GENETIC DIVERSITY OF PERENNIAL RYEGRASS WILD ECOTYPES BASED ON AGRO-BIOLOGICAL TRAITS

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Perennial ryegrasses (Lolium perenne L.) are one of the most valuable forage grasses, characterized by high productivity and good feed quality. Intensive cultivation of ryegrasses has resulted in reduced genetic diversity. Wild ryegrass ecotypes can be used in breeding programmes as parental genotypes with the aim to neutralize this genetic erosion. The objective of this study was to evaluate wild perennial ryegrass ecotypes from Ukraine under Lithuanian climatic conditions. Wild ecotypes were collected in Western and Northern Ukraine in 2008–2009 and planted in 2009–2010 as part of the germplasm collection at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry. Winter survival, re-growth in spring, plant height, beginning of heading, flag leaf length and width, inflorescence length and biomass were assessed. High variation of traits was detected among ecotypes (CV = 22.64–41.41%), except for winter survival, for which the coefficient of variation was average (CV = 18.26%). Principal component analysis was carried out and a dendrogram was computed based on agro-biological traits. In both years of investigation the grouping of ecotypes was mostly influenced by biomass, plant height and date of heading.

Key words: *perennial ryegrass, genetic diversity, wild ecotypes.*

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is one of the most important grass species of temperate climates. It is widely cultivated for forage due to high yield, good digestibility and tolerance to grazing pressures and it is used also for amenity purposes (Wilkins, 1991; Humphreys *et al.*, 2010). Perennial ryegrass is a diploid species ($2n = 2x = 14$) with a two-locus self-incompatibility system, which ensures a high degree of genetic variation in populations. Information on the level and distribution of genetic diversity in crop species is important for the selection of parental materials and optimum strategies of preservation of germplasm in gene banks (Gunter *et al.* 1996).

Intensive cultivation of ryegrasses for feed has resulted in reduced genetic diversity. In practice, the time of flowering is the most important character for the grouping of germplasm to establish gene pools. Following this approach, breeders have combined both closely related and unrelated materials. This might be one of the reasons for the rather low genetic diversity among perennial ryegrass cultivars

(Roldan-Ruiz *et al.* 2001). In order to neutralize this genetic erosion, grass breeders can collect their base materials from natural grassland. Wild ecotypes, collected in their natural habitats, usually are not productive, but some genotypes exhibit high resistance to various biotic and abiotic stresses, and therefore they can be used as parental genotypes in ryegrass breeding (Lemežienė and Kanapeckas, 2008).

The usefulness of the germplasm collections depends on sufficient characterisation. Various approaches can be applied to measure diversity of accessions and to classify them. Genetic diversity can be measured using DNA markers (Roldan-Ruiz *et al.*, 2001; Bolaric *et al.*, 2005; McGrath *et al.*, 2007) or traditionally, by evaluating morphological traits (Bennet *et al.*, 2000; Mirjalili *et al.*, 2008; Sartie *et al.*, 2009). Thorough evaluation of various agro-biological traits of foreign ecotypes under local climatic conditions is especially important for breeding programmes. The objective of this study was to evaluate the diversity of wild perennial ryegrass ecotypes from Ukraine under Lithuanian climatic conditions.

MATERIAL AND METHODS

Plants of 40 wild ecotypes of perennial ryegrass (*Lolium perenne* L.), collected in 2009 and 2010 in natural habitats of grasses in North and North West Ukraine (Lviv, Volyn, Rivne and Zhytomyr regions) (Table 1), were planted in 2010 and 2011 as part of the germplasm collection at the Lithuanian Institute of Agriculture. Lithuanian variety 'Žvilgė' was used as standard. Plants of each genotype were planted at 50 × 50 cm distances. In the autumn of each year, phosphorus and potassium fertilisers (P₆₀ K₉₀) were applied. Nitrogen fertiliser (N₁₅₀) was applied each year of herbage use in several applications: in spring N₆₀, and N₄₅ after the first and second cut.

In the collection trials the following parameters were assessed: winter survival, re-growth in spring, beginning of heading, plant height, length and width of flag leaf, inflorescence length and biomass. The parameters were estimated using the standard UPOV methodology (Anonymous, 2006), where a score of 9 represent very high and 1 is a very low value for the trait.

The spring of 2010 was warm, with sufficient available moisture in the soil. The weather in June was cool. There were days in July–April months when air temperature rose above 30° C. The entire growing season was characterised by abundant rainfall.

The temperature in the spring of 2010 was variable. The summer was very warm and rainy. Mean temperature in summer was 1.7 °C higher than the long-term mean temperature. Soil moisture was sufficient during the season, except in June.

The coefficient of variation (CV) and standard error (Sd) of agro-biological traits were estimated using computer software *STAT* adapted by P. Tarakanovas in Visual Basic of Application as a macro programme to run in EXCEL (Tarakanovas and Raudonius, 2003). NTSYSpc v. 2.2 (Exeter Software, Setauket, NY) (Rohlf, 2005) was used to perform principal component analysis (PCA) and compute dendrograms based on Euclidean distances.

RESULTS

High (22.64–41.41%) variation of most investigated traits was observed among wild perennial ryegrass ecotypes. An exception was winter survival (CV–18.26% in 2009 and 17.29% in 2010). This trait also scored higher (average 8.56 in 2009 and 7.6 in 2010) than other traits. Plant height was the most variable trait in both years of investigation (Table 2).

Principal component analysis was carried out based on agro-biological characteristics. For traits in 2010, the first three principal components (PC) accounted for 58.67% of the total variation among the 27 studied genotypes (Table 3). PC1 accounted for 22.36% of total variation, which according to eigenvector values, was best explained by beginning of heading and biomass. PC2 accounted for 18.86% of total variation and was related to winter survival and plant height. PC3 accounted for 17.45% of total variation and was explained by re-growth in spring and inflorescence length. The ecotypes were grouped according to these measurements. Genotypes No. 3811 and No. 3823 diverged from the rest (Fig. 1). No. 3811 was late-heading, poorly survived winter, exhibited good re-growth in spring but was very low-yielding, while No. 3823 scored highest in the first three traits and produced average biomass yield. Genotypes No. 3816, 3820, 3822, 3825 and 3828 formed a distinct group in the dendrogram. All genotypes were characterised by good winter survival, tall plants and high biomass yield.

Table 1
COLLECTION SITES OF WILD *Lolium perenne* ECOTYPES

Accession No.	Latitude N	Longitude E	Accession No.	Latitude N	Longitude E
3809	49°56'19"	24°57'28"	3832	50°27'99"	25°50'69"
3810	49°57'93"	24°42'51"	3833	50°34'07"	25°33'84"
3811	49°57'93"	24°55'59"	3834	51°01'70"	25°07'25"
3812	49°58'18"	24°53'88"	3879	49°14'92"	23°21'04"
3813	50°11'21"	25°24'94"	3880	49°11'93"	23°18'38"
3814	50°20'09"	25°38'49"	3881	49°11'93"	23°18'38"
3815	50°35'98"	26°28'33"	3882	49°10'79"	23°18'02"
3816	50°36'08"	26°32'24"	3883	49°09'42"	23°16'11"
3817	50°34'94"	26°47'17"	3884	49°10'03"	23°11'73"
3818	50°34'29"	27°12'22"	3885	49°12'09"	23°09'49"
3819	50°33'95"	27°42'78"	3886	49°12'08"	23°04'79"
3820	50°33'95"	27°42'78"	3887	49°29'35"	23°09'35"
3821	50°26'94"	28°05'17"	3888	49°30'01"	23°09'33"
3822	50°20'49"	28°20'33"	3889	49°37'43"	23°24'95"
3823	50°23'45"	28°41'03"	3890	49°52'25"	23°45'78"
3824	50°30'96"	28°39'94"	3891	49°56'23"	23°47'72"
3825	50°39'58"	28°38'02"	3892	49°58'52"	23°49'27"
3826	50°43'82"	28°37'26"	3893	50°17'96"	24°10'76"
3827	50°44'00"	28°37'33"	3894	50°36'89"	24°11'29"
3828	50°58'93"	28°29'71"	3895	50°59'82"	24°17'39"
3829	50°48'07"	27°57'38"	3896	51°20'06"	23°59'85"
3830	50°33'93"	26°13'78"	3897	51°28'29"	23°54'69"
3831	50°27'99"	25°50'69"	3898	51°22'11"	23°58'46"

Table 2
AGRO-BIOLOGICAL TRAITS (POINTS) OF PERENNIAL RYEGRASS WILD ECOTYPES, 2010–2011

Characteristics	2010		2011	
	$\bar{x} \pm Sd$	CV, %	$\bar{x} \pm Sd$	CV, %
Winter survival	8.56 ± 1.55	18.15	7.60 ± 1.31	17.29
Re-growth in spring	6.48 ± 1.53	23.58	6.10 ± 1.37	22.50
Beginning of heading	6.33 ± 1.57	24.77	5.05 ± 1.19	23.58
Plant height	5.33 ± 2.34	43.84	5.50 ± 2.04	37.07
Length of flag leaf	5.37 ± 2.00	37.29	5.70 ± 1.17	20.60
Width of flag leaf	5.89 ± 1.01	17.20	4.20 ± 1.20	28.49
Inflorescence length	5.37 ± 1.24	23.18	5.00 ± 1.45	29.02
Biomass	6.04 ± 1.79	29.59	5.30 ± 1.49	28.12

Table 3

BASIC STATISTICS OF PRINCIPAL COMPONENT ANALYSIS FOR AGRO-BIOLOGICAL TRAITS OF WILD ECOTYPES, 2010¹

Traits	Eigenvector		
	PC1	PC2	PC3
Winter survival	0.211	0.737	0.208
Re-growth in spring	0.455	-0.087	-0.504
Beginning of heading	0.693	-0.278	0.008
Plant height	0.131	0.695	-0.404
Length of flag leaf	0.337	-0.139	0.180
Width of flag leaf	-0.433	0.373	-0.400
Inflorescence length	0.565	-0.181	-0.450
Biomass	0.648	0.456	0.391
Components			
Eigenvalue	1.79	1.51	1.40
% of variation explained	22.36	18.86	17.45
Cumulative variance	22.36	41.22	58.67

¹ PCA loading are given for plant traits

Fig. 1. Dendrogram of wild ecotypes based on agro-biological traits, 2010

In 2011, the first three PCs accounted for 69.83% of the total variation (Table 4). PC1 explained 38.17% of total variation and was related to biomass and beginning of heading. Factors explaining PC2 (16.95% of total variation) were plant height and width of flag leaf. PC3 accounted for 14.71% of total variation; factors associated with this axis were length of flag leaf and winter survival. Genotypes No. 3887 and 3886 formed a distinct group in the dendrogram (Fig. 2). Both started heading earlier than all other genotypes, had short and narrow flag leaves. Genotype No. 3887 was tall and high-yielding, while No. 3886 was short and low-yielding.

Table 4

BASIC STATISTICS OF PRINCIPAL COMPONENT ANALYSIS FOR AGRO-BIOLOGICAL TRAITS OF WILD ECOTYPES, 2011¹

Traits	Eigenvector		
	PC1	PC2	PC3
Winter survival	0.464	0.092	0.738
Re-growth in spring	0.851	0.235	0.115
Beginning of heading	0.891	0.004	-0.056
Plant height	-0.105	-0.677	-0.083
Length of flag leaf	0.443	0.168	-0.765
Width of flag leaf	0.366	-0.646	-0.079
Inflorescence length	0.391	-0.616	0.106
Biomass	0.909	0.094	-0.080
Components			
Eigenvalue	3.05	1.36	1.18
% of variation explained	38.17	16.95	14.71
Cumulative variance	38.17	55.12	69.83

¹ PCA loading are given for plant traits

Fig. 2. Dendrogram of wild ecotypes based on agro-biological traits, 2011

DISCUSSION

High genetic diversity exists in populations of *Lolium perenne*, due to its self-incompatible nature. Even plants of cultivated varieties are not uniform, let alone wild ecotypes (Gharani *et al.*, 2003; Bolaric *et al.*, 2005; McGrath *et al.*, 2007). High variation of agro-biological traits of wild ecotypes from Ukraine was confirmed in this study. Perennial ryegrass has been grown in Lithuania for a long time. It is valued for high productivity and good forage quality (Kanapeckas *et al.*, 1999; Aavola *et al.*, 2003). One of the most restrictive factors to wider usage of perennial ryegrasses is

relatively poor winter survival under Lithuanian climatic conditions. Ukraine is situated to the South of Lithuania, yet most of wild ecotypes used in this study exhibited good winter survival. Winter-hardy genotypes of Ukrainian origin have been identified in previous studies as well (Kemešytė *et al.*, 2010). Biomass yield, another crucial trait, varied markedly among ecotypes. Both tall, high yielding genotypes, suitable for forage and small, narrow-leaved and low yielding genotypes suitable for turf grass breeding were identified.

Various studies of genetic diversity based on phenotypic traits within *Lolium perenne* species and within genus *Lolium* identified different traits to be discriminatory factors in principal component analysis. Leaf type and length, spikelet length and width were found to explain principal components in a phenetic analysis of the genus *Lolium* (Mirjalili *et al.*, 2008). In another study, ear emergence, spike height and flag leaf length and width were the main factors that explained variation in four species of the genus *Lolium* (Bennet *et al.*, 2000). Total tiller number and leaf lamina width discriminated two perennial ryegrass cultivars, whereas leaf length and leaf elongation rate identified individual plants within cultivars in PCA (Sartie *et al.*, 2009). Various traits were found to be discriminatory in this study, yet biomass, beginning of heading and plant height had the major role.

In general, high phenotypic diversity within wild ecotypes and their suitability for different breeding purposes was confirmed in this study. Both forage type and turfgrass type winter-hardy genotypes could be identified in the relatively small collection.

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DAUDZGADĪGĀS AIRENES SAVVAĻAS EKOTĪPU ĢENĒTISKĀ DAUDZVEIDĪBA, PAMATOJOTIES UZ AGRONOMISKO UN BIOLOĢISKO PAZĪMJU ANALĪZI

Raksturoti 40 Ukrainas daudzgadīgās airesnes ekotipi, audzējot tos Lietuvā. Parādīta augsta svarīgo pazīmju mainība, kas norāda uz to, ka pētītais materiāls var būt vērtīgs jaunu šķirņu veidošanas izejmateriāls.