

THE MORPHO-PRODUCTIVE CHARACTERIZATION OF THE PERENNIAL RYEGRASS GERMPLASM IN THE WESTERN PART OF ROMANIA

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Abstract. Perennial ryegrass-dominated grassland ecosystems are typically stable in terms of resilience, as persistence is an important evaluation criterion in perennial breeding programs. The aim of the study was to analyze the morpho-productive characterization of the germplasm collection of the perennial ryegrass collection to identify local provenances that exceed in phenotypic expression under current cultivation conditions in the western part of Romania. For that, numerous observations were made regarding the dynamics of the following traits: plant height (cm), number of leaves per plant, leaf width (cm), leaf length (cm) and the number of shoots. The biological material was made up of local provenances of *Lolium perenne* collected from three geographical areas: the Almaju Mountains, the Mehedinți Plateau and the Blahniței Plain. From the analysis of the correlation relationships highlighted between the five morpho-productive traits investigated, it can be observed the determining role of the height of the perennial ryegrass plant in the dimensioning and phenotypic expression of the traits. Thus, the height of the plant is in a direct interdependence relationship with traits such as the number of leaves per plant and the length and width of the leaf. These interdependencies are subject to the biomorphological composition of the grass species in which the stem comprises nodes and internodes, and a leaf leaves from each node. In other words, the taller the plant is and the more nodes it has, the more leaves it has. The distinctly significant correlation relationship between the number of leaves per plant and the number of shoots where the growth in the form of a bush in perennial ryegrass explains this high correlation is also highlighted. The study provides a detailed analysis of linear regressions for different morpho-productive traits plant height (cm), number of leaves per plant, leaf width (cm), leaf length (cm) and number of shoots, with the aim of understanding how the collected genotypes influence these traits.

Keywords: perennial ryegrass, morpho-productive traits, correlation, linear regression

INTRODUCTION

Among the priorities of breeding programs are defined as the increase in the production and quality of grasslands and the increase in the drought resistance of perennial grasses, and the achievement of these objectives is based on the understanding of the physiology of plant growth and development.

Perennial ryegrass (*Lolium perenne* L.) is one of the forage species in temperate regions, with a high nutritional value, adapted to defoliation and frequent grazing. In practice, there is an obvious focus on the production of crops with high, stable yields, good dry matter quality, and tolerance to drought, frost and other stressful environmental conditions (Jensen et al., 2005; Cunliffe et al., 2004; Sokolovic et al., 2007; Byrne et al., 2009). The species originated in the Near East, with Europe being a secondary centre of origin, spreading across many soil types and at different altitudes (McGrath et al., 2005; Lakić et al., 2015). However, the introduction of this species into cultivation is much more recent, the focus on yield and persistence was only achieved starting in the 1970s. Total dry matter production remains an important objective in obtaining productive and resilient varieties under grazing or silage conditions (McDonagh et al., 2016; Foito et al., 2009).

Perennial ryegrass-dominated grassland ecosystems are typically stable in terms of resilience, as persistence is an important evaluation criterion in perennial breeding programs (Zhang et al., 2016; Salama et al., 2017). The increase in drought tolerance is limited because currently used species must maintain root contact with water to survive. Unfortunately, little is known about the growth physiology of *Lolium perenne* under grazing conditions, with or without water stress (Kemp et al., 1994). The achievement of high growth rates has led to a trend in modern perennial ryegrass genetic lines for high-density cultivation (Pembleton et al., 2016).

Drought survival in perennial ryegrass has been associated with several traits: root length and mass, photosynthetic characteristics and water-soluble carbohydrate concentrations, in other words, drought survival is a quantitative trait which shows high variability and high heritability (Auzanneau et al., 2011).

It has been proven that water stress decreases the photosynthetic capacity due to the reduction of the active leaf area, the increase of stomatal resistance and the decrease of the quantum efficiency of photosynthesis depending on the rate of onset and extension of the stress caused by water shortage (Pearson et al., 2011). Drought is an important environmental stress in agriculture worldwide due to climate change that is destabilizing production, affecting both yield and quality (Jonavičienė et al., 2014; Kemesyte et al., 2017).

In recent decades, mild to moderate summer drought periods have become increasingly common in temperate areas where this species is grown. The effect of severe drought has been the subject of many studies, with responses to severe water stress differing from known observations under mild or moderate stress, which already limits growth (Yates et al., 2019; Simpson et al., 2012; Toporan R.L., 2024).

There are numerous studies stating that *Lolium perenne* tolerates poorly drained soils well, with a pH range between 5.0 and 8.3. Optimal growth occurs between 20 and 25 °C and the ability to survive and grow at low temperatures, to acclimatize to cold, to tolerate wind and snow (Schmidt et al., 2015; Skøt et al., 2002).

Various studies have been conducted on perennial forage plants related to heat stress, but they are usually focused on the production of new cultivars on the physiological and biochemical aspects of the plant response (Wang et al., 2017; Toporan R.L., 2024). Improving cold tolerance is the most important breeding objective, and its implementation by conventional breeding methods has had limited success due to the characteristics of the cold tolerance trait (Zhao et al., 2008; Harrison et al., 1997; Toporan R.L., 2024).

Lolium perenne is characterized by its predominant presence in grassland ecosystems or as a stand-alone crop, especially in temperate regions of the world. The predominant use by grazing is explained by the fact that the species has a high level of metabolizable energy associated with the possibility of providing a major nutrient input for grazing sheep and cattle.

The forage obtained from this species is characterized by the availability in the forage of water-soluble carbohydrates (WSC) composed of glucose as the primary energy source along with fructose, sucrose and fructans, the latter being found in the leaf sheaths and at the base of the leaves (Butnariu and Caunii, 2013; Herridge et al., 2021). For *Lolium perenne* species to be compatible with their role in agricultural systems, they often need to not only tolerate water stress but also persist after drought.

Also, being at the same time a species grazed by animals, they need to regenerate following sudden defoliation of vegetative organs. Resistance to frequent grazing has led to the acquisition of specific physiological adaptations, mainly focused on controlling water losses through stomatal regulation under moderate stress conditions (Hatier et al., 2014).

It is expected that in the future, global climate change will cause decreases in agricultural production due to lack of water during the growing season. To overcome this problem, a genetic improvement of drought tolerance is needed by creating and expanding new varieties in cultivation (Zhao et al., 2016). Consequently, improving the adaptability of perennial forage plants to high and/or low temperatures can increase forage yield and turf quality in regions where their biological development is strongly affected (Wang et al., 2016; Förster et al., 2018).

MATERIAL AND METHODS

Biological material and the purpose of the work

The biological material was made up of local provenances of *Lolium perenne* collected from three geographical areas: the Almaju Mountains, the Mehedinți Plateau and the Blahniței Plain. This distribution covered diverse orographic, edaphic and climatic conditions, generating the premise that the collected genetic material should possess good genetic variability of genotype/phenotype traits as well as valuable biochemical properties in the breeding process.

The purpose of the study

The aim of the study was to analyze the morpho-productive biometric characterization of the germplasm collection under - study conditions of the perennial ryegrass collection originating from natural areas with high intraspecific biodiversity. This was achieved by aiming to identify local provenances that exceed in phenotypic expression under current cultivation conditions in the western part of Romania.

On the *Lolium perenne* provenances described in the initial material, numerous observations were made regarding the dynamics of the following traits: plant height (cm), number of leaves per plant, leaf width (cm), leaf length (cm) and number of shoots.

Cultivation conditions

The collection was located at Lovrin Agricultural Research and Development Station (SCDA Lovrin), on a chernozem typical soil, weakly gleyed, epicalcareous, medium clay-clayey/medium clay-clayey loam type, dominant within the Galațca Plain (Pesac-Lovrin-Teremia) and representative for a significant area of the Low Banat Plain. From the analysis of the main chemical properties of the soil, it is observed that: the pH values oscillate within the limits of the norms, for the parental materials in the area, indicating a weakly alkaline reaction (7.3-8.4) in the range of 20-100 cm, respectively moderately alkaline (8.5-9.0) between 100-130 cm and strongly alkaline (9.1-9.4) between 130-200 cm.

Climatic conditions

The analysis of data on the rainfall regime in the period 2023-2024 shows that overall it was an atypical year, with precipitation amounts well below the multiannual monthly average in most months of the year. The total amount of precipitation in the 2023-2024 agricultural year was 417.4 mm, with a negative deviation of 104 mm from the multiannual average.

During the crop-growing season (April - August), the amount of precipitation was 121.6 mm, with a negative deviation of 134.6 mm from the MMA.

The average annual temperature was 14.6 0C, 3.60C higher than the multiannual average over 70 years (10.90C). During the crop vegetation period, temperatures exceeded the multiannual average monthly. Pronounced atmospheric scorching was also recorded. The largest deviations from the average were reported starting with February and until July. Thus, in February there was a positive deviation of +70C, in March +4.30C, in April +3.10C, in June +3.80C and in July +4.60C.

Statistical processing

Statistical processing was performed in Excel using the following statistical functions: descriptive statistics, correlation, histogram and linear regression.

The Pearson correlation coefficient was calculated to identify the links between the morphological variables used, these correlations were calculated in JASP version 0.18.3 [JASP Team, 2024]. Correlation coefficients are a measure of correlation and are dimensional and have values between -1 and +1 (-1 perfect negative correlation; +1 perfect positive correlation; 0 no correlation or a random association). The p-value shows that significant correlations are marked with: * $p < .05$ if the correlation is significant at $\alpha=.05$ level; ** $p < .01$ if the correlation is significant at $\alpha=.01$ level; and *** $p < .001$ if the correlation is significant at $\alpha=.001$ level.

RESULTS AND DISCUSSIONS

First, the dynamics of the investigated morpho-productive traits were investigated, namely plant height (cm), number of leaves per plant, leaf width (cm), leaf length (cm) and the number of shoots. The experimental analysis of these traits according to living conditions was aimed at grouping and spreading the values of these traits according to the group mean and its standard deviation through descriptive statistical analysis. The following table represents the interdependence between the continuous variables previously stated with scatter diagrams between variables in the entries outside the diagonal and histograms.

Table 1. Descriptive statistics for *Lolium perenne* morpho-productive traits

Plant height (cm)		Number of leaves per plant		Leaf width (cm)		Leaf length (cm)		Number of shoots	
Mean	45.32	Mean	6.55	Mean	4.38	Mean	14.55	Mean	1.147
Standard Error	1.48	Standard Error	0.25	Standard Error	0.11	Standard Error	0.53	Standard Error	0.059
Median	45.4	Median	6.5	Median	4	Median	14.7	Median	1
Mode	53.2	Mode	5	Mode	4	Mode	14.2	Mode	1
Standard Deviation	8.64	Standard Deviation	1.50	Standard Deviation	0.65	Standard Deviation	3.12	Standard Deviation	1.07
Sample Variance	74.75	Sample Variance	2.25	Sample Variance	0.42	Sample Variance	9.74	Sample Variance	1.15
Kurtosis	0.48	Kurtosis	-0.55	Kurtosis	0.01	Kurtosis	0.42	Kurtosis	0.06
Skewness	-0.45	Skewness	-0.03	Skewness	0.11	Skewness	-0.28	Skewness	0.77
Range	39.3	Range	6	Range	3	Range	14.5	Range	4
Minimum	24.7	Minimum	3	Minimum	3	Minimum	7.2	Minimum	0
Maximum	64	Maximum	9	Maximum	6	Maximum	21.7	Maximum	4
Sum	1541.2	Sum	223	Sum	149	Sum	495	Sum	39
Count	34	Count	34	Count	34	Count	34	Count	34
Largest(1)	64	Largest(1)	9	Largest(1)	6	Largest(1)	21.7	Largest(1)	4
Smallest(1)	24.7	Smallest(1)	3	Smallest(1)	3	Smallest(1)	7.2	Smallest(1)	0
Confidence Level(95.0 %)	3.0167	Confidence Level(95.0%)	0.52	Confidence Level(95.0%)	0.2275	Confidence Level(95.0%)	1.0893	Confidence Level(95.0%)	0.375
	14		3841		01		77		719

The average of the analyzed traits expressed on the 95% confidence interval, as well as the distribution of the experimental data, underline normal values of enrollment that make us appreciate that the experimental data were correctly sampled and statistically interpreted (Table 1).

The morpho-productive traits investigated in the selection and improvement process in *Lolium perenne*, namely: plant height (cm), number of leaves per plant, leaf width (cm), leaf length (cm) and the number of shoots, were analyzed through the correlation matrix to estimate the correlation of the collected genotypes. Also, through this procedure, the null hypothesis according to which the population correlation between pairs of variables is equal to 0, was tested and statistically invalidated (Table 2).

Table 2. Pearson's correlation matrix of morphoproductive traits in perennial ryegrass

Variable		Plant height (cm)	Number of leave per plant	Leaf width (cm)	Leaf length (cm)	Number of shoots
1. Plant height (cm)	Pearson's r	—				
	p-value	—				
	Upper 95% CI	—				
	Lower 95% CI	—				
2. Number of leaver per plant	Pearson's r	0.341	—			
	p-value	0.141	—			
	Upper 95% CI	0.681	—			
	Lower 95% CI	-0.120	—			
3. Leaf width (cm)	Pearson's r	0.421	0.246	—		
	p-value	0.064	0.296	—		
	Upper 95% CI	0.728	0.621	—		
	Lower 95% CI	-0.026	-0.221	—		
4. Leaf length (cm)	Pearson's r	0.607 **	0.329	0.542 *	—	
	p-value	0.005	0.156	0.014	—	
	Upper 95% CI	0.828	0.674	0.794	—	
	Lower 95% CI	0.226	-0.132	0.130	—	
5. Number of shoots	Pearson's r	0.282	0.720 ***	0.522 *	0.102	—
	p-value	0.228	< .001	0.018	0.670	—
	Upper 95% CI	0.644	0.882	0.784	0.521	—
	Lower 95% CI	-0.183	0.408	0.103	-0.357	—

* $p < .05$, ** $p < .01$, *** $p < .001$

The following correlations are highlighted and statistically ensured:

- the link between the plant height trait and number of leaves per where the value of the correlation coefficient r is 0.44, considering that the p -value < 0.05 if the correlation is significant at the $\alpha=.05$ level (Figure 1), acceptable degree of association.

- the link between the plant height trait and leaf length (cm) where the value of the correlation coefficient r is 0.43, considering that the p -value < 0.05 if the correlation is significant at the $\alpha=.05$ level (figure 2), acceptable degree of association.

- the link between the plant height trait and leaf width where the value of the correlation coefficient r is 0.34, considering that the p -value < 0.05 if the correlation is significant at the $\alpha=.05$ level, (Figure 3), acceptable degree of association.

- the relationship between the trait number of leaves per plant and number of shoots where the value of the correlation coefficient r is 0.64, considering that the p -value < 0.01 if the correlation is significant at the $\alpha=0.01$ level, (Figure 4), moderate to good correlation.

In the following graphical representations, the scatter diagrams for each possible combination of the variables analyzed in the breeding process were displayed.

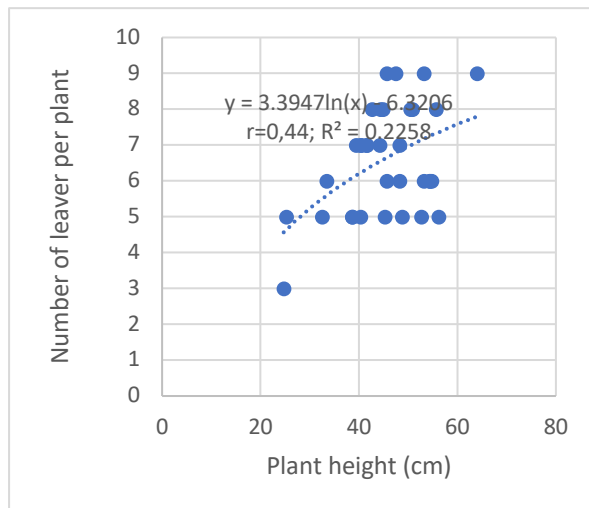


Figure 1. Representation of the correlation between plant height and number of leaves per plant

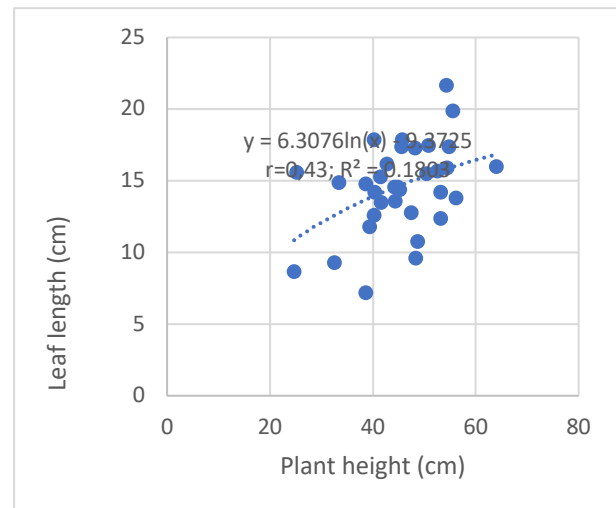


Figure 2. Representation of the correlation between plant height and leaf length

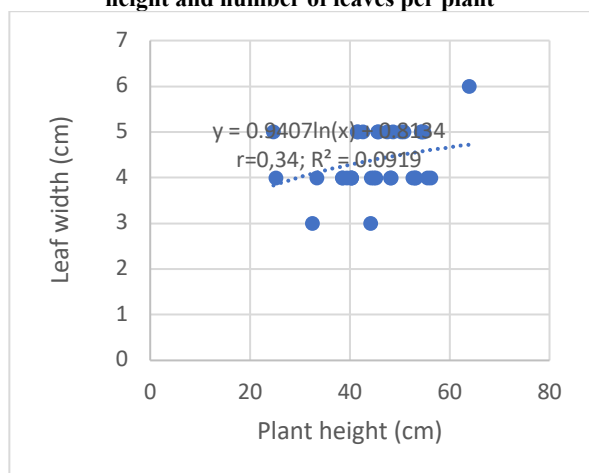


Figure 3. Representation of the correlation between plant height and leaf width

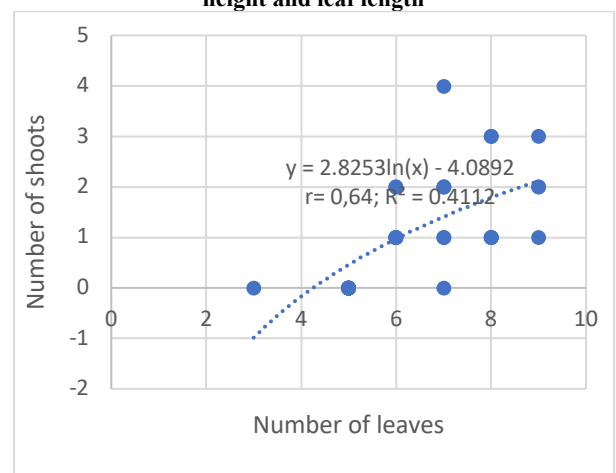


Figure 4. Representation of the correlation between the number of leaves and the number of the shoots

From the analysis of the correlation relationships highlighted between the five morpho-productive traits investigated, it can be observed the determining role of the height of the perennial ryegrass plant in the dimensioning and phenotypic expression of the traits.

Thus, the height of the plant is in a direct interdependence relationship with traits such as the number of leaves per plant, the length and the width of the leaf. These interdependencies are subject to the biomorphological composition of the grass species in which the stem comprises nodes and internodes, and a leaf leaves from each node. In other words, the taller the plant is and the more nodes it has, the more leaves it has. The distinctly significant correlation relationship between the number of leaves per plant and the number of shoots where the growth in the form of a bush in perennial ryegrass explains this high correlation is also highlighted.

In the statistical processing of the results of the average dynamics of the five morpho-productive traits (plant height (cm), number of leaves per plant, leaf width (cm), leaf length (cm) and the number of shoots) in perennial ryegrass, linear regression analysis was also used.

Multivariate linear regression was used because, in this case, several correlated dependent variables are predicted. This type of linear regression focuses on the conditional probability distribution of the response given the values of the predictors, rather than on the joint probability distribution of all these variables, which is the domain of multivariate analysis. The obtained results indicate that the obtained statistical models are nonlinear.

In this case, linear regression as a statistical model was used to estimate the linear relationship between a scalar response (dependent variable) and one or more explanatory variables (regressive or independent variable).

Table 3 shows the multivariate linear regression calculation performed for the traits that have the highest interaction rate expressed by the value of the correlation coefficient r . This concerns the interdependence

between the following traits: plant height /no of leaves/plant, plant height/leaf length and number of leaves per plant/no of shoots.

Table 3. The multivariate linear regression calculation was performed for the traits with the highest interaction rate

	plant height /no of leaves/plant	plant height/leaf length	no of leaves per plant/no of shoots
<i>Regression Statistics</i>			
Multiple R	0.44	0.43	0.64
R Square	0.19	0.18	0.41
Adjusted R Square	0.17	0.16	0.39
Standard Error	7.86	7.91	1.169
Observations	34	34	34

The goal of linear regression is to determine the regression line i.e. "the straight line that best fits the data": a - intercept: the value of y when x is zero (the intersection of the regression line with the Oy axis) and b slope of the regression line: the amount by which the value of y changes when we change the value of x by one unit.

Table 4. The multivariate linear regression between the plant height and the number of leaves/plant

<i>ANOVA</i>					
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	487.586642	487.586642	7.883198	0.00843
Residual	32	1979.243946	61.85137332		
Total	33	2466.830588			

The linear regression between the traits plant height and number of leaves/plant is justified by the previous correlation. Table 4 summarizes the statistical analyses including coefficients that intervene in the regression line equation. This is obtained after statistical processing and reveals that the significance level of the F coefficient value is 7.88 and the F significance is 0.00843 (p-value < 0.05) which rejects the null hypothesis and statistically assures us of the existence of a significant interdependence between the group means.

Table 5. The multivariate linear regression between the plant height and the leaf length

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	460.2091522	460.2091522	7.339049	0.010746
Residual	32	2006.621436	62.70691988		
Total	33	2466.830588			

The linear regression between the plant height/leaf length is shown in Table 5. It is observed that the significance level of the F coefficient value is 7.33, and the F significance is 0.010746 (p-value < 0.05), which statistically ensures the existence of a significant interdependence between the group means.

Table 6. The multivariate linear regression between the plant height and the leaf length

	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>
Regression	1	30.57758738	30.57758738	22.33736	4.4E-05
Residual	32	43.80476556	1.368898924		
Total	33	74.38235294			

The linear regression between the plant height/leaf length is shown in Table 6. It is observed that the significance level of the F coefficient value is 22.33 and the F significance is 4.4E-05 (p value < .05), which statistically ensures the existence of a significant interdependence between the group means. The overall analysis of the linear regression results reveals that the F-critical value coefficient values have a much more grouped distribution with values between 0.00843 and 4.4E-05, in this case, it can be hypothesized that there are no significant differences between the means of the investigated trait groups.

CONCLUSIONS

With climate uncertainty and land use change, a better understanding of the potential of germoplasm collections needs to be developed through species-level descriptive processes and specific objectives and activities for perennial ryegrass breeding. In the case of perennial ryegrass, germoplasm collections are needed to improve the agricultural relevance of forage varieties, as well as to address new challenges related to improving environmental services, as well as water and nutrient use efficiency and carbon sequestration.

This will prioritize future approaches to *Lolium perenne* genotypes for further evaluation in terms of broadening the range of available germoplasm and deepening the biological understanding underlying the approach to future challenges. The continued success of grassland cultivation depends on supporting breeding programs for this species, predominantly spontaneously, in the composition of plant communities.

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