

Identification of Traits with Predictive Value for the Selection of *Trifolium Repens* L. Genotypes

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Abstract. This study aims to identify morpho-productive traits with predictive value for the efficient selection of *Trifolium repens* L. genotypes, a forage legume species of high agronomic and ecological importance. Due to its high protein content, adaptability, and nitrogen-fixing capacity, white clover is a key component of both permanent and sown pastures and represents an important target in breeding programs. The biological material consisted of 57 genotypes collected from Romania's natural flora (forest-steppe area of Banat), tested in 2023 in the experimental fields of SCDA Lovrin. Following the selection process, 24 clones labeled T1–T24 were retained in 2024 for detailed biometric observations and measurements. The analysis of phenotypic variability and trait correlations aimed to identify useful indicators for selection within the breeding program. The results of phenotypic variability analysis revealed significant differences among *Trifolium repens* L. genotypes for most of the morpho-productive traits, confirming valuable selection potential. Traits such as clone diameter, number and height of generative shoots showed significant positive correlations with the number of inflorescences, recommending them as effective predictors of reproductive potential. A perfect correlation ($r = 1.0$) between the number of generative shoots and inflorescences highlights a direct relationship between generative vegetative structure and flowering capacity. Although less influential on yield, stable traits such as the number of leaves per shoot may still have value in indirect selection. The white clover genotypes T11, T12, T13, and T14 stood out through superior performance and are recommended for further testing, due to their high potential expressed through vigorous vegetative and generative development. The study provides a scientific basis for the use of morpho-productive traits as indirect, rapid, and effective tools in white clover breeding, with the potential to accelerate the selection cycle and support the development of productive cultivars well adapted to the agroecological conditions of Western Romania.

Keywords: *Trifolium repens* L., morphoproductive traits, phenotypic variability, phenotypic correlations

INTRODUCTION

Grasslands in Romania are characterized by high biodiversity (Hoancea et al., 2017, Samfira et al., 2010; Simon et al., 2018, Vaida et al., 2021) and host a wide range of valuable species, among which white clover (*Trifolium repens* L.) is frequently found, along with other forage legumes of significant ecological and agronomic importance (Moisuc et al., 1997, Sărățeanu et al., 2023).

In temperate grasslands worldwide, *Trifolium repens* L. includes approximately 240 species (Zoric L. et al., 2012). Through its symbiotic relationship with *Rhizobium* bacteria, it has the ability to fix atmospheric nitrogen, which enhances soil fertility and reduces the need for chemical fertilizers, thereby contributing to sustainable agriculture (Smith et al., 1985).

White clover (*Trifolium repens* L.) is a significant perennial legume in temperate agricultural systems globally. It encompasses approximately 240 species (Zoric L. et al., 2012), is valued for its beneficial agronomic and ecological functions. Through its symbiotic relationship with *Rhizobium* bacteria, it possesses the ability to fix atmospheric nitrogen, which improves soil fertility and decreases the application of chemical fertilizers, making agriculture more sustainable (Smith et al., 1985).

Apart from nitrogen fixation, white clover is also a basis for permanent pastures and forage crops (Nichols P. et al. 2007). It is a good-quality forage crop for animals, being high in protein content, with good digestibility and high palatability (Wilkins & Humphreys, 2003). It ensures a well-balanced diet for animals when blended with grasses, which is suitable for milk and meat production (Egan et al., 2017).

In addition, white clover is a key contributor to the control of soil erosion through its dense cover of the ground and fibrous root system that holds the soil in place. White clover can also be an effective cover crop in conservation agriculture by suppressing weeds and retaining soil moisture (Norton et al., 2021).

There are challenges, however, to realizing the full potential of white clover. Seed production, as required for pasture establishment and rejuvenation, can be unreliable, subject to environmental and genetic influences (Marshall & Humphreys, 1995). Key characteristics like adaptability over a variety of soil and climatic environments, disease and pest resistance, and persistence in mixtures are priority areas for breeding programs (Abberton & Marshall, 2005; Rhodes & Webb, 1998). Therefore, exploitation and understanding of the phenotypic

and genetic diversity of the species are essential to develop new varieties with excellent adaptability to current and future agro-ecological conditions (Annicchiarico & Piano, 2004; Bouton, 2001; López-Luna et al., 2018).

Plant breeding aims to improve the genetic potential of crops to meet the specific demands of modern agriculture. This involves not only increasing yield (Acikgoz, 1990; Rumbaugh & Johnson, 1986), but also enhancing nutritional quality, resistance to diseases and pests, tolerance to abiotic stress factors (e.g., drought, salinity) (Marshall & Laidlaw, 2002), and adaptability to sustainable production systems (Abberton & Marshall, 2005; Sleper & Poehlman, 2006). To achieve these goals, breeders must select superior genotypes from a diverse population and include them in crossing and selection programs. The use of morpho-productive traits as selection tools is applied efficiently, enabling rapid and accurate determination of the genetic potential of plant material (Allard, 1999; Fehr, 1987), thereby reducing both the time and cost required to develop new cultivars (Fehr, 1987).

Consequently, studying variability and the correlations between traits is essential for establishing a solid scientific basis that supports strategic and efficient selection of valuable genotypes (Annicchiarico & Piano, 2004; López-Luna et al., 2018). For instance, a strong correlation between an easily measurable trait (such as clone diameter) and a complex trait like seed yield allows breeders to use the former as a phenotypic predictor, significantly simplifying the early selection process (Falconer & Mackay, 1996; Li et al., 2013).

In the absence of sufficient genetic diversity, breeders' ability to select and create genotypes with improved performance in terms of yield, quality, or environmental resilience is considerably limited (Sleper & Poehlman, 2006). Therefore, the evaluation and conservation of genetic material are fundamental activities in any breeding scheme.

Effective plant breeding inevitably relies on identifying reliable performance indicators. Since measuring complex traits such as seed production is often slow and costly, identifying easily measurable morpho-productive traits that are strongly correlated with reproductive success offers an indirect and early selection method (Falconer & Mackay, 1996; Fehr, 1987). Such indicator traits can significantly accelerate the breeding cycle and, consequently, the development of improved cultivars (Acikgoz, 1990). In genotype evaluation, the breeder must ensure that selected genotypes not only exhibit high reproductive potential but are also capable of surviving and persisting in various agro-ecological environments (López-Luna et al., 2018; Annicchiarico & Piano, 2004).

The aim of this study is to identify morpho-productive traits that may have predictive value in the selection of *Trifolium repens* L. genotypes, with applicability in breeding programs targeting either improved biomass production or the selection of genotypes with superior reproductive potential. By analyzing phenotypic variability and trait correlations, the study seeks to highlight those traits that can serve as efficient indicators for identifying valuable genotypes in terms of yield and adaptability under specific pedoclimatic conditions.

Ultimately, the results of this research will provide a solid scientific foundation for the development of improved *Trifolium repens* L. cultivars, thereby contributing to more productive and resilient forage systems.

MATERIAL AND METHODS

1. Pedoclimatic conditions

The experiments were carried out within the Lovrin Agricultural Research and Development Station, in the improvement fields of the unit, located on a typical chernozem soil. The pedoclimatic conditions of the area provide a representative framework for evaluating the behavior of genotypes for selection and breeding.

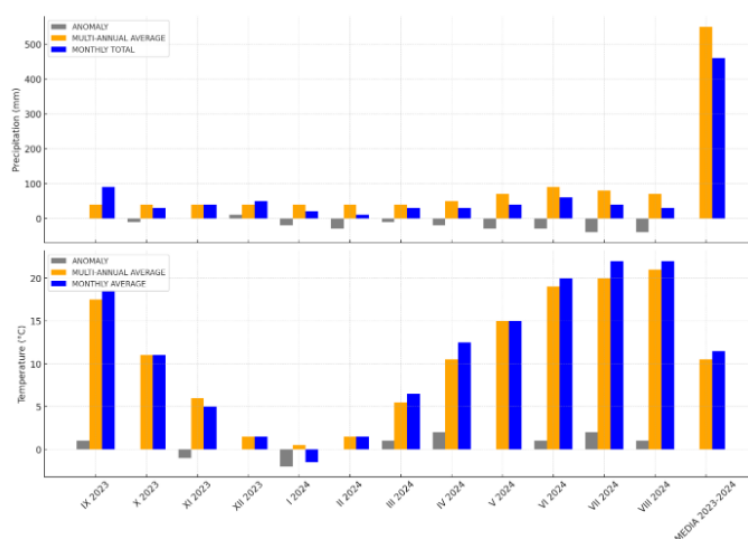


Figure 1. Climatic conditions in 2023–2024 (Meteorological Station – SCDA Lovrin)

During the 2023–2024 period (Fig. 1), monthly temperatures recorded at SCDA Lovrin were generally above the multi-annual average, particularly from spring through autumn, which accelerated the onset of vegetation and active growth in white clover. However, this thermal trend was accompanied by a consistent precipitation deficit in most months, leading to suboptimal moisture conditions for this species, especially during regeneration and peak development stages. Consequently, the combination of elevated temperatures and reduced rainfall increased the mismatch between the plant's physiological demands and the available resources, negatively impacting biomass production potential and the uniformity of the vegetative cover.

2. Biological material and working method

The biological material used in this study consisted of various genotypes of *Trifolium repens* L., collected from Romania's natural flora, specifically from the forest-steppe region of Banat.

In 2023, a total of 57 clones (genotypes) of *Trifolium repens* L. were tested in the collection and selection fields of the Agricultural Research and Development Station (SCDA) Lovrin. These genotypes were selected through multiple stages, according to predefined breeding objectives, and monitored in subsequent generations. Each individual plant represented a separate clone, with a spacing of 1 meter between clones. Following field observations and biometric measurements, the most valuable genotypes were retained and included in the creation of the initial breeding material.

Thus, in the experimental year 2024, 24 clones were selected for detailed biometric evaluation. These 24 white clover (*Trifolium repens* L.) genotypes were labeled using a coded system (T1–T24), in which each identifier corresponds to an internal classification used within the experimental collection to distinguish between genetic forms. This coding system enabled clear and efficient tracking of each genotype throughout morphological observations and statistical analyses.

3. Statistics used

The data collected for the studied *Trifolium repens* L. genotypes were statistically analyzed. 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]. The p-value shows that significant correlations are marked with: * $p < .05$ if the correlation is significant at the alpha level = .05; ** $p < .01$ if the correlation is significant at alpha level = .01; and *** $p < .001$ if the correlation is significant at the alpha level = .001.

RESULTS AND DISCUSSIONS

In order to understand the phenotypic diversity within the analyzed *Trifolium repens* L. genotypes, a descriptive analysis of the main morphoproductive characteristics was performed: clone diameter (cm); number of vegetative shoots/clone; height of vegetative shoots/clone (cm), height of generative clone shoots (cm); number of leaves/shoot; leaf area (cm²); number of inflorescences/clone.

The analysis of morphoproductive traits in *Trifolium repens* L. genotypes highlights important variations between individuals. Table 1 shows the mean and dispersion values for the most relevant features. The coefficients of variation indicate a moderate to high dispersion, being the highest for the number of inflorescences/clone.

Table 1. Analysis of morphoproductive traits

Trait	Mean	Standard Deviation	Coefficient of Variation	Skewness	Kurtosis
Clone diameter (cm)	108.458	16.611	0.153	-0.616	0.567
No. of vegetative shoots/clone	641.667	198.728	0.310	0.202	-1.195
Height of vegetative shoots/clone (cm)	27.458	5.389	0.196	0.417	0.200
Height of generative shoots/clone (cm)	34.750	5.620	0.162	-0.470	0.133
No. of leaves/shoot	3.0	0.0	0.0	nan	nan
Leaf area (cm ²)	7.405	2.277	0.307	0.450	0.046
No. of inflorescences/clone	149.042	85.961	0.577	1.171	1.084

In order to verify the normality of the distribution of morphoproductive traits, the Shapiro-Wilk test was applied. It tests the null hypothesis that the data comes from a normal distribution. The results obtained (e.g. for clone diameter: $W = 0.962$, $p = 0.471$) do not indicate significant deviations from normality, which confirms that the distribution is in accordance with the Gaussian model. Thus, the data are suitable for the use of parametric tests, such as Pearson correlation and linear regression. The only exception was identified in the case of the number of inflorescences/clone ($p = 0.004$), where the distribution differs significantly from normality.

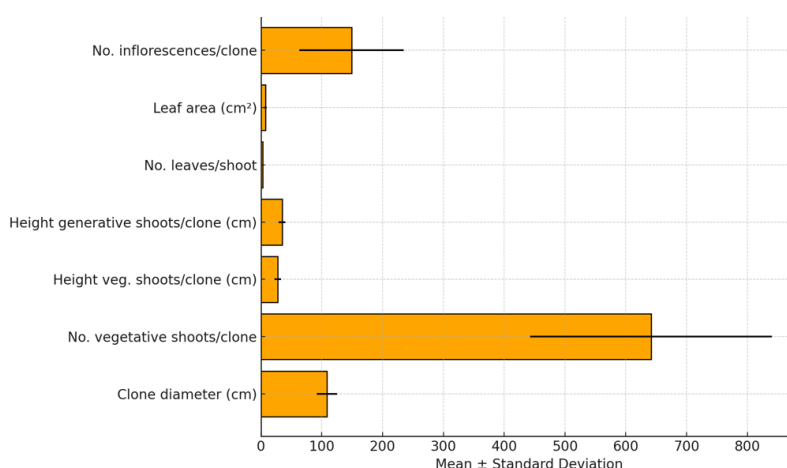


Figure 2. Phenotypic variability within the genotypes of *Trifolium repens* L.

Figure 2. shows the mean values and standard deviations for a number of relevant morphoproductive traits within a set of *Trifolium repens* L. genotypes. These traits include biometric plant sizes (e.g. clone diameter, height of vegetative and generative shoots), architectural elements (number of shoots and leaves), as well as components of reproductive potential (number of inflorescences) and physiological (leaf area).

The analysis of these traits is essential for characterizing the phenotypic variability within the studied populations. The degree of dispersion (represented by the standard deviation) provides information on the stability of the trait under similar environmental conditions and its genetic expression. Traits with high variability, such as the number of inflorescences and the number of vegetative shoots per clone, may reflect a strong influence of the gene pool, but also a greater susceptibility to environmental factors. On the other hand, low dispersion traits (e.g. number of leaves/shoot) can be considered relatively stable and predictable traits in selection.

From the perspective of plant breeding, these preliminary data allow the identification of traits with high practical value, either for improving biomass production or for the selection of genotypes with higher reproductive potential. Also, the observed variability is a valuable starting point for the estimation of genetic parameters (heritability, estimated genetic progress) and for the application of modern methods of marker-assisted selection or genomic improvement.

In order to explore the interdependencies between the evaluated morphoproductive traits, a Pearson correlation analysis was performed, with the aim of highlighting the significant relationships between the different components of *Trifolium repens* L. plant development (Table 2).

The analysis of Pearson correlations revealed significant relationships between several morphoproductive characters of *Trifolium repens* genotypes. The diameter of the clone showed a strongly positive correlation with the number of vegetative shoots per clone ($r = 0.727$, $p < 0.001$), which indicates that plants with a larger diameter tend to form more vegetative shoots. Also, a high correlation was observed between the diameter of the clone and the height of the generative shoots ($r = 0.664$, $p < 0.001$), and between the diameter of the clone and the number of inflorescences there was a correlation of $r = 0.577$ ($p = 0.003$). These results suggest that a larger clone diameter is associated with more intense aerial and reproductive development.

The number of generative shoots per clone was strongly correlated with the number of inflorescences ($r = 1.000$, $p < 0.001$), a relationship that directly reflects the biological link between the development of generative shoots and the appearance of inflorescences.

The height of the vegetative shoots was significantly correlated with both the height of the generative shoots ($r = 0.598$, $p = 0.002$) and the leaf area ($r = 0.442$, $p = 0.030$), which indicates a relationship between the height of the vegetative apparatus and the photosynthetic capacity of the plant.

In contrast, other correlations, such as the one between the number of vegetative shoots and leaf area ($r = -0.105$, $p = 0.625$), were not statistically significant, which shows a lack of clear association between these variables. These results confirm that some traits can be used as indirect indicators for the selection of genotypes with high morphoproductive potential.

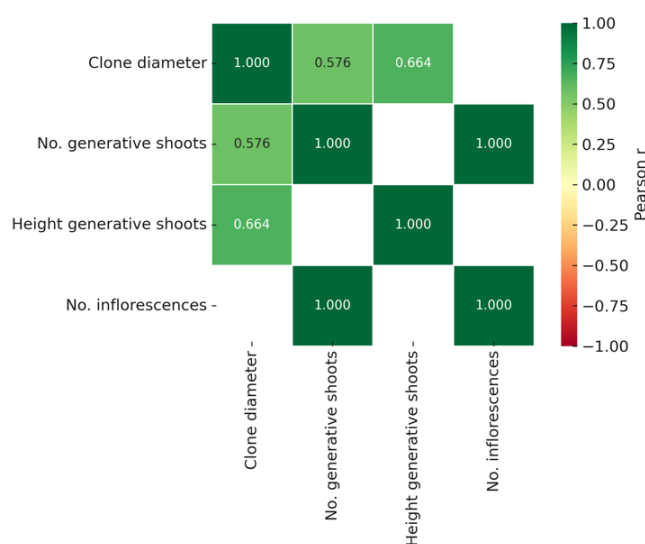
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Table 2. Pearson Correlation Matrix for morphoproductive characteristics of *Trifolium repens* L. genotypes

Variable		Clone diameter (cm)	No. of vegetative shoots/clone	No. of generative shoots/clone	Height of vegetative shoots/clone (cm)	Height of generative shoots/clone (cm)	Leaf area (cm ²)	No. of inflorescences/clone
1. Clone diameter (cm)	Pearson's r	—						
	p-value	—						
2 No. of vegetative shoots/clone	Pearson's r	0.727 ***	—					
	p-value	<.001	—					
3. No. of generative shoots/clone	Pearson's r	0.576 **	0.385	—				
	p-value	0.003	0.063	—				
4. Height of vegetative shoots/clone (cm)	Pearson's r	0.288	-0.086	0.058	—			
	p-value	0.172	0.691	0.786	—			
5. Height of generative shoots/clone (cm)	Pearson's r	0.664 ***	0.298	0.436 *	0.598 **	—		
	p-value	<.001	0.158	0.033	0.002	—		
6. Leaf area (cm ²)	Pearson's r	0.108	-0.105	-0.276	0.442 *	0.313	—	
	p-value	0.614	0.625	0.192	0.030	0.136	—	
7. No. of inflorescences/clone	Pearson's r	0.577 **	0.388	1.000 ***	0.059	0.437 *	-0.276	—
	p-value	0.003	0.061	<.001	0.786	0.033	0.192	—

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

In the realization of the heat map (fig.3) were included those morphoproductive traits that directly or indirectly influence the formation and development of seeds. These include the diameter of the clone (as an indicator of the general vigor of the plant), the number and height of generative shoots (involved in the development of floral organs) and the number of inflorescences (directly correlated with the potential for seed production).

**Figure 3.** Heat map of correlations between morphoproductive traits relevant for seed production in *Trifolium repens* L.

The heat map in Figure 3 illustrates the Pearson correlation coefficients calculated between the analyzed traits. A perfectly positive correlation ($r = 1.0$) between the number of generative shoots and the number of inflorescences is noted, which reflects the natural biological connection between generative shoots and the appearance of inflorescences. Also, the diameter of the clone correlates positively with both reproductive traits (number of shoots and their height), suggesting that more vigorous plants have a greater potential to form inflorescences.

This analysis supports the idea that certain vegetative traits can be used as indirect predictors of seed production capacity, contributing to the efficiency of the selection process in the improvement of *Trifolium repens* L. genotypes.

To highlight the degree of morpho-productive similarity among the 24 white clover (*Trifolium repens* L.) genotypes, a hierarchical cluster analysis (HCA) was performed based on the standardized values of seven quantitative traits (fig. 4). The distances between genotypes were calculated using Euclidean distance, and the grouping method applied was Ward's method, which minimizes within-cluster variance.

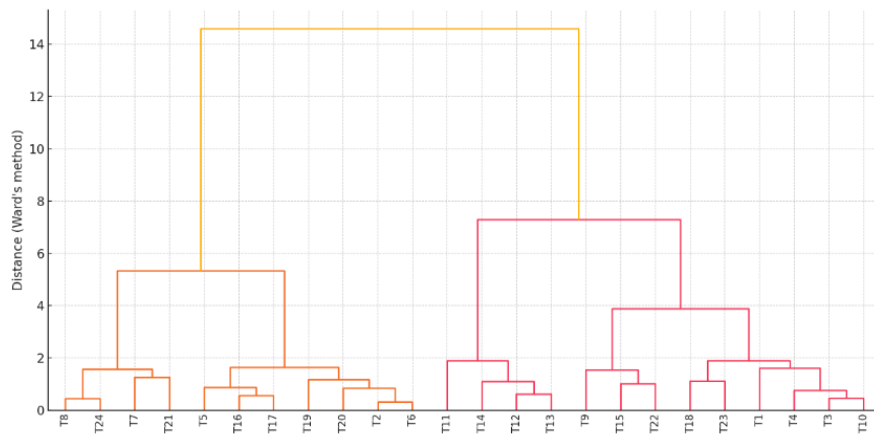


Figure 4. Cluster analysis of 24 white clover genotypes (T1-T24) based on quantitative traits

The results are graphically represented as a dendrogram (Fig. 4), reflecting the phenotypic relationships among the 24 white clover (*Trifolium repens* L.) genotypes, labeled T1–T24. The dendrogram enables the identification of distinct genotype clusters, characterized by high internal homogeneity and significant morpho-productive differences compared to other groups.

Several key observations can be highlighted:

Genotypes T11, T12, T13, and T14 form a homogeneous cluster, indicating similar morpho-productive behavior and high performance across most analyzed traits.

In contrast, genotypes T8 and T24 are positioned at the outermost branches of the dendrogram and appear as isolated entities, suggesting substantial differences from the rest of the experimental set. These may represent valuable genetic resources for introducing variability into breeding programs.

Other compact groups, such as T16–T17 and T5–T6, indicate intermediate forms with potential for targeted selection, depending on breeding objectives (e.g., forage yield or seed production).

The clear differences identified among the analyzed white clover genotypes reflect significant variation in morpho-productive traits, an aspect that is essential to study for both qualitative and quantitative characteristics. This variability provides breeders with a valuable basis for selecting superior forms and improving traits of agronomic interest. The existence of genotypic and phenotypic variability at the species level plays a fundamental role in strengthening improved lines and in effectively initiating breeding programs (Annicchiarico & Piano, 2004; Mohammed et al., 2011; Bhandari et al., 2017; Tahir et al., 2023).

CONCLUSIONS

The analysis of phenotypic variability showed significant differences between the genotypes of *Trifolium repens* for most of the morphoproductive traits analyzed, which confirms the existence of a valuable potential for selection.

Features such as clone diameter, number of generative shoots, and their height showed significant positive correlations with the number of inflorescences, suggesting that they can be used as phenotypic predictors of reproductive potential. The perfect correlation ($r = 1.0$) between the number of generative shoots and the number of inflorescences highlights a direct relationship between the generative vegetative structure and the flowering capacity, an essential aspect in the selection for seed production. The stability of some traits, such as the number of leaves/shoot, indicates a possible value in indirect selection, although they have a lower contribution to reproductive expression.

The *Trifolium repens* L. genotypes that exhibited superior performance for most of the analyzed morpho-productive traits, such as T11, T12, T13, and T14, are recommended to be preserved and included in further testing. These genotypes represent valuable resources for selection and breeding programs due to their high potential expressed through increased clone diameter, a high number of vegetative and generative shoots, optimal plant height, and well-developed leaf area.

The results obtained support the use of morphoproductive traits as useful tools in the early selection of valuable genotypes, providing a scientific basis for breeding oriented towards seed multiplication efficiency and adaptability in various agroecological conditions.

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