ISSN 1392-3196
Zemdirbyste-Agriculture, vol. 96, No. 3 (2009), p. 47–57
UDK 633.265:633.264:575

Isozymes and ISSR markers as a tool for the assessment of genetic diversity in *Phleum* spp.

Kristina JONAVIČIENĖ, Vanda PAPLAUSKIENĖ, Gintaras BRAZAUSKAS

Lithuanian Institute of Agriculture
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: kristinaja@lzi.lt

Abstract

A total of 10 *Phleum* spp. populations, mainly of Lithuanian origin, were investigated at the Lithuanian Institute of Agriculture during 2007–2009 with three isozymes and five ISSR primers to assess genetic variation and relationships among three *Phleum* species: *Phleum pratense* L., *Phleum bertolonii* DC, and *Phleum phleoides* (L.) H. Karst. Isozymes and ISSR primers revealed 47.7 and 57.3% polymorphism, respectively, from 100 individuals. The Shanon index content varied from 0.124 to 0.303 for isozymes and from 0.248 to 0.326 for ISSRs. Genetic distance in the computed matrix between *Phleum* spp. populations ranged from 0.089 to 0.784 for isozyme analysis and from 0.123 to 0.667 for ISSR analysis. Dendrograms constructed from isozymes and ISSR data showed overall similar topologies. The ISSR data precisely organized ten populations into three clusters corresponding to the species and the isozyme data grouped into two clusters. The study demonstrated that isozymes and ISSR methods are an efficient approach for genotyping *Phleum* species. These results provide important baseline for future improvement of grass breeding programs.

Key words: isozymes, ISSR, *Phleum*, species, genetic distance, polymorphism.

Introduction

Over the long term, the ability of a species to respond adaptively to environmental changes depends on the level of genetic variability it contains /Aronson et al., 1987/. A species without an appropriate amount of genetic diversity is thought to be unable to cope with changing environments or evolving competitors and parasites. Therefore, investigations on population genetic diversity within and between the species provide information useful for breeding.

Timothy (*Phleum pratense* L.) is a high yielding, well adapted to Northern European conditions and one of the most productive grass species in Lithuania /Lemežienė et al., 2004/. Timothy is valued for its winter hardiness, good palatability, and moderate nutritional feed value. Due to its finely textured leaves and creeping tillers, *P. bertolonii* could be used as dormant turf grass. *P. phleoides* meant to have highly developed mechanism for growing under drought conditions. There is limited knowledge regarding the genetic diversity and interspecies relationships in *Phleum*, limiting the efficiency of breeding programs.

Molecular markers are very useful tools for the analysis of genetic diversity /Qian et al., 2001; Bolaric et al., 2005; Pivorienė, Pašakinskienė, 2008/. Isozyme and

inter-simple sequence repeat (ISSR) markers are two molecular typing approaches that have been used to detect variation among plants. Each method has been used extensively to identify and determine relationships at the species and cultivar levels /Culley, Wolfe, 2001; Angelov, 2003/. These methods are widely applicable because they are rapid, inexpensive and simple to perform. Isozyme analysis is a quick and effective method for the determination of genetic diversity /Jaaska, 2005; Kull, Oja, 2007/. Isozymes are used as genetic markers to observe the recombination and segregation of linked qualitative and quantitative characteristics /Fleischman, 1990/. ISSR is a developed modification of simple sequence repeat (SSR)-based marker systems /Zietkiewicz et al., 1994/. It offers many advantages, such as requirement of only low quantities of template DNA, no need for sequence data for primer construction, random distribution throughout the genome, the generation of many informative bands per reaction etc. The ISSR technique has been proven to be useful in population genetic diversity studies /Kubik et al., 2001; Camacho, Liston, 2001/. The genetic variability of *P. pratense* has been investigated cytologically /Perný et al., 2008/ and using RAPD and UP-PCR techniques /Guo et al., 2003/. To date, there have been no publications, to our knowledge, on the use of ISSR markers for the characterization of the genetic diversity of *Phleum* spp. and only one other study has directly compared genetic diversity estimates based on allozyme and ISSR data /Esselman et al., 1999; Culley, Wolfe, 2001/. The objectives of this study were to evaluate the applicability of ISSR genotyping in *Phleum* spp. in comparison to isozyme genotyping and to compare the extent of genetic variation in *P. pratense*, *P. phleoides* and *P. bertolonii*.

Materials and methods

Ten populations of *Phleum* spp. were used for this study (Table 1). The seed was sown in plastic pots filled with soil mixture and grown in a greenhouse. When the plants had reached 5–7 cm in height, ten individuals from each population were used for isozymes analyses and after 8 weeks leaf material was harvested for DNA extraction.

Total DNA was isolated from seedling leaves taken from 10 plants of each population as described by Doyle and Doyle (1990). DNA concentration was determined with Biophotometer ("Eppendorf", Germany).

Five selected inter simple sequence repeat (ISSR) primers ("Metabion", Germany) were used to generate ISSRs: three of them were composed of di-nucleotide repeats and two of tetra-nucleotide repeats listed in Table 3. The PCR reaction mixture (15 µl) contained 50 ng of genomic DNA, 1 × Dynazyme reaction buffer, 1.87 mM $MgCl_2$, 0.2 mM dNTP, 0.25 µM of each primer and 2 units of DyNAzyme^{TM} II DNA Polymerase ("Finnzymes", Finland). Amplification was performed by an "Eppendorf Thermal Cycler" ("Eppendorf", Germany) using one of the following profiles, +95^{o}C initial denaturation for 2 min, then 40 cycles of +95^{o}C for 30 s, a 1 minute annealing step at either +45 or +50^{o}C, depending on the annealing temperature value of the primer pair (Table 2), and +72^{o}C for 1 min. The PCR was finished with a 6 min elongation step at +72^{o}C. The amplification products were size-separated by standard horizontal electrophoresis in 1.5% agarose gels and stained with ethidium bromide. The reproducibility of the DNA profiles was tested by repeating the PCR amplifications 2–3 times with each of

the primers analyzed. Only the robust and repeatable bands were considered in this study.

Table 1. Description of *Phleum spp.* populations used for this study
1 lentelė. *Motiejuko (Phleum spp.) populiacijų apibūdinimas*

Species *Rūšys*	Population name *Populiacijų pavadinimai*	Status *Statusas*	Ploidy level *Ploidiškumas*	Origin *Kilmė*
Phleum pratense	ʻGintaras IIʼ	cultivar *veislė*	hexaploid *heksaploidas*	Variety ʻLishoverʼ (Germany) x variety ʻOetofteʼ (Denmark)
	ʻJauniaiʼ	cultivar *veislė*	hexaploid *heksaploidas*	Variety S-352 (England) x variety ʻGintaras IIʼ (Lithuania)
	ʻŽolisʼ	cultivar *veislė*	hexaploid *heksaploidas*	Variety ʻSAMOʼ (Holland) x 2 old unknown varieties (Holland)
	ʻKlonisʼ	cultivar *veislė*	hexaploid *heksaploidas*	Intercross of medium early biotypes of variety ʻGintaras IIʼ (Lithuania)
	ʻVėlenisʼ	cultivar *veislė*	hexaploid *heksaploidas*	Variety ʻSAMOʼ (Holland) x VIR-1 (Russia)
	1724	breeder's line *selekcinė linija*	hexaploid *heksaploidas*	204 (Russia) x variety 437 (Finland) x wild ecotype 432 (Russia) x variety VIR-1 (Russia)
Phleum bertolonii	119	breeder's line *selekcinė linija*	diploid *diploidas*	Germany
	2518	wild ecotype *laukinis ekotipas*	diploid *diploidas*	Kaišiadorys distr., Lithuania
Phleum phleoides	2718	wild ecotype *laukinis ekotipas*	diploid *diploidas*	Joniškis distr., Lithuania
	2754	wild ecotype *laukinis ekotipas*	diploid *diploidas*	Orjalu distr., Estonia

Two or three leaves from 10 plants of each population were crushed in a cooled mortar using a pestle and a liquid extraction buffer (TRIS, pH 7.2) was added. Small Whatman papers soaked in the extract were placed on starch gel. Gels were made of 12% potato starch ("Sigma", Germany). Lithium borate pH 8.3, Tris-citrate pH 8.3 in the proportion 1:9 were used to prepare starch gel and lithium borate (pH 8.3) was used as a running buffer. Samples from all ten populations were run alongside markers of known genotypes to ensure consistent scoring. Three isozymes were tested: phosphoglucoisomerase (PGI), aspartataminotranspherase (AAT) and esterase (EST) (Table 2). The staining recipe for each isozyme was different: EST and PGI were from Pasteur /Pasteur et al., 1988/, and AAT from Taddesse and Bekele /Taddese, Bekele, 2001/. Alleles were designated as letters representing band migration distance, with "a" assigned to the most anodal allozyme.

The molecular size of each fragment from ISSR was estimated relative to GeneRuler™ DNA Ladder Mix ("Fermentas", Lithuania). Isozyme markers as well as ISSR markers were scored as presence (1) or absence (0) of a band, and the data obtained were used in a rectangular matrix. The data matrix was then used to generate a

genetic distance index /Nei, 1972/. Cluster analysis was carried out based on genetic distance, using unweighted pair-group method using arithmetic averages (UPGMA). The resulting clusters were represented as dendrograms. Estimates of the differences between the dendrograms based on isozymes and ISSR-marker analyses were obtained by computing the cophenetic values and constructing the relative cophenetic matrices for each marker type. These cophenetic matrices were compared using Mantel's test for matrix correspondence /Mantel, 1967/. These computations were performed with *NTSYSpc v. 2.2* analysis software. The percentage of polymorphic loci (PPB) and the Shannon index of diversity (*I*) were computed with *PopGene 3.2* assuming all loci to be dominant and in Hardy-Weinberg equilibrium.

Results

Initially, 35 ISSR primers were screened against genomic DNA from ten *Phleum* spp. populations for their ability to amplify DNA fragments. Of the 35 primers, 4 produced no distinct bands on a smeary background and 18 resulted in very faint bands upon a highly smeared background and 4 were monomorphic. The remaining 9 primers produced robust amplification patterns. Of all the amplified profiles, the 5 best and highly polymorphic patterns were selected for further analysis (Table 2).

Table 2. Products generated by ISSR analysis of *Phleum* spp. populations
2 lentelė. *Motiejuko (Phleum spp.) tyrimų rezultatai, gauti naudojant ISSR pradmenis*

Primer *Pradmuo*	Sequence (5→3') *Seka* (5→3')	Annealing temp. *Kaitinimo temperatūra* °C*	*Phleum pratense*		*Phleum bertolonii*		*Phleum phleoides*	
			Fragment size range *Fragmentų dydžių ribos* bp	Fractions of fragments *Fragmentų dalys* **	Fragment size range *Fragmentų dydžių ribos* bp	Fractions of fragments *Fragmentų dalys* **	Fragment size range *Fragmentų dydžių ribos* bp	Fractions of fragments *Fragmentų dalys* **
105H	$(GA)_8CT$	48	500–2000	11/9	500–2000	11/6	550–2000	11/8
155H	$(CA)_7GA$	50	650–1550	12/9	550–1400	12/7	550–1550	12/7
104H	$(GACA)_4GT$	50	700–2000	13/11	500–2000	11/6	500–2000	11/9
78H	$AC(GACA)_4$	50	550–2400	12/8	550–2400	12/8	550–2400	12/10
UBC822	$(TC)_8A$	45	400–1750	10/10	400–1750	10/10	400–1750	10/7
	Total / *Iš viso*			58/47		58/37		58/41

Note / *Pastaba*. * – determined empirically / *apskaičiuota empiriškai*, ** – number of fragments amplified / number of polymorphic fragments / *amplifikuotų fragmentų skaičius / polimorfiškų fragmentų skaičius*.

High genetic variation was observed using ISSR markers. The set of 5 ISSR primers amplified a total of 56 bands from the 10 *Phleum* spp. populations tested (Table 2). Genetic diversity varied greatly among populations with PPB values ranging from 48.3 (2518) to 72.4 ('Gintaras II'). Shannon's index (*I*) ranged from 0.248 to 0.326, with an average of 0.280 at the population level and at the species level *P. pratense* had the highest *I* values – 0.297 (Table 4).

Table 3. Products generated by isozyme analysis of *Phleum* spp. populations
3 lentelė. *Motiejuko (Phleum spp.) tyrimų rezultatai, gauti naudojant izofermentus*

Isozymes *Izofementai*	Fragment size range *Fragmentų dydžių ribos* R_f**	Fractions of fragments *Fragmentų dalys* *	Fragment size range *Fragmentų dydžių ribos* R_f**	Fractions of fragments *Fragmentų dalys* *	Fragment size range *Fragmentų dydžių ribos* R_f**	Fractions of fragments *Fragmentų dalys* *
PGI	0.45–0.23	7/6	0.45–0.38	7/2	0.45–0.38	7/2
AAT	0.70–0.25	6/5	0.7–0.25	6/5	0.7–0.33	6/4
EST	0.87–0.33	18/14	0.87–0.16	18/13	0.87–0.33	18/11
Total / *Iš viso*		31/25		31/20		31/17

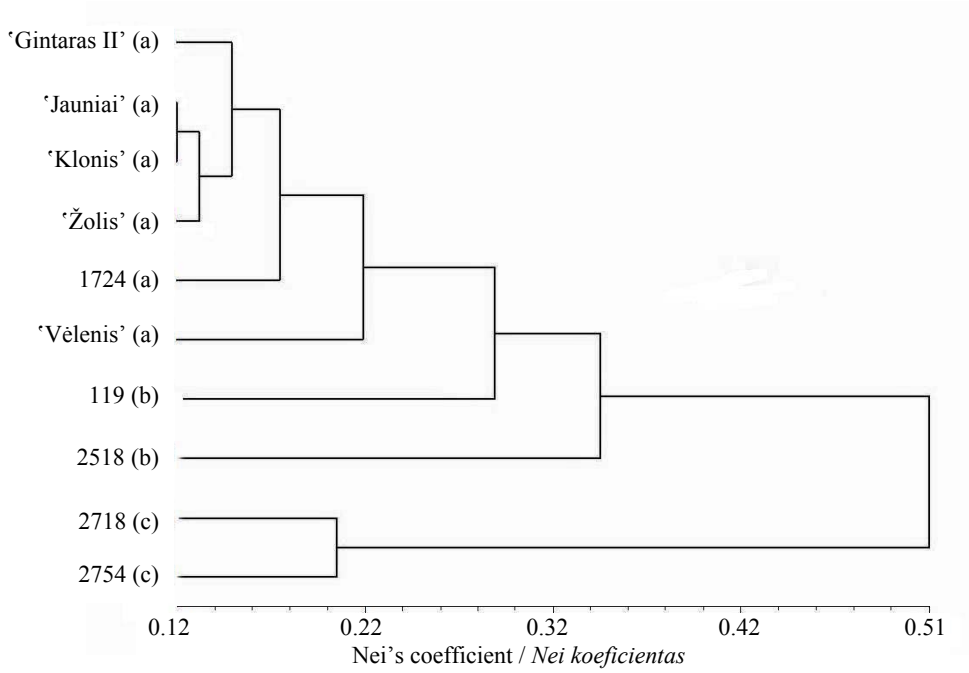
Note / *Pastaba*. * – number of fragments amplified / number of polymorphic fragments / *amplifikuotų fragmentų skaičius / polimorfiškų fragmentų skaičius,* **R_f – relative mobility / *elektroforezinis mobilumas.*

Table 4. Genetic variability of isozymes and ISSRs in *Phleum* spp. populations
4 lentelė. *Motiejuko (Phleum spp.) populiacijų genetinė įvairovė pagal izofermentus ir ISSR*

Species, populations *Rūšys, populiacijos*	Genetic diversity *Genetinė įvairovė*			
	ISSR		Isozymes / *Izofermentai*	
	Shannon index *Šanono genetinės įvairovės indeksas I*	Percentage of polymorphic loci *Polimorfinių fragmentų proc.* PPB %	Shannon index *Šanono genetinės įvairovės indeksas I*	Percentage of polymorphic loci *Polimorfinių fragmentų proc.* PPB %
P. pratense				
ʻGintaras IIʼ	0.326	72.4	0.296	75.0
ʻJauniaiʼ	0.323	65.5	0.289	65.6
ʻŽolisʼ	0.279	60.3	0.263	59.3
ʻKlonisʼ	0.305	63.8	0.230	53.1
ʻVėlenisʼ	0.278	58.6	0.303	65.2
1724	0.271	53.4	0.244	53.1
Mean / *Vidurkis*	**0.297**	**62.3**	**0.271**	**61.8**
P. bertolonii				
119	0.281	56.9	0.253	50.0
2518	0.285	48.3	0.187	37.5
Mean / *Vidurkis*	**0.283**	**52.6**	**0.220**	**43.8**
P. phleoides				
2718	0.277	62.1	0.124	28.1
2754	0.248	51.7	0.220	46.9
Mean / *Vidurkis*	**0.262**	**56.9**	**0.172**	**37.5**
Total mean *Bendras vidurkis*	**0.280**	**57.3**	**0.221**	**47.7**

Table 4 reports the observed values of *I* and PPB using PGI, AAT and EST enzymes. The total of 31 scored loci gave 25 polymorphic alleles in *P. pratense*, 20 in *P. bertolonii* and 17 in *P. phleoides* by isozyme analysis (Table 3). At the species level, the PPB for isozyme analysis in *P. pratense* was 62.3% while that of *P. bertolonii* and *P. phleoides* was 52.6 and 56.9%, respectively. An average of 61.8% of PPB was within populations of *P. pratense*, with individual population values ranging from 53.1 to 75.0%. The populations of *P. pratense* species appear to be more diverse than these of *P. bertolonii* (43.8), and of *P. phleoides* (37.5) ones. The I values were higher in *P. bertolonii* 0.220 than that of *P. phleoides* species (0.172) but lower than in *P. pratense* (0.271).

ISSR analysis revealed extensive polymorphism among the different populations of *Phleum*. A cluster analysis (UPGMA) was used to generate a dendrogram based on the Nei's coefficient /Nei, 1972/ among all 10 populations (Fig. 1). In the UPGMA dendrogram from ISSRs data, major group was shared by six of ten populations analyzed ('Gintaras II', 'Jauniai', 'Klonis', 'Žolis', 'Vėlenis' and 1724). Another cluster consists of two *P. phleoides* populations (2718 and 2754) and the third cluster is composed of two *P. bertolonii* populations (119 and 2518). Nei's genetic distance values between pairs of populations ranged from 0.123 between 'Klonis' and 'Jauniai' to 0.667 between 2718 and 119 (Table 5).



Note / *Pastaba*. The letters represent different species: a – *P. pratense*, b – *P. bertolonii*, c – *P. phleoides* / *Raidėmis pažymėtos skirtingos rūšys: a – P. pratense, b – P. bertolonii, c – P. phleoides.*

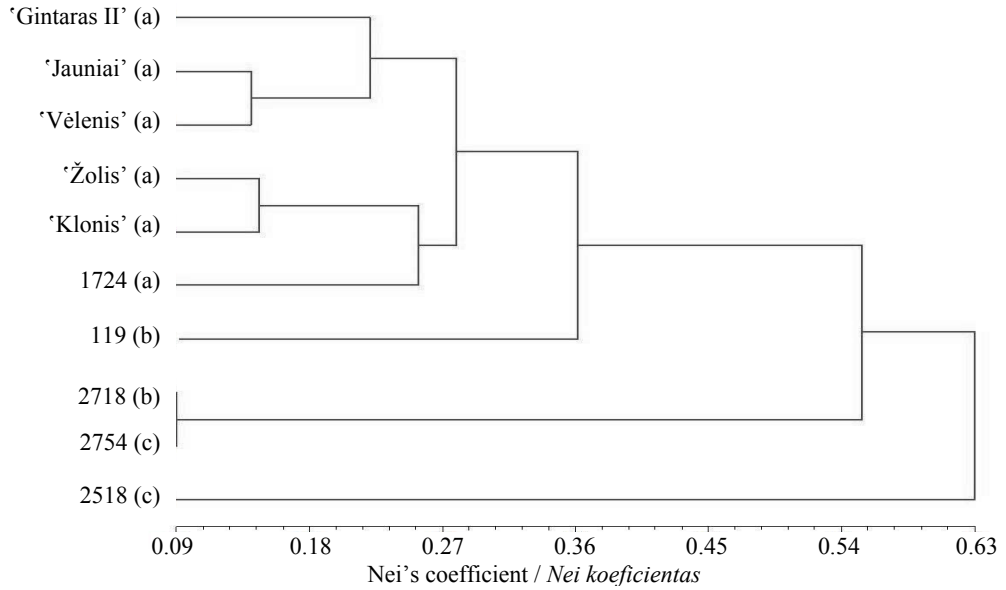
Figure 1. UPGMA dendrogram of *Phleum* spp. populations generated from ISSRs data
1 paveikslas. *Phleum spp. populiacijų dendrogama pagal ISSR duomenis*

Table 5. Genetic Nei's distance between *Phleum* populations using isozymes (upper half of matrix) and ISSRs (lower half of matrix)

5 lentelė. *Genetiniai atstumai pagal M. Nei (1972) tarp Phleum populiacijų, naudojant izofermentus (viršutinė lentelės dalis) ir ISSR (apatinė lentelės dalis) žymenis*

	'Gintaras II'	'Jauniai'	'Žolis'	'Klonis'	'Vėlenis'	1724	119	2518	2718	2754
'Gintaras II'	*	0.262	0.340	0.188	0.177	0.354	0.764	0.282	0.674	0.558
'Jauniai'	0.127	*	0.293	0.198	0.139	0.217	0.518	0.408	0.433	0.381
'Žolis'	0.146	0.138	*	0.145	0.434	0.457	0.371	0.312	0.715	0.469
'Klonis'	0.182	0.123	0.130	*	0.260	0.327	0.618	0.299	0.641	0.469
'Vėlenis'	0.233	0.199	0.219	0.213	*	0.364	0.631	0.446	0.733	0.703
1724	0.189	0.199	0.179	0.137	0.231	*	0.761	0.398	0.380	0.302
119	0.268	0.316	0.295	0.165	0.420	0.260	*	0.438	0.769	0.784
2518	0.323	0.400	0.274	0.378	0.326	0.329	0.369	*	0.600	0.590
2718	0.550	0.465	0.533	0.579	0.562	0.546	0.667	0.409	*	0.089
2754	0.476	0.456	0.562	0.526	0.581	0.507	0.513	0.279	0.206	*

The dendrogram from isozyme data also indicated that the *P. pratense*, *P. bertolonii* and *P. phleoides* populations were distinctly separated into two major clusters (Fig. 2). The first cluster is composed of six *P. pratense* populations ('Gintaras II', 'Jauniai', 'Klonis', 'Žolis', 'Vėlenis' and 1724) and the second one is composed of four populations: two from *P. bertolonii* (119 and 2518) and another two from *P. phleoides* (2718 and 2754). Nei's genetic distance values between pairs of populations ranged from 0.089 between 2718 and 2754 to 0.784 between 119 and 2754 (Table 5).



Note / *Pastaba*. The letters represent different species: a – *P. pratense*, b – *P. bertolonii*, c – *P. phleoides* / *Raidėmis pažymėtos skirtingos rūšys: a – P. pratense, b – P. bertolonii, c – P. phleoides.*

Figure 2. UPGMA dendrogram of *Phleum* spp. populations generated from isozyme data

2 paveikslas. *Phleum spp. populiacijų dendrogama pagal izofermentų duomenis*

The matrices for isozymes and ISSR markers were also compared using Mantel's test /Mantel, 1967/ for matrix correspondence. The correlation between the matrices of cophenetic values for the dendrograms based on isozymes and ISSR data was medium ($r = 0.660$).

Discussion

P. pratense cultivars are heterogeneous populations, most timothy cultivars are synthetics, i.e. the advanced generation of two to many parental clones that were interpollinated (Table 1). Parental clones are selected on the basis of phenotype, the performance of the individual plants, or combining ability, which is determined through progeny testing /Lemežienė, 2006/. Some cultivars have been selected from land races or ecotypes. In order to assess genetic diversity in such plants, a two-way profiling approach was used. The ISSR profiles reveal information about diversity of *Phleum* genomes in regions rich in repetitive sequences while the isozymes profiling provides a single-gene molecular markers (alleles) that are biparentally inherited and that generally adjust to a codominant patterns of expression. Thus it is not surprising that ISSRs have provided more polymorphisms than isozymes. Our ISSR surveys on ten populations revealed 57.3% of polymorphic bands, which are higher than those from the isozyme analysis (47.7%) although 5 primers were used in the ISSR analysis whereas only 3 allozymes were used in the isozyme analysis. This discrepancy is mainly due to the DNA segments targeted by those two methods. First, the greater polymorphism detected by ISSRs than by isozymes may be partially explained by the conservative nature of the coding sequences, primarily housekeeping genes, sampled by allozymes in contrast to the non-coding sequences sampled by ISSRs /Fahima et al., 1999/. Second, micro-satellites are short tandem repetitive DNA sequences with a repeat length of only a few base pairs. These sequences are abundant, dispersed throughout the eukaryotic genome and highly polymorphic due to DNA slippage /Weber, May, 1989/.

Genetic diversity of forage grasses using isozyme analysis have been already reported for orchardgrass /Tosun et al., 2002/, red clover /Lange et al., 2000; Yu et al., 2001/ and perennial ryegrass /Balfourier, Charmet, 1994/. Our diversity indices, presented in Table 4, are consistent with those reported by these authors. The efficiency of ISSR markers in identifying cultivars and genotypes of perennial ryegrass was reported by Posselt et al. /Posselt et al., 2006/. In comparison, Guo et al. /Guo et al., 2003/ indicated that useful segregating markers were found using Random Amplified Polymorphic DNA (RAPD) and universally primed PCR (UP-PCR) in timothy. For the *Phleum* species studied, this is, to our knowledge, the first report of molecular variability evaluation using ISSR and isozyme markers. In this study, *P. pratense* showed the highest level of intra-species polymorphism (Table 5), supporting the work of Guo et al. /Guo et al., 2003/ who reported that *P. pratense* was highly polymorphic species. The different ploidy levels among ten *Phleum* species studied (Table 1) might have affected band number due to the presence of different copies of the same locus in the genome. On the other hand, 10 individuals from each population were analyzed, while more than 15 plants per population in outcrossing species are suggested /Morell et al., 1995/. However, in studies applying other marker systems like RFLP in alfalfa /Labombada et al., 2000/ and SSRs in perennial ryegrass /Kubik et al., 2001/, different optimal

sample sizes were found. This indicates the dependency of the minimum sample size on the marker system.

The distances (0.123 to 0.434) were in a wide range indicating thereby, that *P. pratense* represents a genetically diverse species. The distances between *P. bertolonii* and *P. phleoides* were narrower but these three species were clearly separated in the present study by the five ISSR primers assayed. The populations of *P. pratense* species were clustered together, clearly separating them from the *P. bertolonii* and *P. phleoides* species but also showing intra-species variability (Figure 2). Our genetic- distance estimates within each cluster showed the extensive genetic variation within the species, with *P. phleoides* exhibiting lower levels of genetic variability as compared with *P. pratense.*

Conclusion

In conclusion, isozymes and ISSRs are effective and promising marker systems for detecting genetic variation. Furthermore, ISSR is superior to isozymes in terms of the polymorphic bands detected per primer and the reproducibility involved. Our data provide evidence of a genetic diversity between the tested *Phleum* spp. populations. Cluster analysis (UPGMA) revealed three main groups of *Phleum* spp. accessions and clearly distinguished *P. pratense* populations from those of *P. bertolonii* and *P. phleoides.* These findings are important for further breeding programs, because variation is required for successful breeding of forage crop species to provide farmers with suitable cultivars adapted to different environments and management systems.

Acknowledgments

The study has been supported by grants N-21/2009 and No. T-09162 provided by the Lithuanian State Science and Studies Foundation.

Received 01 07 2009
Accepted 29 07 2009

REFERENCES

1. Angelov G. B. Isoenzyme variation of esterase and acid phosphatase and genetic affinities among *Dasypyrum villosum* (L.) *P. Candargy*, *Elytrigia repens* (L.) *Nevski* and *Elymus caninus* (L.) L. // Turkish Journal of Botany. – 2003, vol. 27, p. 249–254

2. Aronson L. J., Gold A. J., Hull R. J. Cool-season turfgrass responses to drought stress // Crop Science. – 1987, vol. 27, p. 1261–1266

3. Balfourier F., Charmet G. Geographical patterns of isozyme variation in Mediterranean populations of perennial ryegrass // Heredity. – 1994, vol. 72, p. 55–63

4. Bolaric S., Barth S., Melchinger A. E. et al. Genetic diversity in European perennial ryegrass cultivars investigated with RAPD markers // Plant Breeding. – 2005, vol. 124, p. 161–166

5. Camacho F. J., Liston A. Population structure and genetic diversity of *Botrychium pumicola* (*Ophioglossaceae*) based on ISSR // American Journal of Botany. – 2001, vol. 88, No. 6, p. 1065–1070

6. Culley T. M., Wolfe A. D. Population genetic structure of the cleistogamous plant species *Viola pubescens Aiton* (*Violaceae*), as indicated by allozyme and ISSR molecular markers // Heredity. – 2001, vol. 86, p. 545–556

7. Doyle J. J., Doyle J. L. Isolation of plant DNA from fresh tissue // Focus. – 1990, vol. 12, p. 13–15

8. Esselman E. J., Jianqiang L., Crawford D. J. et al. Clonal diversity in the rare *Calamagrostis porteri* ssp. *insperata* (*Poaceae*): comparative results for allozymes and random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers // Molecular Ecology. – 1999, vol. 8, p. 443–451

9. Fahima T, Sun G. L., Beharav A. et al. RAPD polymorphism of wild emmer wheat populations, *Triticum dicoccoides*, in Israel // Theoretical and Applied Genetics. – 1999, vol. 98, p. 434–447

10. Fleischman H. Genetic diversity of aspartate-aminotransferase isoenzymes in natural populations of *Aegilops triuncialis* and its putative progenitors // Biochemie und Physiologie der Pflanzen. – 1990, Bd. 186, S. 189–194

11. Guo Y. D., Yli-Mattila T., Pulli S. Assessment of genetic variation in timothy (*Phleum pratense* L.) using RAPD and UP-PCR // Hereditas. – 2003, vol. 138, p. 101–113

12. Jaaska V. Isozyme variation and phylogenetic relationships in *Vicia* subgenus *Cracca* (*Fabaceae*) // Annals of Botany. – 2005, vol. 96, p. 1085–1096

13. Kubik Ch., Sawkins M., William A. M. et al. Genetic diversity in seven perennial ryegrass (*Lolium perenne* L.) cultivars based on SSR markers // Crop Science. – 2001, vol. 41, p. 1565–1572

14. Kull T., Oja T. Low allozyme variation in *Carex loliacea* (*Cyperaceae*), a declining woodland sedge // Annales Botanici Fennici. – 2007, vol. 44, p. 267–275

15. Labombada P., Pupilli F., Arcioni S. Optimal population size for RFLP-assisted cultivar identification in alfalfa (*Medicago sativa* L.) // Agronomie. – 2000, vol. 20, p. 233–240

16. Lange O., Schifino-Wittmann M. T. Isozyme variation in wild and cultivated species of the genus *Trifolium* L. (*Leguminosae*) // Annals of Botany. – 2000, vol. 86, p. 339–345

17. Lemežienė N., Kanapeckas J., Tarakanovas P. et al. Analysis of dry matter yield structure of forage grasses // Plant Soil Environment. – 2004, vol. 50, No. 6, p. 277–282

18. Lemežienė N. Timothy (*Phleum pratense L.*) breeding: methods and new varieties // 60 years of research at the Latvian Agriculture Institute. – Skriveri, Latvia, 2006, p. 131–135

19. Mantel N. The detection of disease clustering and a generalized regression approach // Cancer Research. – 1967, vol. 27, p. 209–220

20. Morell M. K., Peakall R., Appels R. et al. DNA profiling techniques for plant variety identification // Australian Journal of Experimental Agriculture. – 1995, vol. 35, p. 807–819

21. Nei M. Genetic distance between populations // American Naturalist. – 1972, vol. 106, p. 283–292

22. Pasteur N., Pasteur G., Bonhomme F. et al. Practical isozymes genetics. – New York, USA, 1988, p. 112–143

23. Perný M., Kolarčik V., Majeský L. et al. Cytogeography of the *Phleum pratense* group (*Poaceae*) in the Carpathians and Pannonia // Botanical Journal of the Linnean Society. – 2008, vol. 157, p. 475–485

24. Pivorienė O., Pašakinskienė I. Genetic diversity assessment in perennial ryegrass and *Festulolium* by ISSR fingerprinting // Zemdirbyste-Agriculture. – 2008, vol. 95, No. 2, p. 125–133

25. Posselt U. K., Barre P., Brazauskas G. et al. Comparative analysis of genetic similarity between perennial ryegrass genotypes investigated with AFLPs, ISSRs, RAPDs and SSRs // Czech Journal of Genetics and Plant Breeding. – 2006, vol. 42, No. 3, p. 87–94

26. Qian W., Ge S., Houng D. Y. Genetic variation within and among populations of a wild rice *Oryza granulata* from China detected by RAPD and ISSR markers // Theoretical and Applied Genetics. – 2001, vol. 102, p. 440–449

27. Tadesse W., Bekele E. Isozymes variability of grasspea (*Lathyrus sativus* L.) in Ethiopia // Lathyrus Lathyrism Newsletter. – 2001, p. 43–46

28. Tosun M., Akgün I., Taşpinar M. S. et al. Determination of variations of some enzymes in Orchardgrass (*Dactylis glomerata* L.) ecotypes // Acta Agriculturae Scandinavica. – 2002, vol. 52, p. 110–115

29. Weber J. L., May P. E. Abundant class of human DNA polymorphism which can be typed using the polymerase chain reaction // The American Journal of Human Genetics. – 1989, vol. 44, p. 388–396

30. Yu J., Mosjidis J. A, Klingler K. A. et al. Isozyme diversity in North American cultivated red clover // Crop Science. – 2001, vol. 41, p. 1625–1628

31. Zietkiewicz E., Rafalske A., Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification // Genome. – 1994, No. 20, p. 178–183

ISSN 1392-3196
Žemdirbystė-Agriculture, t. 96, Nr. 3 (2009), p. 47–57
UDK 633.265:633.264:575

Motiejuko (*Phleum* spp.) rūšių genetinės įvairovės atskleidimas, naudojant izofermentinius ir ISSR žymenis

K. Jonavičienė, V. Paplauskienė, G. Brazauskas
Lietuvos žemdirbystės institutas

Santrauka

Genetinė įvairovė ir genetiniai ryšiai tarp trijų *Phleum* rūšių – *P. pratense*, *P. bertolonii* bei *P. phleoides* – tirti naudojant tris izofermentus ir penkis paprastų pasikartojančių sekų intarpų (ISSR) pradmenis. Ištyrus 100 augalų, reprezentuojančių 10, daugiausia lietuviškos kilmės, motiejuko populiacijų, pagal izofermentinius ir ISSR žymenis nustatytas atitinkamai 47,7 ir 57,3 % polimorfiškumas. *Shanon* genetinės įvairovės indeksas izofermentų ir ISSR tyrimų metu kito atitinkamai nuo 0,124 iki 0,303 ir nuo 0,248 iki 0,326. Apskaičiuotas genetinis atstumas tarp populiacijų izofermentų tyrimų metu siekė 0,089–0,784, o ISSR tyrimų – 0,123–0,667. Pagal gautus rezultatus sudarytos izofermentų ir ISSR dendrogramos atskleidė panašią tipologiją. 10 populiacijų, atitinkamai pagal rūšis, išsiskirstė į tris pagrindines grupes ISSR dendrogramoje ir į dvi grupes izofermentų dendrogramoje. Tyrimai parodė, kad izofermentų ir ISSR metodai yra efektyvūs motiejukų rūšių polimorfizmui nustatyti. Gauti duomenys ateityje gali būti pritaikyti kuriant pašarinių žolių selekcines programas.

Reikšminiai žodžiai: izofermentai, ISSR žymenys, *Phleum*, rūšys, genetinis atstumas, polimorfizmas.