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Assessment of Intra-population Morphological Variability in *Lolium perenne*

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ABSTRACT

Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage grasses in temperate regions due to its high productivity, nutritive value and adaptability. The present study aimed to evaluate the morphological variability and yield-related traits of 15 *Lolium perenne* genotypes (breeding lines, varieties and local populations) in order to identify valuable parental material for breeding programs. The experiment was conducted during 2023-2025 at the Research and Development Institute for Grasslands Braşov, Romania, under field conditions, using 540 individual plants. A set of morphological and agronomic traits was recorded, including plant height, number of vegetative and generative tillers, flag leaf dimensions, spike length, seed weight and resistance to rust. Data were processed using correlation analysis and principal component analysis (PCA). Results showed a high level of phenotypic variability among genotypes for all studied traits. Plant height ranged from 48.1 to 85.7 cm, while the number of generative tillers varied between 260.7 and 516.3, indicating important differences in yield potential. High productivity was observed in genotypes such as Barnhem and Barsprinter, which exhibited superior generative tillering capacity. Troian showed the highest seed weight (0.14 g), suggesting improved grain quality potential, while Summit was characterized by reduced plant height, indicating suitability for lodging resistance breeding. Correlation analysis revealed a significant positive association between vegetative and generative tillers (r = 0.63), and moderate relationships between generative tillers and flag leaf traits. PCA indicated that the first two principal components explained 74.86% of the total variance, with tillering capacity, flag leaf morphology and earliness representing the main sources of variation. The study highlights substantial genetic diversity within the evaluated germplasm and confirms the importance of tillering ability and leaf traits in yield formation. The identified genotypes represent valuable genetic resources for breeding *Lolium perenne* varieties with improved productivity and adaptability under changing environmental conditions.

Keywords: correlations, germplasm, genotypes, morphological and physiological characteristics.

List of Abbreviations: h - Plant height (cm), veget.t - Number of vegetative tillers per plant, gener.t - Number of generative tillers per plant, h leaf - Flag leaf length (cm), w. leaf - Flag leaf width (mm), earling/earing - Heading date/inflorescence emergence, PC - Principal Component, PC1, PC2, PC3 - First, second, and third principal components, PCA - Principal Component Analysis, Dev. - Deviation from long-term average, IV-IX - Vegetation period (April-September), I-XII - Annual period (January-December), cm - Centimeters, mm - Millimeters, g - Grams, t/ha - Tons per hectare, °C - Degrees Celsius.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is one of the earliest cultivated forage grasses, the most widespread perennial grass species in the temperate zone, and one of the most valuable species for pastures due to its high resistance to animal trampling and rapid regeneration after grazing (Frame et al., 1998; Humphreys et al., 2010).

The species is characterized by a rapid establishment and growth rate and is widely used in intensive mixtures with white clover (*Trifolium repens*) or fescue (*Festuca* spp.), as well as in complex mixtures with other perennial grasses and legumes for pastures, hayfields, and silage production. Under favorable environmental conditions, these mixtures can be grazed 4-6 times per year (Moga and Schitea, 2000; Hopkins and Wilkins, 2006). Perennial ryegrass is also frequently used for overseeding degraded natural grasslands, contributing to soil improvement through its well-developed root system, which enhances soil structure and fertility (Whitehead, 2000).

In addition, *Lolium perenne* is a key component of most lawn seed mixtures due to its rapid establishment, persistence, and aesthetic qualities (Turgeon, 2011).

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The species has a high production potential, reaching 8-12 t/ha of dry matter, and a high nutritional value, characterized by a crude protein content of 14-17%, high levels of soluble carbohydrates, and relatively low crude fiber content (24-28%), resulting in high palatability and intake by livestock (Wilkins and Humphreys, 2003; Marușca, 2016; Mocanu et al., 2021). Yield performance in *Lolium perenne* is strongly influenced by agronomic factors such as sowing rate and nitrogen fertilization (Jovanović Todorović et al., 2026). These characteristics contribute significantly to improved animal production. Recent studies also show that early root development traits play an important role in biomass accumulation and yield stability, representing key targets in breeding programs (Malinowska et al., 2025).

Perennial ryegrass is included in numerous breeding programs worldwide, with new varieties being registered annually. Current breeding objectives focus on increasing productivity and quality, as well as improving resistance to diseases (especially rust) and tolerance to drought, which increasingly affect plant persistence under current climate change conditions (Humphreys et al., 2010; Sampoux et al., 2011).

Modern genomic approaches, including genomic prediction and genotype-by-environment interaction analyses, are increasingly used to improve selection efficiency for complex traits such as dry matter yield (Zhu et al., 2025).

In the context of accelerating climate change and increasing demands for high-quality forage, breeding of *Lolium perenne* has become a priority in modern agronomic research (Varga et al., 1998). The development of new varieties with improved tolerance to biotic and abiotic stresses, high productivity, and enhanced nutritional value represents a strategic direction for ensuring the sustainability of grassland systems (Sampoux et al., 2011; Ergon et al., 2018).

Previous studies have highlighted the considerable genetic variability of *Lolium perenne* populations and their importance for breeding programs (Mut et al., 2017).

Genetic variability has been extensively explored through quantitative trait loci (QTL) analysis and comparative genomics, enabling the identification of genomic regions associated with fertility, metabolite composition, and agronomic traits (Armstead, et al., 2008; Cogan et al., 2013)

Advances in genome assembly and candidate gene identification, including genes controlling self-compatibility, further support the development of improved breeding strategies (Chen et al., 2024).

The success of breeding programs depends largely on the availability of valuable genetic resources and the diversity of germplasm. The evaluation of germplasm collections through field observations, measurements, and physiological determinations enables the identification of valuable parental forms for the development of new varieties adapted to changing environmental conditions (Annicchiarico et al., 2015). Studies on natural populations and germplasm collections have also confirmed significant phenotypic variability useful for selection (Jităreanu et al., 2014), while traits such as seed retention are increasingly considered important for breeding efficiency and seed production (Sampoux et al., 2024).

According to the Official Catalogue of Varieties and Hybrids in Romania, only two varieties of *Lolium perenne* developed at our institute are currently registered (Mara - 1989 and Brevis - 2025), highlighting the need to intensify breeding efforts in this important species. Therefore, 15 local and foreign genotypes, including varieties, local populations, and candidate varieties under registration, were studied in order to identify valuable parental forms for the creation of new varieties with improved persistence.

MATERIAL AND METHODS

The experiment was conducted in a field trial of the Research and Development Institute for Grasslands - Brașov, during the years 2023-2025. The plant material used in this experiment was represented by 15 accessions - breeding lines and varieties, with a total of 540 individual plants.

The plants were obtained by sowing in March 2023, in the greenhouse, in rows in trays, followed by individual transplanting into small plastic pots. When the seedlings were sufficiently well developed, with vigorous shoots and a well-developed root system, they were transplanted into the field, as individual plants, at equal distances of 50 cm, 10 plants per row and 36 rows/per block. Regarding la vegetation period, 2025 was the third year, in this way we chose the plants with perennial potential.

During the vegetation period of 2025 year, the following observations and determinations have been made heading date (inflorescence emergence), growth habit (before inflorescences emergence), number of vegetative tillers, scale 1-5, number of generative tillers, plant heigh, the distance in cm from the plant base to the of panicle after an thesis, resistance to rust (% of healthy plants) (Tod et al., 2025a).

The morphological and phonological characters were scored by visual inspection or measurements. The recorded data were statistically processed in the Statistica 7 software package.

The meteorological conditions in the years 2024-2025 (Table 1) indicate a warmer period compared to the multiannual average. Considering the recorded temperatures, in both years the values exceeded the multiannual average, with 3.6°C in 2024 and with 1.7°C in 2025. From the point of view of precipitation, the year 2024 recorded a total deficit of -22.6 mm and of -43.2 mm in the vegetation period compared to the multiannual average. In 2025 the temperature exceeded with 1.7°C compared to the multiannual average and during the vegetation period with 2°C. However the precipitation was more abundant with 120.7 mm in the vegetation period. All these meteorological conditions led to optimal plant growth and development.

Table 1. Meteorological conditions from Brașov stationary 2024-2025

Years	Annual average I - XII	Dev.	Vegetation period IV - IX	Dev.
Temperature (°C)				
2024	11.4	+3.6	17.6	+3.4
2025	9.5	+1.7	16.0	+2.0
Average 59 years	7.8	0	14.2	0
Precipitation (mm)				
2024	730.6	-22.6	485.9	-43.2
2025	821.8	68.8	649.8	120.7
Average 59 years	753.2	0	529.1	0

RESULTS AND DISCUSSION

The evaluated germplasms exhibited substantial variability across all analysed morphological and yield-related traits, reflecting a broad genetic diversity suitable for both agronomic evaluation and breeding purposes (Table 2). Such variability is essential for the identification of superior genotypes and for the selection of complementary parental lines (Tod et al., 2024; Tod et al., 2025b).

Plant height varied widely among germplasms, ranging from 48.1 cm in Summit to 85.7 cm in Timis 81. Tall genotypes such as Timis 81, Troian, and Brevis are generally associated with increased biomass production; however, excessive height may increase susceptibility to lodging under high-input or unfavourable weather conditions. In contrast, Summit and Barnhem, which displayed shorter stature, may offer improved lodging resistance and are therefore valuable for breeding programs targeting reduced plant height without compromising productivity.

The number of vegetative tillers showed pronounced genotypic differences, with values ranging from 0 to 33.3 tillers per plant. High vegetative tillers, observed in 11 BR 1804, Barnhem, and Summit, indicates strong early vigour and adaptability. Nevertheless, high vegetative tilling did not always translate into a higher number of fertile tillers, confirming that the conversion efficiency from vegetative to generative tillers is genotype-dependent.

The number of generative tillers, a major determinant of yield, ranged from 260.7 to 516.3. Barsprinter, Barnhem, and Algol recorded the highest values, highlighting their superior reproductive efficiency and yield potential. Conversely, Timis 81, despite its tall stature, showed the lowest number of generative tillers, suggesting a less favourable partitioning of assimilates towards reproductive organs.

Flag leaf size is closely associated with photosynthetic contribution during grain filling. In the present study, flag leaf length ranged from 14.6 cm to 24.1 cm, while leaf width varied between 3.7 mm and 7.0 mm. Germplasms such as P1 2016, P2 2016, and Barnhem exhibited larger flag leaves, which may enhance assimilate supply to developing spikes. In contrast, 11 BR 1804 and Zekol, with narrower leaves, may possess improved tolerance to water stress due to reduced transpiration surface.

Spike length, varied from 20.3 cm to 27.8 cm. Longer spikes were observed in ICDP Brașov, Troian, and 11 BR 1804, which may contribute to a higher number of spikelets and grains per spike. Seed weight showed considerable variation, with Troian exhibiting a markedly higher value (0.14 g) compared to all other germplasms. This suggests a compensatory mechanism whereby lower tiller numbers are offset by heavier grains, a desirable trait for grain quality improvement.

Table 2. Morphological and physiological characteristics of the genotypes

Germoplasm	Height h (cm)	No vegetative tillers	No. generative tillers	Height standard leaf (cm)	Standard leaf width (mm)	Spike length (cm)	Seed weight (g)
P1 2016	67.1	16.0	376.3	23.1	6.3	-	-
P2 2016	73.9	10.0	424.7	24.1	7.0	-	-
Algol	63.3	24.0	432.0	21.0	6.0	-	-
Barsprinter	70.7	13.0	516.3	17.1	4.7	25.3	0.07
Olaf	69.8	0.0	319.3	16.2	4.0	22.3	0.06
Barnhem	61.4	33.0	493.3	20.9	6.7	-	-
Summit	48.1	28.3	335.0	18.3	4.7	-	-
Mara	79.6	0.0	387.0	17.2	5.3	25.6	0.08
ICDP Brasov	73.8	7.7	286.3	14.8	5.0	27.8	0.05
Troian	85.3	10.0	319.3	15.4	4.7	27.1	0.14
Timis 81	85.7	9.7	260.7	16.3	4.0	20.3	0.06
Option	74.1	17.0	386.7	20.5	4.3	25.3	0.06
11 BR 1804	66.9	33.3	389.0	16.7	3.7	26.3	0.03
Zekol	65.0	10.0	275.0	14.6	3.7	21.8	0.08
Brevis	82.1	13.7	330.3	16.3	5.0	20.8	0.07

Overall, Barsprinter and Barnhem combined high numbers of generative tillers with favorable vegetative traits, making them strong candidates for high-yielding cultivars. Troian distinguished itself through superior seed weight, indicating potential use in breeding programs targeting grain size and quality. Summit, with its reduced plant height, represents a valuable source of lodging resistance.

The contrasting trait combinations observed among the evaluated germplasms underline their potential utility as parental material in breeding strategies aimed at combining yield stability, plant architecture, and grain quality.

Correlation analysis revealed several biologically relevant relationships among the evaluated traits (Table X). Plant height showed a moderate positive correlation with flag leaf width ($r = 0.62$) and a weak negative correlation with the number of vegetative tillers ($r = -0.29$), whereas its association with generative tillers and spike length was negligible ($r \leq |0.17|$).

A strong positive correlation was observed between the number of vegetative and generative tillers ($r = 0.63$), highlighting the importance of early tilling capacity for reproductive success. Generative tillers were moderately correlated with both flag leaf length (r = 0.45) and width (r = 0.39), confirming the key role of the flag leaf in assimilate supply. Flag leaf length exhibited a moderate positive correlation with spike length (r = 0.55), whereas flag leaf width was weakly and negatively associated with this trait (r = -0.19) (Table 3).

Table 3. Correlation matrix

Variable	h	veget.t	gener.t	h leaf	w. leaf	earing
h	1.00	-0.29	0.04	0.09	0.62	-0.17
veget.t		1.00	0.63	0.43	0.14	0.02
gener.t			1.00	0.45	0.39	0.05
h leaf				1.00	0.37	0.55
w. leaf					1.00	-0.19
earing						1.00

The results indicate that tilling capacity and flag leaf morphology and the earing date contribute more substantially to yield-related traits than plant height, supporting the feasibility of selecting for reduced plant stature without compromising reproductive performance (Figure 1).

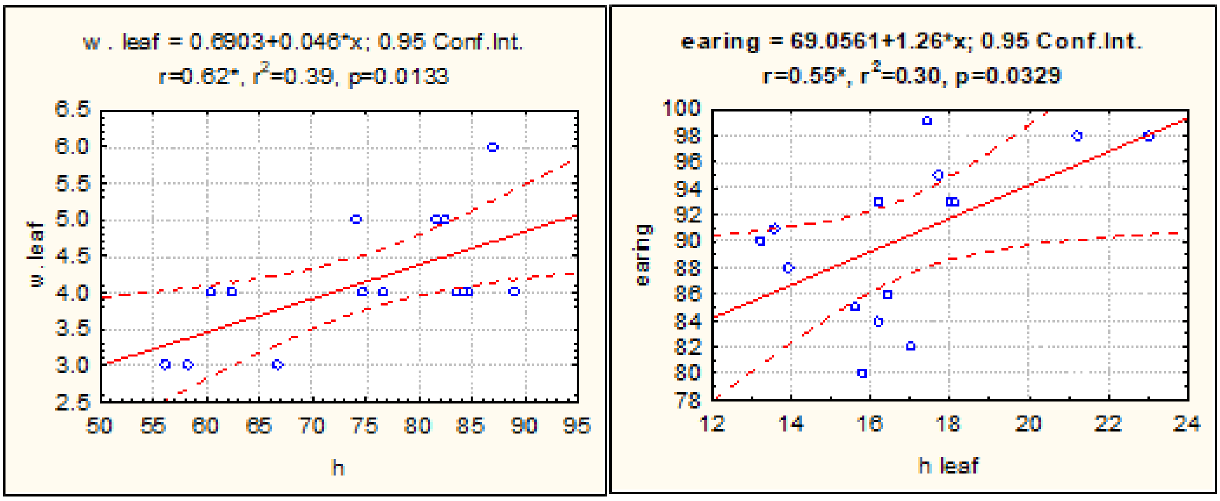


Figure 1. Positive correlation between wight leaf and h plant, and hearing date and h leaf

Principal component analysis (PCA) was conducted to reduce data dimensionality and to identify the main factors contributing to the observed phenotypic variation. The eigenvalues and the proportion of variance explained by each principal component are presented in Table 4.

Table 4. Eigenvalues of correlation matrix, and related statistics

PC	Eigenvalue	% Total variance	Cumulative eigenvalue	Cumulative %
1	3.200384	53.33973	3.200384	53.3397
2	1.291140	21.51900	4.491524	74.8587
3	0.662968	11.04947	5.154492	85.9082
4	0.406171	6.76952	5.560664	92.6777
5	0.314424	5.24040	5.875087	97.9181
6	0.124913	2.08188	6.000000	100.0000

The first principal component (PC1) exhibited an eigenvalue of 3.20 and explained 53.34% of the total variance. This component represents the dominant source of variation and is mainly associated with traits related to tilling capacity and flag leaf development, which were previously shown to be strongly intercorrelated.

The second principal component (PC2) had an eigenvalue of 1.29 and accounted for 21.52% of the total variance. Together, PC1 and PC2 explained 74.86% of the cumulative variance, indicating that the majority of phenotypic variation among germplasms can be effectively summarized in a two-dimensional PCA space. PC2 appears to be related primarily to plant architecture traits, particularly plant height and spike-related characteristics, which showed weaker correlations with the variables dominating PC1.

According to the Kaiser criterion (eigenvalue > 1), only the first two principal components were retained for interpretation, as they capture the most meaningful variation in the dataset. This supports the use of a PCA biplot for visualizing the relationships among traits and the relative positioning of germplasms (Figure 2).

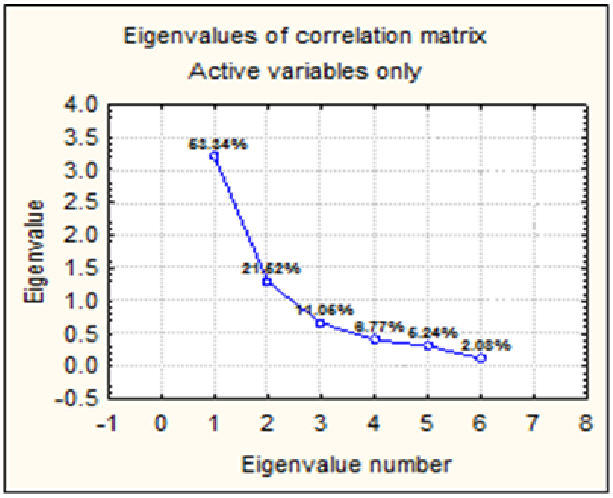


Figure 2. Eigenvalue graph

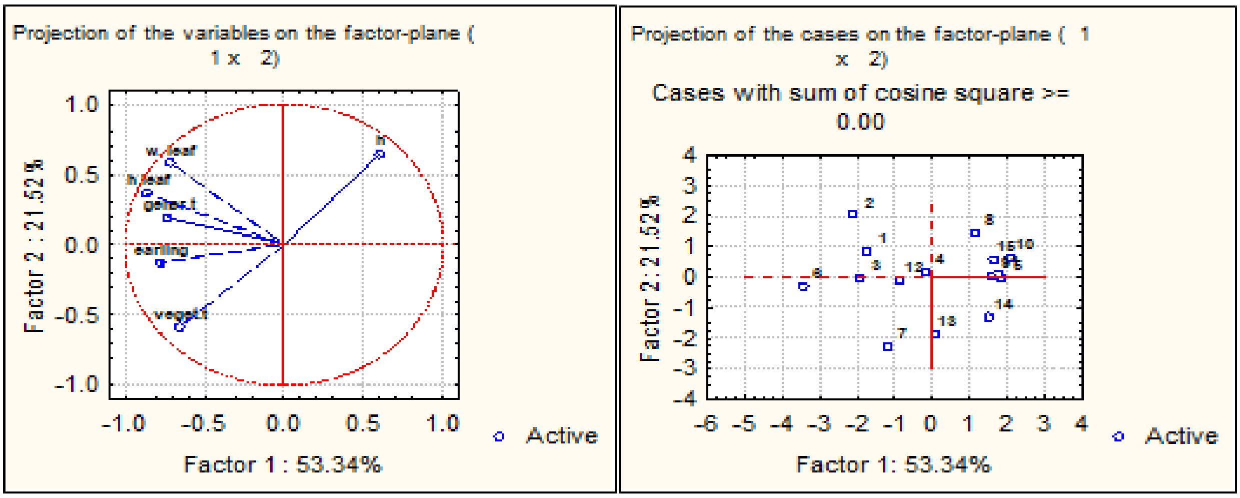
The PCA results corroborate the findings of the correlation analysis, emphasizing that traits related to tillering efficiency and flag leaf morphology contribute most strongly to phenotypic differentiation among germplasms. The relatively minor contribution of plant height to the principal components suggests that selection for reduced stature can be achieved without adversely affecting key yield components. From a breeding perspective, germplasms positioned positively along PC1 are expected to exhibit superior yield potential due to favorable combinations of generative tillers and leaf traits, whereas those differentiated along PC2 may represent contrasting plant architecture types. This multivariate approach facilitates the identification of complementary parental lines and supports the strategic combination of desirable traits in breeding programs.

Principal component loadings indicated that PC1 (53.34% of variance) was mainly driven by flag leaf height (-0.87), earliness (-0.78), and both vegetative and generative tillers (-0.66 to -0.73), representing a productivity-related axis. PC2 (21.52%) was associated primarily with plant height (0.65) and flag leaf width (0.58), reflecting plant architectural variation. PC3 (11.05%) was influenced by generative tillers (0.58) and earliness (-0.50), capturing secondary reproductive and phenological differences among germplasms (Table 5).

Table 5. Factor-variable correlations, based on correlations

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
h	**0.5986**	**0.6452**	0.0018	0.4599	-0.1174	-0.0113
veget.t	**-0.6567**	-0.5876	0.1999	0.2545	-0.3445	0.0094
gener.t	**-0.7297**	0.1929	0.5764	0.1262	0.2856	-0.0251
h leaf	**-0.8665**	0.3680	-0.1944	-0.0065	-0.0291	0.2739
w. leaf	**-0.7161**	0.5824	0.0164	-0.2350	-0.2495	-0.1741
earling	**-0.7841**	-0.1335	-0.5026	0.2423	0.1932	-0.1368

The PCA loadings confirm that tillering capacity, flag leaf morphology, and earliness are the main drivers of phenotypic differentiation among the evaluated germplasms. The limited and contrasting contribution of plant height across components supports the hypothesis that reduced stature can be selected without negatively affecting yield-related traits (Figure 3).



1 - P1 2016, 2 - P2 2016, 3 - Algol, 4 - Barsprinter, 5 - Olaf, 6 - Barnhem, 7 - Summit, 8 - Mara, 9 - ICDP Brasov, 10 - Troian, 11 - Timis 81, 12 - Option, 13 - 11 BR 1804, 14 - Zekol, 15 – Brevis.

Figure 3. Projection of variables and species in the plane of the factorial axes of the two principal components

The variable contributions confirmed the PCA interpretation (Figure 4) PC1 was mainly driven by flag leaf height (23.46%), earliness (19.21%), and generative tillers (16.64%), representing a productivity axis. PC2 was dominated by plant height (32.24%), flag leaf width (26.27%), and vegetative tillers (26.74%), reflecting plant architecture.

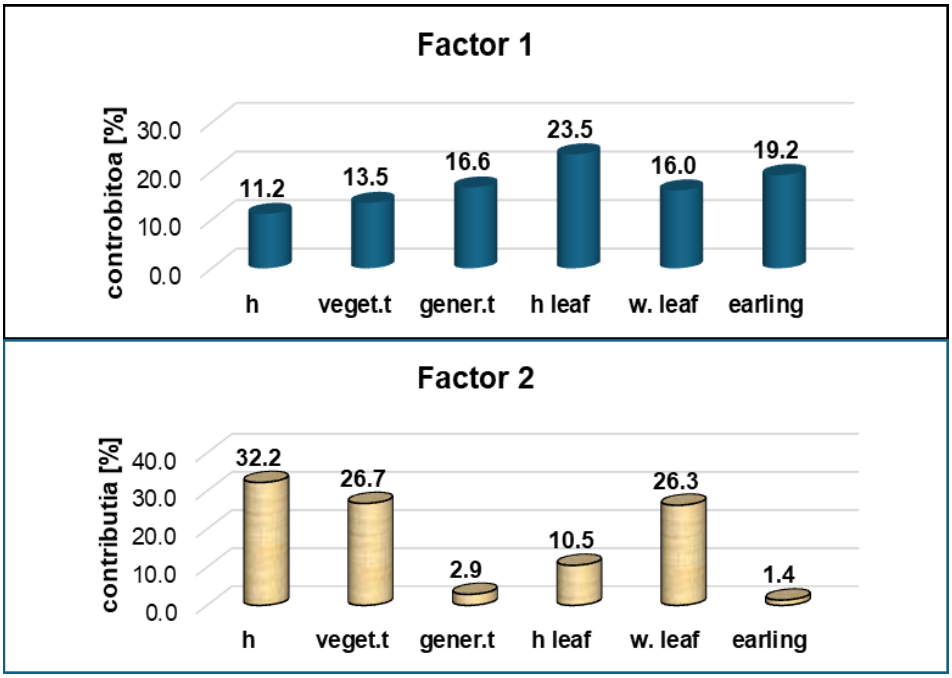
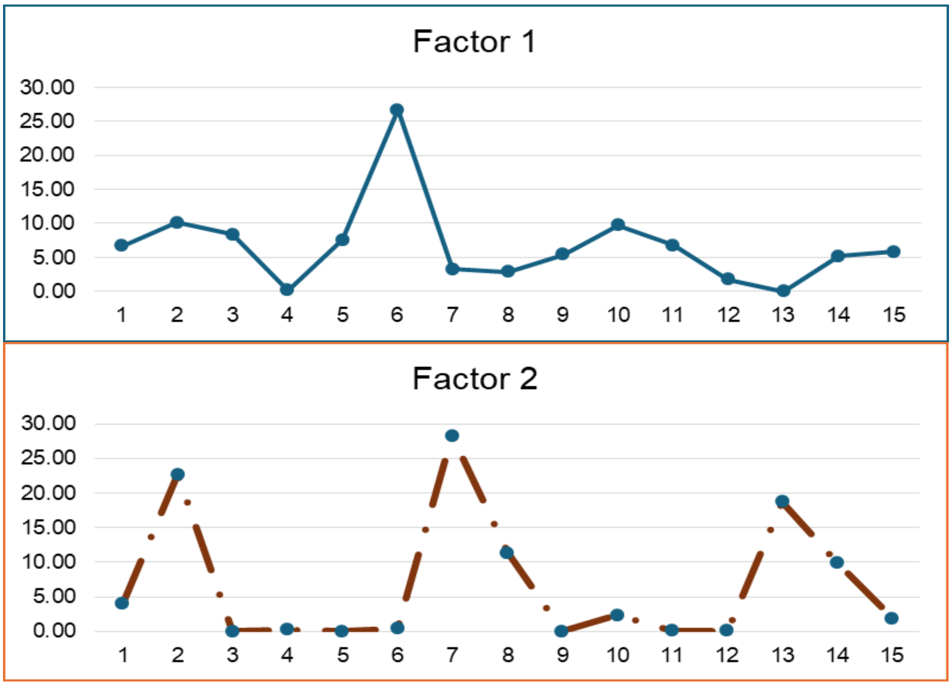


Figure 4. Contribution of variables within CP1 and CP2

The contributions of individual germplasms to the first two principal components (PC1 and PC2) revealed that Barnhem (26.64%), P2 2016 (10.06%), and Troian (9.71%) were the major drivers of PC1, reflecting productivity and reproductive traits. PC2 was dominated by Summit (28.31%), P2 2016 (22.65%), and 11 BR 1804 (18.74%), indicating variation in plant architecture. Low contributors, such as Barsprinter and Algol, had minimal influence, highlighting intermediate phenotypes. These results allow the identification of key genotypes for breeding and germplasm selection (Figure 5).



1 - P1 2016, 2 - P2 2016, 3 - Algol, 4 - Barsprinter, 5 - Olaf, 6 - Barnhem, 7 - Summit, 8 - Mara, 9 - ICDP Brasov, 10 - Troian, 11 - Timis 81, 12 - Option, 13 - 11 BR 1804, 14 - Zekol, 15 – Brevis.

Figure 5. Contribution of varieties within CP 1 and CP2

CONCLUSIONS

The analysis of morphological and yield-related traits revealed substantial variability among the evaluated germplasms, particularly in plant height, tillering capacity, flag leaf dimensions, and earliness. This genetic diversity provides a strong basis for the selection and improvement of *Lolium perenne* genotypes.

Correlation analysis demonstrated that both vegetative and generative tiller numbers are positively associated with flag leaf size, highlighting the key role of leaf development in determining yield potential. In contrast, plant height showed a limited relationship with yield-related traits, indicating that reduced plant stature can be selected without negatively affecting productivity. Principal component analysis identified tillering capacity, flag leaf length, and earliness as the main factors driving phenotypic variation (PC1), while plant height contributed primarily to structural differentiation among genotypes (PC2). These results emphasize the importance of reproductive efficiency and leaf morphology in breeding strategies.

Among the evaluated germplasms, Barnhem, P2 2016, and Troian were the main contributors to PC1, reflecting superior productivity traits, whereas Summit, P2 2016, and 11 BR 1804 were associated with PC2, indicating variation in plant architecture. These genotypes represent valuable parental material for breeding programs aimed at combining high yield potential with desirable plant structure.

Overall, the results suggest that effective selection strategies should prioritize tillering capacity, flag leaf development, and earliness, while plant height can be independently optimized. The combination of genotypes with contrasting positions along the principal components offers a practical approach for developing new varieties with improved performance and adaptability under changing environmental conditions.

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