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GENOTYPE X ENVIRONMENT INTERACTION AND DRY MATTER YIELD STABILITY OF WHITE CLOVER (*TRIFOLIUM REPENS* L.) CULTIVARS AND BREEDING POPULATIONS

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Abstract

Dry matter yield peculiarities in six cultivars and four breeding populations of white clover (*Trifolium repens* L.) were studied at the Lithuanian Institute of Agriculture (Dotnuva) during the period 2001-2003. ANOVA, joined regression and pattern (cluster) analysis were employed to draw the conclusions. Results of ANOVA analysis showed that dry matter yield was essentially ($P < 0.01$) influenced by the cuts, year of trials, cultivars and their interaction. Promising numbers Nos.: 1123 and 1124 combined high annual yield of dry matter (5.80-5.89 t ha^{-1}) with a low variance (S^2_d) of stability (0.0593-0.0956). Cluster analysis revealed the presence of 3 groups of varieties distinguished by a similar genotype - environment response. The results of cluster analysis in general confirmed the breeding value of the varieties and of the population, obtained on the basis of stability evaluation.

Key word: white clover, stability of yield, cluster analysis.

Introduction

White clover (*Trifolium repens* L.) is one of the most nutritious species available in Lithuanian grassland/ruminant production systems. In association with grass, this species increases protein, mineral content, intake and nutrient value of the total forage. Because of its nitrogen fixing capacity, white clover has the potential to reduce, or in the case of organic systems, eliminate the need for inorganic nitrogen fertilizer on grazed grassland /Sprainaitis et al., 2002/. Not only a high yield of dry matter, but also its stability in different conditions of cultivation are important for white clover cultivars. Growing awareness of the importance of genotype-environment (GE) interaction has led crop genotypes to be ordinarily assessed in multi-environment, regional trials for cultivars recommendation or for the final stages of elite breeding material selection /Annicchiarico, 2002/.

Most of the stability measures relate to either of the two contrasting concepts of stability: “static” and “dynamic” /Lin et al., 1986; Becker, Leon, 1988/.

Static stability is analogous to the biological concept of homeostasis: a stable genotype tends to maintain a constant yield across environments. Dynamic stability implies for a stable genotype a yield response in each environment that is always parallel to the mean response of the tested genotypes, i. e. zero GE interaction /Annicchiarico, 2002/.

The presence for varieties of GE interaction can reduce errors in the breeding process, as selection in one type conditions cannot provide advantage in others /Simmonds, 1991/.

In white clover variety populations, adaptability is based on genetic homeostasis which comprises not only heterogeneity, but also homozygocity which is induced by free interpollination in plants. Thus, white clover varieties exhibit a wide range of response to environmental factors. The yield stability of white clover (*Trifolium repens* L.) results in changes in plant habit in response to different environmental stresses /Seeker et al., 2003/. A 30-year study of environmental effects on the persistence of white clover in Australia concluded that late summer moisture stress was the critical factor limiting white clover persistence /Hutchinson et al., 1995/. Knowledge of dry matter yield stability parameters of a new variety allows prognosticating its response to changed conditions of cultivation and recommending the necessary agrotechnics for obtaining optimum yields.

Several methods have been proposed to analyze genotype x environment interactions and phenotypic stability. These methods can be divided into two major groups, univariate and multivariate stability statistics /Lin et al., 1986/. Joint regression is the most popular calculation and application for them /Goncalves et al., 2003/. Finlay and Wilkinson's (1963) b_i considers a cultivar stable if its response to environments is parallel to the mean response of all cultivars in the trial. Varieties with the coefficient of regression b_i=1.0 exhibit a full correspondence between the yield dynamics and environmental changes. The higher the value of the coefficient ($b_i > 1.0$), the higher the level of response of a variety. In the case when ($b_i < 1.0$), a variety shows a weaker response to environmental conditions than the average pool of the varieties under study. Eberhart and Russell's (1966) S^2_d considers a cultivar stable if the residual mean square from Finlay and Wilkinson's regression model is not significant. The less the sum of yield deviation squares is shown by a variety, the higher are its stability characteristics. A stable genotype has a regression coefficient (b_i) value close to 1.0, and deviations from regression are as small as possible ($S^2_d = 0$).

For GE interaction assessment, intricate mathematical methods have been developed. One of them, pattern analysis, is realized in the IRRISTAT computer software.

The aim our investigations was to establish dry matter yield stability parameters in six varieties and four breeding populations of white clover and to select the most valuable ones for the development of an ecologically stable variety.

Materials and methods

As experimental material we used 6 white clover cultivars ('Sūduviai', 'Bitūnai', 'Atoliai' and 'Nemuniai' from Lithuania, 'Milo' and 'Rivendel') from Denmark and 4 breeding populations (Nos. 1123, 1124, 1421 and 1435) developed over the recent years at the Lithuanian Institute of Agriculture. The experiments were carried out during 2001-2003 in central Lithuania (Dotnuva) on a sod gleyic moderately heavy drained loam soil with a pH value in the arable layer varying from 6.4 to 7.2 and humus content from 1.9 to 2.2 %. The following crop rotation was used: 1) black fallow; 2) grasses of the sowing year; 3) grasses of the first year of use; 4) grasses of the second year of use; 5) spring cereals; 6) spring cereals. The experiment was located under numbers 2, 3 and 4 in this rotation.

The white clover populations were sown on 10.0-12.5 m^{-2} plots in the first half of June without a cover crop. The seed rate for all cultivars and numbers was 8 kg ha^{-1}. The experimental design was a randomized complete block with three replications. In the year of use the herbage was cut twice with a Hege 212 field mower, when white clover plants had reached 10 % flowering. After cut, 0.5 kg herbage samples were taken for dry matter content analysis. All samples were weighed and dried to a constant weight in an oven controlled at 105 ^{o}C and the amount of dry matter harvested was determined. As a standard, the 'Sūduviai' white clover cultivar was used.

In the autumn of each year of use, phosphorus and potassium fertilizers were applied.

Table 1. Precipitation and temperature data (April-October) in Central Lithuanian region (Dotnuva), for the study period (2001-2003), with long - term (1924-2003) average

1 lentelė. *Tyrimo metų (2001-2003 m.) kritulių ir temperatūros duomenys (balandžio - spalio mėn.) Vidurio Lietuvoje (Dotnuva), palyginus su vidutiniu daugiamečiu (1924-2003 m.) vidurkiu*

Month *Mėnuo*	Precipitation mm *Krituliai mm*				Mean temperature ^{o}C *Vidutinė temperatūra ^{o}C*			
	2001	2002	2003	1924-2003	2001	2002	2003	1924-2003
April *Balandis*	34.7	21.6	37.6	38.2	8.0	7.9	5.4	5.6
May *Gegužė*	34.6	19.5	36.3	52.1	12.8	15.4	13.6	12.2
June *Birželis*	52.8	53.2	54.9	62.3	14.4	16.8	15.5	15.6
July *Liepa*	102.5	35.7	54.6	73.7	21.0	20.3	20.6	17.6
August *Rugpjūtis*	59.1	23.1	66.5	73.2	17.6	20.3	17.3	16.6
September *Rugsėjis*	76.5	14.6	22.4	54.8	11.9	12.9	12.9	11.9
October *Spalis*	40.4	124.9	56.2	49.4	9.0	4.5	4.9	6.7

Meteorological conditions in the years of study varied rather significantly. In 2001 and 2003, the growing season's conditions favoured the growth and development of white clover plants. In 2002, the second half of summer was droughty, as a result, the dry matter yield of the two cuts was lower (Table 1).

The stability analysis computer software 'YIELDSTAB' developed by P. Tarakanovo in the Visual Basic of Application as macro program to run in the EXCEL was used. This program includes regression /Brewbaker, 1995/ and stability analysis /Kang, Magari, 1995/. Pattern analysis was processed using program IRRISTAT. Pattern analysis module for program IRRISTAT was adapted from program GEBEI developed by Dr. Jan Delasy from University of Queensland, Australia /IRRISTAT 4.3. for Windows, 2002/.

Results and discussion

The data of the analysis of variance showed that the dry matter yield of the first and second cuts and annual had been essentially influenced by the years of condition, genotypes and their interactions. The latter factor was of particular significance, since the presence of reliable genotype x year interactions ($P< 0.01$) allows further analysis. The absence of a reliable covariance (heterogeneity) between variety yield and average annual yield is indicative of the absence of an additive, direct effect between them (Table 2).

Table 2. Mean squares relevant to the study of dry matter yields white clover genotypes

2 lentelė. *Baltųjų dobilų genotipų sausųjų medžiagų derlių dispersinės analizės kvadratų vidurkiai*

Dotnuva, 2001-2004

Source *Šaltinis*	Df *L. l.*	Mean squares (MS) / *Kvadratų vidurkiai*		
		I cut / *I pjūtis*	II cut / *II pjūtis*	Annual / *Metinis*
Genotypes (G) *Genotipai (G)*	9	0.605**	1.081**	3.223**
Years (Y) *Metai (Y)*	4	38,239**	51.545**	169.642**
Interaction G x Y *Sąveikos G x Y*	36	0.096ns	0.136**	0.334**
Heterogeneity *Heterogeniškumas*	9	0.089ns	0.212ns	0.418ns
Residual *Liekanos*	27	0.099	0.111	0.307
Pooled error *Bendra paklaida*	90	0.065	0.044	0.117

** $P < 0.01$; ns – nonsignificant / *nepatikima*

Dry matter yield in white clover varieties was essentially influenced by the weather conditions, especially the sum total of precipitation in the test years. Dry matter yield was the highest in 2001. It was significantly lower (1.7-3.3 times) in

2002 and 2003. The growing period in 2002 was characterized by extremely dry weather, especially in the second half of summer and early autumn, the factor that did not favour the second cut yield. Whether conditions in 2003 were better than in 2002, however, the average white clover yield was below the 2001 level (Table 3).

Variety testers are mostly interested in the combined level of productivity and stability, as they allow selecting the most valuable varieties.

Table 3. Mean dry matter yield of white clover performance (t ha^{-1}) in different years

3 lentelė. *Skirtingų bandymo metų baltųjų dobilų genotipų sausųjų medžiagų vidutinis derlius (t ha^{-1})*

Dotnuva, 2001-2003

Years of sowing / harvesting *Sėjos / derliaus metai*	I cut / *I pjūtis*	II cut / *II pjūtis*	Annual / *Metinis*
2000/2001	4.16*	3.71*	7.87*
2000/2002	1.47	0.93	2.40
2001/2002	1.99	2.41	4.40
2001/2003	2.39	1.37	3.76
2002/2003	3.64*	3.79*	7.43*
Average /*Vidurkis*	2.73	2.44	5.17
LSD_{05} / R_{05}	0.082	0.068	0.111

* $P < 0.05$

Joint regression analysis. The joint regression analysis is widely used by the researchers for the study of genotype x environment interaction and main stability parameters. The stability parameters for all varieties are given in Table 4. The regression coefficient (b_i) measures the increase in the mean yield of a genotype per unit of increase in the environmental index. The mean squared deviation from regression (S^2_d) measures how well the predicted response agrees with that actually observed and includes GE analysis. A genotype with a regression coefficient > 1.0 is responsive to increasingly favourable conditions with respect to site mean yield; a genotype with a regression coefficient < 1.0 is considered not responsive. Small values of S^2_d are considered to be more stable. High values of coefficient of determination (r^2) would be more stable.

In the present study, the regression coefficient of the varieties ‘Bitūnai’, ‘Rivindel’ and ‘Nemuniai’ were significantly different from 1.0. The varieties that significantly exceed an average dry matter yield in the trial were ‘Atoliai’, ‘Nemuniai’ and No. 1123, 1124.

The simultaneous consideration of four parameters of stability (Table 4) for the individual genotype revealed that genotypes 1123 and 1124 gave significant highest yield (5.80 and 5.89 t ha^{-1}) with the regression values 1.02 and 1.07 respectively, low standard deviation from regression (0.059 and 0.095) and high significant ($P< 0.01$) determination coefficient (0.993 and 0.989).

Table 4. Means and estimates of stability statistics for annual dry matter yield of white clover genotypes

4 lentelė. *Baltųjų dobilų genotipų metinio sausųjų medžiagų derliaus vidurkio ir stabilumo įvertinimas*

Dotnuva, 2001-2003

Name of variety *Veislė / numeris*	Means (t ha^{-1}) *Vidurkis (t ha^{-1})*	b_i	S^2_d	r^2
'Sūduviai' St.	5.14	1.01	0.0079	0.999**
'Bitūnai'	4.56	0.88*	0.0207	0.996**
'Atoliai'	5.36*	1.02	0.0257	0.997**
'Nemuniai'	5.52*	1.13*	0.2575	0.974**
'Milo'	5.07	1.01	0.0393	0.995**
'Rivindel'	4.62	0.87*	0.1890	0.968**
1123	5.80*	1.02	0.0593	0.993**
1124	5.89*	1.07	0.0956	0.989**
1421	4.81	0.98	0.0222	0.997**
1435	4.96	0.97	0.2018	0.972**
Average *Vidurkis*	5.176	1.0	0.09196	
LSD_{05} / R_{05}	0.166	0.0694		

*,** Significant at the 0.05 and 0.01 levels respectively

, Patikimumo lygis atitinkamai 0,05 ir 0,01*

The diagrammatic presentation (Fig. 1) of good stability parameters showed those genotypes Nos. 1123 and 1124. These genotypes differ insignificantly in regression coefficient from 1.0 and dry matter yield > grand mean. Therefore, they appeared to be the best varieties with regard to stability. These two breeding populations have wide adaptation and may be recommended for cultivation in different environments across the country. By dry matter yield (5.52 t ha^{-1}) the variety 'Nemuniai' significantly exceeded the average yield in the trial and regression coefficient b_i was significant ($P < 0.05$) > 1.0, therefore it may be suitable for specific adaptation in favourable environments. The varieties 'Bitūnai' and 'Rivindel' had yield significantly below grand mean dry matter yield and had regression coefficients below the unity, therefore it may be suitable for specific adaptation in poor environments. Similar results were shown by the breeding populations Nos. 1421, 1435, and varieties 'Milo', 'Sūduviai' has below average dry matter yield and regression coefficient $b_i < 1$ and are specially adapted to poor environments.

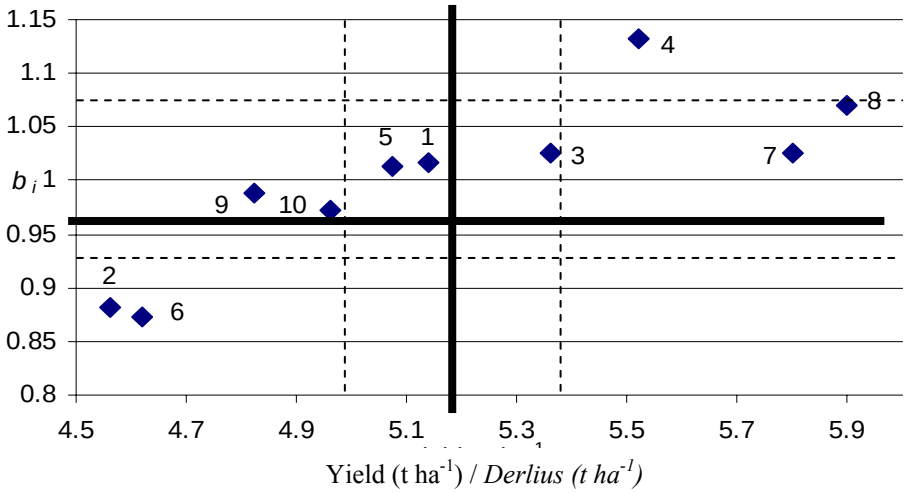

Figure 1. Scattered diagram for annual dry matter yield of white clover and regression coefficient. 1 – 'Sūduviai', 2 – 'Bitūnai', 3 – 'Atoliai', 4 – 'Nemuniai', 5 – 'Milo', 6 – 'Rivendel', 7 – No. 1123, 8 – No. 1124, 9 – No. 1421, 10 – No. 1435

1 paveikslas. *Baltųjų dobilų genotipų metinio sausųjų medžiagų derliaus ir regresijos koeficientų erdvinė diagrama. 1 – 'Sūduviai', 2 – 'Bitūnai', 3 – 'Atoliai', 4 – 'Nemuniai', 5 – 'Milo', 6 – 'Rivendel', 7 – No. 1123, 8 – No. 1124, 9 – No. 1421, 10 – No. 1435*

Pattern analysis. To elucidate the groups of varieties with a similar genotype – environments interaction, investigators use cluster analysis /Hausmann et al., 2001/. Pattern analysis using cluster classification techniques for grouping genotypes and locations on the basis of similarity GE effects and main effects was employed /Annicchiarico, 2002/. A squared Euclidean distance as the dissimilarity measure and Ward's clustering method is normally recommended /DeLacy et al., 1996/. It was successfully used for analysis of GE interaction in multi location trials with wheat /Robert, 1997/, sorghum /Haussmann et al., 2001/, sunflower /Ghafoor et al., 2005/ and other crops.

Figure 2 shows the results of cluster analysis of annual dry matter yield of white clover varieties and numbers. For variety grouping the Euclidean distances below 2.42 were taken.

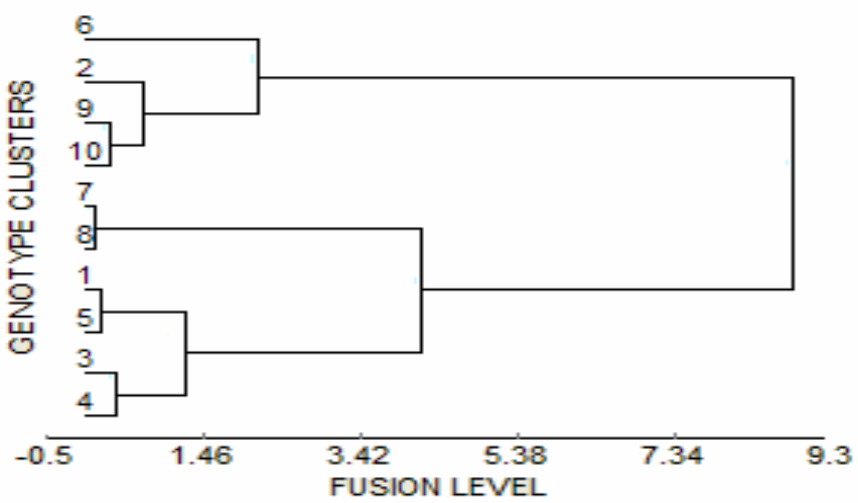

Figure 2. The cluster dendrogram for annual dry matter yield of white clover genotypes: (1 – 'Sūduviai', 2 – 'Bitūnai', 3 – 'Atoliai', 4 – 'Nemuniai', 5 – 'Milo', 6 –'Revendel', 7 – No. 1123, 8 – No. 1124, 9 – No. 1421, 10 – No. 1435)
2 paveikslas. *Baltųjų dobilų genotipų metinio sausųjų medžiagų derliaus klasterinė dendrograma: (1 – 'Sūduviai', 2 – 'Bitūnai', 3 – 'Atoliai', 4 – 'Nemuniai', 5 – 'Milo', 6 – 'Revendel', 7 – No. 1123, 8 – No. 1124, 9 – No. 1421, 10 – No. 1435)*
Dotnuva, 2001-2003

In total, three groups of varieties, united on this level were determined. Varieties and numbers of the same group exhibit a similar reaction of the genotype – environment interaction.

The first group comprised the low – productive cultivars: 'Bitūnai', 'Rivendel' and No. 1425, 1435, the last derived from wild – growing forms of Lithuanian clover.

The second group contained Nos. 1123 and 1124, which combined high productivity and stable dry matter yield.

The third group included medium – productive cultivars 'Atoliai', 'Sūduviai', 'Nemuniai' from Lithuania and 'Milo' from Denmark.

The results of cluster analysis based on the pattern analysis confirmed the conclusions on the selective value of the cultivars and numbers studied, obtained by a Joined regression analysis. Thus, a Joined regression analysis assessment and cluster analysis allowed us to highlight white clover Nos. 1123 and 1124 as the most valuable for plant breeding. They exhibit high stable dry matter yield both in the first and second cuts, which is formed in the conditions of limited soil moisture content.

Conclusions

1. Employment of the plant-breeding-specified software allows a more reasonable selection of the most valuable white clover numbers that realize their genetic potential in different environmental conditions.

2. Dry matter yield of the first and second cuts and per year was essentially influenced by the years conditions of experiment, cultivars and their interactions ($P < 0.01$). Good stability parameters were shown by genotypes Nos. 1123 and 1124. These genotypes differ in significantly in regression coefficient from 1.0 and have dry matter yield > grand mean. Therefore, they appeared to be the best varieties with regard to stability. These two breeding populations have wide adaptation and may be recommended for cultivation in different environments across the country. The cultivars 'Bitūnai', 'Rivendel' and No. 1421, 1435 are specially adapted to poor environments. The cultivar 'Nemuniai' was found to be suitable for specific adaptation in favourable environments.

3. Cluster analysis revealed three groups of cultivars showing a similar genotype – environment reaction. The results of cluster analysis in general confirmed the conclusions concerning the value of the cultivars and numbers studied for plant breeding, which had been based on a general estimate.

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BALTŲJŲ DOBILŲ VEISLIŲ IR POPULIACIJŲ SAUSŲJŲ MEDŽIAGŲ DERLIAUS STABILUMAS

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Santrauka

Lietuvos žemdirbystės institute 2001-2003 m. įvertintas 6 veislių baltųjų dobilų (*Trifolium repens* L.) ir 4 selekcinių numerių sausųjų medžiagų derlius. Tyrimams naudotos ANOVA, regresinė ir klasterinė analizės. Nustatyta patikima ($P > 0,01$) tyrimų metų sąlygų įtaka, pjūčių, genotipų ir jų tarpusavio sąveikos sausųjų medžiagų derliaus kitimui. Perspektyviniai Nr.1123 ir 1124 pasižymėjo dideliu sausųjų medžiagų derliumi (5,80 ir 5,89 t ha^{-1}) ir žema stabilumo variansa (0,0593 ir 0,0936). Klasterinė analizė išryškino tris grupes genotipų, besiskiriančių panašiu reagavimu į sąveiką genotipas – aplinka. Klasterinės analizės rezultatai sutampa su genotipų įvertinimu, gautu pagal sausųjų medžiagų derliaus stabilumo analizę.

Reikšminiai žodžiai: baltieji dobilai, derliaus stabilumas, klasterinė analizė.