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IMPACT OF SEEDING DISTANCE BETWEEN ROWS ON STOLONS FEATURES IN *TRIFOLIUM REPENS* L.

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Abstract. *Trifolium repens (white trefoil) is an important perennial leguminous species from permanent and artificial pastures. White trefoil is usually cultivated in mixture with a grass species, respectively Lolium perenne because they are forming together a balanced source of forage from nutritional point of view. The research analyses the variation of the seasonal growth of Trifolium repens and Lolium perenne in pure stands and as mixture in the conditions of grazing with sheep and cutting. The research was performed at Research and Development Station for Sheep and Goats Breeding Caransebeş on an albic luvisol, pseudo-gleyic, low acid, moderate provisioned with phosphorus and potassium. The formation, growth and development of Trifolium repens stolons was assessed at every grazing cycle and after every cut. Also, there was assessed the plant height before every cutting or grazing cycle. The experimental factors considered were exploitation mode (cutting, grazing 2 LU/ha and 4 LU / ha) and distance between rows at seeding (12.5 and 25 cm). The assessed features for Trifolium repens were: length of the stolons per plant, number of nodes per stolon and the distance between two nodes per 10 plants from each replicate. Distance between rows hasn't been influenced by the analysed stolons features. Also, once with the increase of the nodes number decreases the length of an internode, these two features of the stolons being corelated negatively.*

Keywords*: Trifolium repens, stolons, mixture, grazing, cutting.*

INTRODUCTION

It is considered that genus *Trifolium* originates from the mediterranean area WILLIAMS et al., 2011). Other literature resources are considering that the members of this genus are originating from temperate and subtropical areas, except south-east Asia and Australia (ELLISON ET AL., 2006; PUTNAM ET ORLOFF, 2014).

Trifolium repens L. belongs to the class *Magnoliopsida*, subclass *Rosidae*, order *Fabales*, family *Fabaceae*, tribe *Trifolieae*, genus *Trifolium* (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=info&id=3899, accessed on 16 Mai 2021).

White trefoil is used in general in mixtures with grasses, the most frequently used mixture being with perennial ryegrass (*Lolium perenne*) (BROCK et HAY, 1988).

In the research developed by GUY et al. (2021) was highlighted that white trefoil is in disadvantage with perennial ryegrass if is cultivated in mixture, mostly due to the slow growing rate of the white trefoil at low temperatures, this fact determining the obtaining of a small amount of forage early in spring. Also, there was noticed that the ploidy degree of the perennial ryegrass is influencing significantly the elongation of the stolons and the number of leaves from the stolons.

In other research NÖLKE et al. (2021) have shown also that the architecture of the white trefoil plants and the production is highly influenced by the accompanying species and by the season.

The rate of the vegetative dissemination and seed production in white trefoil is relatively insufficiently known, but it is considered that the vegetative spreading is based on the base of the stolons that are growing at the top and decompose at the base, instead to the radial spread of the stolons from a crown. Thus, the stolons can be considered parental plants because they are wearing ramifications, from which some become independent when are separated by the parental plant (BĂRBULESCU et MOTCĂ, 1987).

Stolons and roots of white trefoil are considered the main depositing organs for nitrogen during winter. In the literature are researches referring to the features of the stolons (HAY, 1983; DAVIES, 1992) and their survival during winter (COLLINS, 1991; COLLINS et RHODES, 1995; FRANKOW-LINDBERG et VON FIRCKS, 1998; STURITE, 2006).

Use of the competition of the white trefoil with grasses and grazing as elements in the selection and breeding process of this species have contributed to the identification of some valuable genotypes used for creation of new varieties with very good perennity and productive in the extreme environmental conditions (CARADUS, 1990).

The purpose of the work was to analyse some elements of the growth and development of the cultivated white trefoil at two different densities. There was considered that such data can be useful for the identification of some useful information underlying the formation and development of the stolons in the white trefoil.

MATERIAL AND METHODS

The biological material was represented by the white trefoil variety Rivendel. The experimental factor considered in this research was distance between rows at seeding, this having two graduations, respectively 12.5 cm (V1) and 25 cm (V2). The experimental variants had three replicates each.

The biometrical features considered in this research were: nodes number, stolon length (cm), and distance between nodes (cm). There were sampled 10 plants from each replicate and each variant.

The collected data were statistically processed using the software JASP 0.17.2.1 (JASP Team, 2023). The statistical calculations used were: descriptive statistics, one-way ANOVA, there being also applied the bootstrapping method, Bravais-Pearson correlation and linear regression.

RESULTS AND DISCUSSIONS

The initial analysis of the experimental data was done using the descriptive statistics (Table 1).

The mean number of nodes per stolon was 11.27 in the variant sown at 12.5 cm between rows (V1) and 12.13 in the variant sown at 25 cm (V2). The coefficient of variation of the number of stolons was higher in V1 (Cv = 0.46) than in V 2 (Cv = 0.40).

In the case of stolon length, the average value was greater in V2 (17.4 cm) than in V1 (15.4 cm). The same trend was noticed for the coefficient of variation, which was higher in V2 sown at 25 cm between rows (Cv = 0.37) than in V1 sown at 12.5 cm between rows (Cv = 0.34).

The average internode length was greater in V1, respectively 1.96 cm, compared to V2 which was 1.70 cm. The same trend was noticed as well in the case of coefficient of variation, which was also higher in V1 (Cv = 0.76) than in V2 (Cv = 0.54).

Table 1

Descriptive statistics regarding the analysed elements of the stolons in white clover

	Nodes no.		Stolon length (cm)		Internode length (cm)	
	1	2	1	2	1	2
Mean	11.26	12.13	15.40	17.40	1.98	1.70
Std. Deviation	5.19	4.88	5.20	6.37	1.49	0.92
Coefficient of variation	0.46	0.40	0.34	0.36	0.76	0.54
Variance	26.96	23.84	27.05	40.57	2.23	0.84
Shapiro-Wilk	0.96	0.95	0.96	0.95	0.74	0.91
P-value of Shapiro-Wilk	0.29	0.14	0.29	0.21	< 0.001	0.02
Minimum	3.00	4.00	6.06	6.99	0.43	0.49
Maximum	24.00	20.00	27.91	31.88	7.67	4.06

The following is the one-way ANOVA for the factor row spacing at white clover seeding. Thus, the initial information obtained in the analysis of variance applied to the stolon analysed features is presented in Table 2.

Thus, according to the preliminary results obtained from the statistical processing of the data for the considered factor (row spacing), the null hypothesis was confirmed, respectively we do not have confirmation that there are statistically significant differences between the variants for any of the variables of the stolons analysed.

Table 2

Variance analysis (ANOVA) for the analysed variables of the stolons (nodes no., stolon length and internodes length)

Cases	Sum of Squares	df	Mean Square	F	p
ANOVA - Nodes no.					
Variant	11.267	1	11.267	0.444	0.508
Residuals	1473.333	58	25.402		
ANOVA - Stolon length (cm)					
Variant	60.020	1	60.020	1.775	0.188
Residuals	1961.102	58	33.812		
ANOVA - Internodes length (cm)					
Variant	1.151	1	1.151	0.750	0.390
Residuals	88.996	58	1.534		

Note. Type III Sum of Squares

However, in order to check whether the null hypothesis is confirmed there was used bootstrapping modelling as an alternative to the traditional method based on the initial data set only. The model applied in the paper is based on random multiplication of the mean of the differences, in the present case by 500 times to simulate a larger number of data (Table 3), based on the original experimental data and their normality, which also tests the null hypothesis, but without taking into account the sample size of the data and their distribution. In fact, by boostrapping we increase the precision in terms of testing the null hypothesis, even for smaller data sets.

Table 3

Bootstrapped Post Hoc Comparisons - Variant (V1 and V2)

Nodes no.

Variant		Mean Difference	95% bca† CI Lower	95% bca† CI Upper	SE	bias	t	p_{tukey}
1	2	-0.972	-3.366	1.883	1.317	-0.110	-0.666	0.508

Stolon length (cm)

Variant		Mean Difference	95% bca† CI Lower	95% bca† CI Upper	SE	bias	t	p_{tukey}
1	2	-1.977	-5.105	0.964	1.439	0.044	-1.332	0.188

Internodes length (cm)

Variant		Mean Difference	95% bca† CI Lower	95% bca† CI Upper	SE	bias	t	p_{tukey}
1	2	0.279	-0.284	0.995	0.315	-0.003	0.866	0.390

† Bias corrected accelerated.
Note. Bootstrapping based on 500 successful replicates.
Note. Mean Difference estimate is based on the median of the bootstrap distribution.

The statistical results obtained by bootstrapping suggest, as in ANOVA, that seeding *Trifolium repens* at 12.5 or 25 cm between rows does not influence the morphological characteristics of the stolons analysed in this work.

In the graphs below the characters of white clover runners are compared in terms of the variants analysed (V1 and V2) (Figure 1). On the graph are represented the mean and standard deviation values and the outliers, the latter being present in the case of the node distance feature, while in the other two features analysed, we didn't have such discrepant values.

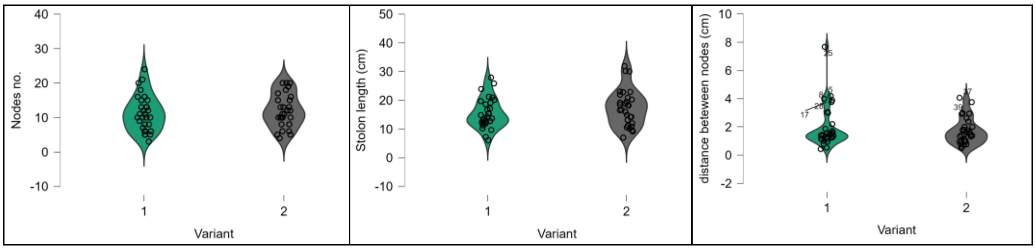

Figure 1. Comparison of the analysed features of the white clover stolons (V1 and V2); a) nodes number; b) stolon length (cm); c) distance between nodes (cm)

Figure 2 shows the patterns of the interactions between the analysed morphological features of the white clover analysed on the two experimental variants analysed. From the model it is clear that this interaction is different depending on the row spacing at sowing of white clover in the case of pairs nodes number and stolon length. Between the features nodes number and distance between nodes the interaction is similar for both variants of row spacing. In the case between the features nodes number and distance between nodes, it appears that this interaction is different and is largely influenced by the row spacing of white clover at seeding. In V1, the two traits are correlated and are more interdependent than in V2.

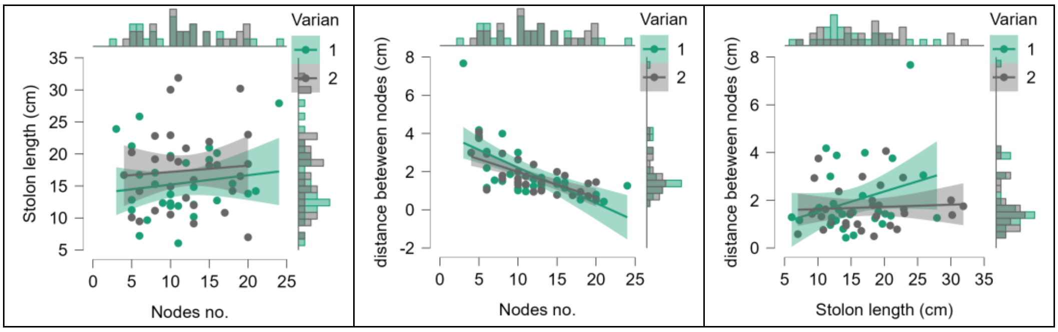

Figure 2. Model of the interaction between the analysed features of the white clover stolons (V1 and V2)

The following is an analysis of correlations (Pearson) between the features of the white clover stolons, respectively number of nodes, stolon length (cm) and internode length (cm) (Table 4), without considering the distance between rows.

Table 4

Correlations matrix between the analysed features of the white clover stolons (nodes number, stolon length and internode length

Variable		Nodes no.	Stolon length (cm)	distance beteween nodes (cm)
1. Nodes no.	Pearson's r	—		
	p-value	—		
2. Stolon length (cm)	Pearson's r	0.120	—	
	p-value	0.359	—	
3. distance beteween nodes (cm)	Pearson's r	-0.661 ***	0.163	—
	p-value	< .001	0.214	—

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

Table 4 shows that there is a highly significant negative correlation (Pearson's r = -0.661***) between the number of nodes and internode length regardless of the sowing distance.

In order to determine the existence of a possible linear relationship between the analysed features of the stolons, linear regression was analysed, where the explanatory variables considered were the number of nodes and the internode distance (predictors) and the continuous dependent variable (stolon length).

Hypothesis testing (Table 5) confirmed the validity of the null hypothesis and indicated autocorrelation between the residual values of the variables analysed.

Table 5

Examination of the regression coefficients

Model Summary - Stolon length (cm)

					Durbin-Watson		
Model	**R**	**R²**	**Adjusted R²**	**RMSE**	**Autocorrelation**	**Statistic**	**p**
H_1	0.952	0.906	0.899	5.527	-0.062	2.085	0.849

To confirm the results based on the experimental data, 500-fold random multiplication of the data was also applied to obtain the estimated regression coefficients (Table 6).

Table 6

Estimated regression coefficients estimated by boostrapping

Model		**Unstandardized**	**Bias**	**Standard Error**	**p***
H_1	Nodes no.	0.478	-0.014	0.197	0.044
	distance beteween nodes (cm)	2.111	-0.030	0.627	0.006
	Variant (1)	5.835	0.197	3.070	0.030
	Variant (2)	8.185	0.330	3.201	0.016

Note. Bootstrapping based on 500 replicates.

Note. Coefficient estimate is based on the median of the bootstrap distribution.

* Bias corrected accelerated.

The following is the regression model table (Table 7), which includes partial correlations and semipartial correlations between the predictor variables and the independent variable, showing the degree of association between two random variables. Thus, the table shows the extent to which the dependent variable stolon length is most influenced by internode length (0.336), followed by the number of nodes (0.3), but also by the distance between rows (variant) (0.187).

Table 7

Partial and semi-partial correlations between the considered variables of the white trefoil stolons

Model		**Partial**	**Part**
H_1	Nodes no.	0.310	0.100
	distance beteween nodes (cm)	0.343	0.112
	Variant	0.326	0.106

The investigations applied to the white trefoil stolons show a great complexity, the obtained results suggesting the necessity to extension the number of the investigated features, even in relationship with the accompanying plants.

CONCLUSIONS

On the basis of the experimental results that have been obtained on the behaviour of white trefoil stolons features under sowing conditions at different row spacings the following conclusions could be drawn:

- the sowing mode of *Trifolium repens* at 12.5 or 25 cm row spacing did not influence the morphological features of the stolons such as: number of nodes, stolon length and internode length;
- as the number of nodes increased, the distance between nodes decreased, and vice versa, these features being strongly negatively correlated;
- when quantifying the degree of influence on the dependent variable by the means of the multiple correlations, it was found that stolon length is the most impacted feature by the internode length (0.336), followed by the number of nodes (0.3), but also by the distance between rows (variant) (0.187), but the variant has a lesser impact.

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