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Main additive effect and multiplicative interaction analysis of white clover genetic resources

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Dry matter content in overground biomass in six varieties and four breeding populations of white clover (*Trifolium repens* L.) were studied at the Lithuanian Institute of Agriculture (Dotnuva) during the period 2001–2003. In this study, the AMMI model was used with the objective of assessing dry matter content in the overground biomass of white clover genotypes, selecting stable genotypes and investigating genotype by environments (G × E) effects. The effects of environments, genotypes and G × E interaction were highly significant ($P < 0.01$). The first two bilinear AMMI model terms accounted for 88%. The biplot showed three groups of genotypes: Nos. 1123 and 1124 unstable but high-yielding; varieties 'Suduviai', 'Bitunai', 'Atoliai' and No. 1421 stable but low or medium yielding; variety 'Nemuniai', 'Rivendel' and No. 1435 unstable, low or medium yielding. To breed a new ecologically stable variety of white clover we intend to use the variety 'Nemuniai' and No. 1124 as initial material for hybridization. They have a high level of dry matter productivity but differ by GE response.

Key words: white clover, AMMI model, biplot, yield stability

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 and mineral content, intake and nutrient value of forage. Because of its nitrogen fixing capacity, white clover has the potential to reduce, or in the case of organic systems to eliminate the need for inorganic nitrogen fertilizer on grazed grassland [1]. For a white clover variety it is important that it not only has a high yielded of dry matter, but also shows its stability in different conditions of cultivation. The growing awareness of the importance of genotype–environment (GE) interaction has led crop genotypes to be ordinarily assessed in multi-environment, regional trials for variety recommendation or for the final stages of the elite breeding material selection [2].

The presence for varieties of GE interaction can reduce errors in the breeding process, as selection in one type of conditions cannot provide advantage in others [3]. White clover variety population adaptability is based on genetic homeostasis; it 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 [4]. 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 [5].

The stability methods can be divided into two major groups – univariate and multivariate stability statistics [6]. From the latter group, additive main effect and multiplicative interaction analysis (AMMI) are widely used for GE analysis. This method has been shown to be effective, because it captures a large portion of the GE sum of square, it clearly separates the main and interaction effects that present agricultural researchers with different kinds of opportunities, and the model often provides agronomically meaningful interpretation of the data [7]. The results of AMMI analysis are useful in supporting breeding program decisions such as specific adaptation and selection of environment [8]. Usually, the results of AMMI analysis shown in common graphs are called biplot [9]. A biplot shows the value of the genotypes and the environments and their relationships using the sinqular vectors technique [10].

The aim our investigations was to establish dry matter content in overground biomass 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 six white clover varieties ('Suduviai', 'Bitunai', 'Atoliai', and 'Nemuniai' from Lithuania, 'Milo' and 'Rivendel' from Denmark, and four 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 varieties 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 reached 10% of flowering. After cut, 0.5 kg of 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 °C, and the amount of dry matter harvested was determined. As a standard, the 'Suduviai' white clover variety was used.

In the autumn of each year of use, phosphorus and potassium fertilizers ($P_{60}K_{90}$) were applied.

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, dry matter content in overground biomass of the two cuts was lower (Table 1).

AMMI analysis was carried out using the IRRISTAT program. This module for the IRRISTAT program has been adapted from the GEBEI program developed by Dr. Jan Delasy from Queensland University, Australia [11]. In AMMI analysis, the model for phenotypic performance of genotype *j* tested in environment *i* can be expressed as

$$Y_{ger} = \mu + a_g + b_e + Sl_n \, g_{gn} \, d_{en} + r_{ge} + E_{ger},$$

where Y_{ger}id the yield of genotype g in environment *e* for replicate *r*, μ is the grand mean, a_g is the mean deviation of the genotype *g* (genotype mean minus grand mean), and b_e is the mean deviation of environmental mean; l_n is the singular value for IPCA axis *n*; g_{gn} is the genotype *g* eigenvector value for IPCA axis n; d_{en} is the environment *e* eigenvector value for the IPCA axis *n*; r_{ge} is the residual, and E_{ger} is the error.

The genotype × environment interaction effects were calculated using the formula $(G \times E)_{ij} = \bar{y}_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..}$ (where $\bar{y}_{ij}$ is the mean of the i_{th} genotype on the j_{th} environment and $\bar{y}_{i.}, y_{j}$, and $\bar{y}_{..}$ are the mean of the i_{th} genotype, the mean of the j_{th} environment, and the overall mean, respectively [12].

RESULTS AND DISCUSSION

Data of the analysis of variance showed that dry matter content in the overground biomass of the first and second cuts and annual biomass yield were essentially influenced by the years of testing, genotypes and their interactions. The latter factor was of particular significance, since the presence of a reliable genotype × 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).

Dry matter content in overground biomass in white clover varieties was essentially influenced by the weather conditions, the sum total of precipitation in the test years in particular. Dry matter content in

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

Month	Precipitation (mm)				Mean temperature (°C)			
	2001	2002	2003	1924–2003	2001	2002	2003	1924–2003
April	34.7	21.6	37.6	38.2	8.0	7.9	5.4	5.6
May	34.6	19.5	36.3	52.1	12.8	15.4	13.6	12.2
June	52.8	53.2	54.9	62.3	14.4	16.8	15.5	15.6
July	102.5	35.7	54.6	73.7	21.0	20.3	20.6	17.6
August	59.1	23.1	66.5	73.2	17.6	20.3	17.3	16.6
September	76.5	14.6	22.4	54.8	11.9	12.9	12.9	11.9
October	40.4	124.9	56.2	49.4	9.0	4.5	4.9	6.7

Table 2. **Mean squares relevant to the study of dry matter content in overground biomass of white clover genotypes**
Dotnuva, 2001–2003

Source	Df	Mean squares (MS)		
		I cut	II cut	Annual
Genotypes (G)	9	0.605**	1.081**	3.223**
Environments (E)	4	38.239**	51.545**	169.642**
G × E interaction	36	0.096ns	0.136**	0.334**
Heterogeneity	9	0.089ns	0.212ns	0.418ns
Residual	27	0.099	0.111	0.307
Pooled error	90	0.065	0.044	0.117

** $P < 0.01$; ns – nonsignificant.

Table 3. **Mean dry matter content in overground biomass performance (t ha^{-1}) in different years**
Dotnuva, 2001–2003

Years of sowing / harvesting	I cut	II cut	Annual
2000/2001 (A)	4.16*	3.71*	7.87*
2000/2002 (B)	1.47	0.93	2.40
2001/2002 (C)	1.99	2.41	4.40
2001/2003 (D)	2.39	1.37	3.76
2002/2003 (E)	3.64*	3.79*	7.43*
Average	2.73	2.44	5.17
LSD$_{05}$	0.082	0.068	0.111

* $P < 0.05$.

Table 4. **Analysis of variance for annual dry matter content in overground biomass AMMI model**
Dotnuva, 2001–2003

Source	DF	SS	MS	F	Probability
Total	49	239.873			
Genotypes (G)	9	9.670	1.074		
Environments (E)	4	226.189	56.547		
G × E	36	4.0121	0.111		
AMMI Comp. 1	12	2.2696	0.189*	2.605	0.022
AMMI Comp. 2	10	1.2767	0.128*	3.838	0.011
AMMI Comp. 3	8	0.4262	0.053*	8.088	0.011
AMMI Comp. 4	6	0.0395	0.006		

* $P < 0.05$.

overground biomass was highest in 2001. It was significantly lower (1.7–3.3 times) in 2002 and 2003. The growing season in 2002 was characterized by the extremely dry weather, especially in the second half of summer and in early autumn, the factor that did not favour dry matter content in overground biomass of the second cut. The whether conditions in 2003 were better than in 2002, however, the average white clover dry matter content in overground biomass was below the 2001 level (Table 3).

The first bilinear interaction term of the AMMI analysis of the G × E accounted for 56% of the G × E sum of squares, the second accounted for 32% and the third for 11%, using 12, 10 and 8 df respectively (Table 4). The first two bilinear terms accounted for 88% of the G × E sum of squares and used 22 of the total 36 df available in the interaction. The obtained data confirm adequacy to the AMMI model. This has presented a possibility of constructing of the biplot and calculating the genotype and environment effects.

Table 5 shows the effects of genotypes and site values from the additive genotype × environment model.

The main highly significant positive effects have environments A (2.805 t ha^{-1}) and E (2.257 t ha^{-1}). Other environments have significant negative main effects. The genotypes No. 1124 and 1123 had the greatest main positive effects (0.723 t ha^{-1} and 0.625 t ha^{-1}, respectively). Other genotypes had a significant negative or an insignificant low positive main effect. Specific GE interaction positive highly significant effects were shown by the variety 'Nemuniai' in environment A (0.935 t ha^{-1}) and 'Rivendel' in environment B (0.800 t ha^{-1}). In Figure, the IPCA 1 scores for both the genotypes (numbers) and environments (upper case) were plotted against the mean dry matter content in overground biomass for the genotypes and the environments respectively. We can clearly see an association between the genotypes and the environments plotting on the same graph. The IPCA scores of a genotype in the AMMI analysis are indicative of the adaptability over environments. The graph space of Figure is divided into

Table 5. **Effects of the annual dry matter content in overground biomass (t ha^{-1}) from the AMMI additive GE model** Dotnuva, 2001–2003

Accession designation	Years of sowing / harvesting (environments)					
	2000/2001 (A)	2000/2002 (B)	2001/2002 (C)	2001/2003 (D)	2002/2003 (E)	Genotype Effects
1124	–0.1642	–0.2730	0.1330	–0.1898	0.4941	0.7229***
1123	–0.1810	–0.2658	0.1938	0.0277	0.2253	0.6250***
Nemuniai	0.9352**	–0.1069	–0.4704	–0.1721	–0.1858	0.3461*
Atoliai	0.2360	–0.0621	–0.1344	0.0641	–0.1033	0.1840
Suduviai St.	0.1005	–0.0749	–0.0902	–0.0673	–0.0485	–0.0378
Milo	–0.0422	0.1450	–0.1864	–0.1331	0.2168	–0.1031
1435	–0.5219	–0.2657	0.4702	0.08186	0.2325	–0.2155*
1421	–0.0527	–0.1055	0.2156	0.0351	–0.0924	–0.3539*
Rivendel	–0.0516	0.7996**	–0.4011	0.0111	–0.3580	–0.5550***
Bitunai	–0.2580	0.2065	0.0896	0.3423	–0.3804	–0.6126***
Environments Effects	2.695***	–2.772***	–0.7725***	–1.407***	2.257**	

* P < .05; ** P < .01 and *** P < 0.001.

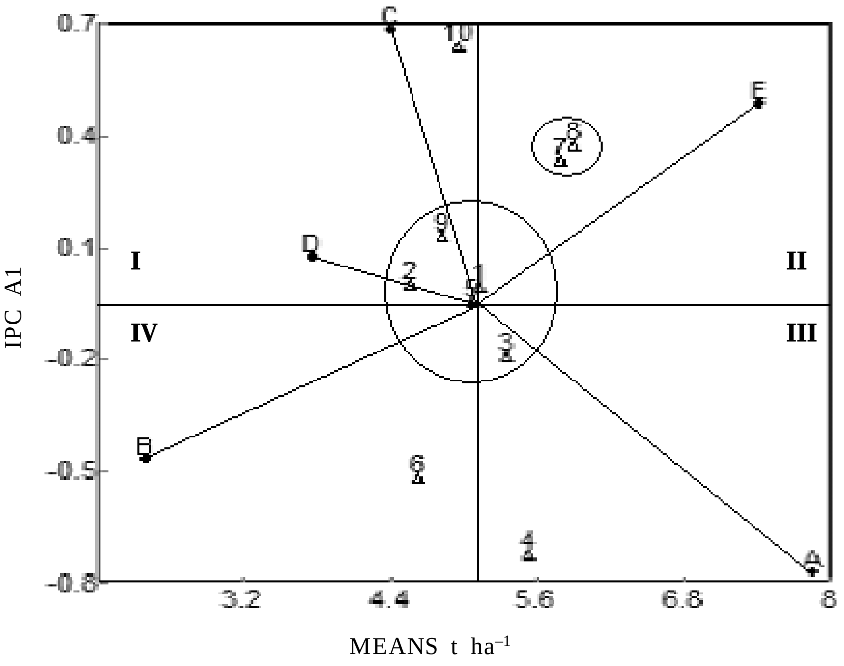

Figure. AMMI model biplot for 10 white clover varieties and breeding populations in 5 environments (Dotnuva, 2001–2003). *1* – Suduviai, *2* – Bitunai, *3* – Atoliai, *4* – Nemuniai, *5* – Milo, *6* – Rivendel, *7* – No. 1123, *8* – No. 1124, *9* – No. 1431, *10* – No. 1435

four quadrants from lower yielding environments in quadrants 1 and 4 to high yielding in quadrants 2 and 3. The breeding populations Nos. 1123 and 1124 posed in quadrant 2 shows that they have specific adaptation to favorable environments. Considering only the IPCA 1 scores it became clear that Nos. 1123 and 1124 were the more unstable genotypes, but they were well adapted to high-yielding or more favourable environments. The varieties 'Sûduviai', 'Bitunai', 'Atoliai' and No. 1421 with a close to zero IPCA 1 scores show that they have a more stable but low yield. These genotypes are better suited for cultivation in low-yielding environments. Stability in field performance of the genotypes is influenced by prevailing biotic and abiotic stresses. The variety 'Nemuniai' was developed by selection of plants resistant to clover rot (*Sclerotinia trifolium* Eriks.) on an infection background. The length of the genotype vectors reflects the amount of interaction for those varieties, thus according to Figure, most of GE interaction is due to the varieties 'Nemuniai', 'Rivendel' and No. 1435. These genotypes have a specific adaptation and seem unstable just considering IPCA 1 scores. The angle between genotype vectors corresponds to the interactions between the interaction residuals. The varieties 'Sûduviai' with 'Milo' and No. 1123 with 1124 are very similar and show a hig positive correlation. No. 1124 with var. 'Nemuniai' and var. 'Rivendel' with No. 1435 have a negative correlation. An alternative model to AMMI for studying and interpreting the interaction includes partial least squares regression [13] and factorial regression [14]. Comparative studies have found that the AMMI, partial least squares regression, and factorial regression models are all useful and may identify similar variety and environmental variables in explaining the interaction [15]. GE interaction patterns revealed by AMMI plots indicate that white clover genotypes are narrowly adapted. No genotype shows a superior performance in either of the environments (Table 5).

For the purpose of breeding a new ecologically stable variety of white clover it is planned to use the variety ‘Nemuniai’ and No. 1124 as initial material for hybridization. They have a high dry matter content in overground biomass but differ by GE response (Figure).

CONCLUSIONS

1. The AMMI model was very effective for studying G × E interaction. The first two bilinear AMMI model terms accounted for 88%.

2. The biplot showed three groupings of genotypes: I) Nos. 1123 and 1124; II) varieties ‘Suduviai’, ‘Bitunai’, ‘Atoliai’ and No. 1421; III) ‘Nemuniai’, ‘Rivendel’ and No. 1435.

3. To develop a new ecologically stable variety of white clover, we intend to use the variety ‘Nemuniai’ and No. 1124 as initial material for hybridization.

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BALTØJØ DOBILØ GENETINIØ IÐTEKLIØ PAGRINDINIØ ADITYVINIØ EFEKTØ IR DIDËJANÈIOS SÀVEIKOS VERTINIMAS

Santrauka

Lietuvos þemdirbystës institute (Dotnuva) 2001–2003 m. buvo ávertintas 6 baltøjø dobilø (Trifolium repens L.) veisliø ir 4 selekciniø numeriø sausøjø medþiagø derlius. Tyrimams panaudotas AMMI modelis, leidþiantis ávertinti atskirø genotipø ir aplinkos sàlygø efektà, jø sàveikà. Nustatytas aukðtas genotipø efektø, aplinkos sàlygø ir jø tarpusavio sàveikos patikimumas ($p < 0,01$). AMMI modelis sudarë 88% sausøjø medþiagø antþeminës biomasës variacijos. Tyrimo metu iðskirtos trys genotipø grupës, pasiþyminèios skirtinga genotipas × aplinka reakcija. Pirmajai grupei priklauso nestabilûs, sausøjø medþiagø antþeminës biomasës iðeiga iðsiskiriantys selekciniai numeriai Nr. 1123 ir 1124. Stabilumu, bet þemu ar vidutiniu derliumi pasiþymëjo ‘Atoliø’, ‘Bitûnø’, ‘Sûduviø’ ir Nr. 1421 dobilø veislës. Tuo tarpu ‘Nemuniai’, ‘Rivendel’ ir Nr. 1435 – nestabilios, vidutinæ sausøjø medþiagø antþeminës biomasës iðeigà formuojanèios veislës. Naujos ekologiðkai stabilios baltøjø dobilø veislës sukûrimui tikslinga panaudoti ‘Nemuniø’ ir Nr. 1124 veisles. Šie genotipai pasiþymi aukštu produktyvumu ir išsiskiria skirtinga genotipas × aplinka reakcija.