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The study of variability of agro-morphological characteristics of white clover accessions from other countries in Latvian climate conditions

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Abstract. White clover (*Trifolium repens L.*) is one of the most important forage legumes in temperate grasslands because of its large spread, good adaptability, high nutritive value combined with a good palatability and by its nitrogen-fixing capacity (Balan, 2007). The evaluation of the white clover germplasm collections under field conditions is recognized as a general method to estimate biological diversity. During the years 2006–08 experimental field trials were performed at the Research Institute of Agriculture in Latvia. A white clover collection of 46 samples with large-scale geographic origins from 19 countries was established. Wild populations gathered in Latvia and Lithuania were also included in the collection. Native cultivar 'Daile' as the standard was used. All three white clover varietas (var.) – *silvestris*, *hollandicum*, and *giganteum* – were represented in the collection. A huge diversity of morphologically different plants was observed among the collected samples. Winter-hardiness, earliness, growth intensity, time of flowering, weight of leaves and flower heads, length of sprouts, size of the central leaflet, etc, are the important traits of the genetic variability. Among the white clover accessions were samples with untraditional reddish-rose-colored flower heads. The aim of the study was to select valuable source material for future breeding with a good possibility of adaptation in the changeable climate and different soils in Latvia, with high productivity, good disease resistance, of many years' standing in the sward, etc. Our research shows that large genetic variability exists within the investigated white clover collection. The evaluation of samples of our white clover collection in the agro-climatic conditions of Latvia shows that some varieties are featured with several morphological qualities and could be appropriate as promising material for the creation of a new variety. These are 'Dubraux' (Czech Republic/Slovakia); 'Gigant' (Belgium); 'Huia' (New Zealand) and local wild population 'Ecotype 2005', which stand out for their winter-hardiness, earliness, production of a large foliage surface and produce high seed yield.

Key words: accessions, agro-morphological characteristics, breeding diversity, white clover

INTRODUCTION

The white clover holds a significant place in the stands of cultivated pastures and meadows in Latvia. It is a polymorphic, plastic species. White clover *Trifolium repens* L. var. *silvestris* is the most widespread in natural biocenosis in Latvia. Smaller foliage and flower heads, shorter stems and many branches characterize it. This varietas (var.) is featured as winter-hardy, relatively resistant to drought and is long-lasting in stands.

It has a great ability to multiply vegetatively and, therefore, is suited to intensive grazing (Holms, 1992).

At the beginning of 20th century white clover seeds were imported into Latvia from Sweden and Denmark. They presented var. *hollandicum.* It is higher yielding than var. *silvestris* but it is not as winter-hardy, its vegetative propagation is lower and it remains in stands for a shorter period of time.

The only Latvian white clover cultivar, 'Daile', was developed in Priekuli some time ago. It belongs to var. *giganteum,* which has the following characteristics: a large number of big leaves on relatively long and thick stems, big flower heads and thick stalks, and good winter-hardiness (Staszewski & Staszewski, 2007). The cultivar 'Daile' is appropriate also as a component of cut swards and in pasture.

The research of morphological traits and biological characteristics of white clover var. *silvestris*, *hollandicum*, and *giganteum* suggests a great diversity not only among, but also within the forms (Paplauskiene, 2006). White clover is widespread all over the world. The wide geographic distribution of this species is as a result of its great adaptability to different soil and climate conditions (Caradus et al., 1990). It is very important to use wild ecotypes in a breeding program because the ecotypes strengthen the ability of new cultivars to survive in grasslands. For the purpose of identifying ecotype variations in order to use them effectively in breeding, much research has investigated and classified ecotype populations by their morphological traits and DNA markers (Woodfield & Caroders, 1994).

The aim of the research of our white clover collection was to evaluate and compare specific features of different samples in the process of growth and development in the sowing process as well as in the years of use. The aim was also to evaluate the economically valuable features in Latvian soil and climatic conditions and to choose more appropriate samples, which could be included in further programs of examination and selection.

MATERIALS AND METHODS

During the years 2006–08 small plot trials were established at the Research Institute of Agriculture. The experiment was conducted on a sandy loam soil with organic matter content 2.3%, P_2O5–154.0 and K_2O–121.0 mg kg^{-1}, pH_{KCl} 6.2. The basic fertilizer was NPK 5:10:25 200 kg ha^{-1}. Forty-six (46) different accessions of white clover were sown in May. A wide number of samples from the following countries were included in the collection: Lithuania, Germany, England, Ireland, New Zealand, Greece; Japan; Sweden; Denmark; Poland; Czech Republic/Slovakia; Hungary; Canada, USA, the Netherlands, Belgium, Italy and Syria. The variety 'Daile' was chosen as a standard. The experiment was comprised of randomised complete block design with 3 replications. Every accession was sown in 2 rows 2.0 m long, with 60.0 cm between rows and 1.0 m between each accession. Seeds were sown by hand. Wild populations from different locations in Lithuania and Latvia were sown in the plot trials.

In the sowing year we assessed several agro-morphological observations: height of sward, leaf and root weight, root length, etc. In the first year of use, winter hardiness, earliness, and growing intensity were evaluated, as well the determined time

of flowering, weight of 10 flower heads per each accession, and length of sprouts. Winter hardiness was evaluated in 1–9 points (after UPOV). In the 1st year of use the plants were treated against monocotyledon weeds.

The vegetation period in 2006 was dry, the soil started to suffer the deficit of moisture and plants faded. Those conditions strongly affected the intensity of white clover growth, the development of phenological phases, etc. In the years 2006–07 the winter was extreme; vegetation continued till October 29 and then started to freeze, but resumed growth in the third decade of November and continued throughout December and January. At the end of January there was a hard frost reaching –30°C, with a thick layer of snow (27 cm); those conditions continued in February. Summer 2007 was characterized by warm weather conditions and excessive moisture throughout the vegetation period.

Microsoft Excel was used for mathematical processing analysis of the data subprogram (Berzins, 2002).

RESULTS AND DISCUSSION

To create the new intensive type and plastic variety, featured by high productivity, disease resistance, and winter-hardiness, rapid growth capacity after mowing or pasturing, competitiveness in stands with grasses and producing stabile seed yield, the breeder must operate with a multi-faceted initial material including both the best wild harvested forms and selected varieties of other countries, which are appropriate for our climatic conditions. However, the selection process faces obstacles because we have to overcome a series of negative correlations among the desirable features of the variety: high productivity, rapid growth, winter-hardiness, vegetative mass and seed yield.

The results of phenological observations made in the sowing year showed different development processes among different varieties. Cultivars of white clover were characterized by different growth and development dynamics in the vegetation season. The sprouting started at approximately the same time but the phase of shooting and flowering started at various times. The following tables summarize the investigated parameters of white clover accessions that are most appropriate for breeding purposes and are more suitable for Latvian climate conditions.

The most rapidly developing varieties in the sowing year were ‘Menna’, ‘Atoliai’, ‘Bituniai’, ‘Suduviai’, ‘Steinacher’, ‘Gigant’, ‘Huia’, ‘Jubileinij’. Some accessions were characterized by greater weight of leaves or a better developed root system. Cultivars ‘Probstheidaer Weiβklee’, ‘Atoliai’, ‘Menna’ had greater average weight of 10 leaves. In the same period, accessions of local wild populations, which represent var. *silvestris* featured small foliage (Table 1). The root system development in the sowing year also varied: it was confirmed by measuring root weight and length; in this parameter the standard variety ‘Daile’ exceeded the cultivars ‘Menna’, ‘Probstheidaer Weiβklee’, ‘Gigant’ and ‘Huia’.

Latvian climate conditions vary widely every year. With changeable air temperatures and black frosts in winter, good winter-hardiness is one of the most important traits of white clover. The essential differences in winter-hardiness between varieties were observed. In spring of the 1st year, from 46 accessions only 14 over

wintered successfully in the white clover nursery. Similarly, in the 2nd year, the highest estimation of winter-hardiness was for the cultivars 'Gigant', 'Dubraux', 'Ecotype 2005', etc. (Table 1). Our trial results showed that accessions from England, USA, Australia, Greece and the Netherlands were unsuitable for our climate. Some were destroyed by frost, others were estimated at 2–3 points (on a 9-point scale).

Table 1. The agro-morphological characteristics of white clover accessions.

N	Accession	Origin	Sowing year			Winter-hardiness	
			Weight of 10 leaves, g	Weight of 10 roots, G	Average length of roots, cm	1st year of use, (1–9)	2nd year of use, (1–9)
1	Daile	Latvia	6.06	4.82	10.7	9.0	6.0
2	Atoliai	Lithuania	9.20	5.53	12.5	9.0	6.0
3	Bitunai	Lithuania	6.26	3.88	13.0	9.0	6.0
4	Suduviai	Lithuania	7.60	5.60	9.2	9.0	5.0
5	Steinacher	Unknown	7.55	4.60	13.6	6.0	5.5
6	Menna	Holland	8.72	6.96	8.2	6.5	6.0
7	Probstheidaer Weiβklee	Germany	10.25	8.29	11.1	8.5	5.0
8	Gigant	Belgium	5.55	4.92	15.2	9.0	7.0
9	Huia	New Zealand	7.96	5.39	13.0	9.0	6.0
10	NZ White Clover Type	New Zealand	7.08	4.06	10.0	6.5	5.0
11	Araw	Ireland	6.02	5.22	12.2	8.5	4.0
12	Jubiļeinij	Russia	4.90	5.75	12.2	9.0	5.0
13	Dubraux	Czech	5.14	5.25	12.9	9.0	8.0
14	Ecotype 2005	Latvia	2.80	3.04	6.2	8.5	8.0
	$LSD_{0.05}$		1.3	0.9	1.2	0.8	0.7

Spring growth intensity and re-growth rate are important feature in assessment of white clover accessions. Height of swards before flowering varied substantially; the following cultivars stood out: 'Atoliai', 'Suduviai', 'Gigant', 'Huia', 'Araw' (Table 2). Some accessions of white clover ('Ecotype 2005', 'Dubraux', 'Gigant') stood out with earliness: flowering began at the end of May, two weeks earlier than the standard variety 'Daile', which was only in budding stage at the same time.

White clover spreads through the sward by branching stems. Warm weather conditions and sufficient moisture throughout the vegetation period in 2007 – the 1st year of use – had positive effects on the growth and yield formation of white clover.

Stem length was measured. Some cultivars, ‘Huia’, ‘Steinacher’, ‘Dubraux’ (Table 2), belonging to var. *giganteum*, substantially overcame the standard variety in this parameter.

The assimilating leaf area is an important factor for evaluating the biological qualities of the accessions. Varieties of white clover are classified according to leaf size, ranging from small to very large leaf ones. Large-leaved varieties were characterized by high yields and improved grazing performance (Jorgensen, 2006). White clover varieties with intermediate-sized leaf produce fewer stems than the small-leaved types. Leaf size is determined by the length of the central leaflet, and varied widely among white clover accessions (Fig. 1, Table 2).

Table 2. The differences of agro-morphological observations between white clover accessions.

N	Accession	Height of sward before flowering, cm	Length of stems, cm	Average length of central leaflet, mm	Weight of 10 flower heads, g	Weight of seeds from 1 m^2, g
1	Daile	20.0	10.50	29.0	7.58	29.5
2	Atoliai	24.0	8.00	35.0	7.35	62.4
3	Bitunai	19.0	5.00	19.0	4.80	72.1
4	Suduviai	27.0	9.50	32.0	5.92	58.0
5	Steinacher	18.0	15.50	30.0	3.71	30.1
6	Menna	23.0	7.50	28.0	6.95	45.5
7	Probstheidaer Weiβklee	22.0	5.50	28.0	5.01	29.8
8	Gigant	33.0	11.50	30.0	6.03	47.0
9	Huia	40.0	15.00	29.0	7.73	35.9
10	NZ White Clover Type	32.0	11.50	27.0	3.61	26.7
11	Araw	30.0	9.00	32.0	6.95	28.2
12	Jubiļeinij	25.0	11.00	21.0	4.72	11.3
13	Dubraux	31.0	23.00	24.0	4.63	50.2
14	Ecotype 2005	19.0	8.50	18.0	6.89	27.1
	$LSD_{0.05}$	7.0	3.3	1.0	0.9	3.1

Large-leaved varieties with a length of central leaflet >30 mm in our collection were the following: ‘Atoliai’, ‘Araw’, ‘Suduviai’, ‘Gigant’. Small-leaved accessions were ‘Bitunai’ and ‘Ecotype 2005’ with a central leaflet length of 19 and 18 mm, respectively. As the trial results showed, the large-leaf varieties also grew tall, therefore they were most suitable for cutting.

One morphological trait is the pattern on the foliage of white clover. Here, too, wide diversity was observed (Fig. 1). The cultivars ‘Steinacher’ and ‘Araw’ were characterized by bright marked patterns on the foliage.

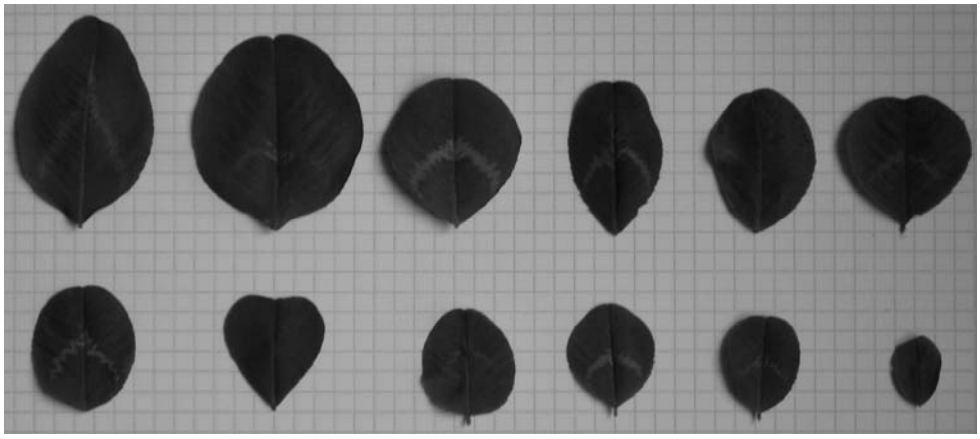

Figure1. Demonstration of differences in leaf form and size among white clover accessions.

The accessions included in the white clover collection differed in weight and size of flower heads (Fig. 2). The average weight of 10 flower heads ranged from 3.6 to 7.73 g. Larger flower heads were found in the cultivars ‘Atoliai’, ‘Huia’ and ‘Menna’ as well as in the standard variety ‘Daile’ (Table 2).

The collection accessions from Lithuania and the Czech Republic/Slovakia were distinguished by their unusual blossom color – light red.

Seed yield is also a significant parameter. The formation of white clover seed yield is dependent upon the number of inflorescences, formation of seeds in inflorescence, weather conditions during flowering time, etc. Number of the ripe inflorescences and the number of valuable seeds in inflorescence mainly determine the biological yield of white clover (Vasko, 2004).

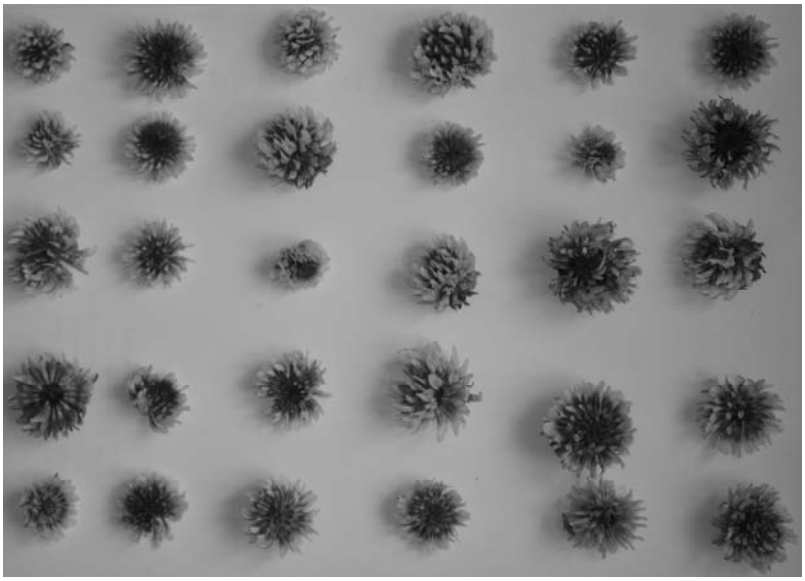

Figure 2. Differences in white clover flower heads' form and size among various accessions.

Comparisons of samples of seed yield also indicated wide variations: from Lithuania, 'Atoliai' – 62.4 g m^{-2}; 'Bitunai' – 72.1 g m^{-2}; 'Suduviai' – 58.0 g m^{-2} produced a high seed yield. There were also good results of the varieties 'Dubraux' – 50.2 g m^{-2} and 'Gigant' – 47.0 g m^{-2} (Table 2).

CONCLUSIONS

The differences in agro-morphological characteristics between white clover accessions were highly significant.

The study of morphological traits and biological characteristics of white clover collection revealed a great diversity between accessions.

Some accessions from Lithuania and the Czech Republic/Slovakia were distinguished by its unusual blossom color – light red.

The evaluation of samples of the white clover collection in the agro-climatic conditions of Latvia shows that some varieties are featured with several economic qualities and could be appropriate as promising material for the creation of a new variety. These are 'Dubraux' (Czech Republic/Slovakia), 'Gigant' (Belgium), 'Huia' (New Zealand) and local wild population 'Ecotype 2005', which excel in winter-hardiness, earliness, have a large foliage surface and produce higher seed yield.

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