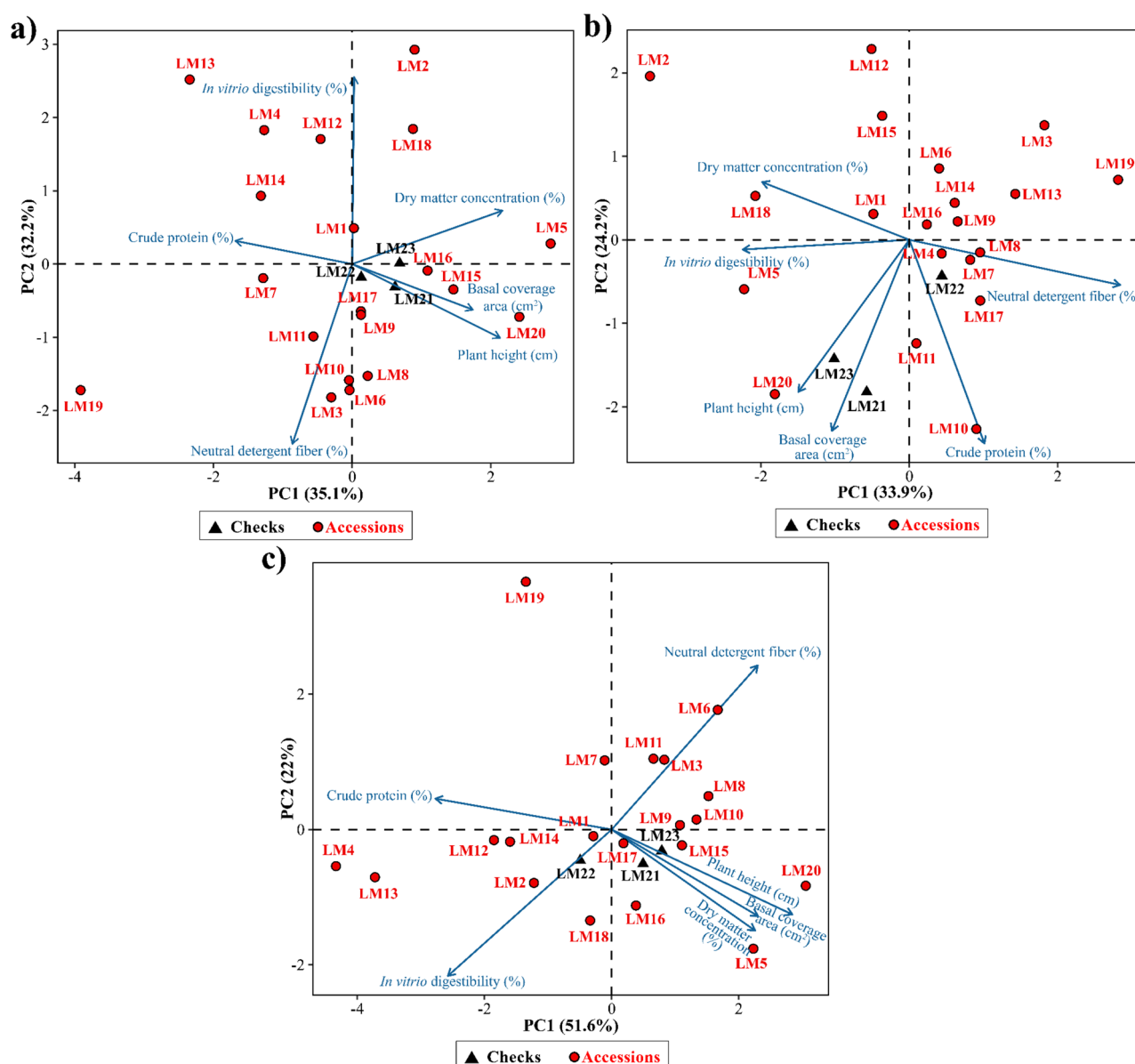


Fig. 4 Principal component analysis of phenotypic selection traits in joint analysis (a), dry season (b), and rainy season (c).

Elston index

The Elston index showed variation in the ranking of accessions depending on the type of analysis (Fig. 5). In the combined analysis (dry and rainy seasons), accessions LM3, LM13, LM15, and LM19 presented an index equal to zero, indicating that they did not exceed the minimum acceptable threshold in at least one of the evaluated traits. Among the remaining accessions, LM20 ranked first (Elston Index = 206.21), followed by LM21 (111.08), LM23 (103.25), and LM5 (79.20), with index values 85.6% and 99.7% higher than those of LM21 and LM23, respectively (Fig. 5a). Although LM5 presented a negative BLUP for crude protein, this did not nullify its index, suggesting that the value remained above the minimum threshold and that the remaining traits contributed to the final index (Fig. 5d; Supplementary Table 3).

During the dry season, LM20 retained the first position (Elston Index = 328.50), followed by LM23 (168.56), LM5 (117.38), and LM21 (106.27); accessions LM3, LM13, LM15, and LM19 again recorded null indices

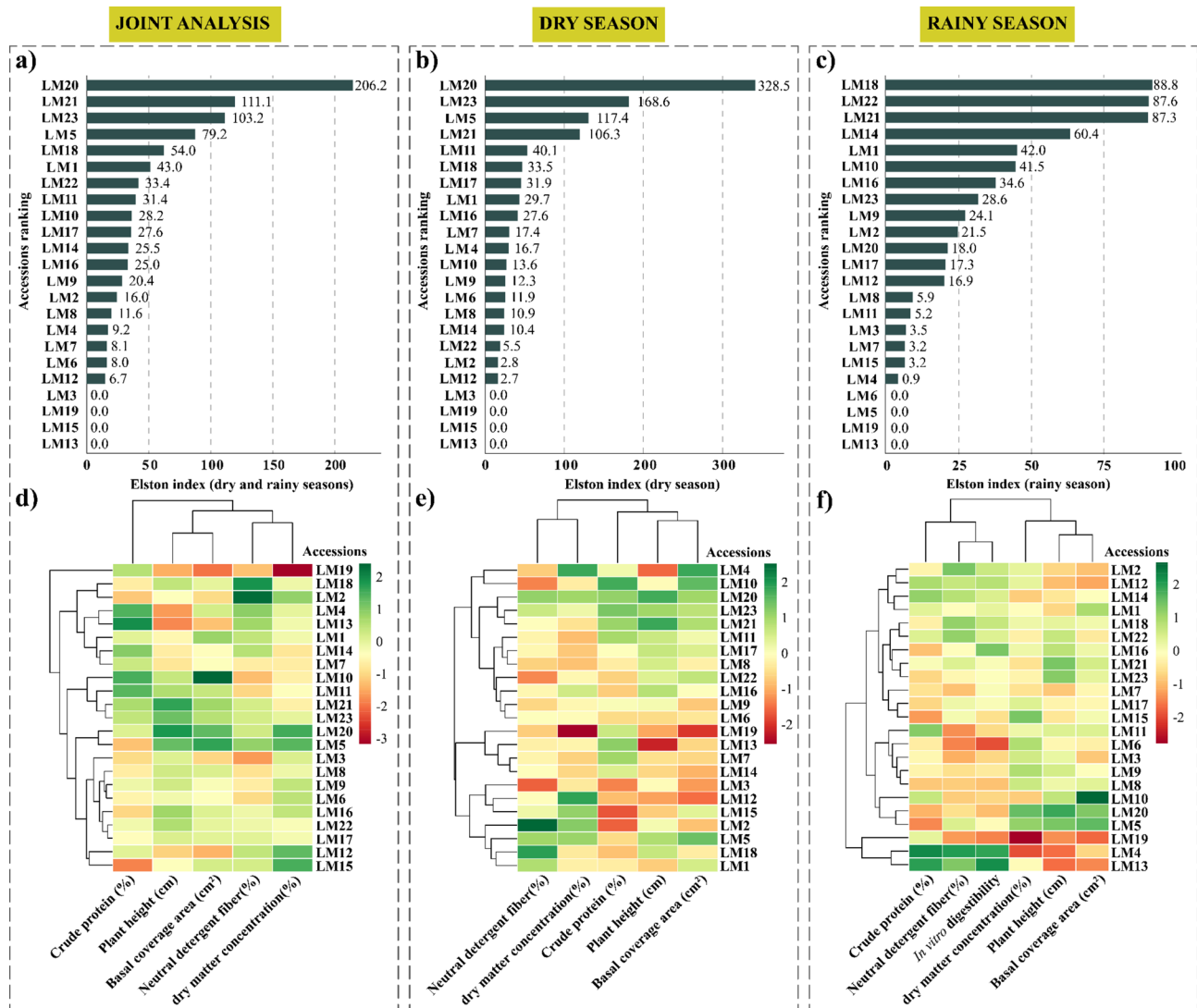


Fig. 5 Ranking of accessions according to the Elston index in the combined analysis (a), dry season (b), and rainy season (c), and performance of the standardized BLUPs for each trait in the Elston index for the combined analysis (d), dry season (e), and rainy season (f).

(Fig. 5b). LM20 exceeded LM23 by 94.9% and LM21 by 209.1% in index values for this season. In contrast, during the rainy season, LM20 dropped to the twelfth position, associated with negative BLUP values for crude protein, neutral detergent fiber, and *in vitro* digestibility (Fig. 5e; Supplementary Table 4). It should be noted that *in vitro* digestibility was excluded from the Elston index in the dry season and in the combined analysis, given that the accession variance component and the estimated heritability for this trait were equal to zero. During the rainy season, LM18 achieved the highest index value (Elston index = 88.79), followed by LM22 (87.60), LM21 (87.34) and LM14 (60.4); LM5 and LM6 presented null indices (Fig. 5c), associated with negative BLUP values for crude protein and *in vitro* digestibility (Fig. 5f; Supplementary Table 5). The unstandardized BLUPs by accession for all traits are presented in Supplementary Table 6.

Discussion

Most agronomic and nutritional traits showed a significant accession effect according to the likelihood ratio test (LRT), indicating that a portion of the observed variation was attributable to differences among accessions, supporting the existence of genetic variation and demonstrating its potential for selecting promising materials tested in this study and under the conditions of the Peruvian Amazonian Andes. These results highlight the broad genetic diversity exhibited by *Lolium multiflorum* Lam. as an allogamous and highly heterozygous species, positioning it as a valuable resource for breeding programs^{1,63,64}.

The accession effect was significant for nearly all agronomic traits in the combined analysis (dry and rainy seasons), with the exception of stem diameter, the only trait that showed no significant accession effect in any analysis. The absence of a significant effect on stem diameter could be attributed to the high phenotypic plasticity of this trait in response to environmental conditions. Research in widely distributed grass species demonstrated that growth traits such as stem diameter are significantly influenced by the growing environment, where plasticity plays a more decisive role than genetic variation in the expression of this trait⁶⁵. Although stem diameter is not typically considered a primary selection trait in breeding programs given that other vigor-related traits such as plant height and basal coverage area fulfill that function⁶⁶, it remains widely employed in morphological characterization⁶⁷.

The accession $\times$ cut interaction was significant for most traits in the combined analysis (except for leaf number and tiller number). These results may be associated with differences in the harvest interval between seasons, given that the cuts were conducted at 60 and 45 days for the dry and rainy seasons, respectively, following the grazing management practices of Andean livestock farmers, who shorten pasture rest periods as forage growth accelerates during the rainy season⁶⁸. Previous research reported that cutting intervals influenced plant height, stem number, and stem diameter in one of our check accessions (INIA 910–Kumymarca), with an optimal cutting interval of 45 days under chemical nitrogen fertilization⁶⁹. In the same vein, Wilkins⁷⁰ reported in *Lolium perenne* that the genotype $\times$ harvest frequency interaction was significant, attributing this response to genetic differences in vegetative and reproductive growth dynamics among cultivars. This suggests that the accessions evaluated respond differentially according to the cutting interval, a pattern that may be associated with genetic differences in their phenology.

The seasonal model in this study was fitted by creating environments with the cuts corresponding to each season within livestock micro-watersheds, with no direct inclusion of the accession $\times$ season interaction effect. Nevertheless, a significant pattern of variation was observed for NDF during the dry season and for in vitro digestibility during the rainy season, which could be explained by previous reports in *Lolium perenne*, where the genetic control of organic matter digestibility and cell wall digestibility has been shown to differ by season of the year⁷¹. This supports the need for further research that explicitly incorporates season as a fixed effect in the model for *Lolium multiflorum* Lam. under Andean-Amazonian conditions and with a greater number of cuts.

The average of in vitro digestibility was slightly higher during the dry season, accompanied by lower neutral detergent fibers values. During the rainy season, the inverse pattern was observed for both traits. In grasslands subject to precipitation variability, it has been reported that reduced water availability decreases neutral and acid detergent fiber concentrations, thereby improving the relative nutritive value of the forage, with plant composition and maturity stage being the primary factors driving these changes in nutritive value⁷². This suggests that the observed changes may be attributable to variability in the days to maturity among accessions, as well as their responsiveness to rainfall. On the other hand, in the combined analysis across both seasons, in vitro digestibility and NDF did not show a significant accession effect. This result partially differs from the findings reported by Matias et al.⁷³, who observed significant genotypic effects in *Brachiaria decumbens* hybrids in the combined analysis of seven harvests grouped by rainy and dry season; however, their findings were consistent with those of the present study in that no significant genotypic effect was detected for in vitro digestibility during the dry season, with significance observed only during the rainy season.

The heritability estimated using the Cullis' method for in vitro dry matter digestibility was zero during the dry season. During the rainy season, however, H^2_{Cullis} was 0.48, which fell within the narrow-sense heritability range reported for temperate forage grasses (0.40–0.60)⁷⁴. When data from both seasons were analyzed jointly, heritability was also zero and consistent with findings reported by Colas et al.³³, who, upon jointly evaluating spring and autumn season data in *Lolium perenne*, obtained zero heritability estimates for organic matter digestibility, suggesting a potential influence of seasonal environmental conditions on the expression of this trait. On the other hand, Cullis' heritability for dry matter concentration during the rainy season was high (0.75), thereby indicating that a substantial proportion of the observed phenotypic variation among accessions may have a genetic basis, and suggesting high selection potential for this trait during that season. This result aligns with evidence reported by Meehan and Gilliland⁷⁵ in a 14-year trial involving 246 cultivars of *Lolium perenne*, in which dry matter concentration was identified as amenable to genetic improvement through selection programs. Crude protein exhibited moderate Cullis' heritability in both seasons, with values of 0.54 during the dry season and 0.51 during the rainy season. These estimates were higher than the broad-sense heritability reported by Wang et al.⁷⁶ for the same trait in *Lolium multiflorum* ($H^2 = 0.36$); however, this comparison is purely referential given the conceptual and methodological differences between estimators. Similarly, Vogel et al.⁷⁷ reported narrow-sense heritability values of 0.42 for crude protein and 0.50 for in vitro dry matter digestibility in *Sorghastrum nutans*. Considering differences among estimators, species, and experimental conditions, the values obtained in the present study for crude protein can be regarded as comparatively higher on a reference basis, whereas in vitro dry matter digestibility showed a broadly similar pattern during the rainy season (0.48). Biologically, the contrasting behavior of digestibility between seasons suggests that its expression may be shaped more by the environment than by genetic differences among accessions, given that the structural compartment associated with architectural plant development has low digestibility and that its relative weight responds strongly to water availability and maturity stage⁷⁸.

In Significant trait correlations were observed, remaining consistent across both seasons. Plant height showed moderate to strong positive correlations with leaf length, number of spikes, basal coverage area, and spike length. This is consistent with findings reported in populations of *Lolium perenne* ssp. *multiflorum*, where plant height prior to flowering was positively correlated with tiller number, aboveground biomass, and number of spikes per plant⁴⁶. Furthermore, a strong association between leaf length and leaf width was evident across seasons, suggesting genetic coordination in leaf blade development. This aligns with what has been described in *Lolium*, where selection for greater leaf size led to an increased duration of individual leaf elongation, demonstrating that the components of leaf size are genetically coordinated⁷⁹.

The number of stems and the number of tillers were positively and significantly associated with the basal cover area, both in the general model and the by-season model, with the association of stem number ($r=0.74\text{--}0.92$) being stronger than that of tiller number ($r=0.65\text{--}0.77$). This difference can be explained by the nature of each trait. The number of stems represents the developed stems present in the clump at the time of cutting and is therefore directly related to the basal area, since each stem occupies a measurable space on the soil surface and their accumulation determines the expansion of the clump⁸⁰. This strong association indicates that stem density can be considered a reliable indirect indicator of basal cover area⁸¹, and therefore could be used as an indirect selection criterion in breeding programs. This application is particularly useful in the early screening stages, given that conventional methods for estimating ground cover are subjective and time-consuming⁸². The number of tillers, in contrast, reflects the appearance rate of new basal shoots⁸³, which are smaller than the developed stems⁸⁴ and therefore occupy less basal area; this could explain their lower correlation. Nevertheless, they reflect the persistence of the sward against mortality⁸² and constitute the mechanism by which the clump maintains its structure over time⁸⁵. The negative relationship between crude protein and dry matter concentration observed in the evaluated accessions reflects a nitrogen dilution mechanism well documented in forage grasses. As the plant accumulates biomass, an increasing proportion of its tissues corresponds to nitrogen-poor structures associated with plant architecture, while tissues associated with growth and photosynthesis maintain higher concentrations⁸⁶. This process explains the parallel decline of crude protein and digestibility as plant mass increases⁸⁷.

In PCA, the proportion of variance explained by the first two components varied across seasons, with greater cumulative variance observed during the rainy season. The higher cumulative variance captured by the first components during the rainy season may reflect a more integrated covariation structure among traits, characterized by stronger correlations or greater trait-to-trait covariation, which promotes greater concentration of variance along fewer orthogonal axes^{88,89}. These results may be a consequence of shifts in environmental conditions, which could affect both trait values and trait-to-trait correlations⁹⁰. In our study, cumulative precipitation increased during the rainy season, although the magnitude of this increase varied across cattle-ranching micro-watersheds, with Pomacochas showing a relatively greater increase. The intra-annual variability in precipitation, characterized by an increase during the rainy season relative to the dry season, may have acted as a dominant environmental driver simultaneously affecting multiple functional traits of *Lolium multiflorum*, including forage yield and quality⁷². Likewise, studies on the same species report that greater water availability increases dry matter content, in vitro digestibility, and metabolizable energy⁹⁰, suggesting a coordinated physiological response across multiple traits that may account for the stronger covariation structure observed in the PCA during the rainy season.

In vitro digestibility was excluded from both the dry-season analysis and the combined two-season analysis of the Elston index, given that the estimate of genotypic variance for this trait was zero under both conditions. From a methodological standpoint, the prediction of genotypic values using BLUP requires that genotypic variance components be estimable with sufficient precision; when this condition is not met, the reliability of genotypic predictions is substantially compromised⁵¹. In this regard, Santos et al.⁹¹ demonstrated through simulation studies that the accuracy of BLUP values decreases considerably when genotypic variance components yield imprecise estimates, as occurs in situations where such variance approaches or equals zero. Therefore, incorporating a trait with no estimable genotypic variance into the Elston index would not only lack statistical justification but would also introduce unreliable genotypic values that could distort the ranking of accessions. Rameez et al.⁹² noted that restricted maximum likelihood (REML) analysis is grounded in large-sample theory and that, when sample sizes are small, its asymptotic assumptions may not hold, thereby increasing uncertainty in the estimation of variance components and heritability. In this context, a genotypic variance estimated as zero should not be automatically interpreted as a true absence of genetic variation, as it may also reflect an estimate at the boundary of the parameter space or insufficient information to detect it statistically in this season⁹³.

Accession LM20 exhibited the highest Elston index, outperforming the controls during the dry season; however, during the rainy season it declined to twelfth place, associated with negative BLUP values for crude protein, neutral detergent fiber, and in vitro digestibility. These patterns may be linked to changes in precipitation, whose magnitude was greater during the rainy season relative to the dry season. This scenario is consistent with the so-called nutrient dilution effect, a phenomenon reported in forage grasses whereby increased water availability stimulates biomass accumulation while reducing the crude protein concentration of the forage an effect commonly referred to in livestock production as “washy grass” during years of high rainfall⁹⁴. Nevertheless, LM20 maintained a high ranking in the combined two-season analysis, suggesting that its superior performance during the dry season partially offset the decline observed during the rainy season. The high potential demonstrated by accessions LM20 and LM5 during the dry season, which is characterized by seasonal water deficit, suggests that these accessions perform well under these conditions. These results underscore the relevance of evaluating local accessions as candidates for future breeding programs, considering that *Lolium multiflorum* Lam. generally exhibits lower tolerance to abiotic stresses compared to other forage grasses⁹⁵. In this context, research in *Lolium perenne* noticed intraspecific differences in biomass production under water stress among accessions from different geographic origins, attributed to osmotic adjustment mechanisms⁹⁶, thus suggesting that similar variability may exist within *Lolium multiflorum* Lam. and could be exploited through the systematic evaluation of local germplasm. On the other hand, the Elston index revealed that accession LM18 exhibited a more balanced multi-trait performance during the rainy season, with notably strong performance in neutral detergent fiber and in vitro digestibility. From a livestock utilization perspective, this profile is of particular interest given that a 1% increase in dry matter digestibility is associated with a 3.2% increase in daily weight gain in cattle⁹⁷, suggesting that LM18 may be a promising accession for use during the rainy season.

One limitation of this study was that the evaluation covered a single annual growing season across three livestock micro-watersheds. Therefore, the results should be interpreted considering that year-to-year environmental variation was not assessed, which may influence accession performance and the consistency of their responses over time. However, the evaluation included contrasting dry and rainy seasons across multiple target environments, providing valuable preliminary information on accession performance under variable field conditions. Likewise, trait selection relied on a single multi-trait selection index³⁰, with no comparison against alternative approaches. The augmented block design should likewise be considered when interpreting the results, since errors cannot be estimated directly because individual variances and standard errors cannot be calculated for each unreplicated treatment given that each one appears only once per environment; the precision of the comparisons is unequal because contrasts between two unreplicated treatments have a larger standard error than comparisons between an unreplicated treatment and a replicated check; environmental or block interactions cannot be separated from the effects of individual treatments for single-plot entries; statistical power is lower because significance tests for smaller or less heritable trait differences are less sensitive owing to the limited residual degrees of freedom derived solely from the checks; and final selection is inconclusive because performance estimates carry greater uncertainty, which means that the results are more useful for preliminary selection than for final variety release^{98,99,100}. In addition, the harvest interval differed between seasons, so the seasonal contrasts reported here reflect the combined effect of environmental conditions and length of the regrowth period.

Several multi-trait selection methods have been developed over time, each with different data requirements and underlying assumptions²⁹. The present evaluation, however, was conducted as the initial phase of a breeding program and was aimed at identifying a first set of promising accessions rather than at providing an exhaustive methodological comparison of selection approaches. Future studies should therefore include evaluations over multiple years, a larger number of locations, and a broader range of genetic materials. They should also incorporate stability analyses to characterize accession performance across contrasting environments, and apply alternative multi-trait selection approaches as a complement, allowing cross-validation of the traits and accessions prioritized here and thereby supporting their recommendation for breeding programs. Incorporating genomic tools is likewise worth exploring, given that organic matter digestibility and neutral detergent fiber in ryegrass have been predicted from genotyping-by-sequencing data with moderate accuracy¹⁰¹.

Conclusion

Accessions of *Lolium multiflorum* Lam. obtained from the INIA Cajamarca germplasm bank exhibited significant effects on most agronomic traits and on selected nutritional traits, evaluated under field conditions across three livestock micro-watersheds in the Amazonian Andes. Cullis heritability estimates ranged from moderate to high for most characters, particularly for crude protein, plant height, basal cover area, leaf length and width, and the number of spikes and spikelets. The expression of nutritive value traits, specifically in vitro digestibility and neutral detergent fiber, varied between the dry and rainy seasons. Application of Elston's multi-trait selection index identified accession LM20 as the most promising entry in both the combined analysis and the dry season, outperforming the control cultivars established in the study area, and LM5 exhibited performance close to that of the control cultivars, while LM18 stood out during the rainy season for its well-balanced profile of in vitro digestibility and neutral detergent fiber content. Collectively, these findings establish a quantitative basis for the characterization and utilization of *Lolium multiflorum* Lam. germplasm, and provide relevant information for the design of plant breeding programs aimed at developing cultivars with improved forage productivity and nutritional quality, to strengthen Amazonian Andean livestock systems in the face of climate variability. It is recommended to evaluate these accessions across multiple years to perform stability analyses that allow characterizing their performance under different environmental conditions and supporting their recommendation for breeding programs.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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